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Effect of salt stress on muskmelon (*Cucumis melo* L.) seeds

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Abstract

Cucumis melo is one of the most appreciated and consumed vegetables in the world, however, producing areas have been suffering from increased salinity in irrigation water. Therefore, accesses of melon, from the germplasm bank of the Federal Rural Semi-Arid University (UFERSA), were tested for salinity and the physiological responses of the seedlings were evaluated. For the 24 accesses of *C. melo*, salinity of 0.06 and 3.45 $\mathrm{dS\,m^{-1}}$ was applied. For this, four subsamples of 25 seeds per access were used, which were seeded in polystyrene trays having as a substrate washed and sterilized sand, initially moistened at 50 % field capacity. The design was completely randomized in a factorial scheme (two salinity levels and 24 accesses). Salinity affected the physiological quality (germination and mean germination time) of melon seeds, germinating on average 62 %, with an average germination time of 4 days, mean height of 10.5 cm and reduction of dry matter accumulation. The saline treatment increased the electrical conductivity (EC) of the soil saturation extract, indicating the presence of stress. The accesses that presented intolerance to salinity were: A35, A24, A41, A31, A09, A28 and A43. The moderately tolerant accesses were: A16, A19, A15, A17, A34, A25, A27, A18, A42. The salinity tolerant accesses were: A45, A08, A37, A50, A14, A36, A07, A39 which may serve as a basis for genetic improvement.

Keywords: Cucurbitaceae, electrical conductivity, germination, vegetables

1 Introduction

Brazil is one of the largest producers of fruit and vegetables in the world, and among these vegetable crops of great importance to the semi-arid region, muskmelon (*Cucumis melo* L.) stands out for generating employment and income for the local and regional population. The main Brazilian states responsible for this amount in 2017 were Rio Grande do Norte, followed by Ceará and Bahia (IBGE, 2018). The commercialization of the fruit of this vegetable has been increasing gradually thanks to the export to the international market, mainly supplying Europe, which during the months of August 2015 to February 2016, increased 7.9 % over the same period of 2014/15 and generated approximately US$ 142 million (Melão, 2016).

Successful production in the Northeast region of the country is due to the specific climatic conditions for proper development of *C. melo*, among these, low relative humidity of the environment and the high temperatures that enable its production almost the whole year (Campelo *et al.*, 2014). Despite the increase in production and exports reached record highs in recent harvests, shortage of good quality water is a limiting factor in the areas of culture, being a major constraint in the production of this species (Medeiros *et al.*, 2012). It is even more severe when low rainfall is associated with soil salinity, representing a major problem for world agriculture, especially under irrigation conditions, since water is the carrier agent of the salts through the soil profile (Aragão *et al.*, 2009).

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In the Mossoró region of Rio Grande do Norte, the production system uses water from wells whose average depth can reach 900 m, and due to its high cost of implantation and capture, has been one of the main limiting factors for the increase of the irrigated area (Medeiros *et al.*, 2011). An alternative for this problem would be the use of brackish water, due to its high availability in this region, easily accessible (shallow water) and with potential for irrigation, although the high electrical conductivity ($EC > 2\,dS\,m^{-1}$) may limit crop yield due to soil salinization (Porto Filho *et al.*, 2011; Dias *et al.*, 2011; Freitas *et al.*, 2014). The muskmelon production system is a monoculture that generally comprises five stages: soil preparation, melon seed sowing, management, harvesting and field clean up. Polyethylene mulch is used to reduce soil water evaporation and to prevent fruit rot when in prolonged contact with moist soil. Sowing and crop management begins with daily drip fertigation as well as disease and pest control (Santos *et al.*, 2018).

Within the germination process, water is responsible for activating the seed germination process, whose phases are basically summarized in three distinct stages, the first phase corresponding rapid water uptake by the seeds until complete hydration of tissues; the second reactivation of metabolism occurs with a limited amount of water absorption, which the germination process has not been completed; and, finally, the third phase occurs, which coincides with the radicle protrusion, where the seed continues to absorb water to complete the germination process (Bewley, 2013).

