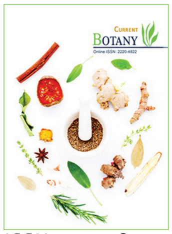
Current Botany 2025, 16: 18-30
doi: 10.25081/cb.2025.v16.9263
https://updatepublishing.com/journal/index.php/cb

Research Article



ISSN: 2220-4822

Changes in germination, early seedling growth and morpho-physiology of sesame under PEG-induced osmotic Stress

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ABSTRACT

The present study investigates the impact of polyethylene glycol (PEG) (0 and 25%) induced osmotic stress on germination, growth, morpho-physiological responses and stress tolerance indices of thirteen sesame (*Sesamum indicum* L.) genotypes at an early seedling stage. The experiments were conducted under lab-based controlled cultural conditions with two levels of PEG-6000 (0% and 25%) and thirteen genotypes using diverse seed colours (white, brown, light brown, and black) and twenty-two variables subjected to multivariable analysis. At 25% concentration, the principle component analysis (PCA) biplot classified thirteen sesame genotypes into two groups according to F values. The PCA biplot placed the tolerant (group-I) and sensitive (group-II) genotypes on opposite sides. Group-I contains eight genotypes viz. with black seed colour (IC-132300, GT-10), white seed colour (IC-96229, IC-132171, IC-205471, IC-203962) and light brown seed colours (IC-204966 and IC-131500) Group II contains five sensitive genotypes with brown seed colours such as YLM-17, YLM-66, YLM-11, Madhavi and Gowri, contributed from fresh weight, dry weight, chl a, chl b, total chlorophyll, carotenoids and chlorophyll stability index. The present study offers breeders a laboratory-based, reliable, and quick method for screening sesame germplasm to identify and develop drought-tolerant genotypes contributed from the rate of germination, germination percentage, root length, shoot length, root/shoot ratio, seedling length, dry weight, germination percentage stress tolerance index, root length stress tolerance index, shoot length stress tolerance index, seedling length stress tolerance index, fresh weight stress tolerance index, dry weight stress tolerance index, seedling vigour index and chl a/b.

Received: November 04, 2024
Revised: February 13, 2025
Accepted: February 14, 2025
Published: March 02, 2025

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KEYWORDS: Early seedling stage, Osmotic stress, Polyethylene glycol, Principal component analysis, Seed germination, Sesame

INTRODUCTION

Compared with cereal crops, research on oilseed crops has been limited, although demand for vegetable oil is expected to increasing almost 200 billion kilograms by 2030 (Langyan *et al.*, 2022). Sesame (*Sesamum indicum* L.; family Pedaliaceae), the ninth most cultivated oil seed in the world, has the potential to meet the rising global demand for vegetable oils and combat nutritional deficiencies in developing countries (Dossa *et al.*, 2017). Sesame is consumed by humans worldwide for edible seeds, oil, paste, cake, and confectionary purposes. Sesame is cultivated in over 70 countries, with India, China, and Myanmar accounting for 52% of global production and the provisional data shows a 6.4 million-ton seed production occupying 13 million hectares.

Sesame seeds are small and flattened and can present varied colours ranging from white to black, passing through to brown to golden yellow. Most current cultivars contain 50-60% oil and 18-24% protein. In addition, sesame oil is rich in antioxidants such as lignans, among lignans, sesamin and sesamolin, which have health benefits and cosmetic applications (Dossa *et al.*, 2017). Drought is a significant abiotic stress, causing soil water volume to decrease and become too tightly bound to soil particles, affecting plant growth, physiological and metabolic changes, and altering the availability of nutrients in the soil (Aqaei *et al.*, 2020). Sesame is cultivated in drought-prone and marginal areas with minimal water and nutrient supply. It is sensitive to drought stress at germination and reproductive stages, leading to reduced growth and loss in seed yield. In addition, the quality of the sesame oil and protein content is also reduced by severe drought stress (Dossa *et al.*, 2019). Therefore, it is essential to select appropriate sesame germplasm and apply breeding techniques for developing drought-resistant sesame cultivars (You *et al.*, 2019; Kouighat *et al.*, 2021).

Sesame has a large number of diverse varieties due to long-term natural and artificial selection coupled with a wide geographic distribution. Efforts have been made to characterize the genetic diversity based on phenotypic traits and molecular markers (Pandey *et al.*, 2015; Strvridou *et al.*, 2021). However, screening drought-tolerant genotypes has been limited due to a lack of understanding of tolerance mechanisms and unreliable screening methods (Pandey *et al.*, 2021). Sesame genotypes have been assessed for drought tolerance using various methods, including biochemical markers (Hota *et al.*, 2019), physiological traits and indices (Gopika *et al.*, 2022), agro-morphological-traits and seed yield-based indices (Dossa *et al.*, 2017; Ravitej *et al.*, 2019; Sravanthi *et al.*, 2021; Baghery *et al.*, 2022) and transcriptomic and metabolomic profiling (You *et al.*, 2019).

The drought experiments in sesame were primarily conducted in fields or pots, with few genotypes at reproductive or terminal stages (Dossa *et al.*, 2017; Sravanthi *et al.*, 2021; Kouighat, *et al.*, 2021; Baghery *et al.*, 2022). Multivariate statistics like correlation analysis, principal component analysis (PCA), and clustering analysis (CA) were used to analyse many variables simultaneously to establish genotype relationships and identify superior genotypes under water stress conditions (Sravanthi *et al.*, 2021). The effect of water deficit was studied in the field using eleven sesame genotypes viz. IC 132171, IC132186, IC 204445, IC 131500, IC 132207, IC 205471, IC 203962, IC 205353, IC 96229, and IC 204966 and GT 10 at reproductive stage. The morpho-physiological and yield traits under water stress (WS) significantly decreased compared to well-water conditions (WW). PCA revealed that traits like capsules/plants, total dry matter, stomatal conductance, and transpiration rate correlated with variation in drought tolerance in sesame genotypes. Two varieties IC 132207 and IC 205471 were stable performers under WS and WW conditions (Sravanthi *et al.*, 2021). The pot/field screening method has the disadvantages of being costly and time-consuming. Nonetheless, drought cannot be easily controlled in the field because rainfall can impede water deficit (Mude *et al.*, 2023).

Osmotic stress at the early seedling stage was commonly achieved using chemical molecules such as polyethylene glycol (PEG), mannitol, sodium chloride (NaCl), sucrose and glucose (Lawlor, 1970). Recent studies have demonstrated the potential of imposing PEG-induced osmotic stress and the use of multivariate statistics for the classification of drought-tolerant and sensitive genotypes of sunflower (Razzaq *et al.*, 2017), finger millet (Mude *et al.*, 2023) and tomato (Sivakumar *et al.*, 2023). While previous research in sesame has explored multivariate analysis for field studies, to date, limited studies on the sesame seed germination and early seedling growth in the presence of PEG-induced osmotic stress under lab-controlled conditions and classification of stress-sensitive and tolerant genotypes by multivariate analysis. Therefore, the present study aims to demonstrate the impact of PEG-induced osmotic stress on germination, early seedling growth, morpho-physiological parameters, stress tolerance indices and the use of multivariate statistics to classify sesame genotypes for differential drought tolerance, aiding future breeding programs and 'omics' research.

MATERIALS AND METHODS

Seed Material and Treatments

Sesame genotypes used in this study were obtained from the Indian Council of Agriculture Research (ICAR)-Indian Institute of Oil Research (IIOR), located in Hyderabad, Telangana, India, as well as from the Regional Agriculture Research Station (RARS), affiliated with Acharya N. G. Ranga University (ANGRAU) in Tirupati, Andhra Pradesh, India. The research was conducted in a plant tissue culture room from 2021 to 2022 at the Department of Botany, Yogi Vemana University, Kadapa, Andhra Pradesh, India. Drought treatments were induced by employing a solution of polyethylene glycol 6000 (PEG-6000) to increase osmotic potentials comparable to those observed in the field. The choice of PEG-6000 was based on the previous studies that found that varying PEG concentrations stimulate drought effect on germination and seedling growth in sesame (Kouighat *et al.*, 2021; Liang *et al.*, 2021). We screened 24 genotypes from various geographical locations and diverse seed colours (white, brown, light brown and black) with two levels of PEG-6000 induced osmotic stress (0% and 25%) under lab-based controlled conditions for germination. Following the overall germination response, 13 genotypes (Table 1) were subjected to PEG-induced osmotic stress and multivariate statistics to assess early seedling growth. The experiments followed a completely randomized design (CRD) with two factors: sesame genotypes and two levels of PEG-6000. Each treatment combination was repeated three times, using twelve seeds.

