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Assessing genotypic diversity in *Gladiolus grandiflorus* for tolerance to saline conditions

EDIGA AMALA[1], KISHAN SWAROOP[1]*, KANWAR PAL SINGH[1], SAPNA PANWAR[1], V K SHARMA[1], SHIV PRASAD[1], AMOLKUMAR U SOLANKE[2], NAMITA[1] and PANCHAL SANGMESH[1]

ICAR-Indian Agricultural Research Institute, New Delhi 110 012, India

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ABSTRACT

Salinity is a major abiotic stress affecting crop productivity, particularly in commercial cut flower like gladiolus (*Gladiolus grandiflorus* L.), which is highly sensitive. The present study was carried out during 2022–23 and 2023–24 at ICAR-Indian Agricultural Research Institute, New Delhi to evaluate the morphological, physiological and biochemical responses of 10 gladiolus genotypes (Peter Pears, Pusa Red Valentine, Warang Pink, Pusa Srijana, Urmi, Little Fawn, Urvashi, Gulal, Pusa Suhagin and Yellow Stone) under different salinity levels (0, 45 and 90 mM NaCl). Growth parameters, including plant height (22.1%), leaf area (37.8%) and number of corms (22.17%) were significantly reduced due to highest salinity stress level (90 mM NaCl) and it varied with the genotypes. Genotypes Little Fawn and Yellow Stone exhibited superior performance, maintaining higher relative water content (86.22 and 83.75%, respectively) at 90 mM NaCl, while chlorophyll levels (declined up to 10.5% at 45 mM and 23.4% at 90 mM NaCl) compared to more salinity sensitive genotypes like Pusa Srijana (68.1% reduction in RWC and 55.8% reduction in total chlorophyll). The salinity-induced oxidative stress, indicated by increased electrolyte leakage rate (83.3% in Little Fawn and 443.7% in Pusa Srijana) and malondialdehyde content (108.1%), were notably lower in the tolerant genotypes. Principal component analysis (PCA) revealed a strong correlation between growth traits and stress bio-markers, highlighting the resilience of Little Fawn and Yellow Stone. These results demonstrated the potential of selecting and evolving salt-tolerant gladiolus genotypes, offering a pathway for sustainable cultivation in salt-affected areas, thereby supporting the flower industry.

Keywords: Biochemical responses, Gladiolus genotypes, Growth traits, Osmotic stress, Physiological, Salinity stress

Abiotic and biotic stresses significantly challenge crop productivity, with several of the commercial plant varieties are often susceptible to pests, diseases and viruses, with marked reduction in the flower quality (Singh and Dubey 2007, Kakade *et al.* 2008). Amongst the abiotic stresses, salinity is particularly severe, affecting plant growth, physiological and biochemical traits, resulting in yield losses exceeding up to 50% in some crops (Ferdosi *et al.* 2021, Vanlalruati *et al.* 2019). Soil salinity, driven by presence of excess chloride and sodium ions, induce osmotic pressure and ionic stress, impairing the plant health (Ahir and Singh 2017). Globally, salinity is projected to impact 50% of arable land by 2050 (Machado and Serralheiro 2017), with 6.73 million hectares in India is already affected (Mandal *et al.* 2010).

Salinity stress on floricultural crops, including *Gladiolus grandiflorus* L. remains understudied. Gladiolus, a high-value cut flower renowned for its vibrant spikes and long shelf-life, is widely cultivated across the tropical to temperate regions. However, the mechanisms underlying the salinity tolerance in this crop remain poorly understood and no studies have been reported till date on development of salt-tolerant varieties. Salinity issues are exacerbated by irrigation with saline water, particularly in semi-arid and arid regions, leading to salt accumulation, impaired growth and flowering traits (Rozema and Flowers 2008). Salinity-induced oxidative stress further damages sensitive genotypes via lipid peroxidation (Abogadallah 2010, Hu *et al.* 2012). Studies revealed that even low NaCl levels adversely affect the gladiolus growth, flowering and corm traits (Ahir and Singh 2017). However, genetic variability offers practical options to identify salt-tolerant genotypes. Mechanisms such as osmolyte accumulation, including proline, enhance salt tolerance in crops such as chrysanthemum (Rai *et al.* 2017, Rohman *et al.* 2024). Thus, this study investigated the morphological, physiological and biochemical responses of ten gladiolus genotypes to graded salt (NaCl) induced stress in semi-arid regions like Delhi. Insights from this

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[1]ICAR-Indian Agricultural Research Institute, New Delhi. [2]ICAR-National Institute for Plant Biotechnology, New Delhi. *Corresponding author email: swaroopkishan15@gmail.com

research will aid in identifying salt-tolerant genotypes, ensuring sustainable gladiolus production in salt-affected areas, while supporting economic gains for growers.

