Indian Journal of Agricultural Sciences **91** (1): 128–31, January 2021/Article
https://doi.org/10.56093/ijas.v91i1.110941

Protocol for salt stress screening of African marigold under hydroponic system

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Received: 30 June 2020; Accepted: 20 November 2020

ABSTRACT

The present study was carried out at ICAR-IARI to standardize the protocol for screening of African marigold against saline stress under hydroponic system. Seedlings of African marigold cv. Pusa Narangi Gainda were grown under hydroponics system. They were treated with different levels of NaCl, viz. 0 mM 50 mM, 80 mM, 100 mM, 120 mM, 140 mM, 150 mM, 160 mM, 200 mM, after 7 days of transplanting to standardize the threshold level for the further rapid screening of germplasms at nursery stage. Observations on mortality, growth, morphological, physiological, biochemical parameters and tissue nutrient status were recorded and found to decrease with increasing in saline stress. Mortality in seedlings was not observed up to 80 m M NaCl stress. 150-160 mM qualified as the indicator concentration for the screening of African marigold genotypes based on the performance of marigold genotypes with regard to different morphological, physiological, biochemical parameters and tissue nutrient contents.

Key words: African marigold, Hydroponics, NaCl, Saline stress

Marigold is a common ornamental and herbaceous plant belonging to the family Asteraceae. The species such as *T. erecta* (African marigold) and *T. patula* (French marigold) are mainly cultivated in India. It is used for floral decoration, religious offerings, also for making garlands. African marigold and French marigold are grown mostly for loose flower production and in landscaping as a garden plant. It is said to deter some common insect pests as well as nematodes. Hence it is used as companion plant for tomato, eggplant, chilli etc. *T. erecta* is rich in orange yellow carotenoid (lutein) and is used as a food flavour and in poultry Industry as an additive to feed. In India it accounts for more than half of the nation's loose flower production. Major growing states are Karnataka, Gujarat, Maharashtra, Haryana, Andhra Pradesh, Uttar Pradesh, West Bengal and Tamil Nadu.

Salinity is a worldwide problem affecting plant growth, yield, and quality. In India, salt affected soils are source of concern in over 6.73 m ha (Singh 2009). Saline soils extensively occur in Punjab, Haryana, Uttar Pradesh, Gujarat, Rajasthan, West Bengal, Maharashtra, Odisha, Andhra Pradesh, Karnataka and Tamil Nadu. Salt affected soils have higher amount of chlorides and sulphates of the calcium, sodium and magnesium. The general characteristics of saline soil is the occurrence of high concentration of soluble salts or high exchangeable sodium (Na) (ESP>15%, *p*H> 8.5) which interferes with normal plant growth. Marigold is moderately tolerant to salinity because growth is affected when EC of irrigation water exceeds 8 dS/m (Anguilar *et al*. 2009).

Recent studies on salt tolerance of plants is focused on crops such as rice, wheat, groundnut and other horticultural crops but research on salt tolerance of marigold has not been widely reported. Screening of genotypes/varieties under hydroponic system is a very fast and accurate method to test the genotypes for tolerance to salt stress. The present investigation was designed to standardize the protocol for screening of African marigold against saline stress under hydroponic system.

MATERIALS AND METHODS

The experiment was conducted at National Phytotron Facility (NPF) and Division of Floriculture and Landscaping, ICAR-IARI New Delhi during 2016. African marigold cv. Pusa Narangi Gainda was taken for standardization of protocol for screening under hydroponic system.Healthy and vigorous seeds were sown in plastic protrays filled with cocopeat, vermiculite and perlite in 3:1:1 ratio. These protrays were kept in green house in Phytotron and optimum temperature was maintained which resulted in healthy and disease-free seedling production. Thermofoat, plastic nets, crates were used for raising seedlings under hydroponics system. Seedlings were sown in punch holes made on

