DOI : https://doi.org/10.58993/ijh/2023.80.2.10

Indian J. Hortic. 80(2), June 2023: 190-196

Differential response of chrysanthemums on osmolyte accumulation, chlorophyll content and growth attributes under salinity stress

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ABSTRACT

Chrysanthemum is moderately sensitive to salt stress, and salinity largely affects its production. The experiment was undertaken at the research farm of the Division of Floriculture and Landscaping, ICAR-IARI, New Delhi, during the winter season. Ten chrysanthemum varieties were screened to understand their response to salt stress (150 mM NaCl) and tolerance mechanisms. Salt stress significantly affected osmolyte accumulation and physio-biochemical attributes of chrysanthemum varieties against control. Red Gold variety performed well in terms of growth and yield attributes, indicating its ability to tolerate the salt level of 150 mM NaCl. The highest leaf proline content was recorded in var. White Prolific followed by var. Tata Century. NaCl stress caused a 3/2-fold and 1/2-fold increase in the level of Na^+ and K^+ ions in leaf tissues of most varieties against control Shoot K^+/Na^+ ratio was recorded highest in variety Tata Century followed by variety Discovery. From the experiment, vars. White Prolific, Red Gold, Tata Century, and Discovery were found to be more tolerant due to better osmotic adjustments through the accumulation of proline, relative performance under salt stress for plant growth, flower yield, photosynthetic pigments and high K^+/Na^+ concentration to better sustain under saline condition than the susceptible varieties. PCA analysis revealed that the sum of principal components PC1 and PC2 explained 59.0% of the variations among the varieties. Plant height, chlorophyll content, and fresh weight number of branches had the highest positive loading value, i.e., ~0.7, and leaf proline content had the lowest loading value, i.e., ~0.08, indicating the strongest influence on PC1 and PC2.

Keywords: *Chrysanthemum morifolium*, Proline, Chlorophyll, Salinity

INTRODUCTION

Salinity affects more than 6% of the world's total land area, and salt-affected soils are a concern in over 8.5 million hectares in India. By the year 2050, it has been estimated that more than half of the arable land will be salinized (Jamil *et al.*, 6). Saline soils are affected by the presence of sodium, chloride, and sulfate ions, with high SAR (Sodium Absorption Ratio), high pH, and electrical conductivity (EC > 4.0 dS/m). Salinity-induced ion toxicity alters plant morphology, physiology, and metabolism resulting in decreased availability of essential nutrients. The ability of plants to tolerate salt stress is correlated with the Na^+/K^+ ratio, and tolerant varieties have lower contents of Na^+ and moderate ratios of Na^+/K^+ in young tissues in rice (Yong Gao *et al.*, 16). Plants with a higher K+/Na+ ratio are considered more tolerant to salt stress. Sodium (Na) excess in roots hampers potassium (K) absorption, thereby reducing plant growth as potassium maintains cell turgidity, membrane potential, and enzyme activity. Plants accumulate some compatible osmolytes with proline playing a critical role in resisting salinity's toxic effects (Skriver and Mundy, 14). Given the negative effects of salinity on plant growth, understanding the absorption mechanism, movement within plants and translocation of major toxic ions is crucial for improving salt tolerance in crops.

Chrysanthemum (*Chrysanthemum morifolium* Ramat.) is one of the commercial cut flowers cultivated widely in India. It is a plant of halophytic origin and is said to grow satisfactorily in a moderately saline environment (Rahi and Singh, 12). Salinity stress significantly impacts chrysanthemum productivity and quality; therefore, improving its tolerance to salinity is a top concern for breeders. Previously, the differential response of chrysanthemum varieties to varied salt levels was examined (Chen *et al.*, 4; Guan *et al.*, 5). It has been reported that even very low (10 mM) NaCl concentrations significantly affected chrysanthemum var. 'Amiko Red' (Paraskevapoulu *et al.*, 10). The objective of the present study was to investigate the salt stress responses and identify the salt-tolerant chrysanthemum varieties based on the expression of physiological and morphological traits under saline stress.