Germination Studies

The seeds were immersed in water for five hours at room temperature (30±2 □), and surface sterilized with 1% (v/v) aqueous sodium hypochlorite for five minutes. The disinfected seeds were rinsed five times with sterilized distilled water for 5 min and dried on filter paper before planting in nursery trays (L: 37 cm × B: 27 cm × H: 3 cm) with 70 cylindrical tubes (10 cm diameter and 12 cm depth), each filled with 30 g of sterilized coco-peat (Sri Balaji Agro-Services, Madanapalli, Andhra Pradesh, India). The trays were placed in a culture room, covered, and incubated at 25±2 □ in the dark. The average germination period was between 4 to 7 days for all seed varieties under control (0%) and 25% PEG-induced osmotic conditions. The seeds have started germination on the fourth day of sowing. Upon germination, the radicle ruptures the coleorhiza (root sheath) and emerges from the seeds. Seeds were removed gently from the coco peat and laid on the grid paper, and each radicle was measured. The seeds whose root/radicle length is 2 mm or more are considered germinated. The germination percentage (G%) is calculated after seven days as follows; the number of germinated seeds/the total number of seeds × 100. The rate of germination (ROG) is determined

Table 1: Impact of PEG-induced osmotic stress on germination in Sesame genotypes

Genotypes	Seed Color	Type	Geographical Origin	PEG (%)	Germination	
					Germination (%)	Rate of Germination
GT-10	Black	National Check	Gujarat	0	88.88±2.77	8.66±0.88
				25	74.99±8.33^{cde}	7.00±1.15^{cd}
IC-132300	Black	Land race	Arunachal Pradesh	0	86.11±5.55	7.33±0.33
				25	83.33±4.81^{cde}	7.00±0.57^{bcd}
IC-96229	White	Other	Karnataka	0	80.55±10.01	7.33±0.66
				25	74.99±4.81^{bcde}	6.33±0.88^{bcd}
IC-132171	White	Land Race	Tamil Nadu	0	86.11±2.77	7.66±1.20
				25	83.33±4.81^{cde}	6.66±1.33^{bcd}
IC-203962	White	Unknown	Uttar Pradesh	0	91.66±4.81	8.00±0.57
				25	77.77±7.34^{cde}	7.00±0.57^{bcd}
IC-205471	White	Unknown	Himachal Pradesh	0	91.66±4.81	9.66±0.88
				25	80.55±2.77^{de}	6.67±0.66^{d}
IC-131500	Light Brown	Unknown	Punjab	0	83.33±8.33	7.66±1.45
				25	80.55±2.77^{cde}	6.66±0.33^{bcd}
IC-204966	Light Brown	Unknown	Maharashtra	0	100.0±0.00	9.33±0.88
				25	88.88±2.77^{e}	8.00±1.15^{d}
Gowri	Brown	Elite Line	Andhra Pradesh	0	80.55±5.55	4.00±0.57
				25	55.55±2.77^{bcd}	2.66±1.45^{a}
Madhavi	Brown	Elite Line	Andhra Pradesh	0	66.66±4.81	5.00±0.57
				25	51.94±6.56^{abc}	4.00±0.57^{ab}
YLM-11	Brown	Elite Line	Andhra Pradesh	0	74.99±4.81	2.66±1.45
				25	36.11±7.34^{ab}	1.33±0.66^{a}
YLM-17	Brown	Elite Line	Andhra Pradesh	0	61.11±2.77	3.66±0.88
				25	19.44±10.0^{a}	1.66±1.20^{a}
YLM-66	Brown	Elite Line	Andhra Pradesh	0	94.44±2.77	6.66±0.33
				25	44.44±55.8^{bcde}	3.00±1.52^{abc}

Data are mean of three replicates±standard error of three replications; all statistical differences were presented relative to the 0% PEG (control); Means with different alphabetical letters (a and b) within the same column are significantly different ($p<0.05$) according to Tukey's Multiple Comparison Test; ns=non-significant

using the following formula as described by (Carleton *et al.*, 1968). ROG = [(Ni/Di)], where 'Ni' represents the number of seeds germinated between counts and Di represents the day of counting.

Measurement of Growth and Morphological Parameters

Upon germination after seven days, seedlings were incubated in 16 h of light and 8 hours of darkness to encourage growth. The seedlings were supplied distilled water when required to serve as a control (0%), while a 25% concentration of PEG-6000 was applied to each tray hole for osmotic treatment. Seedlings were harvested on the 21st day to assess growth and morpho-physiological studies. The growth characteristics, such as fresh (FW) and dry weights (DW), were evaluated. The seedlings were gently removed from the coco peat medium. The roots were gently cleaned with water to eliminate moisture and then placed on tissue paper. An electronic balance (ELICO Company, India) was used to measure the FW of the seedlings. Afterwards, the seedling was placed on aluminium foil and kept in a hot air oven (LG-1 Wave MEZ 64, 748, 904) at 70 □ for 48 h to obtain DW. The morphological parameters such as seedling length (SLL), shoot length (SL), and root length (RL) were determined using the straight ruler method and the measurements were taken in centimetres (cm). The root-to-shoot ratio (RSR) was calculated as root length (RL)/shoot length (SL).

PHYSIOLOGICAL STUDIES

Relative Water Content (RWC)

The RWC of sesame leaves was measured according to the method described by Barrs and Weatherly (1962). The leaves weighed fresh (FW, g) and placed in distilled water in the refrigerator (4 °C) until fully rehydrated for 8 h in the dark, weighed to obtain turgid weight (TW, g). The leaves were dried in the hot air oven at 70 °C for 24 h, and dry weight (DW, g) was obtained. The RWC was calculated using the following formula;

$$\text{Relative Water Content (\%)} = (\text{FW-DW})/(\text{TW-DW}) \times 100$$

Photosynthetic Pigments

Pigments chlorophyll 'a' (chl a), chlorophyll 'b' (chl b), and total chlorophyll (T. chl) and carotenoids (car.) of sesame seedlings were determined by the method of Lichtenthaler (1987) and Wellburn (1994), respectively. Pigments were extracted from fresh leaves (200 mg) using 80% acetone and centrifuged at 4000 ×g for 5 minutes. The absorbance of the extracts was measured at 663 and 645 nm and calculated using the following equations: Chl a. (mg/g FW) = 12.7 (O.D. at 663) - 2.69 (O.D. at 645) × (v/w × 1000), Chl b. (mg/g FW) = 22.9 (O.D. at 645) – 4.68 (O.D. at 663) × (v/w × 1000), T. chl. (mg/g FW) = 20.2 (O.D. at 645) + 8.02 (O.D. at 663) × (v/w × 1000), The chlorophyll a/b ratio (chl a/b) was calculated as chl a/chl b and Car (μg/g FW) = [1000 (O.D. at 470) - 2.86 (chl a)]/221

Stress Tolerance Indices

The seedling vigour index (SVI) was determined using the formula described by Abdul-Baki and Anderson (1973). Seedling Vigour Index (%) = (the seedling's length/the germination percentage) ×100. The other stress tolerance indices, such as germination percentage stress index (GPSI), root length stress index (RLSI), shoot length stress index (SLSI), seedling length stress index (SLLSI), fresh weight stress index (FWSI) and dry weight stress index (DWSI) were determined according to the International Seed Testing Association (ISTA) protocols (ISTA, 2019). Germination Percentage Stress Index (GPSI) (%) = (Germination percentage under stress condition/germination percentage under control) × 100, Root Length Stress Index (%) = (the root length under stress conditions/the root length under control) × 100, Shoot Length Stress Index (%) = (the shoot length under stress conditions/the shoot length under control) × 100, Fresh Weight Stress Index (FWSI) (%) = (fresh weight under stress conditions/fresh weight under control) × 100, Dry Weight Stress Index (DWSI) (%) = (dry weight under stress conditions/dry weight under control) × 100. The Chlorophyll Stability Index (CSI) was calculated using the method of Murthy and Mujumdhar (1962); Chlorophyll Stability Index (%) = (the total chlorophyll content under stress conditions/ the total chlorophyll content under control) × 100.

