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In vitro screening of chrysanthemum (*Chrysanthemum morifolium*) varieties for salt tolerance

HENUKA RAI[1], NAMITA[2*], D V S RAJU[3], M K SINGH[4], KANWAR P SINGH[5], GUNJEET KUMAR[6], SUBODH KUMAR SINHA[7], LEKSHMY S[8], R N PANDEY[9] and BIBIN POULOSE[10]

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

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ABSTRACT

The present study was carried out to screen six promising varieties of chrysanthemum (*Chrysanthemum morifolium* Ramat.), *viz*. Pusa Chitraksha, Lalit, Pusa Aditya, Basanti, Himanshu, and Pusa Sona in MS (Murashige and Skoog) media supplemented with 0, 50, 100 and 150 mM/NaCl. As the concentration of NaCl increased, there was a corresponding decrease in shoot length, fresh weight, number of leaves and rooting percentage. Number of shoots per plant exhibited not much significant difference. Maximum reduction in plant growth was recorded in the variety Pusa Sona (60.60%) while minimum reduction was observed in variety Pusa Aditya (35.60%). The highest fresh weight was recorded in variety Pusa Aditya and lowest in the varieties Himanshu and Pusa Sona, when the medium was supplemented with 150 mM NaCl. Variety Pusa Sona (57.00%) had the maximum reduction in the number of leaves per plant and minimum was found in the variety Lalit (29.10%). Increasing salt stress had an adverse effect on rooting abilities of all the varieties. Under the control with no added NaCl, the rooting percentage was 100% among all the varieties. In medium supplemented with 150 mM/l NaCl, the maximum rooting was recorded in the variety Pusa Aditya (63.80%)) and Basanti (63.80%), whereas least was observed in the varieties Pusa Sona and Himanshu (22.00% and 41.80%, respectively).

Key words: Chrysanthemum, *In vitro,* Murashige and Skoog media, NaCl, Salt tolerance

Salinity is a major environmental factor affecting the performance of many crop plants (Munns 2002) which is posing a serious challenge to several crops. The problem of soil salinity is due to continuous use of nutrient solution (fertilization) in the greenhouse as well as the use of reclaimed water for irrigation due to the limited supply of fresh water, especially in arid and semi-arid regions of the world. Salinity inhibits the growth and reduces the yield by altering various biochemical and molecular processes. Under salinity conditions, the occurrence of high concentrations of soluble salts or high exchangeable sodium (Na) interferes with the normal plant growth by altering uptake and transport of many nutrient elements. Due to severe stress, there will be the production of reactive oxygen species, resulting in oxidative damage and ultimately leading to cell injury (Sairam *et al.* 2002). Salt stress causes reduction protein synthesis and disturbs nucleic acid metabolism (Boyer 1965; Kaiser 1987). A vast tract of land suitable for horticultural crops, is suffering from problems of salinity. However, very limited efforts have been made for screening various ornamental plants suitable for cultivation in salt affected soils to meet the increasing demand for flowers and bring diversification.

Chrysanthemum (*Chrysanthemum morifolium* Ramat.) belongs to the family Asteraceae and is native to China. It is one of the most popular ornamentals across the world. It is also known as “Queen of the East” and “Autumn Queen”. It has earned tremendous popularity due to its diversity in terms of growth habit, size, colour and flower type. There are approximately 30,000 cultivars of chrysanthemum in the world (Chen 2001). In India, it is equally popular as cut flower and as pot mum, however it is sensitive to salinity. Development of salt tolerant plants using *in vitro* selection has been reported in a wide range of plant species including cereals, vegetables, fruits, and other commercially important plant species. Tissue culture technique has emerged as a feasible and cost-effective alternative tool for developing

[1]Research Scholar (henukathulung@gmail.com), [2]*Corresponding author and Senior Scientist (namitabanyaliari@gmail.com), [4]Scientist (singh_markandey@yahoo.com), [5]Principal Scientist (kanwar_iari@yahoo.co.in), [6]Principal Scientist (gunjeet234@gmail.com), [10]Research Scholar (bibinnilgris@gmail.com), Division of Floriculture and Landscaping, [8]Senior Scientist (lekshmy@iari.res.in), Division of Plant Physiology [9]Principal Scientist (rnpandey@iari.res.in), Division of soil science and Agricultural Chemistry, ICAR-IARI, New Delhi. 110 012; [3]Principal Scientist (rajivalex@gmail.com), ICAR- Directorate of Floricultural Research, Pune; [7]Senior Scientist (subsinha@gmail.com), ICAR-NIPB, Pusa, New Delhi.

