

## Quality of soursop (*Annona muricata* L.) seedlings under different water salinity levels and nitrogen fertilization

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### Abstract

The edaphoclimatic characteristics of the Brazilian Northeast region favor the exploitation of soursop, a crop that is appreciated by the population of the whole country. However, due to the water limitation in this region, it is necessary to study techniques that make saline water feasible in the cultivation of the species. The present study aimed to evaluate phytomass production and quality of soursop cv. 'Morada Nova' seedlings, irrigated with waters of different salinity levels and fertilized with nitrogen (N) doses. The experiment was carried out in a greenhouse at the Center of Sciences and Agri-food Technology of the Federal University of Campina Grande (Pombal – PB), using a randomized block experimental design, in 5 x 4 factorial scheme, which corresponded to five levels of water electrical conductivity— ECw (0.3; 1.1; 1.9; 2.7 and 3.5 dS m<sup>-1</sup>) and four N doses (70, 100, 130 and 160 mg of N dm<sup>-3</sup> of soil), with four replicates and two plants per plot. At 90 days after applying the treatments, the following variables were evaluated to assess the quality of the seedlings: fresh and dry phytomass of stem and leaves, dry phytomass of shoots (aerial parts), roots and total, and Dickson's quality index. Irrigation water with ECw of up to 2.0 dS m<sup>-1</sup> allows the production of soursop seedlings, cv. 'Morada Nova', with acceptable reduction of growth. The N dose of 70 mg dm<sup>-3</sup> stimulates phytomass production and quality of the seedlings. There was no significant interaction between the factors on the studied variables of seedlings at 90 days after application of the treatments. Water of 2.0 dS m<sup>-1</sup> associated with nitrogen fertilization using 70 mg of urea dm<sup>-3</sup> of soil should be used in the formation of soursop seedlings.

**Keywords:** Mineral fertilization, *Annona muricata* L., salt stress, saline water management and seedling production.

**Abbreviations:** DAT\_days after applying the treatments; StFP\_stem fresh phytomass; LFP\_leaf fresh phytomass; StDP\_stem dry phytomass; LDP\_leaf dry phytomass; RDP\_root dry phytomass; TDP\_total dry phytomass; DQI\_Dickson's quality index; PH\_plant height; SD\_stem diameter; DAS\_days after sowing; ECw\_ electrical conductivity of the irrigation water.

### Introduction

Soursop (*Annona muricata* L.) belongs to the Annonaceae family and is considered the most tropical among these species. It occupies a promising position in Brazilian fruticulture mainly in the Northeast region, where its consumption has increased, either fresh or industrially processed, due to the nutritional importance and forms of use in human food, besides the medicinal properties of its leaves, fruits, seeds and roots (Campos et al., 2008).

Despite the importance of this fruit crop for the Northeast Brazil, in the semi-arid region there are irregular rainfalls and the water resources available for irrigation are frequently scarce and with variation of spatial and temporal distribution. In addition, the quality of these water resources

also varies, and it is common to find water sources with high concentration of salts (Bezerra et al., 2010). The concentrations of salts in the irrigation water and/or soil in amounts higher than those tolerated by plants may cause reduction in the soil osmotic potential and thus reduce water availability to plants, besides leading to toxicity and nutritional imbalance (Nobre et al., 2010). Thus, the use of saline water in irrigation is conditioned to the tolerance of the crops to salinity, besides management practices of irrigation and fertilization, which should avoid environmental impacts and consequent damages to the crops (Amorim et al., 2010; Ribeiro et al., 2015). In the productive process, besides the importance of adequate

water supply in terms of quantity and quality, fertilization emerges as a preponderant factor to obtain positive result and, among the micronutrients required by plants, N is one of the most important, because it participates in their structure, being a component of amino acids, proteins, enzymes, RNA, DNA, ATP, chlorophyll, among other compounds. Furthermore, it is a nutrient directly related to the characteristics associated with plant growth (Chaves et al., 2011). Additionally, studies have demonstrated that the accumulation of this organic solute increases the osmotic adjustment capacity of the plants to salinity (Silva et al., 2008).