MATERIALS AND METHODS

The experiment was conducted at the Floriculture Research Farm, Division of Floriculture and

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Landscaping, ICAR-IARI, New Delhi, during the winter season (November to March) of 2016-2017 and 2017-2018. Ten chrysanthemum varieties *viz*., Pink Star, Lal Pari, Pusa Anmol, Ajay Orange, White Prolific, Tata Century, Red Gold, Pusa Aditya, Jubilee, and Discovery maintained at the above research farm were selected as plant material. Terminal softwood cuttings of these varieties were raised in 96 (12 × 8) celled plug trays filled with media mix consisting of sand: vermiculite: perlite (1:1:1) in June and transplanted to earthen pots (10-inch size) filled with 5.0 kg sandy loam soil having a pH 7.32, an electrical conductivity (EC) of 0.29 dS m^{-1}pots after attaining pencil-sized thickness and 8-10 cm height. Two salt stress levels *viz.* 0 mM NaCl (control) and 150 mM (EC = 14 to 15.5 dS/m) NaCl were treated to rooted cuttings of uniform height at fortnightly intervals thirty days after transplanting. Na+ and K+ were extracted from 0.2 g samples of various leaf tissues to determine ion concentrations. Samples were washed in an oven at 600°C for 5 hours. The ashes were dissolved in 2ml concentrated nitric acid with gentle heating. This suspension was adjusted to a volume of 50ml with double distilled water and filtered through a paper filter. After that, Na+ and K+ concentrations were analyzed in the filtrate (PE-2100; Perkin Elmer Corporation, USA) using Atomic Absorption Spectrometry.

A fully expanded fourth leaf from the apex was used to estimate given biochemical parameters. The chlorophyll content of leaves was estimated with a chlorophyll meter (SPAD-502, Minolta, Japan). SPAD (Soil Plant Analysis Development) readings are relatively proportional to the chlorophyll in the leaf. The determination of free proline content was performed as Bates et al. (2) described. Leaf tissue (250mg) was homogenized with 10 ml of 3 % sulpho salicylic acid and centrifuged at 2000g for 5min. Two ml of acid-ninhydrin and 2 ml of glacial acetic acid were added to 2mL of supernatant, and the reaction was incubated for 1 hour at 100°C. Proline was extracted with 4 ml of toluene, and proline concentration was assessed by measuring A520 of the toluene phase. The leaf proline content was calculated using standard dilutions of L-proline and expressed µ mol per gram on a fresh weight basis. Shoots and roots were separated to measure fresh weight and expressed in grams (g). Data of growth parameters viz, plant height (cm), number of branches per plant, number of flowers per plant, flower yield/pot, and plant fresh weight (g) of fully grown plants were recorded two months after planting.

The experimental design was factorial CRD (Completely Randomized Design) including ten varieties and two salinity levels. The pot experiment was replicated thrice with five plants per replication. Treatment means were compared by Duncan's Multiple Range Test (DMRT) at 0.05 probability level. Recorded data were subjected to two-way analysis of variance (ANOVA) using statistical software SAS version 9.2 (SAS Institute, 22). Principal Component Analysis (PCA) was performed for plant growth parameters (plant height and number of branches per plant) and physiological traits (plant fresh weight, proline content, and chlorophyll content) using statistical software 'R'.