Table 1 Salient features of chrysanthemum varieties used in the study

Genotype	Source	Developed by	Parentage	Flower form	Type	Flower colour
Pusa Chitraksha	ICAR-IARI, New Delhi	ICAR-IARI, New Delhi	Open pollinated seedling of cv. Lal Pari	Single	Spray	58A, Red Purple group
Lalit	ICAR-IARI, New Delhi	CSIR-NBRI, Lucknow	Gamma rays induced mutant of Portulaca Double	Semi-Double	Spray	NN155D, White group
Pusa Aditya	ICAR-IARI, New Delhi	ICAR-IARI, New Delhi	Open pollinated seedling of cv. Jaya	Semi-Double	Spray	5 A, Yellow Group
Basanti	ICAR-IARI, New Delhi	CSIR-NBRI, Lucknow	Gamma rays induced mutant of E-13	Anemone	Spray	3 A, Yellow Group
Himanshu	ICAR-IARI, New Delhi	CSIR-NBRI, Lucknow	Open pollinated seedling of small flowers	Single	Spray	NN155D, White Group
Pusa Sona	ICAR-IARI, New Delhi	ICAR-IARI, New Delhi	Open pollinated seedling of cv. Sadhbhawana	Single	Spray	9A, Yellow Group

stress-tolerant plants in recent years. This technique can operate under controlled conditions with limited space and time (Sakhanokho and Kelley 2009) and has the potential for selection of stress-tolerant variants using a low-cost laboratory set up. *In vitro* selection can considerably shorten the time for the selection of desirable traits under selection pressure with minimal environmental interaction, and can complement field selection (Jain 2001; Hossain *et al.* 2007; Zenk 1974). Few attempts have been made to produce salt tolerant plants using *in vitro* techniques (Singh *et al.* 2003; Zair *et al.* 2003; Gandonou *et al.* 2006; Hossain *et al.* 2007) such as callus, suspension cultures, somatic embryos, shoot cultures, etc which are screened for variation in their ability to tolerate relatively high levels of salt (NaCl) in media (Woodward and Bennett 2005). Since less systematic research has been carried out on screening of chrysanthemum varieties in India for salt tolerance, therefore, an experiment was conducted to screen chrysanthemum varieties for salt tolerance under *in vitro* conditions.

MATERIALS AND METHODS

The present investigation was conducted at Central Tissue Culture Laboratory, Division of Floriculture and Landscaping, ICAR-Indian Agricultural Research Institute, New Delhi during 2016–18.

Plant material: Six chrysanthemum varieties, *viz.* Pusa Chitraksha, Lalit, Pusa Aditya, Basanti, Himanshu, and Pusa Sona were used in the present studies. These varieties were selected from our previous study on screening of chrysanthemum varieties in glasshouse conditions at National Phytotron facility, ICAR-Indian Agricultural Research Institute, New Delhi (Rai *et al.* 2017). The ray