Thus, this study aimed to evaluate the phytomass production and quality of soursop seedlings, cv. 'Morada Nova', irrigated with different levels of water salinity and different doses of N fertilization. Thus, agricultural producers who have a source of salt water in their rural property could acquire knowledge on techniques that will make viable the use of low quality water in their soursop seedlings.

## Results and discussion

### *Effect of saline stress and nitrogen doses on phytomass production of soursop*

As indicated in Table 1, irrigation water salinity had significant effect on all studied variables, i.e., fresh phytomass of leaf (LFP), stem (StFP), dry phytomass of leaf (LDP), stem (LDP), root (RDP), total (TDP) and on Dickson's quality index (DQI) of seedlings at 90 DAT. In contrast, N fertilization and its interaction with water salinity did not interfere significantly with any of the analyzed variables, which indicates that the different N doses showed similar behaviors at different levels of irrigation water salinity.

The fresh and dry phytomass accumulations in leaves and stem of soursop seedlings were significantly ( $p < 0.01$ ) affected by irrigation water salinity (Table 1) and the data adjusted to quadratic equations (Fig 1). The highest fresh phytomass of stem and leaves were obtained in seedlings irrigated using water of  $1.6 \text{ dS m}^{-1}$  (8.23 g) and  $1.2 \text{ dS m}^{-1}$  (13.06 g), respectively, which led to increases of 1.15 and 0.78 g, in comparison to those irrigated with public-supply water.

The reduction of LFP and StFP, from the previously described saline levels, may be attributed to the decrease in the osmotic potential of the soil solution, due to the increase in salt content, hampering water absorption by plants, causing them to spend more energy to absorb water and nutrients, thus reducing their growth (Nobre et al., 2010). In addition, the effect of salts usually leads to the occurrence of ionic toxicity, and plants tend to close the stomata to reduce water losses through transpiration, resulting in lower photosynthetic rate and, consequently, decrease of phytomass production in the species under stress (Chen & Jiang, 2010).

The increase in salinity of the irrigation water significantly ( $p < 0.01$ ) influenced the dry phytomass of leaves and stem and, according to the regression equations (Fig 1B), the data fitted best to the quadratic model, exhibiting an increase of phytomass up to the levels of  $1.2 \text{ dS m}^{-1}$  (3.56 g) for StDP and  $1.5 \text{ dS m}^{-1}$  (2.27 g) for LDP. The reduction of dry phytomass from these ECw levels is closely related to the effects of the accumulation of the concentration of soluble

salts, which is a limiting factor to the development of most crops. Thus, this behavior can be understood as a possible adjustment mechanism of the plant to reduce the effects of salinity, because plants undergo morphological and physiological changes such as reduction of biomass, when subjected to salt stress (Centeno et al., 2014). Similar result was reported by Távora et al. (2004), who found reduction of dry phytomass in young soursop plants subjected to salt stress.

According to the regression equations (Fig 2A), the dry phytomass of shoots and roots showed quadratic response as a function of the increase in electrical conductivity from 0.3 (public-supply water) to  $3.5 \text{ dS m}^{-1}$ , with maximum values of 6.42 (ShDP) and 1.54 g (RDP) when plants were irrigated using water with ECw of 1.3 and  $1.5 \text{ dS m}^{-1}$ , respectively. When plants were subjected to ECw of  $3.5 \text{ dS m}^{-1}$ , there were respective reductions of 2.80 and 0.64 g in ShDP and RDP. Therefore, based on the regression equations, the highest effect of salinity occurs on shoots, evidenced by the greater reduction in ShDP, compared with RDP.

According to the behavior of shoot and root phytomass of soursop seedlings at 90 DAT (Fig 1A), this species showed tolerance up to a mean ECw of  $1.3 \text{ dS m}^{-1}$ , because the highest values of phytomass were obtained until this level. In addition, as the ECw increased beyond this level, there was a reduction in phytomass production, since the increase of salt concentration in the soil solution reduces the osmotic potential of the soil, compromising water absorption by plants, causing negative effects of nutritional and toxicity nature and/or interfering with the availability of other ions (Lima et al., 2014).