The seed germination is a crucial and vulnerable stage in the life cycle of higher plants and determines the establishment of seedlings (Zhang *et al.*, 2014). The salting process is considered as a major abiotic stress since high salt concentration can limit the growth of the seedlings, inhibiting seed germination, causing the reduction of the root system and fresh weight of seedlings and compromise the establishment of plants in the production fields due to ionic and osmotic toxicity, mainly affecting the physiological machinery (Aflaki *et al.*, 2017, Kusvuran *et al.*, 2014).

At high levels, salinity can cause irreversible effects due to accumulation of ions. This toxicity during the germination process, can cause various physiological and biochemical disorders such as hormonal imbalance and reduced use reservations (Yacoubi *et al.*, 2013).

In view of this environmental aspect lived in semi-arid areas, it is necessary to search for resistant/tolerant accesses to the use of saline water, because according to Araujo *et al.* (2016), it is of great importance to increase the list of the potential genotypes of salinity tolerance and capable of providing high yields, even with use of lower quality water, such as saline. Thus, the objective was to evaluate the physiological responses of rustic accesses of *C. melo* according to the irrigation water, deriving from artesian well with salinity of $3.45\,dS\,m^{-1}$.

2 Materials and methods

The experiment was conducted at Seed Analysis Laboratory (LAS), Agricultural Science Center at the Federal Rural University of Semi-Arid (UFERSA) in November 2016. To this end, we used 24 *C. melo* accesses hits from the Germplasm Bank (GERMEV) of UFERSA Mossoró-RN-Brazil (Table 1). The seeds were subjected to treatments of 0.06 (distilled water) and 3.45 dS.m-1 (well water). The water used in the experiment was obtained from tubular well, exploring the aquifer Calcário Jandaíra located in Mossoró-RN-Brazil (UFERSA), with electrical conductivity of $3.45\,dS\,m^{-1}$ and, as a control, distilled water was used of $0.06\,dS\,m^{-1}$ (Table 2). The seeds were sown in polystyrene trays (14 × 14 × 7 cm), having as a substrate sand washed and sterilized, moistened 50 % of field capacity with each solution. Physiological tests were conducted in a laboratory environment (25 to 30 °C and 60/70 % RH), and the following variables were analysed:

Table 1: *Muskmelon access group (Cucumis melo) belonging to the Germplasm Bank (GERMEV).*

A07- Cantaloupensis	A28- Cantaloupensis
A08- NI	A31- Cantaloupensis
A09- Conomon	A34- Cantaloupensis
A14- Cantaloupensis	A35- NI
A15- Momordica	A36- Cantaloupensis
A16- Conomon	A37- Momordica
A17- Conomon	A39- Cantaloupensis
A18- Cantaloupensis	A41- Cantaloupensis
A19- NI	A42- Momordica
A24- Cantaloupensis	A43- inodorus
A25- Cantaloupensis	A45- inodorus
A27- NI	A50- inodorus

NI = Not identified

Germination

Performed with four subsamples of 25 seeds per access what were seeded on polystyrene trays (14 × 14 × 7 cm) as substrate washed and sterilized sand. Initially, the substrate was moistened with distilled water at 50 % retention capacity. After sowing, the trays remained at laboratory ambient (25 to 30 °C and 70 % RH). The counts were performed on the

Table 2: *Chemical characteristics of the water used in the experiment.*

Chemical characteristics	*Units*	*Well water*	*Distilled water*
EC	dS m^{-1}	3.45	0.06
pH	-	6.80	7.50
K+	mmol L^{-1}	0.41	0.02
Na+	mmol L^{-1}	19.41	0.34
Ca+	mmol L^{-1}	11.20	0.40
Mg+	mmol L^{-1}	10.60	1
Cl-	mmol L^{-1}	27.60	1.20
CO3	mmol L^{-1}	0.00	0.00
HCO3	mmol L^{-1}	5.80	0.60
RAS	-	5.9	0.4
Toughness	mg L^{-1}	1090	70
Cations	mmol L^{-1}	41.6	1.8
Anions	mmol L^{-1}	33.4	1.8

fifth and eighth day after sowing, and the results expressed as a percentage of normal seedlings (BRASIL, 2009).