Statistical Analysis

The study analyzed data using mean values±standard errors. The analysis of variance (ANOVA) was conducted for each trait under absence (0%) and 25% PEG-induced stress using a two-way ANOVA and Tukey's multiple comparison tests to determine significant genotypic differences at $p<0.05$ (IBM SPSS-16.0 version). Statistical methods like the Pearson method, cluster analysis (CA), and principal component analysis (PCA) were used to determine significant genotypic differences. XL-STAT 2022.2.1.1304 was used for multivariate analysis and graphical representations, ensuring accurate and reliable results (Addinsoft, 2019). The study analysed quantitative traits like germination parameters viz. germination percentage (G%); rate of germination (ROG), growth parameters viz. fresh weight (FW), dry weight(DW), morphological parameters viz. seedling length (SLL); root length (RL); shoot length (SL); root to shoot ratio (RSR); and physiological traits viz. relative water content (RWC); pigment composition viz. chlorophyll a (chl a); chlorophyll b (chl b); chlorophyll a/b ratio (chl a/b); total chlorophyll (T. chl) and carotenoids (car) parameters and stress tolerance indices; germination percentage stress index (GPSI), seedling vigour index (SVI); root length stress index (RLSI); shoot length stress index (SLSI), seedling length stress index (SLLSI), fresh weight stress index (FWSI) and dry weight stress index (DWSI) and chlorophyll stability index (CSI) of sesame genotypes using multivariate analysis. Based on Ward's (1963) method, the CA used Euclidean distance to cluster sesame genotypes. PCA defined trait variance for both control and 25% PEG-induced drought stress conditions. Eigen values and factor scores were utilised to determine stress-tolerant and susceptible genotypes (Allel *et al.*, 2016).

RESULTS

Germination Studies

The data presented in Table 1 reveal that seeds of thirteen genotypes began to germinate after the 4th day under control and PEG-induced osmotic stress conditions. Germination percentage (G%) varied among genotypes with diverse seed colours such as white, light brown, brown and black. The G% ranged from 100% to 61.11% under control conditions. Adding 25% PEG concentration to coco peat significantly ($p<0.05$) reduced G% in all genotypes. The genotype IC-204966 had significantly ($p<0.05$) the highest G% (88.88%) and YLM-17 had significantly ($p<0.05$) the lowest G% (19.44%) under PEG-induced osmotic stress (Table 1). The genotype IC-204966 with light brown seed colour had significantly ($p<0.05$) higher G% than brown, black and white seed colours under control and PEG-induced osmotic stress conditions. The genotypes (YLM-17 and YLM-66) with brown seeds had significantly ($p<0.05$) lower G% than other seed types. An interaction between genotype and PEG-induced osmotic stress was observed for germination (ROG) rate. The ROG was higher under control conditions, ranging from 2.66 to 9.66. The ROG was significantly ($p<0.05$) reduced at 25% PEG concentration than control and ranged from 1.33 to 8.0. The genotype YLM-11 had significantly ($p<0.05$) the lowest ROG (1.33) and the genotype IC-204966 had significantly ($p<0.05$) the highest ROG (8.00). Brown seeds showed significantly ($p<0.05$) lower ROG than other seed types (Table 1).

Early Seedling Growth and Morphological Studies

The early seedling growth parameters such as fresh weight (FW) and dry weight (DW) and morphological parameters such as root length (RL), shoot length (SL), root-to-shoot ratio (RSR) and seedling length (SLL) under controlled and 25% PEG induced conditions reported in Table 2. The 25%-PEG induced osmotic stress significantly ($p<0.05$) affected all early seedling growth and morphological parameters, including FW, DW, RL, SL, RSR and SLL (Table 2). Comparatively, the shoot growth was less affected than the root length. At 25% PEG-induced stress, significantly ($p<0.05$) the highest FW (130 mg), DW (23 mg) and SLL (5.90 cm) were observed in the IC-132171 genotype. Significantly ($p<0.05$) the lowest FW (119 mg) and DW (15 mg) were observed in the YLM-11 genotype. The highest RL (1.70 cm) and SL (4.18 cm) values were recorded in the genotype IC-132171. A significant ($p<0.05$) decrease in RL (0.33 cm) and SL (0.68 cm) was found in the genotype YLM-17. The RSR values were varied under PEG-induced osmotic stress with the genotype. Significantly ($p<0.05$), the lowest value of RSR (0.16) was found in the genotype YLM-17, and the highest RSR was with GT-10 (1.04) (Table 2).

Table 2: Impact of PEG-induced osmotic stress on growth and morphology in Sesame at early seedling stage

		Growth and Morphology Parameters					
		Fresh Weight (mg)	Dry Weight (mg)	Root Length (cm)	Shoot Length (cm)	Root to Shoot Ratio (%)	Seedling Length (cm)
GT-10	0	131±1.2	23±1.4	1.54±0.0	4.38±0.4	0.35±0.01	5.93±0.5
	25	130±1.8^{b}	23±0.3^{a}	1.16±0.1^{a}	3.55±0.0^{ab}	1.04±0.68^{a}	4.71±0.1^{ab}
IC-132300	0	131±1.7	22±2.0	1.46±0.1	3.95±0.1	0.36±0.03	5.42±0.3
	25	123±2.6^{ab}	21±1.4^{bc}	0.77±0.1^{a}	2.85±0.1^{ab}	0.27±0.03^{a}	3.62±0.2^{ab}
IC-96229	0	132±3.4	26±1.2	1.27±0.0	3.78±0.0	0.33±0.02	5.01±0.0
	25	126±1.1^{ab}	22±1.1^{bc}	1.01±0.0^{a}	3.28±0.1^{ab}	0.30±0.02^{a}	4.29±0.2^{ab}
IC-132171	0	136±1.7	28±1.1	1.88±0.1	4.7±0.2	0.39±0.02	6.58±0.3
	25	126±1.2^{b}	22±1.4^{c}	1.70±0.1^{a}	4.18±0.2^{b}	0.41±0.01^{a}	5.90±0.4^{b}
IC-203962	0	127±2.3	23±0.8	1.37±0.1	3.62±0.0	0.62±0.05	5.00±0.1
	25	122±0.6^{ab}	20±2.3^{bc}	1.19±0.0^{a}	3.0±0.0^{ab}	0.39±0.01^{a}	4.19±0.1^{ab}
IC-205471	0	128±1.7	22±2.0	1.67±0.1	4.10±0.2	0.40±0.04	5.77±0.3
	25	122±1.7^{ab}	18±1.4^{bc}	1.29±0.0^{b}	3.38±0.0^{ab}	0.38±0.10^{a}	4.67±0.0^{ab}
IC-131500	0	124±2.5	22±0.6	1.23±0.1	3.27±0.2	0.37±0.09	4.50±0.3
	25	124±1.1^{a}	21±2.4^{b}	0.66±0.1^{a}	2.32±0.2^{ab}	0.28±0.03^{a}	2.98±0.2^{ab}
IC-204966	0	127±1.5	22±1.1	1.70±0.0	5.13±0.1	0.33±0.00	6.25±0.1
	25	121±0.8^{ab}	18±1.5^{bc}	1.43±0.0^{a}	3.89±0.2^{b}	0.36±0.01^{a}	5.33±0.3^{ab}
Gowri	0	136±1.7	19±2.0	1.44±0.1	3.18±0.9	0.55±0.16	4.63±1.1
	25	125±0.5^{b}	17±2.3^{c}	1.09±0.5^{a}	2.46±1.3^{ab}	0.30±0.15^{a}	3.55±1.8^{ab}
Madhavi	0	130±2.1	18±2.8	1.43±0.7	3.77±1.9	0.24±0.12	5.21±2.6
	25	123±3.2^{ab}	15±0.3^{bc}	0.94±0.7^{a}	1.55±0.7^{ab}	0.40±0.20^{a}	2.49±1.8^{ab}
YLM-11	0	132±3.5	20±0.8	1.32±0.6	2.77±1.4	0.29±0.15	4.09±2.1
	25	119±2.0^{ab}	15±2.0^{bc}	0.76±0.7^{a}	0.80±0.8^{a}	0.31±0.31^{a}	1.56±1.5^{ab}
YLM-17	0	129±0.0	24±2.0	1.30±0.8	2.39±1.2	0.34±0.19	1.85±1.1
	25	128±3.1^{ab}	23±3.5^{bc}	0.33±0.3^{a}	0.68±0.6^{a}	0.16±0.16^{a}	0.67±0.6^{a}
YLM-66	0	133±1.1	27±0.8	3.32±0.4	4.71±0.7	0.71±0.02	8.04±1.2
	25	127±1.8^{ab}	19±2.6^{bc}	0.76±0.7^{a}	0.80±0.8^{ab}	0.31±0.31^{a}	1.56±1.5^{ab}