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extruded polystyrene foam floats fitted with a nylon wire mess on the bottom side and suspended on trays filled with Hoagland's solution. Seedlings were transplanted after 15 days of sowing. Seedlings grown in thermofoat fitted with plastic nets which were floated over trays were treated with different levels of NaCl, i.e. 0 mM (control), 50 mM, 80 mM, 100 mM, 120 mM, 140 mM, 150 mM, 160 mM and 200 mM after 7 days of placing in hydroponic system. Solutions were changed at weekly interval to ensure the fresh solution ladder with all essential nutrients. Different morphological parameters were taken after 15 days of salt treatment. Shoot and root length were measured by scale. Shoot diameter was measured with digital Vernier Callipers. Fresh weight of roots and shoots was measured with the help of electronic balance. The dry weight of shoot and roots of seedlings were recorded after drying in hot air oven at 70ºC for 48 h or more till constant weight is obtained and expressed in grams.LICOR-3000 leaf area meter was used for measuring leaf area. The number of affected leaves per plant was counted from the seedlings from date of salt treatment at regular interval and final percentage of leaves affected was calculated. Mortality percentage was calculated in terms of percentage. The chlorophyll content (chlorophyll a, b and total chlorophyll) of the leaves was analyzed by following the method suggested by Hiscox and Israelstam (1979). Ratio of chlorophyll a and b was calculated by dividing chlorophyll 'a' by chlorophyll 'b'. Both shoot and root samples from each treatment were harvested for nutrient analysis. The data were recorded for K, Na, Ca, and Mg. Potassium and sodium content was determined by flame photometer using a compressor and LPG flame. Calcium and magnesium content were estimated by Versenate (EDTA) titration method.

The experiment was laid out in completely randomized design. The data on different parameters were analysed by using Analysis of variance (ANOVA) as suggested by Gomez and Gomez (1984).

RESULTS AND DISCUSSION

Saline stress significantly impacted shoot length, root length, shoot diameter, biomass thereby leading to development of necrosis and in case of severe stress leading to mortality. Significant reduction in shoot and root length was observed with increase in salinity levels (Table 1). Reduced height with increasing salinity may be attributed to the capacity of NaCl to inhibit growth of plants and reduction in shoot growth is commonly expressed by a reduced leaf area and stunted shoots (Lauchli 1990). These results were in conformity with the observations made in *Tagetes erecta* (Sayyed *et al.* 2014) and oleander seedlings (Banon *et al.* 2001).The root system is the direct injured part caused by salt and alkaline medium. Similar result on decrease in root length was observed by Sayyed *et al.* (2014) in *Tagetes erecta* and Rehman *et al.* (2014) in *Rosa hybrida*. There was significant reduction in fresh and dry weight of shoots and roots of marigold with increasing concentration of NaCl. One of the initial effects of salt stress on plant

Table 1 Effect of NaCl stress on morphological characteristics and pigment content of marigold seedlings

NaCl conc. (mM)	Shoot length (cm)	Root length (cm)	Shoot fresh weight (g)	Root fresh weight (g)	Shoot dry weight (g)	Root dry weight (g)	Shoot diameter (mm)	Leaf area (cm^2)	Mortality percentage (%)	% of affected leaves	Chlorophyll a (mg/g FW)	Chlorophyll b (mg/g FW)	Total chlorophyll (mg/g FW)	Carotenoid (mg/g FW)	chlorophyll a:b
0	15.90	23.43	3.55	0.42	0.26	0.06	4.06	15.00	0.00	0.00	2.28	1.07	3.34	0.15	2.13
50	17.30	21.27	2.58	0.04	0.20	0.05	3.53	13.48	0.00	2.56	1.78	0.58	2.36	0.12	3.09
80	12.00	18.67	2.16	0.35	0.17	0.04	3.27	13.10	0.00	7.10	2.28	1.15	3.43	0.15	1.98
100	11.60	16.87	2.17	0.33	0.15	0.03	3.12	11.51	17.91	18.37	2.15	0.63	2.78	0.14	3.38
120	9.70	14.43	1.71	0.27	0.13	0.02	3.11	10.11	28.48	25.25	1.71	0.48	2.20	0.13	3.55
140	8.63	12.37	0.82	0.14	0.09	0.01	2.32	8.27	33.45	35.56	1.65	0.36	2.01	0.12	4.62
150	8.20	10.87	0.57	0.03	0.06	0.01	2.07	6.69	50.59	53.03	1.07	0.51	1.58	0.07	2.09
160	7.30	7.53	0.22	0.04	0.03	0.01	1.84	4.21	68.45	82.83	0.75	0.25	1.01	0.06	1.96
200	6.37	5.67	0.07	0.01	0.02	0.00	0.32	0.61	100.00	100.00	0.42	0.17	0.59	0.02	2.62
CD ($P<0.05$)	0.35	0.64	0.29	0.02	0.02	0.004	0.19	0.39	3.33	4.44	0.03	0.04	0.03	0.00	0.47

