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ORIGINAL ARTICLE

Growth performance and changes in physiological attributes of khirni (Manilkara hexandra L.) seedlings under salinity stress

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ABSTRACT

Khirni or *rayan* (*Manilkara hexandra* L.) fruit is known as "protein capsule" in the tribal belt of western and central India, well adopted to arid and semi-arid conditions. It can tolerate drought conditions; however it is its salinity tolerance. Therefore, a pot experiment was carried out at Fruit Research Station, Navsari Agricultural University, Gandevi (Gujarat) during 2017 under polyhouse condition to find out "Effect of different salinity levels on growth and physiological characters of rayan (*Manilkara hexandra* L.)". The experiment consisted of six different salinity levels of irrigation water *viz.*, 0.5, 2, 4, 6, 8 and 10 dS m^{-1} which laid out in a completely randomized design (CRD) with three repetitions. The sea water has been used to impose the treatments. Results revealed that all growth parameters including plant height, number of leaves, fresh and dry weight of seedling; survival percentage and extent of reduction in dry biomass od seedling were found in increasing trend as the salinity level increased from 0.5 to 10 dS m^{-1}. Looking to the extent of reduction in dry biomass and survival percentage in dry biomass and survival percentage, it is revealed that rayan seedlings could grow well up to 8 dS m^{-1} salinity level whereas higher growth of seedlings was obtained up to 4 dS m^{-1}.

Keywords: Khirni, Salinity levels, Growth, Photosynthetic rate, Stomatal conductance, RWC

INTRODUCTION

Rayan or *khirni* (*Manilkara hexandra* L.) is a slow evergreen tree with spreading crown and straight bowl, well adapted to arid and semi-arid conditions and can tolerate drought conditions. The different parts of this plant are known for their nutritional and medicinal properties among the tribal population of western and central India. It bears oval, sweet edible berry fruits with one or more seeds. Khirni fruits are milky, sweet, cooling, aphrodisiac, appetizer, emollient and tonic. Fresh fruits provide good nutritional value especially the requirement of vitamin A (675 IU) for children. However, it has been proved to be the best rootstock for sapota grafting. It is commercially propagated by seeds and has been used in inarch grafting, side grafting and softwood grafting of sapota.

Due to salinity, productivity of sapota is decreasing day by day, for that rayan is recommended as saline tolerant rootstock. The main objective of present studies that to know the saline tolerance of rayan seedlings. Saline soils contain soluble salts in sufficient quantities that affect plant growth adversely and the electrical conductivity of the soil saturation extract exceeds 4 dS m^{-1}. Ions that contribute to salinity include chlorides, sulphates, bicarbonates, sodium, calcium and magnesium (Palaniappan and Chadha, 1993). Salt stress reduces water potential, causes ion imbalance or disturbances in ion homeostasis, and toxicity. Since salt stress involves both osmotic and ionic stress. Growth suppression is directly related to total concentration of soluble salts or osmotic potential of soil water. Salt stress affects all the major processes such as growth, photosynthesis, protein synthesis, and energy and lipid metabolism (Rahimi and Biglarifard, 2011).

MATERIALS AND METHODS

The present investigation was carried out under polyhouse during 2017 at the Fruit Research Station, Navsari Agricultural University, Gandevi (South Gujarat) which situated in agro-ecological situation-III under heavy rainfall zone. One month old rayan seedlings (2 leaf stage) of uniform size were selected for the experiment. Soil : FYM (1:1) were used for preparation of media (Table 1).

Media pH and media EC were determined by pH meter and EC meter. Soil organic carbon (%) was determined by walkley and black's rapid titration method (Jackson 1973). Available N was determined by alkaline $KMnO_4$ method (Subbiah and Asija, 1956). Available P was determined by spectrophotometric, ascorbic acid reagent (Olsen et al., 1954). Available K and extractable Na were determined by flame photometery. Extractable Ca and Mg were determined by complexometric titration method with EDTA (Table 1).

For preparing different salinity treatments *viz.,* 0.5, 2, 4, 6, 8 and 10 dS m^{-1}, sea water was added in tape water / rain water with the help of EC meter (Table 2 and 3).