Data are mean of three replicates±standard error of three replications; all statistical differences were presented relative to the 0% PEG (control); Means with different alphabetical letters (a and b) within the same column are significantly different ($p<0.05$) according to Tukey's Multiple Comparison Test; ns=non-significant

Table 3: Impact of PEG-induced osmotic stress on Photosynthetic pigment composition in Sesame genotypes

Genotypes	PEG (%)	Photosynthetic Pigment Composition				
		Chlorophyll a (mg/g FW)	Chlorophyll b (mg/g FW)	Chlorophyll a/b (:)	Total Chlorophyll (mg/g FW)	Carotenoids (μg/g FW)
GT-10	0	20.69±2.20	16.15±0.49	1.27±0.1	65.4±5.3	1217.0±56.58
	25	16.76±3.44^{c}	9.30±0.77^{c}	1.84±0.41^{a}	47.6±5.2^{c}	1048.7±177.4^{b}
IC-132300	0	13.16±0.26	17.34±0.18	0.75±0.0	59.6±0.5	1133.8±69.28
	25	15.71±0.78^{bc}	6.32±2.05^{bc}	2.98±0.77^{a}	39.3±4.0^{bc}	1167.1±7.97^{b}
IC-96229	0	6.20±0.79	4.70±0.70	1.32±0.0	20.4±2.8	465.9±62.31
	25	4.94±0.58^{a}	5.23±1.15^{ab}	1.03±0.24^{a}	19.5±3.1^{a}	432.6±53.63^{a}
IC-132171	0	8.38±3.70	12.34±3.61	1.01±0.5	40.8±6.5	602.8±226.9
	25	9.55±1.80^{ab}	8.63±1.46^{abc}	1.15±0.21^{a}	34.5±4.9^{abc}	416.5±254.0^{ab}
IC-203962	0	18.50±0.47	10.37±1.42	1.87±0.3	52.7±2.6	745.7±20.54
	25	10.14±0.39^{bc}	4.69±1.11^{abc}	2.40±0.52^{a}	26.7±3.0^{abc}	789.2±25.76^{ab}
IC-205471	0	13.12±1.88	2.21±0.52	7.12±2.5	26.0±2.5	1251.8±285.5
	25	10.50±0.72^{abc}	6.62±0.38^{ab}	1.60±0.16^{ab}	31.5±1.0^{ab}	382.2±132.9^{ab}
IC-131500	0	10.15±0.51	9.15±0.46	1.11±0.1	36.6±0.5	788.2±42.36
	25	6.39±0.73^{ab}	3.83±0.67^{abc}	1.87±0.60^{a}	18.7±0.2^{ab}	493.5±65.22^{ab}
IC-204966	0	14.30±0.63	0.99±0.34	21.3±10.1	25.2±1.7	1140.8±47.68
	25	6.99±1.69^{abc}	6.15±0.71^{a}	1.24±0.47^{b}	24.8±1.1^{ab}	605.9±150.3^{ab}
Gowri	0	13.27±1.75	10.22±2.42	1.52±0.5	47.3±3.6	375.5±142.7
	25	15.58±2.19^{bc}	9.59±0.39^{abc}	1.61±0.16^{a}	46.3±4.3^{bc}	759.1±81.51^{ab}
Madhavi	0	21.07±0.47	17.61±0.92	1.20±0.0	72.9±2.8	1538.7±108.5
	25	5.01±0.96^{abc}	5.52±0.33^{bc}	0.89±0.12^{a}	20.3±2.2^{bc}	374.9±43.47^{ab}
YLM-11	0	12.14±4.34	8.97±2.86	1.26±0.11	39.4±13.3	580.8±182.6
	25	13.32±1.20^{abc}	10.15±1.35^{abc}	1.30±0.07^{a}	43.9±4.9^{abc}	954.5±97.09^{ab}
YLM-17	0	7.23±3.72	5.64±2.40	1.14±0.1	24.1±11.3	350.5±111.4
	25	8.24±4.99^{ab}	6.87±3.99^{abc}	1.23±0.18^{a}	28.4±16.8^{ab}	626.9±412.9^{ab}
YLM-66	0	18.43±1.10	13.78±1.40	1.32±0.0	60.1±4.8	966.4±190.9
	25	16.64±0.32^{c}	12.07±1.04^{c}	1.37±0.12^{a}	53.3±2.5^{c}	936.4±178.3^{ab}

Data are mean of three replicates±standard error of three replications; all statistical differences were presented relative to the 0% PEG (control); Means with different alphabetical letters (a and b) within the same column are significantly different ($p<0.05$) according to Tukey's Multiple Comparison Test; ns=non-significant

Physiological Studies

Relative water content

Under control conditions, leaves of all genotypes maintained higher relative water content (RWC) in the range from 84% to 95% (Figure 1). However, when exposed to 25% PEG-induced osmotic stress, leaves of all varieties exhibited significant (p<0.05) levels of decrease in RWC values. The lowest RWC showed by IC-204966 was 76.31% and the highest RWC was by IC-131500, which was 92.79% under 25% PEG-induced drought stress conditions (Figure 1).

Photosynthetic Pigments

The differences in chl a, chl b, chl a/b ratio, total chlorophyll and carotenoid values were observed among genotypes (Table 3). Imposing drought stress by applying 25% PEG caused a decrease in the contents of chl a, chl b, chl a/b ratio, total chlorophyll and carotenoids (Table 3). A significant (p<0.05) reduction in chl a content was observed in Madhavi, chl b content was in IC-132300 and Madhavi genotypes, chla/b ratio in IC-204966, total chlorophyll in Madhavi and carotenoids in IC-205471 and Madhavi genotypes. Among the genotypes tested, GT-10 retained significantly (p<0.05) high chl a (16.76 mg/g FW) when exposed to 25%-PEG-induced stress. At the same time, YLM-66 recorded significantly (p<0.05) the highest chl b (12.07 mg/g FW) and total chlorophyll (53.3 mg/g FW). 25%-PEG-induced stress also significantly (p<0.05) decreased carotenoid content in all the genotypes, ranging from 374.9 μg/g FW (Madhavi) to 1167.1 μg/g FW (IC-132300).

Stress Tolerance Indices

Different stress tolerance indices such as seedling vigour index (SVI), germination percentage stress index (GPSI), root length stress index (RLSI), shoot length stress index (SLSI), seedling stress index (SLLSI), fresh weight stress index (FWSI), dry weight stress index (DWSI) and chlorophyll stability index (CSI) had different estimates of genotypes under PEG-induced osmotic stress conditions (Table 4). At 25% PEG-induced stress, A significant (p<0.05) decrease in SVI was observed in the genotype YLM-17 (0.68). The GPSI and RLSI were also significantly (p<0.05) reduced in YLM-11, YLM-17 and YLM-66 than control. Significantly (p<0.05) lower values of SLSI and SLLSI were found in YLM-66, YLM-11, YLM-17 and Madhavi genotypes under 25%-PEG induced stress. A significant (p<0.05) decrease was found for FWSI in the genotype YLM-11 (89.78). The 25% PEG-induced osmotic stress significantly (p<0.05) affected the chlorophyll stability index (CSI) in Madhavi (27.89) (Table 4).