RESULTS AND DISCUSSION

ANOVA results showed a significant influence of salt stress on all examined morphological and physiological parameters. In this investigation, there was a significant decrease in the expression of growth parameters at a salinity level of 150 mM NaCl compared to the control. However, no negative effect of applied saline stress was observed on plant life or growth. All of the rooted cuttings in the pots survived completely. In the first year (2016-2017) and second year (2017-2018), there was a decremental effect on plant height, number of branches per plant, number of flowers per plant, flower yield/pot, and plant fresh weight with increased salt concentration. Plant height differed significantly between varieties and salt concentration except for vars. Discovery and Lal Pari. Under salt stress, maximum plant height was recorded in var. Discovery (54.33 cm and 37.57 cm) in the first and second years, respectively. Pooled analysis (Fig. 1) recorded the maximum plant height for var. Red Gold (43.67 cm) and lowest for var. Pink Star (23.67 cm) under salt stress. Plants height of var. Red Gold was severely affected by salt stress though it had larger plants. There was a significant difference between varieties and salt concentration for the number of branch/plant in which var. Pink Star recorded maximum branch numbers (9.01 and 10.86) for both years. The pooled analysis (Fig. 1) also revealed that Pink Star var. differed significantly from the rest varieties for more branches per plant and recorded the highest number of branches per plant (9.94) under saline stress. The degree of growth decline is an essential criterion for assessing ornamental crop salt stress tolerance. Salinity inhibited water and nutrient intake, which may have resulted in a slower growth rate. The detrimental effect of high NaCl on chrysanthemum vegetative development is consistent with the findings of Lee *et al*. (8). Hydraulic conductivity was observed to be significantly reduced in Melon plant roots treated with NaCl (Carvajal *et al*., 3).

The number of flowers/plant differed significantly among the varieties and between salinity treatments,