Growth parameters *viz.,* plant height, number of leaves, fresh weight, dry weight and survival percentage were recorded periodically. Plant height measuring from the base of stem to the growing tip of the plant with the help of a meter scale in centimetre and number of leaves per plant were recorded at 40, 80 and 120 days after treatment and the mean data were calculated for statistical analysis.

For fresh weight, dry weight of shoot and root of rayan seedlings fifteen random samples per treatment were collected. The roots were washed to remove the soil adhering to it and dry weight of roots was recorded in grams with the help of analytic balance after oven drying

at 65°C for 24 hours till the constant dry weight was attained at 120 days after imposing treatment. The mean dry biomass of shoot, roots and whole plant were calculated from oven-dried samples. The reduction in dry biomass due to different salinity levels over control treatment *i.e.* 0.5 dS m^{-1} (T_1) was computed using following formula:

$$\text{Reduction in dry biomass (\%)} = \frac{\text{Check } (T_1) \text{ treatment value} - \text{Salinized treatment value}}{\text{Check treatment value}} \times 100$$

Survival percentage was recorded at the end of experiment with following formula.

$$\text{Survival percentage (\%)} = \frac{\text{No. of survived seedlings}}{\text{No. of total seedlings}} \times 100$$

The rate of photosynthetic parameters *viz*., photosynthetic rate, transpiration rate and stomatal conductance were measured with the help of LCi- SD Portable Photosynthesis System at 40, 80 and 120 days after treatment.

Relative water content was calculated with the help of formula at 120 days after imposing treatment

$$\text{RWC (\%)} = \frac{\text{Fresh weight (g)} - \text{Dry weight (g)}}{\text{Turgid weight (g)} - \text{Dry weight (g)}} \times 100$$

The data collected were analyzed statistically as per completely randomized design (CRD) described by Panse and Sukhatme (1967).

RESULT AND DISCUSSION

EFFECT OF DIFFERENT SALINITY LEVELS ON GROWTH AND SURVIVAL PERCENTAGE

1 plant height and number of leaves

The plant height and number of leaves of rayan seedlings were significantly influenced by different salinity levels (Table 4). The seedling growth was found in decreasing trend as salinity level increased. However, the maximum plant height (9.38, 18.75 and 24.27 cm, respectively) and number of leaves per seedling (6.40, 10.20 and 12.33, respectively) were recorded in 0.5 dS m^{-1} (T_1) at 40, 80 and 120 days after imposing treatment. Higher salinity level (10 dS m^{-1}) showed maximum reduction in plant height (6.88, 10.61 and 11.05 cm, respectively) and number of leaves (5.47, 7.00 and 7.20) at all periodical observations. In saline habitate, presence of NaCl that alters the nutritional balance of plants, resulting in high ratio of Na^+/Ca^{+2}, Na^+/K^+, Na^+/Mg^{+2}, Cl^-/NO_3^-, $Cl^-/H_2PO_4^-$, which may cause reduction in growth (Grattam and Grieve, 1992). The reduction may also be due to the osmotic effect of salt on root and toxic effect of accumulated ion in plant tissues (Lea-cox and Syvertsen, 1993; Storey, 1995). The similar results were finding with Haq et al. (2011) in banana; Pandey et al. (2014) in mango, Sa et al. (2016) in guava and Desouky et al. (2015) in grape.

2 fresh weight and dry weight (g)

The data pertaining to fresh weight, dry weight of shoot and root and whole rayan seedlings as influenced by different levels of saline water are shown in Table 5. It revealed that the highest fresh weight of plant (9.30 g); and dry weight of shoots, (2.57 g) roots (0.92 g) and whole plant (3.49 g) were noted in 0.5 dS m^{-1} (T_1). All these parameters were showed reducing trend with increasing of salinity levels of irrigation water. The higher salinity level (10 dS m^{-1}) recorded the lowest fresh weight of plant (2.95 g); and dry weight of shoots (0.94 g), roots (0.39 g) and whole plant (1.34 g). Probably it is due to the negative effect of salinity on plant, which provoked osmotic potential by salt in growing media. Hence, root cells could not obtain required amount of water from the media which restricts the uptake of water in plants. Thus, the growth and development of plants are inhibited due to defect in metabolism (Pandey et al., 2014). As the salt concentration increases, the nutrient imbalance, hyperosmotic stress and ion disequilibrium plays a pivotal role in disturbing the cellular functions of plant (Foolad, 2004). These results are confirmed by Pandey et al. (2014) in mango and Haq et al. (2011) in banana.