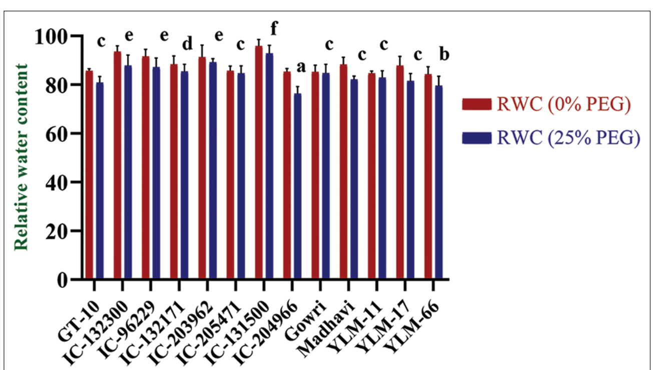

Figure 1: Impact of PEG-induced osmotic stress on relative water content (RWC) of sesame genotypes. Bars represent mean values of three biological replicates±standard error

Multivariate Analysis for Identification of Tolerant and Sensitive Genotypes

The study reveals that the genotypes showed distinct responses to control (0%) and 25% PEG concentration at the germination and early seedling stage. Multivariate analysis was conducted on the 0% and 25% PEG data to identify tolerant and sensitive sesame genotypes. The relationships among germination, growth and morpho-physiological parameters of sesame under control (data not shown) and 25% PEG-induced osmotic stress conditions were evaluated through correlation analysis (Table 5). Under 25%-PEG induced osmotic stress condition, eighteen parameters such as ROG, G%, RL, SL, SLL, FW, DW, Chla, Chl b, Chl a/b, T chl, GPSI, RLSI, SLSI, SLLSI, FWSI, DWSI and CSI were highly and positively correlated with G%, RL, SL,SLL, GPSI, RLSI, SLSI, SLLSI and SVI at p<0.05. However, no significant correlations (p<0.05) were noted between car, SVI, RWC and CSI. Highly significant (p<0.05) and positive correlations included ROG with G%, SL, SLL, GPSI, RLSI, SLSI, SLLSI, and SVI; G% with SL, SLL, GPSI, RLSI SLSI, SLLSI and SVI; RL with SL, SLL, RLSI and SVI; SL with SLL, GPSI, RLSI, SLSI, SLSI and SVI; SLL with GPSI, RLSI, SLSI, SLLSI and SVI; chl a with T chl and carotenoids. Our findings suggested that early seedling growth traits were significantly associated (at p<0.05) with germination and pigment composition among different traits tested.

Based on Ward's method, unsupervised agglomerative hierarchical clustering (AHC) was used to group data on germination, growth, morpho-physiological parameters and stress tolerance indices under control (Data not shown) and 25% PEG-induced-osmotic stress conditions into clusters of increasing dissimilarity (Figure 2). The dendrogram Figure 2 shows that the 13 genotypes were grouped into two major clusters as C1 and C2 at germination and early seedling stage. The C1, represented the 8 tolerant genotypes GT-10, IC-132300, IC-132171, IC-204966, IC-96229, IC-131500, IC-203962 and IC-205471. The C2 consisted of five sensitive genotypes: YLM-17, YLM-11, YLM-66, Madhavi and Gowri (Figure 2). The genotypes GT-10, IC 203962, and IC 205471 were already considered tolerant cultivars at the reproductive stage.

The PCA analysis examined the correlations between 13 genotypes and thirteen variables related to germination, growth and morpho-physiological parameters under control (Data not shown). PCA is a method used to convert original variables into new uncorrelated variables called 'components', which are linear combinations of actual variables not associated with each other.

Table 4: Impact of PEG-induced osmotic stress on stress tolerance indices of sesame genotypes

Genotypes	PEG (%)	Stress indices							
		SVI	GPSI (%)	RLSI (%)	SLSI (%)	SLLSI (%)	FWSI (%)	DWSI (%)	CSI (%)
GT-10	0	525.1±35.7	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0
	25	91.9±18.0[d]	84.2±8.1[a]	75.89±10.9[a]	83.11±10.3[a]	81.1±9.9[a]	99.48±0.6[a]	98.79±7.5[a]	72.41±1.9[a]
IC-132300	0	131.2±22.6	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0
	25	66.1±4.7[a]	98.3±12.2[a]	52.59±4.4[a]	72.22±5.4[a]	67.1±5.2[a]	94.46±2.8[a]	97.43±3.8[a]	65.85±6.0[a]
IC-96229	0	104.5±6.3	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0
	25	80.0±10.6[a]	97.2±16.8[a]	79.59±1.8[a]	79.13±3.5[a]	85.0±3.2[a]	95.15±3.3[a]	88.06±2.7[a]	99.21±17.7[a]
IC-132171	0	167.5±20.5	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0
	25	146.1±6.7[a]	97.2±8.2[a]	91.65±4.5[a]	88.85±2.2[a]	89.5±2.0[a]	92.90±0.8[a]	80.79±1.9[a]	92.08±27.0[a]
IC-203962	0	129.4±12.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0
	25	95.63±6.4[a]	85.9±12.0[a]	88.59±9.1[a]	82.73±2.4[a]	83.9±0.8[a]	96.38±1.8[a]	87.69±10.5[a]	51.30±8.0[a]
IC-205471	0	158.2±24.3	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0
	25	108.2±10.2[a]	88.0±2.3[a]	80.79±14.6[a]	83.11±5.1[a]	81.9±7.0[a]	94.62±2.6[a]	83.89±5.5[a]	95.48±22.1[a]
IC-131500	0	107.1±19.5	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0
	25	55.74±9.5[a]	99.2±13.1[a]	56.06±12.4[a]	72.97±12.5[a]	68.1±11.7[a]	97.86±0.9[a]	93.68±7.7[a]	51.35±1.5[a]
IC-204966	0	175.4±3.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0
	25	130.8±4.4[a]	88.8±2.7[a]	87.87±6.0[a]	76.14±6.1[a]	78.1±5.9[a]	95.57±1.5[a]	81.71±0.9[a]	99.17±6.2[a]
Gowri	0	117.4±7.6	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0
	25	66.0±33.7[a]	70.0±7.7[a]	69.07±36.1[a]	60.46±34.3[a]	62.8±34.7[a]	91.93±0.7[a]	88.65±11.0[a]	98.86±11.6[a]
Madhavi	0	96.33±49.3	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0
	25	44.4±29.5[a]	77.3±4.2[a]	39.30±25.2[a]	26.01±18.2[c]	29.7±20.4[b]	94.34±0.9[a]	89.02±11.7[a]	27.89±3.0[a]
YLM-11	0	98.68±45.6	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0
	25	19.88±19.8[a]	47.3±6.6[b]	30.70±30.7[a]	16.00±16.0[d]	20.8±20.8[c]	89.78±2.1[a]	73.93±8.3[a]	94.03±41.34[a]
YLM-17	0	86.08±56.6	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0
	25	0.68±0.6[a]	33.3±17.1[c]	11.90±11.9[b]	16.43±16.4[c]	14.5±14.5[c]	99.67±4.2[a]	95.23±22.2[a]	84.23±10.7[a]
YLM-66	0	318.9±47.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0
	25	26.24±26.2[d]	47.7±17.3[b]	18.35±18.3[b]	13.89±13.8[d]	15.7±15.7[c]	95.75±1.7[a]	71.12±10.9[a]	89.50±3.6[a]

Data are mean of three replicates±standard error of three replications; all statistical differences were presented relative to the 0% PEG (control); Means with different alphabetical letters (a and b) within the same column are significantly different ($p<0.05$) according to Tukey's Multiple Comparison Test; ns=non-significant

The PCA biplot revealed 59.46% of the total variation, where the first axes (F1) and second axes (F2) account for 38.07% and 21.39% of variation under control, respectively. The PCA analysis examined the correlations between 13 genotypes and 22 variables related to germination, growth, morpho-physiological parameters and stress tolerance indices under 25%-induced osmotic stress conditions (Figure 3). The PCA biplot revealed 61.58% of the total variation, with F1 and F2 axes accounting for 44.67% and 16.90% under 25% PEG-induced osmotic stress. The analysis classified 13 sesame genotypes and 22 variables into two groups, with tolerant (Group-I) and sensitive (Group-II) genotypes placed on diametrically opposite sides in the PCA biplot (Figure 3). A specific behaviour towards PEG-induced stress characterises each group (Figure 3). The group-I includes eight genotypes viz. GT-10, IC-96229, IC-205471, IC-203962, IC-204966 and IC-132171, IC-131500 and IC-132300 with contribution from the rate of germination, germination percentage, root length, shoot length, root/shoot ratio, seedling length, seedling vigor index. RLSI, SLSI, SLLSI, FWSI, DWSI and chl a/b. The group-II contains five genotypes viz. YLM-17, YLM-11, YLM-66, Gowri and Madhavi contributed from fresh weight, dry weight, chl a, chl b, total chlorophyll, carotenoids and CSI.