The increasing salinity of the irrigation water also caused quadratic response for the total dry phytomass (Fig 2B), the highest value (7.36 g) was obtained in plants irrigated using water with ECw of  $1.4 \text{ dS m}^{-1}$ , from this point on, and there was a decrease in this variable. It should be emphasized that the salt stress can cause nutritional and physiological imbalance, with direct influence on the conversion of carbon assimilated by plants and promoting reductions in growth and biomass accumulation of the crops (Taiz & Zeiger, 2013), which can be observed in the present study through the TDP data.

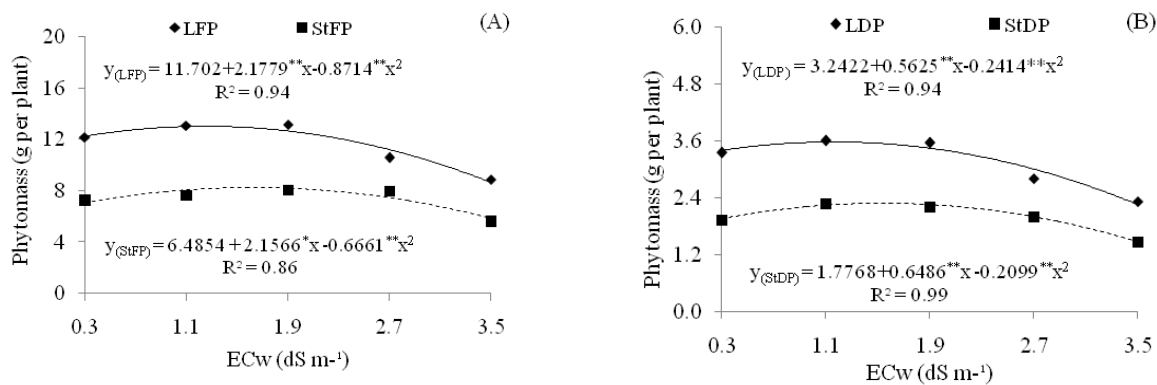
### *Effect of saline stress and nitrogen doses on quality of soursop seedlings*

On the variable related to the quality of soursop seedlings (DQI), there was significant effect ( $p < 0.01$ ) of the levels of irrigation water salinity and, according to Fig 3, the data fitted best to the quadratic mathematical model and the highest DQI (0.73) was obtained when the seedlings were irrigated using water with ECw of  $1.5 \text{ dS m}^{-1}$ , with a subsequent reduction, while plants subjected to ECw of  $3.5 \text{ dS m}^{-1}$  showed DQI of 0.41. This fact is interesting because, even under salt stress conditions, the soursop seedlings, cv. 'Morada Nova', showed DQI higher than 0.2, which characterizes seedlings with good quality for transplanting, according to the criteria established by Gomes (2001), because the higher the DQI value, the better the quality of seedling.

**Table 1.** Summary of the analysis of variance for leaf fresh phytomass (LFP), stem fresh phytomass (StFP), leaf dry phytomass (LDP), stem dry phytomass (StDP), root dry phytomass (RDP), shoot dry phytomass (ShDP), total dry phytomass (TDP) and Dickson quality index (DQI) of soursop seedlings irrigated with waters of different saline levels and under nitrogen fertilization doses at 90 DAT.