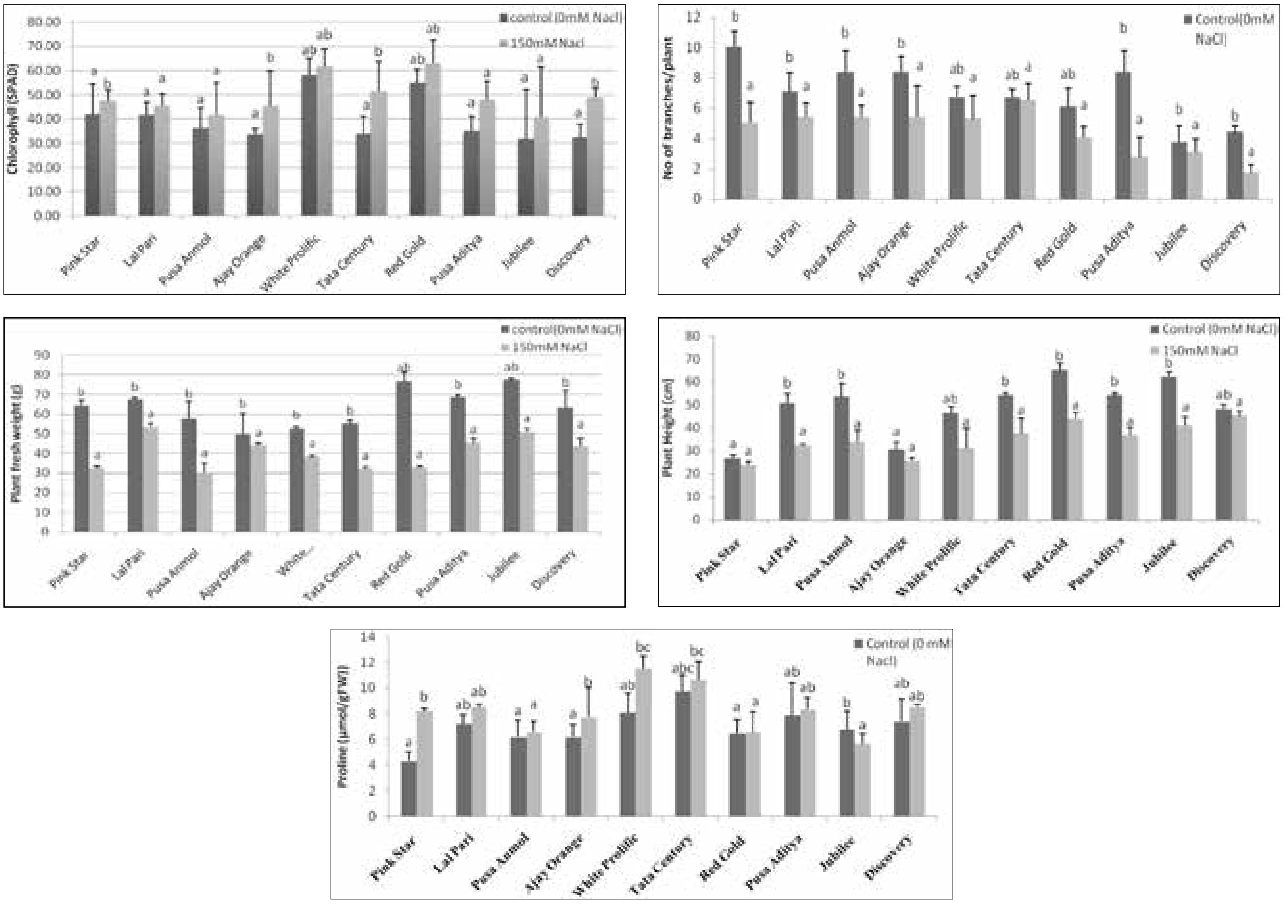

Fig. 1. Effect of NaCl (Pooled) on plant height, number of branches per plant, plant fresh weight and total leaf chlorophyll content, biochemical constituents (leaf proline content) in chrysanthemum varieties.

where Red Gold and Pusa Aditya varieties recorded the maximum number of flowers for the first (2016-2017) and second year (2017-2018), respectively. In pooled analysis (Table 1), the treatment and interactive effects of treatment and varieties were significant except for vars. Tata Century, Pusa Aditya and Ajay Orange when treated with 150mM NaCl and control. NaCl-treated plants showed comparatively few flowers per plant than the control. Under salt stress, the number of flowers per plant was highest in var. Red Gold whereas var. Pink Star had the lowest number of flowers per plant. Increased salinity affects flower yield regarding the number of flower/plant. Further, salinity caused a proportional decline in flower yield in most varieties compared to the control (Table 1).

There was a significant difference between varieties and salt treatments for flower yield/pot. Red Gold and Discovery varieties recorded maximum flower yield/pot (805.10 and 730.00 g) in the first and second years, respectively. In pooled analysis (Table 1), the treatment and the interactive effects of treatment and varieties were significant. The highest flower yield/pot was measured in var. Red Gold while var. Ajay Orange recorded the lowest flower yield/pot. Variety Red Gold performed better in flower yield indicating its ability to tolerate the salt level of 150 mM NaCl. The results presented in our study conform with the earlier report in Rose (Savvas, 13). The reduction in flowering branches and flower numbers per plant under saline stress caused yield reduction. It is opined from the results that impaired plant growth at higher salt concentrations is due to nutrient imbalance. Aside from nutritional imbalance, hyperosmotic stress and ion disequilibrium play an important role in disrupting plant cellular activities during the rise in salt stress. Yield parameters of var. Red Gold in treatment with and without NaCl indicated its salt tolerance ability without compromising its yield potential.

Plant fresh weight of all the varieties differed significantly under control and salt stress (Fig 1). Lal Pari variety recorded maximum plant fresh weight for both years. Pooled analysis revealed that the interactive effects of treatments and varieties were

Table 1. Effect of salt stress on yield attributes of chrysanthemum varieties.

S. No.	Varieties	Number of flowers/plant (Pooled Analysis)		Flower yield/pots (g) (Pooled Analysis)	
		Control (0 mM)	NaCl (150 mM)	Control (0 mM)	NaCl (150 mM)
1.	Pink Star	29.00 ± 1.02a	11.50 ± 1.44a	518.3 ± 62.01a	472.2 ± 04.11a
2.	Lal Pari	34.50 ± 0.43b	17.00 ± 1.62b	642.7 ± 06.16ba	505.0 ± 61.50b
3.	Pusa Anmol	33.17 ± 0.28c	29.50 ± 0.03c	854.2 ± 40.44b	617.8 ± 19.08bc
4.	Ajay Orange	23.50 ± 0.69c	22.50 ± 0.08c	655.4 ± 33.05bc	425.0 ± 03.06bd
5.	White Prolific	39.50 ± 0.35d	30.50 ± 0.39cd	892.2 ± 50.43bcd	648.6 ± 11.38d
6.	Tata Century	23.00 ± 0.16d	22.00 ± 0.54d	870.9 ± 60.20cde	546.9 ± 05.46cd
7.	Red Gold	38.50 ± 0.33d	35.00 ± 0.67df	805.8 ± 11.02cd	795.5 ± 43.26cde
8.	Pusa Aditya	23.16 ± 0.55d	23.00 ± 0.32df	648.5 ± 02.48d	428.8 ± 62.48d
9.	Jubilee	35.62 ± 0.74df	21.50 ± 0.02f	872.1 ± 21.13df	696.4 ± 10.77df
10.	Discovery	28.23 ± 0.30f	24.07 ± 0.11g	848.5 ± 60.87f	750.5 ± 05.01f
$LSD_{0.50}$		1.53	1.37	4.05	14.49
F-value (germplasm)		*	*	*	*
F- value (treatment)		*	*	*	*
F-value (germplasm × treatment)		*	*	*	*