MATERIALS AND METHODS

Experimental site and planting material: The present study was carried out during 2022–23 and 2023–24 at ICAR-Indian Agricultural Research Institute, New Delhi. The objective was to evaluate the effects of salinity stress on the growth, physiological and biochemical traits of 10 gladiolus genotypes, namely, Peter Pears, Pusa Red Valentine, Warang Pink, Pusa Srijana, Urmi, Little Fawn, Urvashi, Gulal, Pusa Suhagin and Yellow Stone obtained from the ICAR-Indian Agricultural Research Institute, New Delhi. Healthy and uniform sized corms (5 cm) were planted in plastic pots (40 cm × 30 cm) filled with 14 kg potting mixture comprising of sandy loam soil and farmyard manure (3:1). The resultant soil *p*H and electrical conductivity (EC), prior to start of the experiment were 7.43 and 0.34 dS/m, respectively. Three corms were planted/pot in October each season. Initially, the plants were irrigated with normal irrigation water (EC: 1.07 dS/m).

The graded salinity treatments, namely, T_1, Control, (EC: 1.07 dS/m); T_2, 45 mM, and T_3, 90 mM NaCl were given one month after planting (November) till harvesting of corms based on plants requirement and were maintained by adding calculated sodium chloride (NaCl) quantities to the irrigation water. Growth traits, including plant height (cm), leaf number, leaf length (cm), total leaf area (cm^2), stem dimeter (mm), shoot fresh weight (SFW) and shoot dry weight (SDW) were measured at flowering stage (February). Corm traits, including number of corms/plant, single corm weight (g) and diameter of corm (mm) were taken 45–50 days after flowering (April). Relative water content (RWC) was determined using Weatherley's (1950) formula:

$$\text{RWC} = [(\text{Fresh weight - Dry weight}) / (\text{Turgid weight - Dry weight})] \times 100$$

Electrolyte leakage rate (ELR) was calculated as a percentage using formula (Sairam *et al.* 1997):

$$\text{ELR (\%)} = (C_1/C_2) \times 100$$

The total leaf chlorophyll content was measured as SPAD values using a SPAD-502 chlorophyll meter (Minolta, Japan). Biochemical traits such as proline content were quantified following Bates *et al.* (1973) and malondialdehyde (MDA) content, an indicator of lipid peroxidation, was analysed as suggested by Dhindsa *et al.* (1981) with minor modifications.

The experiment followed a two-factorial completely randomized design (CRD) with ten genotypes and three salinity treatments (27 plants in each treatment). Data from the two seasons were pooled and analysed using two-way ANOVA in R software (version 4.3.2). Duncan's Multiple Range Test (DMRT) was applied for mean comparisons ($p<0.001$). Pearsons's correlation and Principal Component Analysis (PCA-Biplot) was performed by Metan and FactoMineR, Factoextra packages respectively to evaluate plant growth, physiological and biochemical traits.