Seedling height

On the eighth day the seedlings were measured lap of the base to the apex of the sheet with the aid of a ruler graduated in millimeters. The value of the average length of the seedlings was obtained by the arithmetic average of the number of normal seedlings emerged for each repetition.

Mean germination time

Obtained by daily count of germinated seeds to the eighth day after sowing and calculated using the formula proposed by Labouriau (1983), the results being expressed in days.

$$\text{MGT} = \sum (ni \times ti) \,/\, \sum ni$$

where:

MGT = mean germination time (days);

ni = number of seeds germinated in each count interval;

ti = time elapsed between the onset of germination and the i-th counting.

Dry mass of the aerial part of seedlings

At the end of the germination test performed on the eighth day, normal seedlings were cut in the neck region, packed in plain paper bags and placed in a forced air circulation oven (65 °C) to dry until constant weight. The results were expressed in g total dry matter of the aerial part of seedlings per repetition, for each access.

Soil extract saturation

Finalised physiological assessments of seedlings, samples of the substrate from each experimental unit were collected for verifying the electrical conductivity of the saturation extract (Richards, 1954).

Salinity tolerance index

It was determined according to the methodology proposed by Fageria, Stone & Baeta (2011) using the dry mass content of melon seedlings, classifying the accesses as intolerant (0 – 0.5), moderately tolerant (0.51 – 0.99) and tolerant (>1) for this index.

$$\text{STI} = (\text{TDMcont} - \text{TDMsalt}) \,/\, \text{TDMcont}$$

where:

STI = salinity tolerance index;

TDMcont = total dry mass of the control treatment;

TDMsalt = total dry mass of the salt treatment.

Statistical analysis

The design was completely randomized in a factorial scheme with 24 accesses and two treatments (well and distilled water). Data were submitted to ANOVA and means were compared by the Scott-Knott test ($p < 0.05$) at 5 % probability, using the statistical program ASSISTAT 7.7 beta (Silva & Azevedo, 2009).

3 Results

It was found that the salinity of soil saturation substrate was higher in the saline treatment, indicating the presence of salts when compared to the control treatment. A significant interaction between the levels of salinity factors and accesses was found in relation to the physiological performance. This fact was verified for the variables of germination, mean germination time and total dry mass of seedlings, but is not found significant effect on seedling height (Table 3). Germination of accesses under salinity ranged between 27 and 94 % for the access A28 and A39, respectively (Table 4). Generally, 16 % of the accesses (Table 4) were statistically different, presenting average germination of 62 % when compared to the EC irrigation water. Access A08, A24 and

Table 3: *Analysis of variance (ANOVA) using the melon (Cucumis melo) accesses and salinity doses.*

		MS			
Source of variation	*DF*	*G*	*MGT*	*SH*	*TDM*
Accesses (A)	23	2896.27**	3.79**	31.08**	0.05**
Salinity (B)	1	58.52^{ns}	11.02**	64.44**	0.02**
A × B	23	418.40*	0.96*	1.56^{ns}	0.004*
Error	144	237.93	0.58	2.51	0.002
Average		62.69	4.73	10.03	0.162
CV%		24.60	16.16	15.81	31.76

(MS) mean square; (DF) degrees of freedom; (G) germination; (MGT) mean germination time; (SH) seedling height; (TDM) total dry matter; (ns) not significant; (**) $p < 0.001$ and (*) $p < 0.05$.

