

Original Research Article

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Physio-biochemical Variability of Pomegranate Cuttings Collected from Northern India

Rajkumar*, Anshuman Singh, Anita Mann and R. K. Yadav

ICAR- Central Soil Salinity Research Institute, Karnal-132001(Haryana), India

*Corresponding author

ABSTRACT

Pomegranate (*Punica granatum* L.) is one of the hardy crop and may have potential to be grown under salt affected areas although salinity could be a limiting factor for growth and yield in its cultivation. Thus, screening of salt tolerant genotype of pomegranate is one of the most important aspects for the best utilization and improving the productivity of salt affected soils. To assess the variability among different genotypes on the basis of vegetative growth characteristics, biochemical and physico-chemical parameters etc. twenty genotypes of pomegranate were evaluated under nursery conditions. The pomegranate genotypes collected from Jaipur (03), Ajmer (05), Rajasmand (04), Udaipur (03), Pali (01), Jodhpur (01), Nagour (01), Bikaner (02), respectively were grown in polybags filled with partially reclaimed sodic soils. Different biochemical and physiological parameters like proline ($\mu\text{g/g}$ FW), RWC (%), chlorophyll (mg/gFW), Na^+ (%), K^+ (%), K^+/Na^+ and vegetative growth parameters like number of roots, length of roots (cm), number and length of shoots and number of leaves were recorded after four months of planting the cuttings. Significant variations among these genotypes indicated inherited gain against salinity tolerance. On the basis of the above said parameters, this experiment may provide indicators for selection of salt tolerant pomegranate genotypes for salt affected soils.

Keywords

Physio-biochemical variability, Pomegranate, Salt tolerant

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Introduction

Salt affected soils are an important ecological entity of Indian agriculture. With increasing population pressure and competition for good quality lands, intensive agriculture is being pushed more and more into marginal environments. The arable lands are shrinking due to developmental activities and offer little scope for raising horticultural crops. There is no option other than bringing marginal and salt affected lands under cultivation and

utilizing them judiciously through horticultural practices. By growing horticultural crops on highly deteriorated salt affected soils with saline water may improve economic conditions of farmers. Several salt tolerant fruit trees have been identified for saline and alkaline lands like Ber (Hooda *et al.*, 1990), Date-palm (Furr *et al.*, 1966), Fig (Patil and Patil, 1983a), Guava (Patil *et al.*, 1984), Jamun (Patil and Patil, 1983b), Olive (Taha *et al.*, 1972) and Pomegranate (Maas and Hoffmann, 1976; Munns, 2002) etc.

Pomegranate (*Punica granatum* L.) belonging to family *Punicaceae* is a delicious and desert table fruit of tropical and subtropical regions of the world. It is native to Persia (Iran), Afghanistan and Baluchistan. In India, it is being grown from Kanyakumari to Kashmir but the commercial plantations of pomegranate exists only in Maharashtra, Gujarat, Rajasthan, Karnataka and to a limited extent in Andhra Pradesh, Madhya Pradesh, Uttar Pradesh, Punjab, Haryana and Tamil Nadu etc. Owing to its xerophytic characteristics it is very suitable crop for dry, rainfed, pastured and undulating land, where other fruit crops cannot grow successfully. It provides high yield and more income per unit area with low input in arid and semi-arid regions. At present India's pomegranate production is 2845 thousand MT from an area of 234 thousand hectare in Maharashtra, Karnataka, Tamil Nadu, Rajasthan, Punjab and Haryana etc. with an average productivity of 12.16 MT/ha (Anonymous, 2019) and there is further scope for its expansion in adjacent areas of saline soils in India. India ranks first in the world with respect to pomegranate area and production while in productivity, Spain ranks first (18.5 t/ha) followed by the USA (18.3 t/ha). Iran contributes maximum to exports (60,000 t/year) followed by India (35,176 t). During 2016-17, India exported 49.76 thousand MT pomegranate of worth Rs. 49041.55 Lakh. Despite Spain having very little area (2000 ha), its export share is 37.8% of total production (37,000 t) followed by Israel (23.5%) and the USA (15.5%) while India has the lowest share (Silva *et al.*, 2013). Due to wider adaptability of pomegranate in tropical, subtropical, arid and temperate regions it can also be one of the most important fruit crop for salt affected soils. Salt affected soils and irrigation water are currently most severe abiotic limiting factors for the cultivation of horticultural crops. Where the salinization has been developed through irrigation, that causes

decreasing crop yields and land degradation as a result of excess salts being present in water during irrigation (Ayers and Westcot, 1985). Currently, most of pomegranate cultivation is in arid and semi-arid regions of the world, where soil salinity and water stress are the main limitations of appropriate yield production. There is an increasing attention towards salinity and more than 800 million hectare of lands has been affected that is equivalent to 6% of total (Munns, 2002).