RESULTS AND DISCUSSION

Effect of salt stress on vegetative traits: Salinity stress significantly ($p<0.001$) impacted the vegetative growth, physiological performance and biochemical traits of gladiolus genotypes, with pronounced genotype × salinity treatment interactions. Increasing salinity levels (45 and 90 mM NaCl) led to marked reductions in vegetative growth parameters, including plant height, leaf number, leaf length, total leaf area, stem diameter, SFW and SDW (Table 1 and Supplementary Table 1). These changes highlight the critical role of osmotic pressure and ionic stress in impairing water uptake and nutrient absorption, consequently disrupting cell division and elongation (Cassaniti *et al.* 2009). The observed growth inhibition can be attributed to the dual stress mechanisms, Osmotic stress resulting from reduced water potential and ionic stress caused by Na^+ and Cl^- accumulation in plant tissues, which disrupts cellular homeostasis and enzyme function. Plant height decreased significantly with salt stress from 74.38 cm (control) to 63.33 cm (45 mM) and 57.92 cm (90 mM), representing 14.9% and 22.1% reductions, respectively. Genotypic variation was evident, with Little Fawn demonstrating superior salt tolerance (3.7% and 12.6% height reduction at 45 mM and 90 mM) compared to Pusa Srijana (24.7% and 40.7% reduction at corresponding salt concentrations). This differential genotypic response suggests variations in Na_+ exclusion mechanisms and tissue tolerance, where salt-tolerant genotypes like Little Fawn likely possess enhanced Na^+/H^+ antiporter activity and improved K^+/Na^+ selectivity (Kumar *et al.* 2018). Similar patterns were observed in other ornamental species like *Nerium oleander* (Banon *et al.* 2010) and marigold (Valdez-Aguilar *et al.* 2009), which emphasized the consistency of salinity-induced growth inhibition across plant species. Salinity significantly reduced leaf growth parameters across all genotypes. Leaf number decreased by 12.5% (45 mM) and 18.2% (90 mM) compared to controls (6.81), while leaf length declined by 12.8% and 23.6% from the control value of 59.73 cm. Genotypic variation was evident, with the salt-sensitive Pusa Srijana showing greater reductions (leaf number: 6.14→5.34→4.17; leaf length: 57.51→47.72→39.22 cm) than the tolerant Little Fawn (leaf number: 6.93→6.72→6.47; leaf length: 62.83→60.21→56.53 cm) across control, 45 mM, and 90 mM treatments, respectively (Table 1). The reduction in leaf parameters can be explained by salt-induced alterations in cell wall extensibility and turgor pressure, which are essential for cell expansion (Guo *et al.* 2023). Furthermore, salt stress disrupts auxin transport and gibberellin biosynthesis, leading to impaired leaf initiation and development. Total leaf area showed substantial reduction from 246.90 cm² (control) to 188.16 cm² (45 mM) and 153.76 cm² (90 mM), representing 23.8% and 37.7% decreases, respectively. This reduction was significantly correlated with lower chlorophyll content and biomass accumulation, consistent with the findings of Bybordi (2012) and Rai *et al.* (2017). The substantial reduction in total leaf area represents a critical adaptive strategy to minimize transpirational water loss, though it simultaneously reduces the photosynthetic capacity of the plant. Little Fawn maintained the highest leaf area (307.08 cm² control vs 281.45 cm² at 45 mM and 222.77 cm² at 90 mM; 8.3% and 27.5% reduction), while Pusa Srijana showed the greatest decline (231.45 cm² control vs 117.39 cm² at 45 mM and 91.62 cm² at 90 mM; 49.3% and 60.4% reduction).

Salt stress progressively reduced stem diameter (18.35 to 15.71 and 13.76 mm), shoot fresh weight (SFW: 50.28 to 37.35 and 25.73 g), and shoot dry weight (SDW: 10.54 to 7.09 and 4.52 g) at 45 and 90 mM treatments, representing 14.4–25.0%, 25.7–48.8%, and 32.7–57.1% reductions, respectively. Genotype Little Fawn exhibited superior salt tolerance with minimal reductions in stem diameter (9.2–17.1%), SFW (4.9–21.9%), and SDW (22.9–44.3%) compared to Pusa Srijana, which showed substantial decreases in stem diameter (21.3–32.8%), SFW (48.8–78.2%), and SDW (59.5–73.8%) under identical stress conditions (Supplementary Table 1). The disproportionate reduction in dry weight compared to fresh weight indicated cellular dehydration and metabolic disruption, where salt-sensitive genotypes experience greater impairment in carbon assimilation and allocation (Wang *et al.* 2021). The maintenance of higher biomass in tolerant genotypes is associated with enhanced osmoregulation through accumulation of compatible solutes and better protection of photosynthetic machinery. The reduced biomass aligns with findings on chrysanthemum, where salt-tolerant genotypes exhibited the minimal decline under salinity (Vanlalruati *et al.* 2019).

Flowering parameters: Increasing salinity levels had marked effect on flower induction and further development. The spike was in general reduced and floret opening was very sparse except Little Fawn and Yellow stone where it was up to 90 Mm. In other genotypes, there was restricted spike emergence with poor growth, few or no floret opening and culminating into necrosis of the florets followed by spike (data not presented).

Corm traits under salinity stress: Traits including the number of corms/plant, single corm weight and corm diameter were significantly ($p<0.001$) affected by the salinity levels (Table 2). The number of corms/plant decreased from 2.21 (control) to 1.89 (45 mM) and 1.72 (90 mM), representing 14.5% and 22.2% reductions, respectively. Little Fawn showed minimal reduction (2.60 to 2.45 to 2.33; 5.8% and 10.4% decrease), in contrast to Pusa Srijana (1.53 to 1.17 to 1.07; 23.5% and 30.1% decrease). Single corm weight declined from 53.02 g (control) to 42.18 g (45 mM) and 34.64 g (90 mM), showing 20.4% and 34.7% reductions with reduction exceeding 50% in sensitive genotypes. Little Fawn maintained better performance with only 9.0% and 21.3% reduction (57.52 g to 52.33 g to 45.25 g). Corm diameter decreased from 48.91 mm (control) to 42.32 mm (45 mM) and 38.63 mm (90 mM), representing 13.5% and