Source of variation	FD	Mean squares							
		LFP	StFP	LDP	STDP	RDP	SHDP	TDP	DQI
Salinity (S)	4	74.99**	22.65**	6.76**	2.19**	1.03**	15.25**	23.82**	0.27**
Linear regression	1	188.85**	24.36**	18.26**	3.42**	1.47*	38.34**	53.88**	0.40**
Quadratic regression	1	105.44**	54.03**	8.33**	5.29**	2.55**	22.27**	41.16**	0.60**
N dose (ND)	3	16.71 <sup>NS</sup>	6.77 <sup>NS</sup>	1.11 <sup>NS</sup>	0.21 <sup>NS</sup>	0.07 <sup>NS</sup>	1.91 <sup>NS</sup>	4.00 <sup>NS</sup>	0.01 <sup>NS</sup>
Linear regression	1	0.24 <sup>NS</sup>	0.17 <sup>NS</sup>	0.01 <sup>NS</sup>	0.04 <sup>NS</sup>	0.06 <sup>NS</sup>	0.06 <sup>NS</sup>	0.20 <sup>NS</sup>	0.01 <sup>NS</sup>
Quadratic regression	1	0.224 <sup>NS</sup>	0.60 <sup>NS</sup>	0.01 <sup>NS</sup>	0.02 <sup>NS</sup>	0.01 <sup>NS</sup>	0.26 <sup>NS</sup>	0.08 <sup>NS</sup>	0.001 <sup>NS</sup>
Interaction (S X ND)	12	5.81 <sup>NS</sup>	3.32 <sup>NS</sup>	0.43 <sup>NS</sup>	0.16 <sup>NS</sup>	0.26 <sup>NS</sup>	1.40 <sup>NS</sup>	1.67 <sup>NS</sup>	0.04 <sup>NS</sup>
Blocks	3	33.92**	43.08**	3.27**	5.16**	2.08**	1.29 <sup>NS</sup>	27.31**	0.29**
CV (%)		27.19	28.01	26.41	28.76	34.73	25.94	24.93	28.90

<sup>NS</sup>, \*\*, respectively not significant, significant at  $p < 0.01$  and  $p < 0.05$

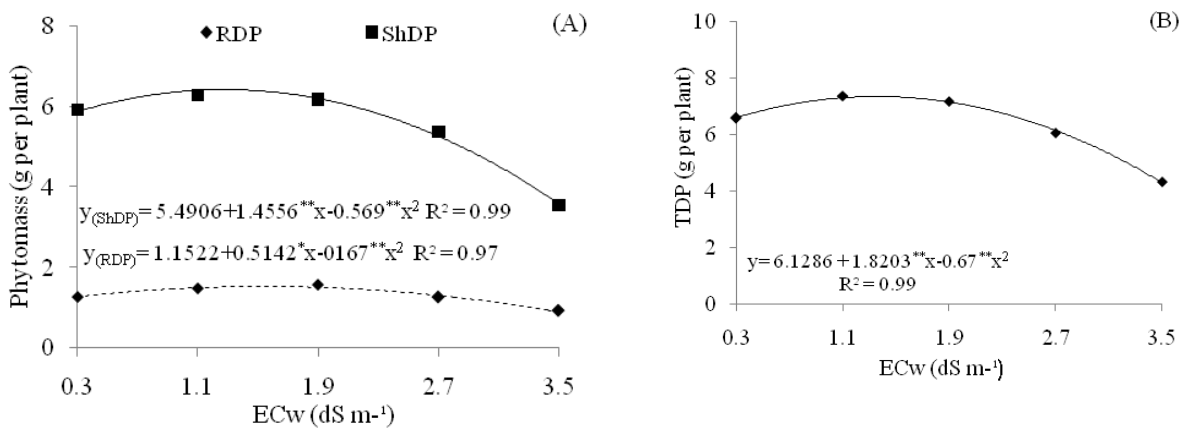


**Fig 1.** Stem fresh phytomass – StFP and leaf fresh phytomass - LFP (A), and stem dry phytomass - StDP and leaf dry phytomass - LDP (B) of soursop seedlings as a function of irrigation water salinity at 90 days after applying the treatments - DAT.

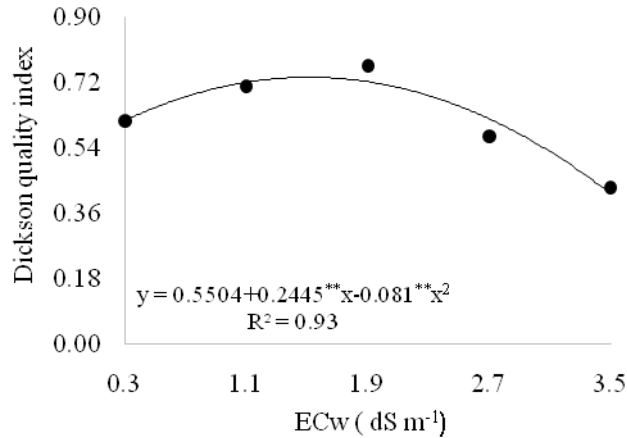
**Table 2.** Physical and chemical characteristics of the substrate used in the experiment.