3 extent of reduction in dry biomass

It is clear from Table 6 that the reduction in dry biomass increased with increasing salinity levels. The lowest reduction in dry weight of shoots (14.01 %), roots (8.73 %) and whole plant (12.70 %) was observed in treatment T_2 (2 dS m^{-1}) over check *i.e.* 0.5 dS m^{-1} (T_1). As the salinity levels increased the extent of dry biomass was increased up to 10 dS m^{-1} (T_6). The maximum reduction in dry weight of shoots (63.42 %), roots (57.09 %) and whole plant (61.89 %) was recorded in treatment T_6 (10 dS m^{-1}) over check *i.e.* 0.5 dS m^{-1} (T_1). However, the permissible extent of decrease in dry biomass (50 %) was recorded in 8 dS m^{-1} salinity level (T_5) with values of 48.64, 53.09 and 49.85 % in shoots, roots and whole plant, respectively. Similar findigs were also observed in teak (Anon., 2017) and in purslane (Alam *et al.*, 2015).

4 survival percentage

The survival (%) of rayan seedlings was also significantly reduced as influenced by salinity levels. Survival percentage was recorded which ranges from 69.09 to 97.58 % (Table 7). The higher survival percentage was obtained up to 8 dSm^{-1}(T_5). It might be due to soil salinity exerts a detrimental effect on plants through ion toxicity and osmotic stress which may cause nutritional imbalance, reduce the respiration rate, induce the hormonal imbalance or damage the plant cells and cytoplasmic organelles. It ultimately causes mortality of plant. The similar results was also obtained in mango (Srivastav et al., 2009).

EFFECT OF DIFFERENT SALINITY LEVELS ON PHYSIOLOGICAL PARAMETERS

1 Physiological parameters

All the physiological parameters were significantly influenced by application of various salinity levels (Table 8 and 9). The maximum photosynthetic rate (26.14, 27.31 and 29.79 µmol CO_2 $m^{-2}s^{-1}$, respectively), stomatal conductance (0.036, 0.061 and 0.066 mmol CO_2 m^{-2} s^{-1}, respectively) and transpiration rate (0.54, 0.57 and 0.62 mmol H_2O m^{-2} s^{-1}, respectively) were recorded in 0.5 $dS.m^{-1}$ (T_1) at 40, 80 and 120 days after imposing treatment. Whereas the minimum values of physiological parameters were noted in 10 $dS.m^{-1}$ (T_6). It might be due to the decreased rate of transpiration may be due to the growth of plant under salinity condition which fails to activate the dehydration avoidance mechanism like impermeability of root membranes to toxic ions of Na^+ and Cl^-. Therefore, sensitive plant do not maintain stomatal conductance up to desired rate (Abbruzzese et al., 2009). The presence of salt leads to the closure of stomata and decreased

transpiration rate resulting in reduced stomatal conductance and decrease in uptake of CO_2 used in carboxylation reactions respectively (Brugnoli and Bjorkman, 1992) and thus photosynthetic rate is reduced. The above results are in conformity with the findings of Byrbordi, (2012) in grape; Singh et al. (2014) in citrus and Singh et al. (2015) in bael.

2 Relative water content (%)

RWC was observed in decreasing trend with increasing salinity levels (Table 10). The minimum relative water content (63.76 %) was noted in higher salinity level treatment T_6 (10 dS m^{-1}). It significantly reduced due to the high salt concentration in root zone, that causes osmotic stress, restricts water absorption by the plants and causes cellular dehydration which seems to be primarily responsible for decrease in relative water content (Greenway and Munns, 1980). The similar results are reported by Singh *et al.* (2014) in citrus and Singh *et al.* (2015) in bael.