Table 1 Effect of NaCl induced salt stress levels on vegetative growth traits in gladiolus genotypes

Genotype (G)	Plant height (cm)*				No. of leaves/plant*				Leaf length (cm)*				Total leaf area (cm^2)*			
	Salinity treatment (T)			Mean	Salinity treatment (T)			Mean	Salinity treatment (T)			Mean	Salinity treatment (T)			Mean
	Control	45 mM	90 mM		Control	45 mM	90 mM		Control	45 mM	90 mM		Control	45 mM	90 mM	
Peter Pears	70.10^{gh}	54.35^{l}	47.10^{n}	57.18^{F}	6.51^{def}	5.23^{m}	4.93^{n}	5.56^{F}	58.51^{cdefg}	44.52^{q}	40.54^{r}	47.86^{E}	231.05^{ef}	134.44^{l}	116.82^{m}	160.77^{F}
Pusa Red Valentine	77.57^{b}	60.92^{k}	63.15^{jk}	67.21^{E}	6.94^{b}	6.24^{fgh}	5.47^{lm}	6.22^{D}	59.43^{bcdef}	52.52^{jk}	48.24^{no}	53.40^{C}	249.91^{c}	200.62^{i}	178.79^{j}	209.77^{D}
Warang Pink	76.60^{bc}	72.07^{efg}	64.02^{ij}	70.90^{C}	7.34^{a}	6.21^{gh}	5.86^{jk}	6.47^{B}	60.56^{abcd}	55.83^{hi}	50.77^{klm}	55.72^{B}	256.33^{c}	231.54^{e}	168.84^{k}	218.90^{C}
Pusa Srijana	69.85^{gh}	52.61^{lm}	41.40^{o}	54.62^{G}	6.14^{hi}	5.34^{lm}	4.17^{o}	5.22^{G}	57.51^{fgh}	47.72^{op}	39.22^{r}	48.15^{E}	231.45^{e}	117.39^{m}	91.62^{o}	146.82^{H}
Urmi	72.97^{def}	65.95^{i}	60.75^{k}	66.56^{E}	6.91^{bc}	5.91^{ij}	5.85^{jk}	6.22^{CD}	57.89^{efgh}	50.63^{klmn}	45.51^{pq}	51.34^{D}	214.34^{gh}	199.82^{i}	172.22^{jk}	195.46^{E}
Little Fawn	80.60^{a}	77.62^{b}	70.41^{gh}	76.21^{A}	6.93^{b}	6.72^{bcd}	6.47^{defg}	6.71^{A}	62.83^{a}	60.21^{bcde}	56.53^{gh}	59.86^{A}	307.08^{a}	281.45^{b}	222.77^{fg}	270.43^{A}
Urvashi	71.75^{efg}	54.52^{l}	$4\ 8.60^{n}$	58.29^{F}	6.84^{bc}	5.61^{kl}	5.35^{lm}	5.93^{E}	60.91^{abc}	48.72^{lmno}	36.22^{s}	48.62^{E}	222.74^{fg}	137.96^{l}	103.28^{n}	154.66^{G}
Gulal	74.00^{de}	68.70^{h}	64.10^{ij}	68.93^{D}	6.64^{cde}	6.38^{efgh}	6.12^{hij}	6.38^{BC}	59.53^{bcdef}	53.73^{ij}	48.18^{no}	53.81^{C}	237.09^{de}	207.02^{hi}	174.75^{jk}	206.29^{D}
Pusa Suhagin	73.05^{def}	51.65^{m}	48.20^{n}	57.63^{F}	6.93^{b}	5.48^{lm}	5.37^{lm}	5.93^{E}	58.91^{bcdefg}	48.32^{mno}	39.81^{r}	49.01^{E}	234.47^{de}	130.11^{l}	105.05^{n}	156.54^{FG}
Yellow Stone	77.35^{bc}	74.90^{cd}	71.45^{fg}	74.57^{B}	6.87^{bc}	6.52^{de}	6.14^{hi}	6.51^{B}	61.22^{ab}	58.41^{defg}	51.12^{kl}	56.92^{B}	284.56^{b}	241.24^{d}	203.45^{i}	243.08^{B}
Mean	74.38^{A}	63.33^{B}	57.92^{C}		6.81^{A}	5.96^{B}	5.57^{C}		59.73^{A}	52.06^{B}	45.61^{C}		246.90^{A}	188.16^{B}	153.76^{C}	
LSD $p<0.001$	G = 1.43; T = 0.78; G×T = 2.48				G = 0.16; T = 0.09; G×T = 0.28				G = 1.44; T = 0.80; G×T = 2.50				G = 4.88; T = 2.67; G×T = 8.45			

*Values (mean of two consecutive years, 2022–2023 and 2023–2024) followed by different uppercase letters in the vertical column represent significant differences between genotypes and in the horizontal row represent significant differences between salinity treatments. Different lowercase letters represent significant differences in the interaction between genotypes and salinity treatments.