Values within a column followed by different letters indicate significant differences among treatments of different concentrations of NaCl at P ≤ 0.05 (Duncan's multiple range test) ; Each value represents the mean of three replicates ± SE (m).

significant at *p*-value <0.05, and high fresh weight at 150 mM NaCl was recorded in var. Lal Pari whereas fresh weight was low in var. Pusa Anmol. Salt stress significantly affected fresh weight of most chrysanthemum varieties; however, with the least influence on var. Lal Pari. It has been described that ion toxicity and reduced osmotic potential at the root zone may reduce plants' fresh and dry weight. Plant biomass reduction due to increased concentration of NaCl was also reported in rose by Massa *et al*. (9).

There was a significant variation in chlorophyll content. In the first year, the maximum was recorded in var. Red Gold. Red Gold and White Prolific recorded the highest value in the second year and were statistically at par under saline conditions. In the pooled analysis, leaf chlorophyll concentration did not differ significantly in most chrysanthemum varieties except for var. Pink Star, var. Ajay Orange, var. Tata Century and var. Discovery under both control and NaCl stress (Fig. 1). Total chlorophyll was highest in var. Red Gold Under a salinity level of 150 mM NaCl which was at par with chlorophyll level of var. White Prolific. The lowest chlorophyll content was recorded in var. Jubilee These findings substantiate our previous report on chrysanthemums grown in hydroponics, where it is indicated that salt resistance of *Chrysanthemum morifolium* germplasm was due to enhanced proline and chlorophyll levels.

There was significant variation among the chrysanthemum varieties with leaf proline content where var. White Prolific recorded the highest value (10.97 and 12.10 µmol/g FW) in the first and second years, respectively. In pooled analysis (Fig. 1), we observed a significant increase in leaf proline content under saline stress compared to control in most varieties. However, no significant differences were observed in Lal Pari, Pusa Anmol, Red Gold, and Discovery varieties. The highest leaf proline content (pooled) was recorded in var. White Prolific (11.53 µmol/g FW) was at par with var. Tata Century (10.67 µmol/g FW) and minimum leaf proline content was recorded in var. Jubilee (5.9 µmol/g FW). In a similar line of study, enhanced shoot proline levels under saline stress were reported in our previous work on chrysanthemums. The proline level in plant tissues indicates the plant's ability to tolerate or adapt to salinity stress. The beneficial effect of proline on alleviating the negative effects of salt stress is most likely connected to improved ionic homeostasis, maintaining a favourable leaf water content, and chloroplast pigment concentration.

PCA analysis revealed that the sum of principal components PC1 and PC2 explained 59.0 % of the variations among the varieties (Fig. 2). Plant height, chlorophyll content, fresh and weight number of branches had the highest positive loading value, i.e., ~0.7, and leaf proline content had the lowest loading value, i.e., ~0.08, indicating the biggest influence on PC1 and PC2. Based on the loading plot value, the number of branches per plant had