Table 4: *Physiological responses of different melon seeds (Cucumis melo) accesses conducted under EC irrigation water for germination (G), mean germination time (MGT), and total dry mass (TDM).*

	G (%)		*MGT* (*days*)		*TDM* (*g*)	
Accesses	*S*	*C*	*S*	*C*	*S*	*C*
A07	68^{bA}	74^{bA}	5^{aA}	4^{bA}	0.32^{bA}	0.36^{aA}
A08	66^{bA}	35^{dB}	4^{bA}	4^{cA}	0.19^{cA}	0.10^{dB}
A09	80^{aA}	73^{bA}	5^{aA}	5^{aA}	0.10^{dA}	0.08^{dA}
A14	54^{bA}	54^{cA}	5^{aA}	6^{aA}	0.26^{bA}	0.23^{bA}
A15	55^{bA}	56^{cA}	5^{aA}	4^{cA}	0.15^{cA}	0.10^{dA}
A16	86^{aA}	91^{aA}	4^{bA}	4^{bA}	0.13^{dA}	0.12^{cA}
A17	67^{bA}	81^{aA}	5^{aA}	4^{cA}	0.11^{dA}	0.15^{cA}
A18	62^{bB}	89^{aA}	6^{aA}	5^{bA}	0.14^{cA}	0.17^{cA}
A19	73^{aA}	83^{aA}	4^{bA}	4^{cA}	0.16^{cA}	0.10^{dA}
A24	44^{cA}	22^{dB}	5^{aA}	5^{aA}	0.09^{dA}	0.03^{dA}
A25	77^{aA}	45^{cB}	5^{aA}	5^{aA}	0.20^{cA}	0.08^{dB}
A27	66^{bA}	64^{bA}	6^{aA}	4^{bB}	0.17^{cA}	0.14^{cA}
A28	27^{cA}	44^{cA}	4^{bA}	3^{dB}	0.07^{dA}	0.12^{cA}
A31	56^{bA}	47^{cA}	4^{bA}	4^{cA}	0.09^{dA}	0.07^{dA}
A34	65^{bA}	65^{bA}	5^{aA}	4^{dA}	0.16^{cA}	0.13^{cA}
A35	28^{cA}	24^{dA}	5^{aA}	5^{bA}	0.04^{dA}	0.03^{dA}
A36	71^{bA}	75^{bA}	3^{bA}	3^{dA}	0.26^{bA}	0.28^{bA}
A37	63^{bA}	48^{cA}	5^{aA}	4^{cA}	0.27^{bA}	0.17^{dA}
A39	94^{aA}	98^{aA}	4^{bA}	4^{cA}	0.41^{aA}	0.38^{aA}
A41	35^{cA}	15^{dA}	4^{bA}	3^{dB}	0.08^{dA}	0.04^{dA}
A42	76^{aA}	81^{aA}	6^{aA}	4^{dA}	0.17^{dA}	0.15^{cA}
A43	58^{bA}	63^{bA}	4^{bA}	4^{cA}	0.11^{dA}	0.09^{dA}
A45	65^{bA}	72^{bA}	5^{aA}	5^{bA}	0.15^{cA}	0.18^{cA}
A50	81^{aA}	91^{aA}	4^{bA}	3^{dA}	0.21^{cA}	0.23^{bA}
CV (%)	24.60		16.16		31.76	

S = saline water treatment and C = control.
Averages followed by the same lowercase letter in the column and uppercase in the line do not differ statistically from each other by the Scott-Knott test at 5 % probability level.

A25 showed an increase of 88, 100 and 71 % of germination respectively, and only access A18 reduced germination by 30 % when compared to EC irrigation water (Table 4). Among the materials, it was found that accesses A09, A16, A19, A25, A39, A42 and A50 presented the highest average, not differing statistically from each other in saline treatment, with an average germination of 81 % (Table 4).