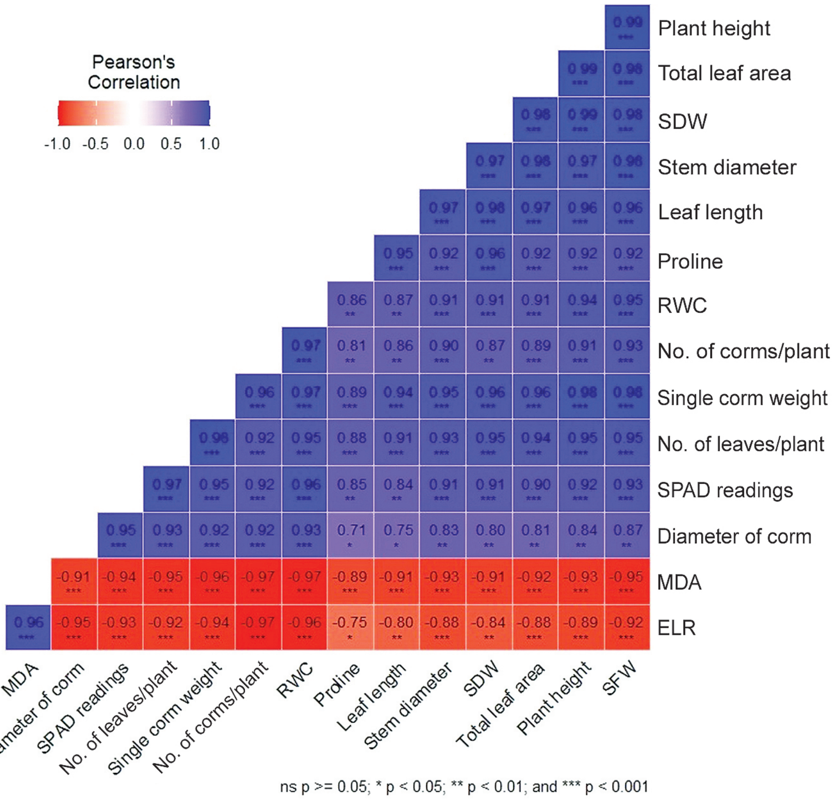
21.0% reductions, respectively. Little Fawn showed the smallest reduction (51.88 mm to 47.99 mm to 42.47 mm; 7.5% and 18.1% decrease), while Pusa Srijana exhibited the largest decline (46.88 mm to 30.55 mm to 25.91 mm; 34.8% and 44.7% decrease). These findings aligned with the previous reports of 30–60% reductions in the corm traits under similar salinity conditions (Ferdosi *et al.* 2021).

Physiological and biochemical responses to salinity stress: Salinity-induced osmotic stress resulted in significant decline in RWC and SPAD chlorophyll readings, while ELR, MDA content and proline accumulation increased (Supplementary Fig. 1 and 2). Relative water content, a key indicator of water status, declined by 12% at 45 mM and 27.2% at 90 mM NaCl, with Little Fawn and Yellow Stone maintaining higher water content (86.22 and 83.75%, respectively). In contrast, Pusa Srijana exhibited a 68.1% reduction (Supplementary Fig. 1a), reflecting impaired osmotic adjustment. The maintenance of higher RWC in tolerant genotypes is attributed to enhanced aquaporin expression and efficient osmotic adjustment through the accumulation of compatible solutes such as glycine betaine, trehalose and proline (Roy *et al.* 2014, Margaryan *et al.* 2024). Reduced RWC was strongly correlated with decreased leaf area and chlorophyll content, exacerbating growth limitations (Koksal *et al.* 2016). Membrane integrity, assessed via ELR and MDA, was markedly compromised under salinity. The ELR increased by 197.6% on average, with Little Fawn showing the lowest rise (83.3%) and Pusa Srijana the highest (443.7%) (Fig. 1). Elevated ELR correlated with higher MDA content (108.1% average increase) (Supplementary Fig. 2b), indicating greater oxidative damage in sensitive genotypes (Katsuhara *et al.* 2005). The increased membrane permeability and lipid peroxidation under salt stress result from the overproduction of reactive oxygen species (ROS), particularly superoxide anion, hydrogen peroxide, and hydroxyl radicals. Chlorophyll content, as measured by SPAD readings, declined by 10.5% at 45 mM and 23.4% at 90 mM NaCl (Supplementary Fig. 2a). The reduction in chlorophyll content is attributed to multiple factors including salt-induced damage to chloroplast structure, inhibition of δ-aminolaevulinic acid synthesis and increased chlorophyllase activity. Furthermore, salt stress disrupts the chloroplast electron transport chain and reduces the efficiency of photosystem II, leading to photo inhibition and chlorophyll degradation. Genotypes Little Fawn and Yellow Stone retained higher leaf chlorophyll levels, which supported better photosynthetic efficiency resulting in higher biomass production. Conversely, Pusa Srijana showed a 55.8% reduction, which correlated with the inhibition of the chlorophyll biosynthesis and enhanced degradation (Guan *et al.* 2012). Proline accumulation, a critical adaptive response, increased by 313% and 294% in Little Fawn and Yellow Stone, respectively, compared to the mean increase of 151.3% (Supplementary Fig. 2c). Higher proline levels correlated negatively with MDA and ELR, highlighting its role in osmotic adjustment, membrane stabilization and oxidative damage mitigation (Zhu *et al.*