The evidence of salinity tolerance depends on the physiological and biochemical processes occurring in plants. Thus, the complex physiology of salt tolerance and the variation between species on the basis of biochemical mechanisms of salinity tolerance may be the promising selection criteria (Ashraf and Harris, 2004). Despite the economic importance of pomegranate and its by-products, the study on the response of pomegranate cultivars to salinity stress has not been studied in India so far. Also, the most resistant native cultivars of pomegranate to soil salinity are unknown for gardeners in the saline soil regions. Therefore, with the objective to identify and introduce the most-tolerant Indian cultivars, rootstocks of pomegranate to different salinity levels have been analyzed in this study.

Materials and Methods

The experiment was carried out from August 2015 to February, 2016 at Experimental Farm of ICAR-Central Soil Salinity Research Institute, Karnal, located at the latitude 29.43, longitude 76.58, altitude 245 m elevation above the mean sea level. The experimental design was laid out in randomized blocks, in a 20 x 3 factorial scheme, for twenty genotypes of Pomegranate with three replications. During last week of August, 2015 cuttings of twenty (18 unknown and 02 known) genotypes were collected from Jaipur (03),

Ajmer (05), Rajasmand (04), Udaipur (03), Pali (01), Jodhpur (01), Nagour (01), Bikaner (02), respectively as shown in Table 1. These cuttings were grown in partially reclaimed sodic soils filled polybags which were kept on polyethene sheets to avoid leaching of salts. Different growth parameters such as number of roots, length of roots (cm), number of shoots, length of shoots and number of leaves as well as physico-biochemical parameters i.e. RWC (%), chlorophyll (mg/g FW), proline ($\mu\text{g/g}$ FW), Na^+ (%), K^+ (%), K^+/Na^+ were recorded after four months of planting the cuttings. Analysis of data was done using at 5% level of significance with the help of SAS (Version 9.3, SAS Institute Inc., Cary, NC, USA).

Results and Discussion

Vegetative growth parameters

Significant variability among different pomegranate genotypes were recorded among number of roots as depicted in table 2. Genotype, Jaipur 1 had maximum number of roots (76.67), followed by 'Ajmer 2' (70.67) and 'Jaipur 2' (70.33), while minimum number of roots were found in 'Pali 1' (56.33). The length of root was measured with measuring scale. Maximum length was recorded in genotype 'Rajasmand 3' (20.90 cm) followed by 'Nagaur' (20.73 cm) and 'Rajasmand 2' (20.70 cm), however, minimum root length was found in 'Ajmer 5' (19.10 cm). Similar findings have been reported earlier about decrease in growth biomass of roots, shoots and leaves due to loss of turgor and its associated cell wall tension relaxation with increasing NaCl level in many agricultural crops (Anthraper and Dubois, 2003, Aragües *et al.*, 2004, Sairam and Tyagi, 2004, Hajer *et al.*, 2006).