CONCLUSION

From the present study, it can be concluded that khirni or *rayan* seedlings can tolerate salinity upto 8 dS m^{-1} by showing higher survival percentage and minimum reduction in dry biomass of plant. Besides, the seedling growth as well as physiological parameters has been recorded unaffected considerably up to 4 dS m^{-1}.

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Table 1 Initial physico-chemical properties of media

Particulars	**Initial**
pH	6.23
EC (dS m^{-1})	0.78
Soil organic carbon (%)	7.2
Available N (mg/kg)	185.6
Available P_2O_5 (mg/kg)	47.2
Available K_2O (mg/kg)	432.3
Extractable Na (me/100 g)	0.56
Extractable Ca (me/100 g)	17.12
Extractable Mg (me/100 g)	9.87

Table 2 Different levels of salinity

Serial No.	**Salinity levels (dS m^{-1})**	**Tap water (lit)**	**Sea water (ml)**
1	0.5	1	1000*
2	2	1	25
3	4	1	75

4	6	1	90
5	8	1	105
6	10	1	130

*Rain water was used to make salinity 0.5 dS m^{-1}

Table 3 Chemical analysis of irrigation water used while experimentation (mean of three periodical saline water)

Particulars	0.5 dS m^{-1}	2 dS m^{-1}	4 dS m^{-1}	6 dS m^{-1}	8 dS m^{-1}	10 dS m^{-1}
pH	7.29	7.42	7.49	7.41	7.45	7.44
EC (dS m^{-1})	0.53	1.98	4.04	5.98	8.02	9.97
HCO^{-}_3 (me l^{-1})	3.00	3.46	3.56	3.56	3.65	3.68
CO_3^{-2} (me l^{-1})	0.32	0.40	0.40	0.35	0.45	0.45
Cl^{-} (me l^{-1})	2.14	15.92	34.52	48.42	73.64	88.66
Na^{+} (me l^{-1})	1.84	15.29	26.82	48.16	60.06	80.67
Ca^{+2} +Mg^{+2} (me l^{-1})	3.34	5.48	7.46	9.40	12.22	15.32
K^{+} (me l^{-1})	0.02	0.03	0.12	0.23	0.42	0.59
RSC	-0.1	-1.76	-3.8	-6.41	-9.61	-11.19
SAR	1.42	9.23	13.89	22.61	24.3	32.22

Table 4 Effect of different salinity levels on plant height and number of leaves of rayan seedlings

Treatments	Plant height (cm)			Number of leaves		
	40 DAT	80 DAT	120 DAT	40 DAT	80 DAT	120 DAT
T_1 0.5 dS.m^{-1}	9.38	18.75	24.27	6.40	10.20	12.33
T_2 2.0 dS.m^{-1}	9.03	17.71	22.68	6.33	9.53	11.80
T_3 4.0 dS.m^{-1}	8.16	15.98	20.10	6.07	9.27	10.67
T_4 6.0 dS.m^{-1}	7.65	14.61	17.16	5.87	8.60	9.53
T_5 8.0 dS.m^{-1}	7.33	13.30	14.29	5.73	8.00	8.33
T_6 10.0 dS.m^{-1}	6.88	10.61	11.05	5.47	7.00	7.20
S. Em ±	0.17	0.43	0.59	0.09	0.18	0.23
C.D. at 5 %	0.53	1.31	1.81	0.28	0.56	0.70
CV %	3.68	4.87	5.58	2.62	3.57	3.95

Table 5 Effect of different salinity levels on fresh weight; and dry weight of shoots, roots and whole plant (g) of rayan seedlings after 120 days

Treatments	Fresh weight (g/plant)	Dry weight (g)		
		Shoots	Roots	Whole plant
T_1 0.5 dS m^{-1}	9.30	2.57	0.92	3.49
T_2 2.0 dS m^{-1}	8.02	2.21	0.84	3.05
T_3 4.0 dS m^{-1}	7.20	2.09	0.76	2.85
T_4 6.0 dS m^{-1}	5.67	1.63	0.64	2.26
T_5 8.0 dS m^{-1}	4.45	1.32	0.43	1.75
T_6 10.0 dS m^{-1}	2.95	0.94	0.39	1.34
S.Em ±	0.16	0.06	0.03	0.06
C.D. at 5 %	0.50	0.17	0.09	0.18
CV %	4.49	5.39	8.29	4.10