Table 2 Effect of NaCl induced salt stress on corm traits in gladiolus genotypes

Genotype	No. of corms per plant*				Single corm weight (g)*				Diameter of corm (mm)*			
	Salinity treatment (T)			Mean	Salinity treatment (T)			Mean	Salinity treatment (T)			Mean
	Control	45 mM	90 mM		Control	45 mM	90 mM		Control	45 mM	90 mM	
Peter Pears	1.63^{h}	1.20^{jk}	1.00^{l}	1.28^{G}	51.33^{efg}	30.14^{p}	24.14^{r}	35.20^{G}	48.16^{bcde}	33.76^{n}	29.13^{o}	37.02^{d}
Pusa Red Valentine	2.33^{d}	2.00^{e}	1.80^{g}	2.04^{C}	52.41^{de}	42.33^{kl}	37.61^{o}	44.12^{D}	48.11^{bcde}	44.58^{fg}	41.84^{ijk}	44.84^{b}
Warang Pink	2.50^{c}	2.32^{d}	2.29^{d}	2.37^{B}	56.75^{ab}	49.25^{hi}	42.60^{k}	49.53^{B}	50.18^{ab}	46.32^{ef}	42.73^{ghij}	46.41^{a}
Pusa Srijana	1.53^{i}	1.17^{k}	1.07^{l}	1.26^{G}	49.89^{gh}	30.33^{p}	21.13^{s}	33.78^{H}	46.88^{de}	30.55^{o}	25.91^{p}	34.45^{e}
Urmi	2.01^{e}	1.98^{e}	1.83^{fg}	1.94^{D}	49.67^{gh}	40.67^{lmn}	39.40^{no}	43.25^{D}	47.46^{cde}	44.27^{fgh}	41.03^{jk}	44.25^{b}
Little Fawn	2.60^{b}	2.45^{c}	2.33^{d}	2.46^{A}	57.52^{a}	52.33^{def}	45.25^{j}	51.70^{A}	51.88^{a}	47.99^{bcde}	42.47^{ghijk}	47.45^{a}
Urvashi	2.02^{e}	1.88^{f}	1.78^{g}	1.89^{E}	53.12^{de}	39.33^{no}	27.42^{q}	39.96^{E}	48.03^{bcde}	43.18^{ghij}	40.28^{kl}	43.83^{b}
Gulal	2.67^{b}	2.33^{d}	2.01^{e}	2.34^{B}	53.83^{cd}	47.67^{i}	41.53^{klm}	47.68^{C}	49.56^{bc}	47.05^{de}	42.28^{hijk}	46.30^{a}
Pusa Suhagin	1.98^{e}	1.25^{j}	1.07^{l}	1.43^{F}	50.51^{fgh}	39.98^{mn}	22.01^{s}	37.50^{F}	48.92^{bcd}	38.58^{lm}	36.72^{m}	41.41^{c}
Yellow Stone	2.80^{a}	2.33^{d}	2.00^{e}	2.38^{B}	55.21^{bc}	49.75^{gh}	45.34^{j}	50.10^{B}	49.95^{ab}	46.94^{de}	43.95^{ghi}	46.95^{a}
Mean	2.21^{A}	1.89^{B}	1.72^{C}		53.02^{A}	42.18^{B}	34.64^{C}		48.91^{A}	42.32^{B}	38.63^{C}	
LSD $p< 0.001$	G = 0.04; T = 0.02; G×T = 0.07				G = 1.08; T = 0.59; G×T = 1.87				G = 1.28; T = 0.70; G×T = 2.21			

*Values (mean of two consecutive years, 2022–2023 and 2023–2024) followed by different uppercase letters in the vertical column represent significant differences between genotypes and in the horizontal row represent significant differences between salinity treatments. Different lowercase letters represent significant differences in the interaction between genotypes and salinity treatments.