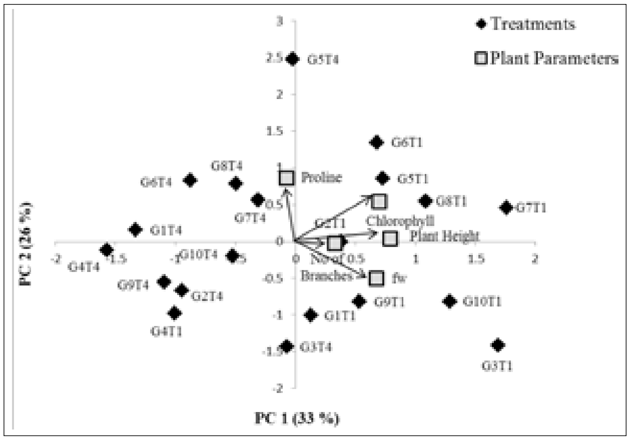

Fig. 2. Principal component analysis of chrysanthemum varieties' physio-biochemical attributes (pooled).

the lowest influence on PC1 and PC2 (Fig. 2). The Eigenvalues of PCA indicated the relative importance of these traits for classifying chrysanthemums. Plant height, chlorophyll content, fresh weight and number of branches had high potential as discriminant variables. At the same time, leaf proline concentration and the number of branches per plant was not distinct enough to be considered a discriminant variable.

Analysis of leaf tissues indicated gradual absorption of Na^+ and decreased K^+ ions due to increased concentration of NaCl. There was a significant difference in Na^+ and K^+ ions levels with the cultivars and different salinity concentrations in the first year and second year where var. Pusa Aditya recorded maximum Na^+ ion level and var. Tata Century has the highest K^+ ion level for both years, respectively. In pooled analysis (Table 2), the treatment and the interactive effects of treatment and varieties were significant. Na^+ concentration was maximum in var. Pusa Aditya and minimum in var. Discovery. NaCl stress caused a 3/2-fold increase in sodium ions in leaf tissues compared to the control. Na^+ ions concentration in leaf tissues of var. Discovery and var. Pink Star did not differ significantly under saline stress against control. At 150 mM NaCl, the highest shoot K^+ concentration was recorded in var. Tata Century, which was on par with White Prolific and Red Gold varieties. However, var. Discovery had the least level of K^+. Like Na^+, K^+ ion level decreased by 1/2-fold in most varieties except vars. Pusa Anmol and Tata Century against control. Due to their ability to restrict Na^+ and Cl^- transport to shoots, salt-tolerant varieties have lower shoot Na^+ and Cl^- concentrations. Extreme salinity alters a plant's morphology, physiology and metabolism, resulting in decreased availability of essential elements. Potassium maintains turgidity of cell, membrane potential and enzyme activity ion and is responsible for cell enlargement, osmoregulation and

Table 2. Salt stress-induced changes in ion content (Na^+, K^+, and Na^+/K^+) in leaves of chrysanthemum varieties.