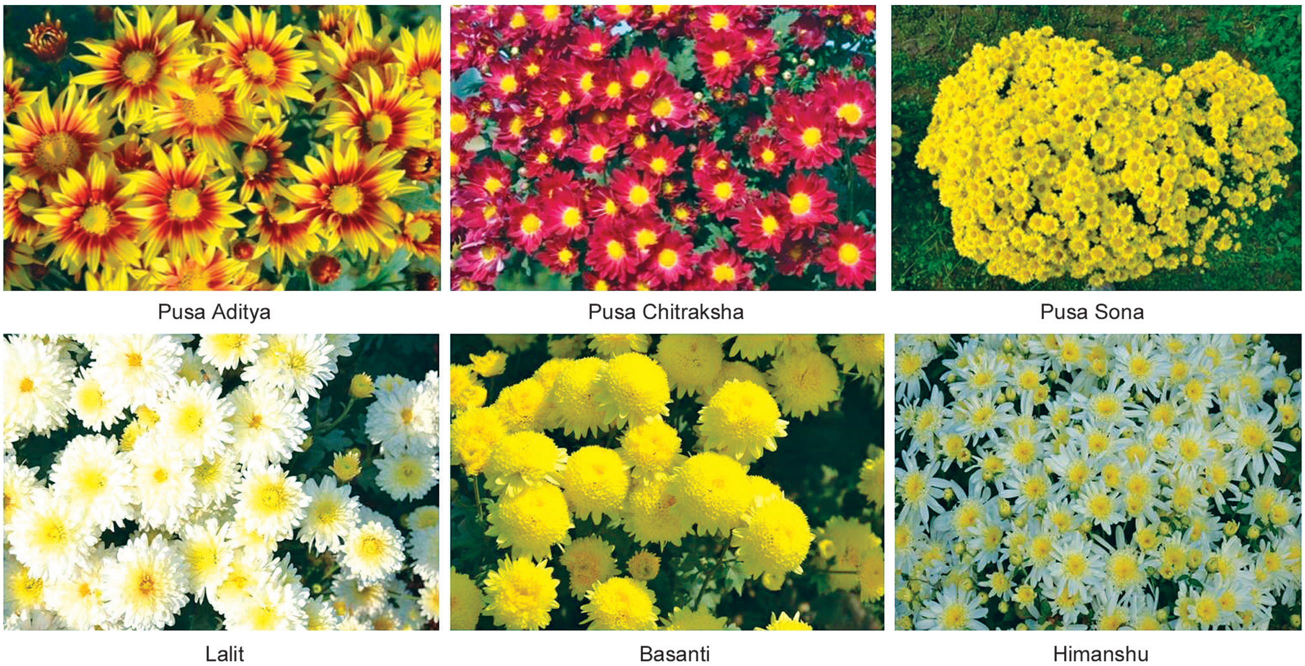

Fig. 1 Chrysanthemum varieties used in the study.

Fig 2 *In vitro* selection of chrysanthemum varieties under different treatments of NaCl: 1). T1-Control; 2) T2-50mM NaCl; 3) T3-100mM NaCl; 4) T4-150mM NaCl.

florets of the healthy mother plants of the above mentioned varieties were used as explant.

Pre-treatment, surface sterilization and explant preparation: The collected explants were washed with Teepol (0.1%) solution for 5 min. followed by washing under running tap water for 20 min. to remove the microbial load and dust particles adhering to the surface of the explant. These explants were pretreated in a solution containing 0.2% carbendazim, 0.2% mancozeb and 8-hydroxyquinoline citrate (8-HQC) (200ppm) before inoculation to minimize the contamination in the cultures for 2.5 hr followed by washing with 0.1% mercuric chloride ($HgCl_2$) solution for 4 min. followed by washing with the double distilled water.

Culture establishment, multiplication and salt treatment: For callus induction, MS media (Murashige and Skoog 1962) along with 6-benzyl amino purine (BAP) 4 mg/l and μ- naphthalene acetic acid (NAA) 1 mg/l with 7 g/l of agar powder was used. The cultures were incubated in culture room after inoculation and provided with a constant photoperiod of 16/8 hr light/dark regime at 25± 1°C temperature having a light intensity of 3000 lux at plant level provided by cool white fluorescent tube lights. The multiplied shoots from the proliferation media were separated and individual micro shoots were transferred to the elongation media comprising of basal MS medium supplemented with GA_3 (0.5 mg/l). The elongated shoots were individually separated and transferred to rooting media consisting of half strength MS basal medium supplemented with sucrose (60 g/l) and NAA (0.5 mg/l) (Fig 1). The salt treatment (0 mM, 50 mM, 100 mM, 150 mM NaCl) was given after the shoot proliferation elongation treatment. Observations on different traits such as shoot length (cm), whole plant fresh weight (g), number of shoots, shoot height (cm), number of leaves and rooting percentage were recorded at the end of the experiment.

Statistical analysis: The experiment was laid out in completely randomized design (CRD). Each treatment had five replications and each replication had 20 units/plants.