	MDA	Diameter of corm	SPAD readings	No. of leaves/plant	Single corm weight	No. of corms/plant	RWC	Proline	Leaf length	Stem diameter	SDW	Total leaf area	Plant height	SFW
Plant height														0.99 ***
Total leaf area													0.99 ***	0.98 ***
SDW												0.98 ***	0.99 ***	0.98 ***
Stem diameter											0.97 ***	0.98 ***	0.97 ***	0.98 ***
Leaf length										0.97 ***	0.98 ***	0.97 ***	0.96 ***	0.96 ***
Proline									0.95 ***	0.92 ***	0.96 ***	0.92 ***	0.92 ***	0.92 ***
RWC								0.86 **	0.87 **	0.91 ***	0.91 ***	0.91 ***	0.94 ***	0.95 ***
No. of corms/plant							0.97 ***	0.81 **	0.86 **	0.90 ***	0.87 **	0.89 ***	0.91 ***	0.93 ***
Single corm weight						0.96 ***	0.97 ***	0.89 ***	0.94 ***	0.95 ***	0.96 ***	0.96 ***	0.98 ***	0.98 ***
No. of leaves/plant					0.98 ***	0.92 ***	0.95 ***	0.88 ***	0.91 ***	0.93 ***	0.95 ***	0.94 ***	0.95 ***	0.95 ***
SPAD readings				0.97 ***	0.95 ***	0.92 ***	0.96 ***	0.85 **	0.84 **	0.91 ***	0.91 ***	0.90 ***	0.92 ***	0.93 ***
Diameter of corm			0.95 ***	0.93 ***	0.92 ***	0.92 ***	0.93 ***	0.71 *	0.75 *	0.83 **	0.80 **	0.81 **	0.84 **	0.87 **
MDA		-0.91 ***	-0.94 ***	-0.95 ***	-0.96 ***	-0.97 ***	-0.97 ***	-0.89 ***	-0.91 ***	-0.93 ***	-0.91 ***	-0.92 ***	-0.93 ***	-0.95 ***
ELR	0.96 ***	-0.95 ***	-0.93 ***	-0.92 ***	-0.94 ***	-0.97 ***	-0.96 ***	-0.75 *	-0.80 **	-0.88 ***	-0.84 **	-0.88 ***	-0.89 ***	-0.92 ***

Fig. 1 Pearson's correlation matrix depicting the relationships among vegetative, corm, physiological, and biochemical traits.
MDA, Melon dialdehyde; RWC, Relative water content; SDN, Shoot dry weight; SFW, Shoot fresh weight; ELR, Electrolyte leakage role.

2013, Amirjani 2010). The enhanced proline accumulation in salt-tolerant genotypes results from upregulation of proline biosynthesis genes (P5CS1 and P5CS2) and downregulation of proline degradation gene (ProDH), creating a metabolic shift favouring proline accumulation. Beyond osmotic adjustment, proline functions as a molecular chaperone, protecting protein structure and enzyme activity under salt stress, and serves as a ROS scavenger, contributing to cellular redox homeostasis. The genotypic variability underscores the role of inherent genetic differences in the salinity tolerance.

Correlation amongst the traits and their Principal Component analysis: Pearson's correlation analysis revealed significant relationships among growth, physiological, and biochemical traits (Fig. 1). Growth parameters, including plant height, total leaf area, and stem diameter, showed strong positive correlations (e.g. plant height vs. total leaf area, r = 0.99, $^{***}p< 0.001$). Physiological traits like RWC and proline levels were positively correlated with growth traits (e.g. RWC vs. plant height, r = 0.91, $^{***}p< 0.001$), indicating their roles in cellular hydration and stress resilience. The high correlation between RWC and growth parameters indicates that water status serves as a primary determinant