is the reduction of growth rate. Fresh and dry biomass of plant exhibited reduction in high salinity by suppression of plant growth under saline conditions may either be due to decreased availability of water or to the toxicity of sodium chloride. The reduction in dry weight under salinity stress may be attributed to inhibition of hydrolysis of reserved foods and their translocation to the growing shoots (Feng *et al.* 2000). Zhani *et al*. (2012) reported that the fresh biomass of *Capsicum frutescens* L. decreased with increasing salinity. Decrease in dry weight of shoot and leaves was observed with salt stress in capsicum (Zhani *et al.* 2012) and marigold (Aguilar *et al.* 2009).

There was increasing decline in fresh weight and dry weight of roots with increase in saline stress. There was dramatic reduction in fresh weight and dry weight above 100 mM. In saline environment, plant growth is affected negatively in various ways such as osmotic effect, specific ion effect and nutritional imbalance, probably all occurring simultaneously. Shoot diameter was affected significantly by salinity. Highest shoot diameter was found in control and the lowest in 200 m M (Table 1). Due to stunted and weak growth it was found to be less.The effect of NaCl stress on leaf area after 10 days of salt treatment was significant and leaf area reduced with increasing levels of salinity (Table 1). The tip and marginal necrosis of leaves was more evident in NaCl treated seedlings. Increase in salt stress significantly affected the number and length of leaves in a concentration dependent manner. Leaf area reduced due to growth reduction. Salinity causes loss of cell turgor and reduces leaf expansion rates. This in turn, leads to reduction in the leaf area available for photosynthesis and a loss of productivity. Jesche and Stelter (1983) observed significant decrease in leaf area with increase in salinity level in irrigation water for lilac.

With increasing levels of NaCl, mortality percentage increased significantly (Table 1). There was 100% mortality with 200 mM and nil in control.Mortality was not observed till 80 mM. The percentage of affected leaves increased dramatically after 80 mM. Mortality percentage was more than 50% and percentage of affected leaves increased above 50% with 150 mM. Mortality was due to reduced availability of water, toxic ionic effects, leaf necrosis, ion imbalance, high Na/K ratio. Pronounced effect of NaCl was observed on chlorophyll content. The NaCl treatment resulted in significant reduction in chlorophyll a, b, total chlorophyll and carotenoid pigments above 80 mM (Table 1). The ratio of chlorophyll a and b clearly indicated that NaCl treatment significantly increased the chlorophyll a and b ratio up to a certain level, i.e. 140 mM and thereafter it declined.Photosynthesis one of the most important metabolic pathways in plants, is a target of salt stress. Chlorosis of leaves is the first visual symptoms of stress leading to senescence and is associated with a decline in concentration of photosynthetic pigments. Parida and Das (2005) suggested that decrease in chlorophyll content in response to salt stress is a general phenomenon which led to disordering synthesizing chlorophyll and appearing chlorosis in plant. The results were in accordance with several reports of decreasing content of chlorophyll and carotenoids by salinity as reported in a number of glycophytes (Agastian *et al.* 2000). Sayyed *et al.* (2014) observed reduction of plant height, root length, no of leaves, fresh and dry biomass Chlorophyll a, b and carotenoids content was reduced under higher concentration of NaCl in *Tagetes erecta*.