Table 2 Effect of NaCl stress on shoot length (cm) in different chrysanthemum varieties

Variety	Different concentrations of salt solution (mM/l)				
	0 (control)	50	100	150	Mean
		Shoot length (cm)			
Pusa Chitraksha	7.06 ± 0.43^{abc}	4.83 ± 0.2^{fg}	4.70 ± 0.2^{g}	3.10 ± 0.2^{h}	4.92
Lalit	6.43 ± 0.44^{bcd}	5.16 ± 0.2^{efg}	4.50 ± 0.28^{g}	3.33 ± 0.44^{h}	4.85
Pusa Aditya	7.50 ± 0.28^{ab}	7.20 ± 0.43^{abc}	7.23 ± 0.72^{abc}	4.83 ± 0.6^{fg}	6.69
Basanti	8.06 ± 0.52^{a}	7.06 ± 0.29^{abc}	5.90 ± 0.2^{def}	3.33 ± 0.44^{i}	6.08
Himanshu	7.20 ± 0.17^{abc}	6.16 ± 0.44^{cde}	5.10 ± 0.55^{efg}	2.83 ± 0.33^{h}	5.32
Pusa Sona	4.73 ± 0.39^{g}	4.63 ± 0.29^{g}	4.60 ± 0.2^{g}	2.90 ± 0.2^{h}	4.21
Mean	6.83	5.84	5.33	3.38	

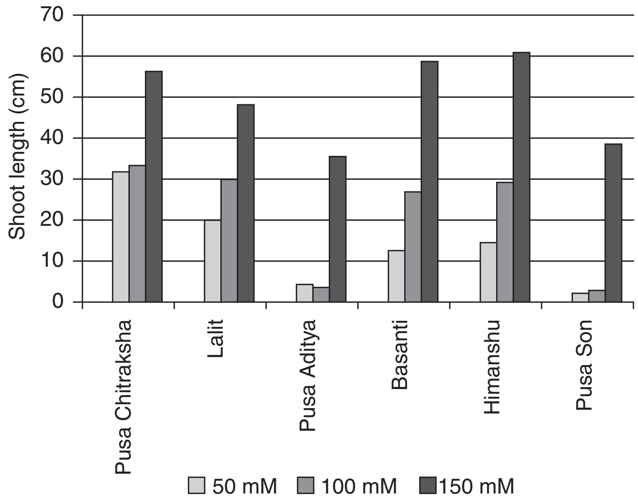

Fig 3 Reduction percentage in shoot length with increase in NaCl stress.

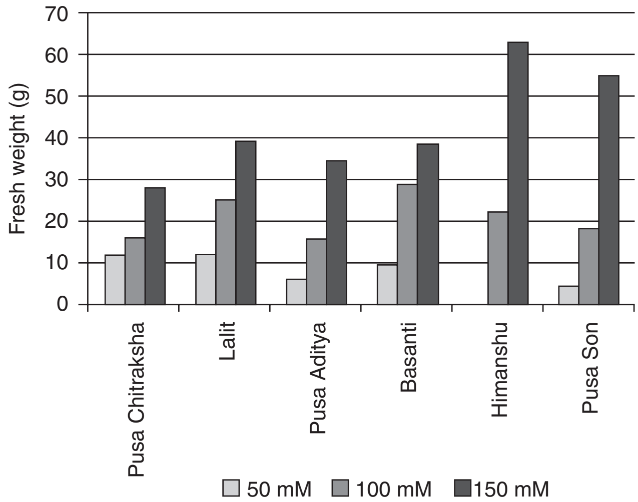

Fig 4 Reduction percentage in fresh weight with increase in NaCl stress.

Each experiment was repeated twice and the reported data are the means of two experiments. All the percentage data was subjected to angular transformation before calculating ANOVA. The data were analyzed statistically using analysis of variance technique (ANOVA). Statistical significance was tested by Duncan's New Multiple Range Test ($p<0.05$).