Maximum number of shoots were found in genotypes Udaipur 2 (5.33), followed by

'Jaipur 3' (5.13) and 'Ajmer 3' (4.77), respectively; however minimum number of shoots were found in 'Nagour' (3.50). Increase in number of shoots might be increased nutrient uptake that could affects the cell division in the vascular cambium, cell expansion and control of differentiation into different types of cambial (Devi *et al.*, 2016). Plant growth under salt stress might be due to osmotic stress, leaf injuries, and reduced photosynthesis (Wahome *et al.*, 2001). The growth of pistachio seedlings under salt stress conditions significantly reduced shoot height, crown diameter, leaf number, and dry mass of the seedlings under 100 and 150 mMNaCl treatments (Mirfatahiet *al.*, 2017). Maximum shoot length was observed in genotype Jaipur 1 (15.73 cm), followed by 'Udaipur 1' (14.87cm) and 'Ajmer 2' (14.17cm), respectively; however minimum length of shoots were found in Bhagwa (8.63 cm). Such changes appear to be related to changes in protein composition of cell walls (Bozarth., 1987; Singh *et al.*, 1987). However, the relationship between reductions of growth rate be more complex as growth is actively controlled independently of the sensing of turgor pressure (Bressan *et al.*, 1982; Boyer *et al.*, 1985), via changes in cell wall extensibility, osmotic inhibition of water availability, toxic effects of high salts ions and disturbance of the uptake and translocation of nutritional ions (Ates and Tekeli, 2007). Maximum numbers of leaves were found in genotype 'Jodhpur 1' (185.00), followed by 'Jaipur 1' (173.33) and 'Ajmer 1' (172.50), with minimum numbers of leaves in 'Rajasmand 3 & 4' (115), respectively. The number of leaves is related to the number and length of sprouts of cutting which depends on hydrolysis of reserve food materials for the maintenance of root and shoot balance. The results of the present study are in line with the findings of Chauhan and Maheswari (1970), Stancatoet *al.*, (2003) and Kaur and Kaur (2016).

Physico-biochemical traits

The first physiological indicator of abiotic stress on plants is relative water content (Kumar *et al.*, 2017; Pooja *et al.*, 2019). In pomegranate, maximum RWC (table 3) was found in genotype 'Jaipur 1' (92.55 %), followed by 'Jaipur 2' (92.07 %), and 'Ajmer 3' (91.54 %), however minimum RWC (81.46 %) was reported in Bhagwa. The maintenance of RWC is also related to osmoregulation under stress conditions since water deficiency or osmotic stress induces changes in the cell metabolic activity (Kumar *et al.*, 2018; Makarana *et al.*, 2019) and reduces cell division and expansion (Taiz and Zeiger, 2006) leading to plant growth inhibition. Similarly, Hokmabadi *et al.*, (2005) reported that maintenance of RWC in pistachio leaves was due to accumulation of osmolytes and osmotic adjustment under salt stress. On the other hand, Mirfattahi *et al.*, (2017) reported that relative water content (RWC) and water potential of the leaves were not affected by the NaCl treatments in pistachio genotypes in soilless culture. The morphological observations revealed little variation in leaf greenness which was further confirmed by non-significant difference in chlorophyll content among different genotypes (table 3). However, the maximum chlorophyll content was found in genotype 'Udaipur 3' (0.62 mg g⁻¹ FW), followed by 'Ajmer 4' (0.56 mg g⁻¹ FW), and 'Rajasmand1' (0.55 mg g⁻¹ FW), with minimum chlorophyll content (0.34 mg g⁻¹ FW) in 'Udaipur 2'. A negative correlation of chlorophyll concentration with salinity *i.e.* a decline in chlorophyll content in response to an increasing salt stress has been reported in citrus (Melgar *et al.*, 2008) and in pomegranate (Mastrogiannidou *et al.*, 2016). Salinity may influence absorption of ions, such as Mg²⁺ and Fe²⁺, which are involved in chlorophyll formation and salinity leads to depression of Mg concentration in plants under saline environment (Sivstev *et al.*,