Textural classification	Apparently density kg dm <sup>-3</sup>	Total porosity %	Organic matter g kg <sup>-1</sup>	P mg dm <sup>-3</sup>	Sortive complex					
					Ca <sup>2+</sup> cmol <sub>c</sub> dm <sup>-3</sup>	Mg <sup>2+</sup> cmol <sub>c</sub> dm <sup>-3</sup>	Na <sup>+</sup> cmol <sub>c</sub> dm <sup>-3</sup>	K <sup>+</sup> cmol <sub>c</sub> dm <sup>-3</sup>		
Sandy franc	1.38	47.00	32	17	5.4	4.1	2.21	0.28		
Saturation extract										
pHes	ECse dS m <sup>-1</sup>	Ca <sup>2+</sup> mmol <sub>c</sub> dm <sup>-3</sup>	Mg <sup>2+</sup> mmol <sub>c</sub> dm <sup>-3</sup>	K <sup>+</sup> mmol <sub>c</sub> dm <sup>-3</sup>	Na <sup>+</sup> mmol <sub>c</sub> dm <sup>-3</sup>	Cl <sup>-</sup> mmol <sub>c</sub> dm <sup>-3</sup>	SO <sub>4</sub> <sup>2-</sup> mmol <sub>c</sub> dm <sup>-3</sup>	CO <sub>3</sub> <sup>2-</sup> mmol <sub>c</sub> dm <sup>-3</sup>	HCO <sub>3</sub> <sup>-</sup> mmol <sub>c</sub> dm <sup>-3</sup>	Saturation %
7.41	1.21	2.50	3.75	4.74	3.02	7.50	3.10	0.00	5.63	27.00

Contents of P, K, and Na in soil determined in Mehlich-1 extractor; Exchangeable Ca and Mg extraction by 1.0 mol L<sup>-1</sup> KCl; Organic matter: Walkley-Black wet digestion; pH<sub>s</sub> = pH of the saturation paste; EC<sub>se</sub> = Electrical conductivity of the saturation extract of the substrate at 25°C.



**Fig 2.** Root dry phytomass - RDP and shoot dry phytomass - ShDP (A) and total dry phytomass - TDP (B) of soursop seedlings as a function of irrigation water salinity at 90 days after applying the treatments - DAT.



**Fig3.** Dickson quality index of soursop seedlings as a function of irrigation water salinity at 90 days after applying the treatments - DAT.

## Materials and methods

### **Location, experimental procedure, treatments and plant material**

The study was carried out under greenhouse conditions at the Center of Sciences and Agri-food Technology (CCTA) of the Federal University of Campina Grande (UFCG) in the municipality of Pombal-PB, situated at 6°48'16" S, 37°49'15" W and at mean altitude of 144 m.

The experiment was set in a completely randomized design, in 5 x 4 factorial scheme, with four replicates and two plants per plot. The treatments consisted in the combination of five levels of irrigation water salinity - ECw (0.3; 1.1; 1.9; 2.7 and 3.5 dS m<sup>-1</sup>) associated with four doses of N fertilization (70; 100; 130 and 160% of the dose recommended for the crop, according to Novais et al. (1991), and the dose of 100% corresponded to 100 mg of N dm<sup>-3</sup> of soil.

The saline levels were selected based on citations of Cavalcante et al. (2001), who classified the soursop crop in its initial growth stage as moderately sensitive to salinity, i.e., the biological performance of the plants increased, with the ECw level of up to 3.0 dS m<sup>-1</sup>.

The waters of different saline levels for irrigation were obtained through the addition of different amounts of salts of NaCl, CaCl<sub>2</sub>·2H<sub>2</sub>O and MgCl<sub>2</sub>·6H<sub>2</sub>O, in the equivalent proportion of 7:2:1, a ratio that prevails in the main water sources available for irrigation in Northeast Brazil (Medeiros, 1992).

The experiment used the soursop cv. 'Morada Nova', which is the genetic material preferred by the farmers in the Northeast region according to São José et al. (2014), besides being the most used by seedling producers. The utilized seeds came from ripe fruits harvested in a commercial orchard (Boi Bravo Farm), located in the municipality of Sousa - PB. The seeds were manually extracted and, subsequently, they were air-dried and the dormancy was broken.