RESULTS AND DISCUSSION

Shoot length

There are significant differences amongst the genotypes in salt stress for the different growth parameters. The results indicated that the shoot length was significantly influenced by the NaCl (Table 2). The highest shoot length was recorded in variety Basanti (8.06 cm) under control, whereas least shoot growth was recorded in the variety in Himanshu (2.83 cm) at 150 mM/l NaCl concentration. Reduction in growth is an adaptive feature for plant survival under stress and the extent of salt tolerance often appears to be inversely related to growth rate (Queiros *et al.* 2007). In our study, the maximum reduction in plant growth was recorded in the variety Himanshu (60.62%) and minimum reduction (35.60%) in variety Pusa Aditya at 150 mM/l NaCl (Fig 3). The depressive effect on plant height by salinity might be due to the reduction in cell division and enlargement, water stress induced by salinity, stomata closure which reduced the supply of carbon dioxide for photosynthesis (Mazhar *et al.* 2012). The reductions in plant growth could be attributed to the altered assimilate partitioning such as an increase in energy consuming processes. It might be due to the combination of factors such as ion toxicity and moisture stress. Similar findings were also reported by (Thongpukdee *et al.* 2014) in chrysanthemum var. Money Maker when grown on media supplemented with NaCl stress under *in vitro* conditions, in *Catharanthus roseus* (Jaleel *et al.* 2007, *Brassica juncea* (Jain *et al.* 1990) and palmarosa (Patnaik and Debata 1997). This reduction in shoot growth had impacted the plant fresh weight. Under 150 mm NaCl treatment, the highest fresh weight and shoot growth were recorded in Pusa Aditya (2.10g and 4.83 cm, respectively) and lowest in the variety Himanshu (1.00 g and 2.83 cm). Our results are in agreement with findings of Thongpukdee *et al.* (2014) in chrysanthemum.

Number of leaves and shoots per plant

The number of leaves per plant exhibied significant differences between the varieties under salinity treatment (Table 4). The number of leaves per plant reduced with

Table 3 Effect of NaCl stress on fresh weight in different chrysanthemum varieties

Variety	Different concentrations of salt solution (mM/l)				
	0 (control)	50	100	150	Mean
	Fresh weight (g)				
Pusa Chitraksha	2.5 ± 0.13^{bcdef}	2.2 ± 0.07^{efgh}	2.1 ± 0.04^{fgh}	1.8 ± 0.21^{h}	2.15
Lalit	2.8 ± 0.05^{abcd}	2.5 ± 0.24^{bcdefg}	2.1 ± 0.05^{efgh}	1.7 ± 0.07^{h}	2.27
Pusa Aditya	3.2 ± 0.28^{a}	3.0 ± 0.13^{ab}	2.7 ± 0.29 ^{abcdef}	2.1 ± 0.49^{efgh}	2.75
Basanti	3.1 ± 0.18^{a}	2.8 ± 0.22^{abc}	2.2 ± 0.07^{defgh}	1.9 ± 0.1^{gh}	2.50
Himanshu	2.7 ± 0.38^{abcde}	2.7 ± 0.26^{abcdef}	2.1 ± 0.02^{fgh}	1.0 ± 0.36^{i}	2.12
Pusa Sona	2.2 ± 0.06^{cdefgh}	2.1 ± 0.01^{fgh}	1.8 ± 0.2^{h}	1.0 ± 0.12^{i}	1.77
Mean	2.75	2.55	2.16	1.58	

Table 4 Effect of NaCl stress on number of leaves in different chrysanthemum varieties

Variety	Different concentrations of salt solution (mM/l)				
	0 (control)	50	100	150	Mean
	Number of leaves				
Pusa Chitraksha	8.6 ± 0.89^{bcd}	8.3± 1.21^{bcde}	7.3± 0.89^{bcdef}	6.0 ± 0.58^{def}	7.55
Lalit	10.3 ± 0.89^{ab}	9.6 ± 2.03^{abc}	8.3 ± 2.19^{bcde}	7.3 ± 0.67^{bcdef}	8.87
Pusa Aditya	12.0 ± 1.16^{a}	10.3± 0.89^{ab}	9.0 ± 1.53^{abcd}	8.3 ± 1.46^{bcde}	9.90
Basanti	10.3 ± 0.34^{ab}	9.6 ± 1.21^{abc}	8.0 ± 1.16^{bcde}	6.6 ± 0.89^{cdef}	8.62
Himanshu	8.0 ± 1.16^{bcde}	7.3 ± 1.34^{bcdef}	6.6 ± 0.89^{cdef}	5.3 ± 0.34^{ef}	6.80
Pusa Sona	10.0 ± 1.16^{ab}	9.3 ± 0.34^{abc}	6.0 ± 1.16^{def}	4.3 ± 0.34^{f}	7.40
Mean	9.86	9.06	7.53	6.30	

increase in the salinity treatment and maximum reduction in the number of leaves was found under the NaCl concentration of 150 mmol/l. It was found that that the number of leaves per plant was maximum under the control without NaCl treatment. The maximum reduction in the number of leaves per plant was in the variety Pusa Sona (57.00%) and minimum was found in the variety Lalit (29.10%) (Fig 5). Highest number of leaves under 150 mM/l was exhibited by the var. Pusa Aditya.