1973; Mastrogiannidou *et al.*, 2016). The chlorophyll degrading enzyme chlorophyllase is more active under salt stress (Singh *et al.*, 2016, Kumar *et al.*, 2016). Papadakis *et al.*, (2007) reported significant reductions of chlorophyll content in NaCl-treated cherry plants containing Na and Cl higher than 5 mg g⁻¹ and 20 mg g⁻¹, respectively. Whereas, Doring and Ludders (1986b) observed lowest chlorophyll contents of pomegranate leaves at medium salt level (60 mMNaCl or Na₂SO₄); though Mastrogiannidou *et al.*, (2016) indicated a noticeable decline in the chlorophyll concentration of plants treated with 120 mMNaCl or Na₂SO₄. Proline, an indicator of the stress condition and its accumulation is important for water conservation in tissues and preventing cytoplasm dehydration under osmotic stress (Lata *et al.*, 2017, 2019; Pooja *et al.*, 2019). Although variation in proline content was seen in different genotypes but it was non-significant. Maximum proline content was found in genotype 'Jaipur 1' (212.97 µg g⁻¹ FW) followed by 'Ajmer 2' (187.73 µg g⁻¹ FW) and 'Ajmer 3' (177.96 µg g⁻¹ FW), however, minimum proline content (113.17 µg g⁻¹ FW) was reported in 'Pali 1' (table 3). As per earlier reports, proline accumulation occurs under stress conditions and proline being an osmoprotectant, provides tolerance to plants towards adverse growth conditions (Singh *et al.*, 2018). Taking this into consideration, the genotypes having higher proline show possibility of better growth under salt affected conditions and hence can be selected for further studies. Many investigators found increased proline accumulation in various plants exposed to salt stress (Karimi *et al.*, 2005; Chatzissavvidis *et al.*, 2014). Similarly, Mirfattahi *et al.*, (2017) found significant increase in leaf proline concentration under severe salt stress in *pistachio* genotypes. A significant difference was reported in Na⁺ and K⁺ contents among different pomegranate genotypes as shown in

Table 2. The minimum Na⁺ content was found in genotype ‘Ajmer 2’ (0.15 %), followed by ‘Jaipur 2’ (0.17%), whereas maximum Na⁺ content (0.35%) was reported in ‘Ajmer 4’ & ‘Ajmer 5’, ‘Pali 1’ and ‘Jodhpur 1’, respectively. The maximum K⁺ content was found in genotype ‘Jaipur 1’ (8.71 %), followed by ‘Rajasmand 3’ (6.27) and ‘Rajasmand 1’ (5.96 %), with minimum K⁺ content (2.81%) in genotype ‘Pali 1’. The ratio of sodium to potassium or vice-versa determines the tolerance level of plants under

stress conditions, lower the ratio more tolerant is the plant. Maximum K⁺/Na⁺ ratio was found in genotype ‘Jaipur 1’ (45.50 %), followed by ‘Ajmer 2’ (33.28%) and ‘Jaipur 2’ (26.66 %), respectively; however minimum K⁺/Na⁺ ratio was reported in ‘Pali 1’ (8.15 %). These pomegranate genotypes showed significant variations in level of tolerance against adverse effects of Na⁺ ions due to varied uptake capacity and accumulation of these ions into leaf tissues.

Table.1 Survey, collection and morphology of pomegranate genotypes

DISTRICT	LOCATION	COORDINATES	pH	ECe (dsm ⁻¹)
JAIPUR	AKEDA, JAIPUR	27.0983° N, 75.7207° E	7.73	2.3
	6 NO. ROAD, JAIPUR	27.0086°N, 75.4785°E	7.40	4.1
	6 NO. ROAD, JAIPUR	27.0086°N, 75.4785°E	7.07	3.6
AJMER	TABIJI, AJMER	26.2201°N, 74.3548°E	7.20	3.2
	TABIJI, AJMER	26.2204°N, 74.3549°E	7.73	2.7
	TABIJI, AJMER	26.0220°N, 74.0354°E	7.68	2.9
	PUSHKAR, AJMER	26.0290°N, 74.0339°E	7.40	3.2
	PUSHKAR, AJMER	26.0291°N, 74.0338°E	7.90	3.9
RAJASMAND	BALLI JASSA KHERA	25.0483°N, 74.0050° E	7.70	4.1
	SELMA, BALLI JASSA KHERA	24.0344°N, 73.0424°E	7.90	2.4
	SARSONIA, RAJASMAND	25.0667°N, 73.8833°E	7.80	3.5
	AUDAN MIRAJ, RAJASMAND	24.0547°N, 73.0481°E	7.78	2.8
UDAIPUR	UDAIPUR	24.5712°N, 73.6915°E	8.04	2.3
	UDAIPUR	24.5712°N, 73.6915°E	8.07	3.8
	SYRA, UDAIPUR	24.9863°N, 73.4123°E	7.96	3.9
PALI	SAJRI, PALI	25.1326°N, 73.4534°E	8.13	3.8
JODHPUR	NAGAUR ROAD, TOOR KI BADI	25.0094°N, 73.0274°E	7.89	4.1
NAGAUR	RAIDHANU, NAGAUR	27.2024°N, 73.7544°E	7.50	3.2
BIKANER	GANESH	28.0067°N, 73.0208°E	7.96	2.7
	BHAGWA	28.0067°N, 73.0208°E	7.85	2.8