The study utilised principal component and cluster analysis to identify genotypes for stress tolerance, with the results indicating deviations in drought tolerance from control to 25% PEG-induced conditions, as shown in Table 6. The eight genotypes viz. with black seed colour (IC-132300, GT-10), white seed colour (IC-96229, IC-132171, IC-205471, IC-203962) and light brown seed colours (IC-204966 and IC-131500) are placed under a drought-tolerant group, while five genotypes with brown seed colours viz. YLM-17, YLM-66, YLM-11, Madhavi and Gowri are placed under the drought-sensitive group under 25% PEG treatment (Table 6). The contributing parameters for drought tolerance were ROG, G%, RL, SL, RSR, DW, SLL, RWC, GPSI, RLSI, SLSI, SLLSI, FWSI, DWSI, SVI and Chl a/b, whereas for drought susceptibility was contributed by FW, Chl a, Chlb, T. Chl, Car and CSI.

DISCUSSION

Germination (seedling), vegetative and reproductive (flowering, capsule/seed formation) stages are the three principal growth stages in the sesame crop cycle. In general, germination and reproduction stages are more vulnerable to the detrimental effects of drought stress. Sravanthi *et al*. (2021) conducted a field experiment to study the impact of water deficit on eleven sesame genotypes at the reproductive stage and yield characteristics. Compared to other crops, sesame has better tolerance. However, it remains susceptible to drought during germination and seedling stage (Boureima *et al*., 2016). The present study focused on the effect of osmotic stress on germination and early seedling growth of sesame under lab conditions using 13 genotypes, including the seven genotypes from previous studies such as stable performers (IC 205471), moderate performer (IC

Table 5: Correlation matrix for analysis variables under 25% PEG induced osmotic stress

Variables	ROG	G%	RL	SL	RSR	SLL	FW	DW	RWC	Chl a	Chl b	Chl a/b	T Chl	Car	GPSI	RLSI	SLSI	SLLSI	SVI	FWSI	DWSI	CSI
ROG	1																					
G%	**0.953**	1																				
RL	**0.597**	**0.684**	1																			
SL	**0.870**	**0.902**	**0.831**	1																		
RSR	0.367	0.295	0.386	0.412	1																	
SLL	**0.832**	**0.878**	**0.898**	**0.991**	0.418	1																
FW	-0.003	-0.124	-0.106	0.058	0.445	0.021	1															
DW	0.389	0.227	-0.069	0.314	0.248	0.232	**0.723**	1														
RWC	0.251	0.330	-0.124	0.146	-0.229	0.086	-0.121	0.278	1													
Chl a	-0.270	-0.218	-0.163	-0.214	0.291	-0.209	0.328	0.105	-0.166	1												
Chl b	-0.529	-0.440	0.021	-0.312	0.215	-0.243	0.269	-0.195	-0.534	**0.683**	1											
Chl a/b	0.426	0.443	-0.001	0.276	0.099	0.217	-0.040	0.344	0.515	0.405	-0.173	1										
T Chl	-0.370	-0.282	-0.020	-0.218	0.307	-0.178	0.272	-0.084	-0.353	**0.838**	**0.916**	0.207	1									
Car	-0.140	-0.146	-0.290	-0.221	0.270	-0.246	0.119	0.102	-0.130	**0.883**	0.482	**0.577**	**0.727**	1								
GPSI	**0.892**	**0.947**	**0.583**	**0.844**	0.240	**0.808**	-0.088	0.239	0.491	-0.301	**-0.566**	0.388	-0.425	-0.261	1							
RLSI	**0.791**	**0.855**	**0.858**	**0.951**	0.362	**0.958**	-0.093	0.162	0.221	-0.254	-0.335	0.228	-0.253	-0.272	**0.799**	1						
SLSI	**0.880**	**0.915**	**0.686**	**0.951**	0.362	**0.917**	0.057	0.407	0.401	-0.211	-0.429	0.443	-0.259	-0.176	**0.881**	**0.930**	1					
SLLSI	**0.864**	**0.908**	**0.731**	**0.961**	0.360	**0.936**	0.027	0.356	0.371	-0.230	-0.412	0.375	-0.269	-0.210	**0.877**	**0.960**	**0.993**	1				
SVI	**0.868**	**0.908**	**0.881**	**0.987**	0.377	**0.993**	-0.026	0.240	0.094	-0.223	-0.269	0.253	-0.198	-0.231	**0.822**	**0.944**	**0.917**	**0.930**	1			
FWSI	0.286	0.034	-0.290	0.038	0.309	-0.039	**0.618**	**0.709**	-0.013	-0.089	-0.326	0.111	-0.238	0.003	0.009	-0.075	0.124	0.064	-0.026	1		
DWSI	0.307	0.193	-0.236	0.224	0.276	0.121	0.411	0.542	0.358	-0.015	-0.524	0.418	-0.291	0.086	0.331	0.120	0.354	0.292	0.094	**0.585**	1	
CSI	-0.146	-0.052	0.242	0.165	-0.131	0.188	0.059	0.005	-0.364	0.095	0.499	-0.261	0.378	0.029	-0.201	0.148	0.066	0.102	0.183	-0.298	-0.437	1

Values in bold are different from 0 with a significance level alpha=0.05. ROG: Rate of Germination, G%: Germination Percentage (%), RL: root length (cm), SL: shoot length (cm), RSR: root/shoot ratio (:), SLL: seedling length (cm), FW: fresh weight (mg/g), DW: dry weight (mg/g), RWC: relative water content (%), chl a: chlorophyll a (mg/g FW), chl b: chlorophyll b (mg/g FW), chl a/b: chlorophyll a/b (:), T chl: total chlorophyll ((mg/g FW), Car: carotenoids (μg/g FW). GPSI: germination percentage stress tolerance index (%), RLSI: root length stress tolerance index (%), SLSI: shoot length stress tolerance index (%); SLLSI: seedling length stress tolerance index (%); SVI: seedling vigour index (%), FWSI: fresh weight stress tolerance index (%); DWSI: dry weight stress tolerance index (%); CSI: chlorophyll stability index (%)

203962), tolerant (GT 10) and others (IC 132171, IC 131500, IC 96229, IC 204966) varied under depending upon conditions (Sravanthi *et al.*, 2021). The study demonstrated that applying 25% PEG led to a decline in germination percentage (G%) and germination (ROG) rate. Similarly, germination percentage and germination rate were reduced in sesame (El Harfi *et al.*, 2016; Kouighat *et al.*, 2021), safflower (Zraibi *et al.*, 2011) and rapeseed (Channaoui *et al.*, 2019).

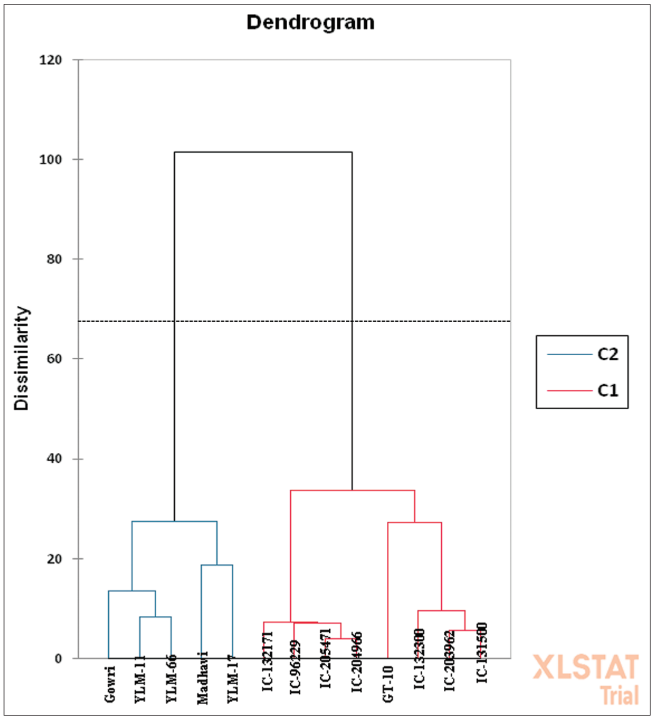

Figure 2: Dendrogram grouping analysis of thirteen sesame genotypes under 25% PEG-induced osmotic stress at germination and early seedling stage based on Euclidean Distance cluster analysis.