In the mean germination time (MGT), 16 % of accesses showed significant differences when compared to the EC irrigation water. It was found that accesses A27, A28, A41 and A42 presented an increase of 75, 33, 33 and 50 % respectively, with an average time of 5 days when compared to the EC irrigation water. As the preference is for materials that grow faster and more effective, it was found that the lowest MGT corresponded to 42 % of these materials with an average time of 4 days, which were: A08, A16, A19, A28, A31, A36, A39, A41, A43 and A50 (Table 4) in saline treatment. The other accesses accounted for 58 % with five days MGT in the saline treatment. For total dry mass (TDM) it was found that 12.5 % of the accesses (A08, A25 and A37) increased the TDM in 90, 150 and 158 % respectively, when compared between the EC of irrigation water. When compared between the accesses, it was verified that the A39 access showed significant differences with higher average total dry mass (Table 4). Checking only the isolated effect of seedling height among the accesses, A39 showed significant differences compared to all other accesses with an average height of 14.6 cm. The lowest height was verified in A41, averaging 5.6 cm (Table 5).

The salinity tolerance index separated the accesses into three levels (Table 6), performed according to the accumulation of dry matter between the materials. Among the accesses analysed, the A39 material stands out among the other accesses as one of the best, according to the STI scale analysed. However, according to the resistance classification proposed by Fageria, Stone and Baeta (2011) other accesses

Table 5: *Isolated effect for the height of seedling in different accesses of melon seeds (Cucumis melo).*

Accesses	*Seedling height (cm)*
A07	12.34^b
A08	11.65^c
A09	7.17^e
A14	9.68^d
A15	11.41^c
A16	9.41^d
A17	9.21^d
A18	7.74^e
A19	10.20^c
A24	7.88^e
A25	10.64^c
A27	7.78^e
A28	10.83^c
A31	10.65^c
A34	9.55^d
A35	8.91^d
A36	10.94^c
A37	9.02^d
A39	14.61^a
A41	5.63^f
A42	10.76^c
A43	11.83^c
A45	10.30^c
A50	12.53^b

Averages followed by the same lowercase letter in column do not differ statistically from each other by the Scott-Knott test at 5 % probability level.

Table 6: *Salinity tolerance index in 24 accesses of melon (Cucumis melo).*

Accesses	*Salinity tolerance index**	
A35	0.08	Intolerant
A24	0.19	
A41	0.19	
A31	0.29	
A09	0.37	
A28	0.41	
A43	0.42	
A16	0.64	Moderately tolerant
A19	0.67	
A15	0.67	
A17	0.72	
A34	0.81	
A25	0.81	
A27	0.93	
A18	0.98	
A42	0.99	
A45	1.05	Tolerant
A08	1.19	
A37	1.78	
A50	1.79	
A14	2.31	
A36	2.68	
A07	4.07	
A39	5.69	

* Carried out according to the total dry matter accumulation for each access. Classification of accesses into tolerant, moderately tolerant and intolerant according to the methodology proposed by Fageria *et al.*, 2011.

also fall into the tolerant category: A07, A08, A14, A36, A37, A39, A45 and A50 due to the salinity of 3.45 dS m^{-1}.

4 Discussion

The use of more saline water produces a higher concentration of average salinity in the soil profile (Porto Filho *et al.*, 2011), the same observed in the substrate used in the study when the soil saturation extract was read. The 30 % reduction in the percentage of germination in the A18 access (Table 4) is due to the fact the saline stress causes physiological drought, because when there is an increase in the concentration of salts in the germination medium, there is a decrease in osmotic potential and, consequently, a reduction in water potential. This reduction may affect the process of water absorption by the seeds (osmotic effect), raising to toxic levels the concentration of ions in the embryo (toxic effect) (Betoni *et al.*, 2011). Salinity affects the growth of seedlings at all growth stages and in a differentiated manner, with most cultivars being more sensitive during the emergence of seedlings. This is possibly due to interactions between salts and cell membranes, interfering in several functions of the membrane, such as permeability and transport of solutes, and may cause structural changes (Aragão *et al.*, 2009).