Significant differences were not found in the number of shoots per plant (Table 5). The maximum number of shoots per plant was found to be 1.75, while minimum number of shoot was 1.05 under salinity treatment of 150 mM/l. Though the number of shoots per plant reduced with salinity treatment, there were no significant differences among the varieties for the number of shoots per plant (Fig 6).

Fresh weight

It was found that the fresh weight decreased with increase in the NaCl stress (Table 3). Similar reduction in shoot fresh weight has been reported by Elhindi (2012) in chrysanthemum. Under non-saline treatment, the highest fresh weight was found to be in the variety Pusa Aditya (3.2 g) and lowest in the variety Pusa Sona (2.2 g). The fresh weight decreased with increase in the NaCl stress (Table 3). The highest reduction in the fresh weight was found in the variety Himanshu (62.00%) followed by Pusa Sona (54.00%) while the least reduction in fresh weight was in variety Pusa Chitraksha (28.00%). Under 150 mmol/l NaCl treatment, the highest fresh weight was recorded in Pusa Aditya (2.1 g) followed by Basanti (1.9 g) and least was recorded in Himanshu and Pusa Sona (1.0 g each).

This reduction in fresh weight may be due to the deduction in leaf area with a consequent increase in salinity treatment. Salinity stress has a negative impact on the biomass production due to inhibition of photosynthetic enzymes responsible for the production of food material and energy in the plant body. Salinity causes reduction in leaf area as well as the rate of photosynthesis, which together result in reduced crop growth and yield.

Rooting percentage

Significant differences were found in the rooting percentage (Table 6). The rooting abilities of all the varieties reduced with the increase in the salt concentration. Under the control with no added NaCl the rooting percentage was 100% among all the varieties. With 150 mM/l NaCl treatment, the maximum rooting was found in the variety Pusa Aditya and Basanti (63.80% in both) and least was observed in the variety Pusa Sona (22.00%) followed by Himanshu (41.80%). Rooting percentage reduced with

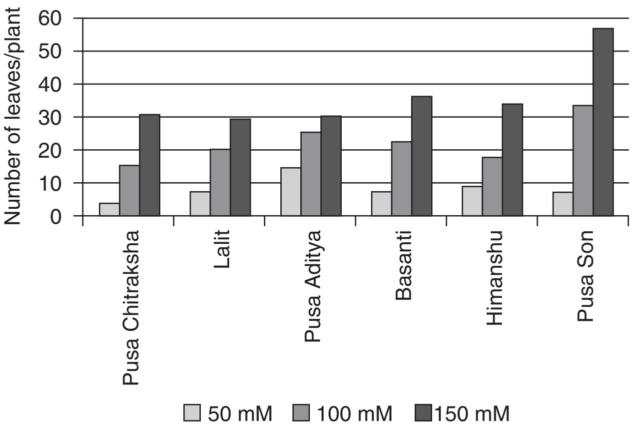

Fig 5 Reduction percentage in number of leaves/plant with increase in NaCl stress.

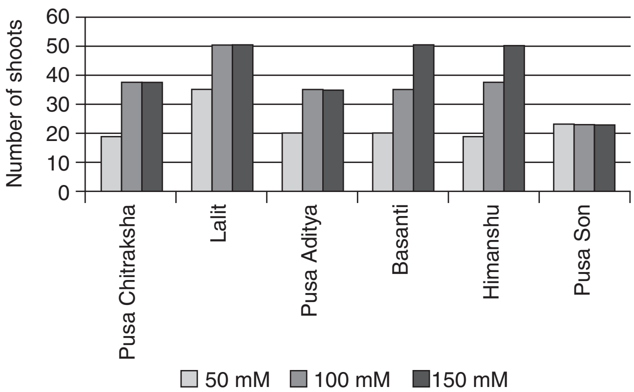

Fig 6 Reduction percentage in number of shoots with increase in NaCl stress.