Sesame genotypes with different seed colours showed differential tolerance to the osmotic stress concerning G% and ROG. Genotypes with white, black and light brown seed colours were least affected under 25% PEG for G% and ROG. In contrast, the genotypes with brown colour seeds were severely affected. Our findings disagree with those of El Harfi *et al.* (2016), who reported that sesame seeds with yellow and dark brown colour recorded better germination percentages than black and white seeds. The seed colour impacts water uptake, gas diffusion, dormancy, germination and seedling emergence in sesame. Seed coat colour is also related to biochemical functions involved in protein and oil metabolism, antioxidant content and stress resistance. Generally, pale colour seeds contain more oil than dark-coloured ones (Cui *et al.*, 2021). Germination is linked to hydration, reserve use, respiration, enzyme and hormone activation, and metabolic pathway alternation (Hegarty, 1977). The highest germination percentage may be attributed to their increased hydration to initiate vital metabolic processes (Hahm *et al.*, 2009; El Harfi *et al.*, 2016).

The growth parameters such as fresh weight, dry weight, root length, shoot length and seedling length were severely inhibited by 25% PEG-induced osmotic stress compared to non-stress conditions, indicating that the growth in sesame is sensitive to water deficit conditions. The suppression of growth may be due to the drop in cellular dehydration, which is closely associated with the multiplication of cells and tissue growth (Hellal *et al.*, 2018). Boureima *et al.* (2016) and Kouighat *et al.*

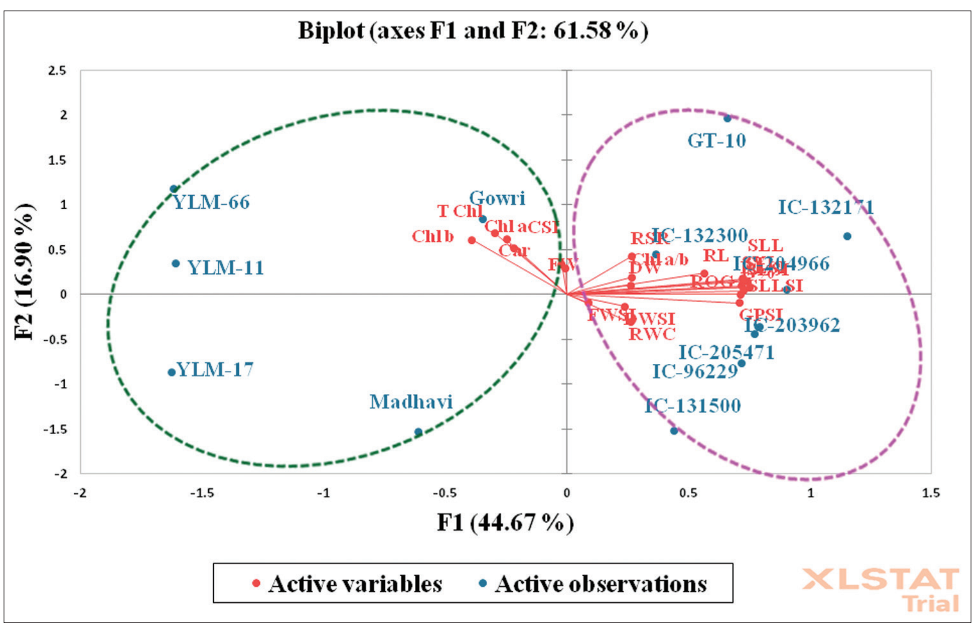

Figure 3: Principal component analysis (PCA) biplot of thirteen sesame genotypes under 25% PEG-induced osmotic stress according to F1 and F2 axes at germination and early seedling growth using twenty-two variables viz. germination, growth, morpho-physiological and stress tolerance indices.

Table 6: Classification of thirteen sesame genotypes into sensitive and tolerant groups factor score and PCA

S. No.	Genotypes	Seed Color	PCA Biplot		Ranking at 25% PEG	PCA Biplot Axis	Cluster Analysis	Contributing Parameters
			Factor score under Control	Factor score under 25% PEG				
1	YLM-17	Brown	-4.641	-5.097	1	F_2 Axis	Cluster II (Sensitive)	FW, Chl a, Chl b, T Chl, Car, CSI
2	YLM-66	Brown	4.764	-5.068	2			
3	YLM-11	Brown	-2.844	-5.050	3			
4	Madhavi	Brown	-0.661	-1.911	4			
5	Gowri	Brown	-1.294	-1.090	5			
6	IC-132300	Black	0.170	1.142	6	F_1 Axis	Cluster I (Tolerant)	ROG, G%, RL, SL, RSR, DW, SLL, RWC, GPSI, RLSI, SLSI, SLLSI, FWSI, DWSI, SVI, Chl a/b
7	IC-131500	Light Brown	-1.659	1.388	7			
8	GT-10	Black	1.825	2.069	8			
9	IC-96229	White	-1.485	2.258	9			
10	IC-205471	White	1.229	2.426	10			
11	IC-203962	White	0.453	2.485	11			
12	IC-204966	Light Brown	2.839	2.837	12			
13	IC-132171	White	1.304	3.611	13			

ROG: Rate of Germination, G%: germination percentage (%), RL: root length (cm), SL: shoot Length (cm), RSR: Root/Shoot ratio (:), SLL: Seedling Length (cm), FW: Fresh Weight (mg/g), DW: Dry Weight (mg/g), RWC: Relative Water Content (%), chl a: chlorophyll a (mg/g FW), chl b: chlorophyll b (mg/g FW), chl a/b: chlorophyll a/b (:), T chl: Total Chlorophyll (mg/g FW), Car: Carotenoids (µg/g FW). GPSI: Germination percentage stress tolerance index (%), RLSI: Root length stress tolerance index (%), SLSI: Shoot length stress tolerance index (%), SLLSI: Seedling length stress tolerance index (%), SVI: Seedling Vigour Index, FWSI: Fresh weight stress tolerance index (%), DWSI: Dry weight stress tolerance index (%), CSI: Chlorophyll Stability Index (%)

(2021) reported similar findings in other sesame genotypes. Root morphology and growth rate can serve as valuable indicators for identifying drought-tolerant varieties, as these traits are the first organs affected by drought stress (Mašková & Herben, 2018). Further, the root growth was more affected than shoot and seedling length in the presence of PEG. This may lower the sensitivity of shoot tissues to water deficit than root tissues (Sravanthi *et al.*, 2021; Gopika *et al.*, 2022) reported similar results in sesame. The tested sesame genotypes' root and shoot ratio (RSR) varied considerably. The mean root-to-shoot (RSR) ratio was observed to be 0.24 to 0.71 under controlled conditions. The 25% PEG-induced stress led to a variation in the RSR; the GT-10, IC132171, IC 204966, Madhavi, and YLM 11 genotypes had an increase in RSR than the controlled condition. The other genotypes showed a decrease in RSR. This result agrees with Kouighat *et al*. (2021), who reported that moderate and severe stresses increased RSR in most sesame genotypes. Therefore, higher RSR under stress conditions is crucial for choosing drought-tolerant varieties (Mašková & Herben, 2018).

The RWC of leaves is a highly responsive indicator of water stress, closely correlated with drought tolerance. It has been proposed as a more accurate measure of plant growth and biochemical parameters (Sinclair & Ludlow, 1985). Under control conditions, leaves of all sesame genotypes maintained higher relative water content (RWC) in the range from 84% to 95% (Figure 1). However, when exposed to 25% PEG-induced drought stress, RWC values were reduced significantly (p<0.05) from 76.31% to 92.79% (Figure 1). The differences in RWC in all genotypes could be correlated with their differential ability to retain water during drought stress. The reduction in RWC causes cellular dehydration and decreased growth and development. Genotypes like IC-204966 had low RWC values when subjected to 25% PEG-induced stress. The high RWC values were found in the IC-131500 genotype even when imposing 25% PEG-induced drought. The study found that sesame genotypes with high RWC values effectively used water, avoiding cellular dehydration due to increased water retention (Sravanthi *et al.*, 2021).