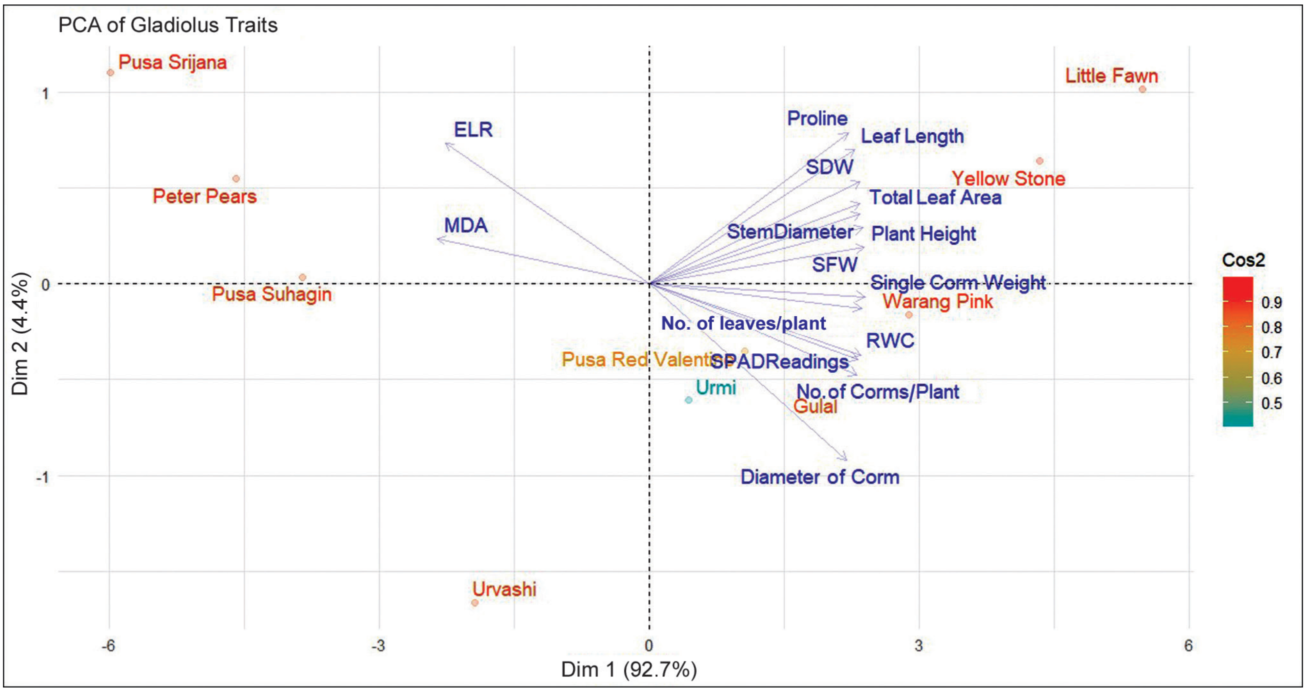


Fig. 2 Principal Component Analysis (PCA) biplot illustrating the distribution of gladiolus genotypes and traits under induced salinity stress conditions.
ELR, Electrolyte leakage rate; MDA, Melon dialdehyde; RWC, Relative water content; SFW, Shoot fresh weight; SDN, Shoot dry weight.

of plant performance under salinity stress (Acosta-Motos *et al.* 2017). SPAD readings, reflecting chlorophyll content, correlated strongly with total leaf area (r = 0.94, $^{***}p< 0.001$), highlighting photosynthetic efficiency's contribution to biomass accumulation. Conversely, MDA, a marker of oxidative stress, exhibited negative correlations with growth traits (e.g. plant height, r = -0.91, $^{***}p< 0.001$), SPAD readings (r = -0.93, $^{***}p< 0.001$), RWC (r = -0.89^{***}), and proline (r = -0.89^{***}), linking oxidative damage to reduced growth and physiological stress tolerance. The Principal Component Analysis (PCA) explained 97.1% of the total variability, with Dim 1 (92.7%) dominated by growth-related traits like plant height, total leaf area, stem diameter, RWC, SDW and single corm weight. Genotypes such as Little Fawn and Yellow Stone clustered for these traits, indicating superior growth. In contrast, stress indicators like MDA and ELR had strong negative loadings on the dimension 1 (Dim 1), with genotypes Peter Pears and Pusa Srijana associated with higher susceptibility to salt stress (Fig. 2).

The study clearly revealed significant variability for salinity tolerance in the gladiolus genotypes. Genotypes, Little Fawn and Yellow Stone showed desired resilience through improved water retention, membrane stability, chlorophyll content and proline accumulation, supporting normal growth under stress. In contrast, Pusa Srijana displayed reduced salt tolerance, highlighting its susceptibility. The key mechanisms included enhanced water status, oxidative stress reduction and proline accumulation, underscoring the need for resilient genotypes in saline environments.

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