Potassium content of shoots declined with increase in saline stress with minimum content in 200 mm and maximum with 0 mM (Table 2). In case of roots the content increased till 100 mM, therafter it declined significantly. Maximum calcium and magnesium content in shoots and roots was observed in control and minimum in 200 mM. Significant reduction in levels of nutrients like potassium, calcium and magnesium was observed in marigold shoots and roots with saline stress. The effect of high concentration of NaCl in the root medium results in suppression of uptake of essential nutrients like K, Ca and N etc. A lower concentration of magnesium was due to high concentration of sodium and chloride in the root medium which suppress the uptake of essential nutrients like potassium, calcium and magnesium and nitrogen (Perviaz and Clement 2002). Sodium competes with potassium for

Table 2 Mineral content of marigold as influenced by NaCl stress

Nacl Conc. (mM)	K content in shoots (%)	K content in roots (%)	Na content in shoot (%)	Na content in roots (%)	Shoot Na:K ratio	Root Na:Kratio	Ca content in Shoot (ppm)	Ca content in root (ppm)	Mg Content in Shoot (ppm)	Mg content in Root (ppm)
0	1.14	3.85	0.04	0.28	0.04	0.07	128.79	47.27	66.55	38.67
50	1.07	2.21	0.07	0.94	0.07	0.43	115.56	39.91	62.92	31.38
80	0.93	4.60	0.09	1.41	0.10	0.31	100.73	32.04	59.22	18.62
100	0.82	5.49	0.11	4.07	0.13	0.74	96.03	23.84	57.66	20.69
120	0.73	4.73	0.12	4.35	0.16	0.92	80.25	19.53	51.86	14.23
140	0.67	2.81	0.14	6.06	0.21	2.16	72.04	16.05	47.72	12.57
150	0.63	1.01	0.15	6.56	0.24	6.50	53.56	13.24	25.48	10.47
160	0.56	2.53	0.16	6.83	0.29	2.70	36.06	11.60	14.79	9.48
200	0.42	1.91	0.16	7.04	0.38	3.69	31.69	9.64	9.64	8.38
C D ($P<0.05$)	0.01	0.68	0.01	0.05	0.01	0.05	2.92	1.00	0.98	0.72

uptake through common transport system and does this effectively as the sodium in saline environment is usually greater than potassium (Dubey *et al.* 2006). The Na contents in both shoot and root was strongly influenced by NaCl. As expected salinity increases sodium content in both shoot and root. Maximum shoot sodium and root sodium was found in 200 mM. Minimum shoot sodium and root sodium was observed in control. It was evident that with increasing concentration sodium content increases.

The increase in sodium content (Na+), Na+–K+ ratio, and decrease in potassium content, was observed under increasing salt stress. The accumulation of Na+ results in ionic imbalance, specific ion effects, nutrient deficiency symptoms and disturbed metabolism by the toxic effects of accumulated ions (Tester and Davenport 2003). Salt stress induced increase in sodium and depletion of potassium contents have been reported earlier in cucumber (Stepien and Kobus 2006). Salinized plants showed higher Na: K ratio compared to control. Significant effect of NaCl on Na: K ratio was found. A high cytosolic K+/Na+ ratio is important for maintaining cellular metabolism. In the present study, the levels of Na+ gradually increased with increasing concentration of salt, while K+ levels decreased with increasing concentration of salinity stress. High levels of Na+ inside the cell inhibits the uptake of K+ thereby increase Na+/K+ ratio which in turns affects plant metabolism (Maathuis *et al.* 1992).

Saline stress had a significant effect on all the morphological and biochemical parameters observed. The percentage of affected leaves and mortality percentage was almost 50% in the range of 150–160 mM NaCl. 150–160 mM can be taken as an indicator dosage of Nacl for screening of African marigold genotypes against saline stress. This hydroponic system is an accurate method for screening of genotypes of marigold against saline stress under controlled conditions.

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