Table 6 Effect of different salinity levels on extent of reduction in dry biomass of shoots, roots and whole plant of rayan seedlings after 120 days

Treatments	Change in dry biomass over T_1 (%)		
	Shoots	Roots	Whole plant
T_1 0.5 dS m^{-1}	-	-	-
T_2 2.0 dS m^{-1}	-14.01	-8.73	-12.70
T_3 4.0 dS m^{-1}	-18.68	-17.45	-18.62
T_4 6.0 dS m^{-1}	-36.58	-30.55	-35.24
T_5 8.0 dS m^{-1}	-48.54	-53.09	-49.85
T_6 10.0 dS m^{-1}	-63.42	-57.09	-61.89

Table 7 Effect of different salinity levels on survival (%) of rayan seedlings at 120 days

Treatments	Survival (%)
T_1 0.5 dS m^{-1}	97.58
T_2 2.0 dS m^{-1}	96.97
T_3 4.0 dS m^{-1}	95.15
T_4 6.0 dS m^{-1}	92.12
T_5 8.0 dS m^{-1}	87.88
T_6 10.0 dS m^{-1}	69.09

S. Em ±	1.399
C.D. at 5 %	4.31
CV %	2.70

Table 8 Effect of different salinity levels on photosynthetic rate (µmol CO_2 $m^{-2}s^{-1}$) of rayan seedlings

Treatments	Photosynthetic rate (µmol CO_2 $m^{-2}s^{-1}$)		
	40 DAT	80 DAT	120 DAT
T_1 0.5 dS m^{-1}	26.14	27.31	29.79
T_2 2.0 dS m^{-1}	24.95	26.03	27.65
T_3 4.0 dS m^{-1}	23.06	23.37	25.63
T_4 6.0 dS m^{-1}	20.56	22.52	24.10
T_5 8.0 dS m^{-1}	18.15	19.83	20.92
T_6 10.0 dS m^{-1}	13.00	16.03	17.02
S. Em ±	0.59	0.70	0.90
C.D. at 5 %	1.82	2.15	2.78
CV %	4.89	5.36	6.46

***** DAT: Days after imposing treatment

Table 9 Effect of different salinity levels on stomatal conductance (mmol CO_2 $m^{-2}s^{-1}$) and transpiration rate (mmol H_2O $m^{-2}s^{-1}$) of rayan seedlings

Treatments	Stomatal conductance (mmol CO_2 $m^{-2}s^{-1}$)			Transpiration rate (mmol H_2O $m^{-2}s^{-1}$)		
	40 DAT	80 DAT	120 DAT	40 DAT	80 DAT	120 DAT
T_1 0.5 dS m^{-1}	0.036	0.061	0.066	0.54	0.57	0.62
T_2 2.0 dS m^{-1}	0.035	0.057	0.064	0.52	0.55	0.58
T_3 4.0 dS m^{-1}	0.032	0.051	0.062	0.49	0.52	0.55
T_4 6.0 dS m^{-1}	0.025	0.044	0.054	0.48	0.49	0.50
T_5 8.0 dS m^{-1}	0.024	0.028	0.043	0.44	0.45	0.45
T_6 10.0 dS m^{-1}	0.022	0.025	0.035	0.41	0.42	0.42
S. Em ±	0.001	0.002	0.003	0.018	0.028	0.016
C.D. at 5 %	0.004	0.007	0.009	0.054	0.086	0.049
CV %	7.64	9.40	9.24	6.35	9.65	5.30

***** DAT: Days after imposing treatment

Table 10 Effect of different salinity levels on relative water content (%) of rayan seedlings at 120 days

Treatments	**Relative water content (%)**
T_1 0.5 dS m^{-1}	86.25
T_2 2.0 dS m^{-1}	81.44
T_3 4.0 dS m^{-1}	78.81
T_4 6.0 dS m^{-1}	73.19
T_5 8.0 dS m^{-1}	72.13
T_6 10.0 dS m^{-1}	63.76
S. Em ±	3.43
C.D. at 5 %	10.59
CV %	7.84