PEG-induced osmotic stress resulted in a significant (p<0.05) decline in chlorophyll values (chl a, chl b, chl a/b ratio, total chlorophyll) and carotenoids compared to their respective controls. The pigment composition of plants depends on their physiological responses and ability to tolerate environmental stresses. The reduction in chlorophyll concentration due to drought stress appears to be a combined effect of disorder in chlorophyll biosynthesis and activation of chlorophyllase, which is involved in chlorosis. The study also found significant (p<0.05) variation in chlorophyll a/b ratio among sesame genotypes, indicating that energy transfer between chl a and chl b was significantly affected by PEG-induced osmotic stress (Khan *et al*., 2019). Carotenoids are light-harvesting compounds that transfer radiant energy to chlorophylls, extending photosynthesis wavelengths. They also play roles in photoprotection, antioxidant, and plant hormone production. Carotenoids are essential for evaluating drought resistance in higher plants. Drought significantly impacts carotenoid biosynthesis and metabolism, affecting carotenoid levels in sesame and other crops like soybeans (Zheng *et al*., 2020).

The study revealed that drought stress reduced the seedling vigour index (SVI), a sensitive indicator of drought tolerance in crops, which agrees with previous reports by (Mude *et al*., 2023) in finger millet and in tomatoes (Sivakumar *et al*., 2023). Except for YLM-66, the other genotypes are non-significantly affected, maintain the higher SVI values under 25%-PEG induced osmotic stress and confirm their better tolerance to the stress. Variations for other stress indices, such as root length stress tolerance index (RLSI) and plant height stress tolerance index (PHSI), were found among sesame genotypes under both control and drought stress conditions. Stress tolerance indices were significantly ($p<0.05$) affected in genotypes Madhavi, YLM-11, YLM-17 and YLM-66 under 25% PEG-induced stress, indicating their vulnerability to water deficit. The differences in RLSI, SLSI, SLLSI, FWSI and DWSI among sesame genotypes could be explained by their genetic variability and differential tolerance to drought stress (Ahmed *et al.*, 2021).

The analysis suggests that the traits related to germination, growth and morpho-physiological parameters under control and 25% PEG-induced osmotic stress conditions showed a high correlation. Pandey *et al.* (2015) and Stavridou *et al.* (2021) have already used correlation studies for genetic diversity assessment in sesame. High positive and significant ($p<0.05$) correlations were observed between germination and the early seedling growth, morphology and pigment composition. Our analysis showed that germination, growth and pigment composition at the early seedling stage could be effectively used for indirect selection of sesame cultivars under drought stress. Similarly, a high correlation coefficient was observed for pigmentation traits in sesame for genetic diversity assessment (Pandey *et al.*, 2015). A study utilised correlation analysis to assess the relationship between sesame's morphological traits under drought and normal conditions, suggesting capsule number and diameter as potential indicators for indirect selection (Baghery *et al.*, 2022). The study found that sesame cultivars in Cluster 1 were more tolerant to 25%-PEG-induced stress than those in Cluster II. These results indicate that AHC analysis can classify sesame genotypes into different drought-tolerant groups based on germination, growth, stress tolerance indices, and morphological and physiological parameters. Like us, cluster analysis has been commonly used in drought stress studies to classify and determine tolerant and susceptible cultivars in sesame (Pandey *et al.*, 2021; Baghery *et al.*, 2022). Similarly, sesame accessions from different geographical origins were scattered through cluster analysis by Baraki *et al.* (2020) and Stavridou *et al.* (2021).

Principal component analysis (PCA) is a widely used multivariate technique that summarises a data set of interrelated observations by several dependent variables, aiming to extract desired information from the data (Kadir *et al.*, 2017; Baraki *et al.*, 2020). Other researchers have successfully used PCA to screen differential tolerance to stress in *Eruca* (Huang *et al.*, 2015) and Barely (Chikha *et al.*, 2016). In the current study, PCA allowed for easy visualisation of complex data, and twenty-two variables among thirteen genotypes were separated into two groups. It was clear that the germination parameters, root length, shoot length, seedling length and root-to-shoot ratio, SVI, RLSI and PHLSI were grouped with favourable loading on the right side of the biplot (group-I; tolerant), suggesting these parameters had a positive correlation among themselves. Fresh weight, dry weight, chl a, chl b, total chlorophyll, carotenoids and CSI were observed on the left side of the biplot (group-II; sensitive). Several reports on other crops agreed with the present findings (Razzaq *et al.*, 2017). Furthermore, several researchers used PCA to sketch biplots and the association of genotypes and their agronomic traits in sesame (Baraki *et al.*, 2020; Gopika *et al.*, 2022). The genotypes with brown seed colour were placed under a sensitive group, whereas those with black, white and light brown were placed under a tolerant group. This result disagrees with El Harfi *et al.* (2016) that Moroccan sesame genotypes characterized by yellow and brown colour seeds were more tolerant to salt and drought stresses than American genotypes with black and white seed colours.

The germination and reproduction stages are more vulnerable to the detrimental effects of drought stress. Sravanthi *et al.* (2021) conducted a field experiment to study the impact of water deficit on eleven sesame genotypes at the reproductive stage and yield characteristics. Their study classified eleven genotypes as stable performers (IC 205471), moderate performers (IC 203962), tolerant (GT 10) and others (IC 132171, IC 131500, IC 96229, IC 204966), showed varied responses. Compared to other crops, sesame has better tolerance; however, it remains susceptible to drought during the germination and seedling stages (Boureima *et al.*, 2016). The present study focused on the effect of osmotic stress on germination and early seedling growth of sesame under lab conditions using 13 genotypes, including the seven genotypes selected from previous study such as IC 205471, IC 203962, GT 10, IC 132171, IC 131500, IC 96229, IC 204966 (Sravanthi *et al.*, 2021). The differential response to drought stress has already been reported in finger millet (Mundada *et al.*, 2020; Mude *et al.*, 2023), rapeseed (Khan *et al.*, 2019) and tomato (Sivakumar *et al.*, 2023) at the seedling stage using either PEG or mannitol. Lab screening has specific advantages over pot/field screening (at the reproductive stage), such as simplicity in scoring shoot/root traits, limited space requirement within a short time, and precise control of the mineral nutrition of the plants (Mude *et al.*, 2023). However, the experiments conducted in the lab/greenhouse are not always representative of field performance (Sivakumar *et al.*, 2023).

The study reveals that PEG-induced osmotic stress can impact seed germination, early seedling growth and morph-physiology of sesame genotypes. Moreover, this study also revealed that various stress tolerance indices can be considered the best indicators for studying drought effects on plants. The PEG-induced osmotic stress, lab screening and multivariate analysis classified thirteen genotypes into Group-I (Tolerant: GT10, IC-132300, IC 96229, IC132171, IC 205471, IC203962, IC 204966, IC131500) and Group-II (Sensitive: YLM-17, YLM 66, YLM-11, Madhavi and Gowri) at germination and early seedling stage. The reliable variables contributing to drought tolerance are the rate of germination, germination percentage, root length, shoot length, root/shoot ratio, seedling length, dry weight, germination percentage stress tolerance index, root length stress tolerance index, shoot length stress tolerance index, seedling length stress tolerance index, fresh weight stress tolerance index, dry weight stress tolerance index, seedling vigour index and chl a/b. To the best of our knowledge, the present study is the first report that uses multivariate analysis, 25% PEG-induced osmotic stress and seed colours for the identification of drought-sensitive and tolerant sesame genotypes based on germination, growth, morpho-physiological parameters and stress tolerance indices. Genotypes with brown seed colours seemed more sensitive than other seed colours (black, white and light brown). Even if our study was a lab-based and PEG-induced osmotic stress experiment, the reported findings could be relevant to the breeders for rapid screening of sesame genotypes for developing drought-tolerant germplasm.

AUTHORS' CONTRIBUTION

Conceptualization: Khadar Basha Shaik, P S Sha Valli Khan, Madakka M; Methodology: Khadar Basha Shaik, S Naseem, V Suneetha, P S Sha Valli Khan, Madakka M; Data analysis and interpretation of results: Khadar Basha Shaik, P S Sha Valli Khan, Madakka M; Writing-original draft preparation: Khadar Basha Shaik, P S Sha Valli Khan, Madakka M; Writing and Editing: P S Sha Valli Khan, Madakka M; All authors have read and approved the submitted manuscript.

ACKNOWLEDGEMENTS

The authors also thank Dr. P. Ratna Kumar, ICAR-Indian Institute of Oil Seeds Research, Rajendra Nagar, Hyderabad, India and Dr. D. Bharathi, Regional Agriculture Research Station (RARS)-ANGRAU, Tirupati, India, for providing seeds of different genotypes of sesame.

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