Table 5 Effect of NaCl stress on number of shoots in different chrysanthemum varieties

Variety	Different concentrations of salt solution (mM/l)				
	0 (control)	50	100	150	Mean
			Number of shoots		
Pusa Chitraksha	1.6 ± 0.33^{ab}	1.3 ± 0.33^{ab}	1.0 ± 0^{b}	1.0 ± 0^{b}	1.25
Lalit	2.0 ± 0^{a}	1.3 ± 0.33^{ab}	1.0 ± 0^{b}	1.0 ± 0^{b}	1.32
Pusa Aditya	2.0 ± 0.57^{a}	1.6 ± 0.33^{ab}	1.3 ±0.33^{ab}	1.3 ± 0.33^{ab}	1.55
Basanti	2.0 ± 0.57^{a}	1.6 ± 0.33^{ab}	1.3 ± 0.33^{ab}	1.0 ± 0^{b}	1.47
Himanshu	1.6 ± 0.66^{ab}	1.3 ± 0.33^{ab}	1.0 ± 0^{b}	1.0 ± 0^{b}	1.22
Pusa Sona	1.3 ± 0.33^{ab}	1.0 ± 0^{b}	1.0 ± 0^{b}	1.0 ± 0^{b}	1.07
Mean	1.75	1.35	1.10	1.05	

Table 6 Effect of NaCl stress on rooting percentage in different chrysanthemum varieties

Variety	Different concentrations of salt solution (mM/l)				
	0 (control)	50	100	150	Mean
			Rooting percentage (%)		
Pusa Chitraksha	100 ± 0^{a}	86.40 ± 3.8^{abc}	75.20 ± 4.6^{bcd}	55.50 ± 5.8 ^{fg}	79.30
Lalit	100 ± 0^{a}	86.60 ± 7.7^{abc}	75.40 ± 8^{bcd}	57.50 ± 7.7^{ef}	79.80
Pusa Aditya	100 ± 0^{a}	88.80 ± 8^{ab}	79.50 ± 10.4^{bcd}	63.80 ± 2.2^{de}	83.40
Basanti	100 ± 0^{a}	79.40 ± 10.4^{bcd}	70.40 ± 5.8^{cde}	63.80 ± 12.2^{de}	78.40
Himanshu	100 ± 0^{a}	79.40 ± 7.8^{bcd}	52.80 ± 7.6^{efg}	41.80 ± 5.8^{fg}	68.52
Pusa Sona	100 ± 0^{a}	57.20 ± 5.8^{efg}	39.60 ± 3.8^{gh}	22.00 ± 2.2^{h}	54.70
Mean	100	79.60	65.50	50.70	

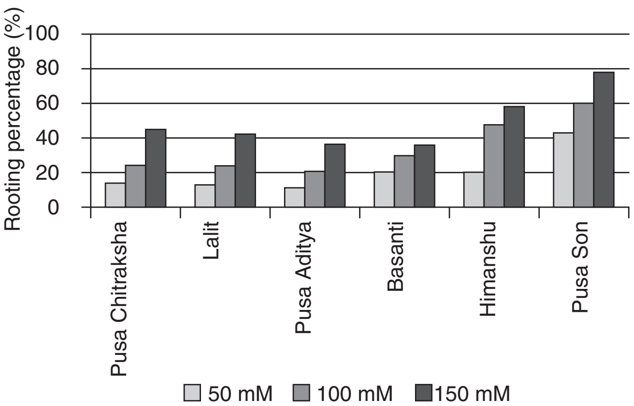

Fig 7 Reduction percentage in rooting percent with increase in NaCl stress.

an increase in salinity levels. Maximum reduction was observed in Pusa Sona (78%) and least was observed in Pusa Aditya and Basanti (both 36.2%) (Fig 7). Our results are in agreement with findings of Hossain *et al.* (2004) and (Thongpukdee *et al.* 2014) in chrysanthemum. Roots are the most sensitive organ in a plant body and are affected first under salinity stress. Inhibition in root growth may be due to the reduction in the length of root tip elongation zone and decline in the rate of cell division. It is evident that NaCl stress causes reduction in many growth parameters such as shoot length, rooting as well as biomass. These parameters can be used as indicators for screening salt tolerant genotypes under *in vitro* conditions.

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