### **Establishment and management of the experiment**

Plants were conducted in plastic bags with capacity for 1.2 dm<sup>3</sup>, filled with substrate composed of 82% of soil, 15% of

sand and 3% of aged bovine manure. The addition of manure aimed to improve soil physical, chemical and biological properties, in order to enhance its water infiltration and retention capacity. The physical and chemical characteristics of the soil (Table 2) used in the experiment were obtained according to Claessen (1997) and determined at the Laboratory of Soil and Plant Nutrition of the CCTA/UFCG.

Sowing was performed on December 13, 2015, by planting two seeds per bag at depth of 1.5 cm. Seedling emergence started at 20 days after sowing (DAS) and continued up to 40 DAS. Five days after total emergence of the seedlings, thinning was performed, leaving only the most vigorous seedling. Prior to sowing, the soil was brought to field capacity and, during the period of germination and emergence, the seedlings were irrigated with public-supply water (ECw = 0.3 dS m<sup>-1</sup>).

The irrigation with different saline levels started at 7 days after emergence (DAE) according to the treatments. Irrigations were applied based on plant water demand, determined by drainage lysimetry, twice a day, in the early morning and late afternoon at 17 h. Biweekly, a leaching fraction of 0.15 was applied, based on the volume applied in this period, in order to reduce the salinity of the substrate saturation extract. N fertilization also started at 7 DAE and was divided into 13 applications, at intervals of 7 days. The N source was urea (45% of N), which was applied through fertigation using water with ECw of 0.3 dS m<sup>-1</sup> for all treatments. Plants were conducted during the seedling stage, i.e., for a period of 100 days (after germination), the adequate time for transplanting.

Cultivation practices performed during the experimental period consisted of manual removal of spontaneous weeds, superficial soil scarification and, for phytosanitary control, plants were sprayed using organophosphate insecticide at the concentration of 150 mL 100L<sup>-1</sup> to control white flies according to the recommendation of the manufacturer.

### **Traits measured**

At 90 days after applying the treatments (DAT), the production of fresh and dry phytomass and quality of soursop seedlings were evaluated. The following variables were determined: stem fresh phytomass (StFP), leaf fresh

phytomass (LFP), stem dry phytomass (StDP), leaf dry phytomass (LDP), root dry phytomass (RDP) and total dry phytomass (TDP).

The stem of each plant was cut close to the soil and separated from the leaves. Both parts were immediately weighed on precision scale (0.001 g), to determine StFP and LFP. After weighing the fresh matter, the different plant parts (leaves, stem and roots) were separately stored in previously identified paper bags and dried in a forced-air oven at temperature of 65 °C until constant weight, to determine LDP, StDP and RDP. The sum of StDP and LDP resulted in shoot dry phytomass (ShDP), which was summed with RDP to calculate TDP. Roots were extracted from the substrate using a 3-mm-mesh sieve and running water.

Seedling quality was determined through the Dickson's quality index (DQI) for seedlings, using the equation of Dickson et al. (1960), described by eq. 1.

Where:

DQI = Dickson's quality index,

PH = plant height (cm),

SD = stem diameter (cm).

TDP = total dry phytomass (g)

ShDP = shoot dry phytomass (g)

RDP = root dry phytomass (g)

### Statistical analysis

The variables were subjected to analysis of variance by F test at 0.05 and 0.01 probability levels and, in cases of significance, linear and quadratic polynomial regression analysis was applied using the statistical program SISVAR-ESAL (Ferreira, 2011). The regression was selected through the best fit, based on the coefficient of determination ( $R^2$ ) and considering a probable biological explanation.

### Conclusion

Irrigation using water with electrical conductivity of up to 2.0 dS  $m^{-1}$  allows the production of soursop seedlings, cv. 'Morada Nova', with acceptable reduction of growth. The N dose of 70 mg  $dm^{-3}$  stimulates phytomass production and quality of soursop seedlings at 90 DAT. There was no interaction between irrigation water salinity and nitrogen fertilization on the variables evaluated in soursop seedlings, cv. 'Morada Nova'.

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