Mean germination time (MGT) is important to evaluate which access germinated faster and is characterized as the most salinity resistant genotypes. As the preference is for materials that grow faster, it was found that the lowest average time corresponded to 42 % of these materials with an average time of 4 days (Table 4). According to Willadino & Câmara (2010), this increase may be related to the condition of mild stress and stimulant that activates the cell metabolism and increases the physiological activity of the plant, which seems to be a positive factor that drives plant growth. According to Cruz *et al.* (2016), the mean germination time (MGT) tends to be higher with higher NaCl concentration present in the substrate, especially at temperatures of 30 °C confirming what was observed in the experiment.

The total dry mass (TDM) showed statistically significant differences between the materials used, stratifying accesses into 4 levels as dry matter accumulation (Table 4). It was observed that only accesses A08, A25 and A37 showed the highest dry matter when applied salinity between the EC irrigation water. According Secco *et al.* (2010) this increase in dry matter may be related to low concentration of salts and an increase in the availability of water in it, while the higher dry matter production in accesses due to the accumulation of nutrients in the root and hence the increase dry weight. The energy expenditure for adaptation to salinity and osmotic potential difference between the soil and plant tissues, are some of the factors that make the newly emerged seedlings in saline environments have less capacity to accumulate dry matter. (Larré *et al.*, 2011). According Aragão *et al.* (2009), biomass production of the plant depends on accumulation of carbon compounds in photosynthesis, which in turn is determined by two main components: net assimilation rate and increased leaf area.

No significant interaction between seedling height and salinity was found (Table 3). Possibly salinity used in this study was not so severe as to cause a decrease in height as reported by Taiz *et al.* (2017) and the same authors report that the increase in EC causes a decrease in seedling height due to the osmotic effect, promoting the physiological drought, as well as toxic effects may occur, resulting from the concentration of ions in the protoplasm. Soares *et al.* (2010) observed that the height of the aerial part of Creole melon seedlings decreased with the increase of water salinity levels, especially from 3.0 salinity $\mathrm{dS\,m^{-1}}$ with significant reduction.

According to Araújo *et al.* (2016) saline waters of up to $1.8\,\mathrm{dS\,m^{-1}}$ can be used for irrigation of melon plants of round Gaucho, and waters of up to $2.4\,\mathrm{dS\,m^{-1}}$ in the irrigation of the cultivars Gaucho Oak Bark and Halles Best Jumbo, during the initial phase of growth by presenting a good tolerance index to salinity (STI). Sarabi *et al.* (2016) also working with wild melon materials observed differences in morphological and physiological responses due to the saline stress used, the authors suggest the salt tolerance index (STI) and principal component analysis as an important tool in determining the salinity tolerance of melon accesses, emphasizing also that the higher wild materials will be used in crosses with existing cultivars to improve the salinity tolerance.

Regional development of melons in arid and semi-arid zones depends on materials adapted to abiotic conditions such as salinity. This selection is important for melon growers and seed companies that work with genetic improvement, because so far there are no salinity tolerant melon cultivars on the market.

5 Conclusion

The salinity affected the physiological quality of melon seed providing average germination of 62 % with an average time of 4 days germination, with an average height of 10.03 cm and weight reduction in dry matter. The saline treatment increased the EC of soil saturation extract indicating the presence of stress.

The accesses that presented intolerant to salinity were: A35, A24, A41, A31, A09, A28 and A43. The moderately tolerant accesses were: A16, A19, A15, A17, A34, A25, A27, A18 and A42. The salinity tolerant accesses were: A45, A08, A37, A50, A14, A36, A07 and A39 which could serve as a basis for genetic improvement.

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Conflict of interest

Authors confirm that there is no conflict of interest.

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