Table.2 Root and shoot characters in pomegranate genotypes at vegetative growth stage

Genotypes	Number of Roots	Root Length (cm)	Number of Shoots	Shoot Length (cm)	Number of Leaves
Jaipur 1	76.67±5.667	20.67±1.245	4.67±0.987	15.73±1.32	173.33±24.253
Jaipur 2	70.33±4.055	19.50±0.2	4.57±0.467	13.80±1.501	132.50±30.721
Jaipur 3	66.67±3.383	19.43±1.313	5.13±0.433	10.93±1.521	132.50±5.0
Ajmer 1	65.00±3.215	20.47±0.524	4.57±0.467	12.63±0.26	172.50±24.109
‘Ajmer 2’	70.67±3.712	20.27±0.865	4.27±0.956	14.17±1.855	155.00±40.927
‘Ajmer 3’	67.33±1.856	19.47±1.471	4.77±0.406	10.17±2.252	137.50±22.22
Ajmer 4	60.67±4.91	20.63±0.549	4.57±0.467	12.80±0.862	132.50±5.0
Ajmer 5	59.33± 0.667	19.10±1.137	4.03±0.667	13.13±0.780	127.50±8.66
Rajasmand 1	64.33±2.404	20.67±0.884	4.10±0.0	11.73±0.521	150.00±22.5
Rajasmand 2	61.67±1.856	20.70±1.002	3.73±1.033	12.60±2.656	155.00±40.927
Udaipur 1	64.33±1.764	19.47±1.004	4.57±0.467	14.87±1.453	127.50±30.311
Udaipur 2	64.67±2.603	20.07±0.949	5.33±0.328	13.80±1.069	137.50±22.22
Rajasmand 3	66.33±1.667	20.90±0.794	4.10±0.808	10.10±1.405	115.00±20.463
Rajasmand 4	65.67± 1.667	19.73±1.44	4.47±0.96	10.20±1.137	115.00±6.614
Udaipur 3	62.67±3.528	20.53±0.935	4.77±0.406	11.13±1.763	132.50±34.731
Pali 1	56.33±3.283	20.10±0.866	4.03±0.067	12.07±0.133	150.00±30.311
Jodhpur 1	57.33±2.667	19.60±1.012	4.67±0.987	12.20±2.103	185.00±5.0
Nagaur	61.33±2.728	20.73±0.41	3.50±0.416	10.57±1.633	127.50±30.311
Ganesh	60.33±2.906	20.57±0.467	3.63±0.467	12.33±2.167	137.50±5.0
Bhagwa	57.67±4.702	20.27±1.703	4.70±0.416	8.63±1.33	150.00±15.612
General Mean	63.83	20.14	4.41	12.18	142.29
CV(%)	8.61	8.94	25.45	22.57	29.50
CD at 5% ± SE (d)	9.14±4.499	NS	NS	NS	NS

The non-significant mean values have at least one common letter at (p<0.05) using Tukey`s Test