S. No.	Germplasm	Na (%) (2017 and 2018 Pooled)		K (%) (2017 and 2018 Pooled)		K^+/Na^+ (2017 and 2018 Pooled)	
		Control (0 mM)	NaCl (150 mM)	Control (0 mM)	NaCl (150 mM)	Control (0 mM)	NaCl (150 mM)
1.	Pink Star	2.33 ± 0.22a	2.30 ± 0.14a	2.12 ± 0.13a	1.10 ± 0.75a	0.92 ± 0.11c	0.44 ± 1.11fg
2.	Lal Pari	2.15 ± 0.24ba	2.85 ± 0.35ba	2.05 ± 0.24a	1.03 ± 0.42ba	0.96 ± 0.50c	0.39 ± 0.05fg
3.	Pusa Anmol	1.97 ± 0.12bc	2.98 ± 0.71bac	1.58 ± 0.33b	1.35 ± 0.14ba	0.80 ± 0.33cde	0.38 ± 0.33fg
4.	Ajay Orange	2.05 ± 0.03bc	3.35 ± 0.36bc	2.05 ± 0.11ba	0.44 ± 0.85bac	0.98 ± 0.71c	0.24 ± 0.52g
5.	White Prolific	1.23 ± 0.44dc	2.73 ± 0.04c	2.22 ± 0.12ba	1.35 ± 0.73bc	1.85 ± 0.32a	0.53 ± 0.04efg
6.	Tata Century	1.55 ± 0.81de	2.76 ± 1.11dc	1.98 ± 1.22ba	1.38 ± 0.54bcd	1.28 ± 0.42b	0.68 ± 0.31cdef
7.	Red Gold	1.06 ± 0.32fe	2.55 ± 0.03ed	2.11 ± 0.04ba	1.33 ± 0.01cd	1.99 ± 0.66a	0.50 ± 0.22fg
8.	Pusa Aditya	1.07 ± 0.04fe	3.92 ± 0.53e	2.25 ± 0.05ba	1.13 ± 0.33d	2.10 ± 0.43a	0.28 ± 0.45g
9.	Jubilee	2.05 ± 0.35fe	3.44 ± 0.74f	1.85 ± 0.11b	1.08 ± 0.45e	0.90 ± 0.83cd	0.27 ± 0.01g
10.	Discovery	2.14 ± 0.25f	2.05 ± 0.52g	2.03 ± 0.31c	1.03 ± 0.32f	0.95 ± 0.21c	0.61 ± 0.33def
$LSD_{0.50}$		0.311	0.342	0.389	0.375	0.428	0.476
F-value (germplasm)		*	*	*	*	*	*
F- value (treatment)		*	*	*	*	*	*
F- value (germplasm × treatment)		*	*	*	*	*	*

Values within a column followed by different letters indicate significant differences among treatments of different concentrations of NaCl at P ≤ 0.05 (Duncan's multiple range test) ; Each value represents the mean of three replicates ± SE (m).

homeostasis. Shoot K^+/Na^+ of all the chrysanthemum varieties was significantly reduced due to salt stress caused by increased NaCl (Table 2). There was a significant difference with Shoot K^+/Na^+ level with the cultivars and different salinity concentrations where var. Red Gold and var. Pink Star had a maximum K^+/Na in the first and second year respectively. In pooled analysis (Table 2), shoot K^+/Na^+ level did not differ significantly in most chrysanthemum varieties except for vars. Ajay Orange, White Prolific, Tata Century and Discovery. However, the highest K^+/Na^+ ratio (pooled) was observed in var. Tata Century, which was at par with var. Discovery. The K^+/Na^+ ratio of these varieties appears to be least affected by salt stress against control. The K^+/Na^+ ratio positively correlated with a crop's salt tolerance. The highest K^+/Na^+ concentration was found in the root compared to the leaf and shoot. Kusvuran *et al*. (7) reported that excess NaCl leads to the loss of potassium due to membrane depolarization by sodium ions. These findings are in harmony with previous reports on melons and legumes (Amador *et al*., 1).

A significant effect of salt stress on different morphological and physio-bochemical traits was observed in chrysanthemums grown through softwood cuttings. It is concluded from the study that varieties namely, White Prolific, Red Gold, Tata Century and Discovery were found to be more tolerant owing to their better osmotic adjustments through accumulation of proline, relative performance in respect of plant growth, flower yield, photosynthetic pigments under salt stress and high K^+/Na^+ concentration to better sustain under saline condition than the susceptible varieties. PCA analysis also revealed that the principal components PC1 and PC2 explained most of the existing variation among the varieties. The study will help identify physiological, morphological, and biochemical attributes in chrysanthemum due to saline stress that may help plant breeders develop salt-tolerant chrysanthemum varieties.

AUTHORS' CONTRIBUTION

Conceptualisation of Research (V, SS); Designing of the experiments (AT, GK), Contribution of Experimental materials and Execution of field/lab experiments, data collection (V, PA), Analysis of data and interpretation (V, VH); interpretation of manuscripts (V).

ACKNOWLEDGEMENTS

The authors acknowledged the facilities provided by the Director, Indian Agricultural Research Institute, Indian Council of Agricultural Research, New Delhi-110012.

DECLARATION

The authors declare no conflict of interest.

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Received : June, 2022; Revised : May, 2023; Accepted : June, 2023