Table.3 Biochemical and Physico-chemical parameters in pomegranate genotypes

Genotypes	Proline ($\mu\text{g g}^{-1}$ FW)	RWC (%)	Chlorophyll (mg g^{-1} FW)	Na ⁺ (%)	K ⁺ (%)	K ⁺ /Na ⁺
Jaipur 1	212.97±14.83	92.55±1.176	0.529±0.12	0.22±0.006	8.71±0.618	39.73±3.516
Jaipur 2	173.90±18.83	92.07±1.055	0.513±0.073	0.167±0.057	3.75±0.673	26.66±8.853
Jaipur 3	157.00±5.696	91.22±2.472	0.418±0.044	0.193±0.041	3.87±0.208	21.80±4.542
Ajmer 1	146.91±27.18	90.14±1.307	0.52±0.07	0.267±0.074	3.93±0.432	20.18±9.843
Ajmer 2	187.73±14.97	90.90±1.528	0.456±0.107	0.147±0.037	4.25±0.279	33.28±8.665
Ajmer 3	177.96±21.904	91.54±2.598	0.473±0.025	0.227±0.027	5.44±0.180	24.52±2.359
Ajmer 4	136.02±3.647	88.86±0.094	0.558±0.055	0.353±0.007	4.51±0.647	12.82±2.066
Ajmer 5	130.33±7.216	87.05±6.563	0.498±0.147	0.347±0.007	4.25±0.073	12.25±0.226
Rajasmand 1	140.27±2.899	90.13±1.185	0.554±0.033	0.333±0.018	5.96±1.46	17.52±3.711
Rajasmand 2	138.40±7.405	87.50±1.442	0.45±0.034	0.26±0.031	4.08±0.26	16.44±3.17
Udaipur 1	141.40±2.795	89.37±2.888	0.496±0.09	0.29±0.041	5.33±1.047	19.96±6.917
Udaipur 2	144.70±3.93	90.69±1.141	0.34±0.057	0.28±0.042	4.73±0.053	17.65±2.594
Rajasmand 3	169.39±17.362	90.24±1.312	0.47±0.051	0.30±0.012	6.27±1.747	20.93±5.829
Rajasmand 4	149.56±5.291	89.68±0.356	0.431±0.017	0.20±0.012	4.03±0.324	20.10±0.82
Udaipur 3	138.37±18.97	90.09±2.162	0.624±0.063	0.33±0.018	5.13±0.324	15.55±1.638
Pali 1	113.17±6.947	81.59±3.853	0.509±0.052	0.35±0.018	2.81±0.407	8.15±1.292
Jodhpur 1	115.95±7.191	83.41±4.855	0.507±0.06	0.35±0.007	3.75±0.428	10.79±1.044
Nagaur	141.29±7.106	87.33±0.725	0.394±0.078	0.28±0.020	4.69±1.042	16.99±4.28
Ganesh	131.37±14.468	81.70±4.661	0.413±0.048	0.32±0.053	4.58±0.252	15.07±2.422
Bhagwa	126.91±7.486	81.46±3.887	0.597±0.09	0.34±0.023	4.26±0.34	12.55±0.77
General Mean	148.68	88.38	0.49	0.28	4.72	19.44
CV (%)	14.788	5.59	26.60	20.418	26.329	42.534
CD at 5% ± SE (d)	36.48±17.95	NS	NS	0.094±0.046	2.06±1.014	13.514±6.65

The non-significant mean values have at least one common letter at ($p < 0.05$) using Tukey's Test

Generally, concentration of Na⁺, K⁺ or K⁺: Na⁺ ions in the plant tissue increases with the rise in salinity which may lead to loss of chlorophyll and ultimately leads to leaf chlorosis and necrosis and also bring interruptions in various enzymatic processes in the cytoplasm like process of protein synthesis as reported by Tattini *et al.*, (1994), Lycoskoufis *et al.*, (2005), Naeini *et al.*, (2006), Neocleous and Vasilakakis (2007),

Khoshgoftarmanesh and Naeini (2008), Parvaiz and Satyawati (2008), Okhovatian-Ardakani (2010).

Based on different observations on pomegranate plants, collected from different locations and grown under salt affected soils, the genotypes could be categorized as salt sensitive or tolerant. It was observed that the genotype 'Jaipur 1' had the most significant

number of roots at seedling stage. The distinctive vegetative characteristics of genotypes 'Jaipur 1', 'Ajmer 2' and 'Jaipur 3' such as root length, number of shoots, shoot length and number of leaves distinguishes these genotypes from the others and show advantage in terms of cultivation under saline conditions and hence, these genotypes can be used as salinity resistant. Besides, 'Nagaur 1', 'Rajasmand 2' and 'Jaipur 1' had the higher root length. However, the reason of salinity tolerance among these genotypes probably comes back to their adaptability in salt affected soils during long periods of growth years. On the basis of various physiological parameters like proline, RWC, Chlorophyll and K^+/Na^+ the genotypes, Jaipur 1, 'Ajmer 2' and 'Ajmer 3' are less prone to salt stress as compared to other genotypes. Therefore, on the basis of these studies, we can conclude that genotypes 'Jaipur 3', 'Pali 1', 'Bhagwa' and 'Rajasmand 4' may have some potential to grow under saline conditions which requires further evaluation to identify better salt tolerant genotypes or root stocks.

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