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Effect of salt stress on germination and initial development of *Ruta graveolens* L.

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ABSTRACT – (Effect of salt stress on germination and initial development of *Ruta graveolens* L.). Salinization occurring through irrigation on arable land has been a growing concern worldwide. This study aimed to evaluate the germination and initial development of common rue subjected to different saline concentrations. Two salts (sodium chloride and calcium chloride) and five saline concentrations (zero, -0.1, -0.2, -0.3, and -0.4 MPa) were utilized. Germination tests, first germination count, seedling length and dry weight were employed to assess seed responses to the studied variations. Based on the results, the excess of salt reduces the germination percentage and the initial development of common rue seedlings, regardless of the substance used for stress induction.

Keywords: germination process, rue, salinity

RESUMO – (Efeito do estresse salino na germinação e desenvolvimento inicial de *Ruta graveolens* L.). A salinização, devido à irrigação em terras cultiváveis, tem sido uma preocupação crescente em todo mundo. Diante disso, objetivou-se com este trabalho avaliar a germinação e o crescimento inicial de arruda submetida a diferentes concentrações salinas. Foram utilizados dois sais (cloreto de sódio e cloreto de cálcio) e cinco concentrações salinas (zero, -0,1; -0,2; -0,3 e -0,4 MPa). As respostas das sementes a essas variações foram avaliadas pelos testes de germinação, primeira contagem de germinação, além do comprimento e massa seca das plântulas. De acordo com os resultados, o excesso de sais reduz a percentagem de germinação e o desenvolvimento inicial das plântulas de arruda, independente da substância utilizada para indução do estresse.

Palavras-chave: arruda, processo germinativo, salinidade

Introduction

Seed germination is an important and vulnerable stage in the plant cycle and determines seedling establishment and plant growth, and is influenced by abiotic factors such as temperature, oxygen and water availability (Carvalho & Nakagawa 2012). Water can be a limiting factor for non-dormant seed germination, affecting the percentage, speed and uniformity of the process. Therefore, water is associated with reserve mobilization and release of energy through respiration, also encouraging the activity of enzymes and growth regulators. In addition, the molecule acts on the dilution of protoplasm, favoring metabolism directed to the resumption of embryonic growth (Marcos Filho 2015).

The study of water relations is important to comprehend the seed germination process in water stress conditions. It is estimated that 20% of all irrigated land is currently affected by salt stress (Taiz *et al.* 2017). Inadequate agricultural production techniques, such as the intensive use of fertilizers that have high concentrations of salts in their composition and the improper development of crop irrigation methods are the main causes of soil salinization (Cruz *et al.* 2017). Salinity decreases crop yield by reducing soil water availability and by the toxicity of chemical elements, especially sodium and chlorine (Souza *et al.* 2014), as well as causing protein denaturation and membrane destabilization, which reduce above-ground plant growth and inhibit photosynthesis (Taiz *et al.* 2017).

Plant propagation in different levels of simulated saline stress has been studied by several researchers. However, there is a lack of information in the literature on the assessment of the effects of salts on common rue seed germination. Common rue (*Ruta graveolens* L.) is a plant of the Rutaceae family, cultivated through seeds and utilized for medicinal purposes due to the presence of secondary metabolites such as flavonoids, coumarins, organic acids, terpenoids, lactones and several classes of alkaloids (Ratheesh *et al.* 2009). One of the flavonoids present in the leaves, i.e., rutin has been described with an antioxidant, anticancer and anti-inflammatory properties (Benazir *et al.* 2011).

The common rue should be cultivated in neutral, well-drained and periodically irrigated soils and under full sun. Once well-established, the plant can tolerate drought periods but does not tolerate waterlogged soils. Common rue development in semi-arid conditions is favorable because it grows well even in very poor soils and tolerates warm temperatures (Corrêa Júnior *et al.* 1994). Unfortunately, this species is still poorly studied in several agronomic aspects, especially under saline stress conditions.

Distinguishing how different plant species react to excess salt exposure is critical as it allows delineating possible salinity control strategies in arid and semi-arid regions, which are constantly more affected by soil salinization processes (Oliveira & Silva 2019). Therefore, this study aimed to evaluate the germination and initial development of common rue subjected to different saline concentrations.

Material and Methods

The experiment was developed at the Plant Genetics Laboratory of the Department of Biology, College of Natural and Exact Sciences of the Federal University of Santa Maria (RS). The common rue seeds used were purchased from the ISLA Seeds Company (Porto Alegre, RS). Initially, there was a pre-cooling at 5 ºC for a period of seven days (methods for breaking dormancy), following the recommendations of the Rules for Seeds Testing (Brasil 2009).

To evaluate the effect of saline stress on common rue seed germination (*Ruta graveolens* L.), aqueous solution of sodium chloride (NaCl) and calcium chloride ($CaCl_2$) at concentrations corresponding to zero; -0.10, -0.20, -0.30, and -0.40 MPa were used. Only distilled water was utilized as the control at the level zero. The amount of solution required to obtain osmotic potentials was calculated by Van't Hoff equation (Braccini *et al.* 1996, Taiz & Zeiger 2013).

The toxic effect of salts on seed germination was evaluated by the following tests:

Germination: test was performed with four replicates of 100 seeds distributed in plastic boxes (gerbox) on three germitest paper sheets moistened with distilled water or saline solution at a ratio of 2.5 times the paper weight. After sowing, the plastic boxes were kept in Bio-Oxygen Demand (BOD) chamber at constant temperature of 20 °C in the presence of constant light. Seed germination counts were performed at seven and 28 days (when the test was ended) and the results expressed as percentage (Brasil 2009).

First count: conducted together with the germination test, where the percentage of normal seedlings was determined on day seven of the test (Brasil 2009).

Seedling length: normal seedlings were obtained by sowing four repetitions of 20 seeds moistened with distilled water or solution. Rolls of paper containing the seeds were kept in a germination chamber for seven days, at a temperature of 20 °C. Total length of 10 normal seedlings were randomly evaluated for each repetition using a millimeter ruler. The average length of the seedlings was obtained by adding the number of measurements of each repetition and dividing this by the number of normal seedlings measured, with the results expressed in centimeters (cm) (Nakagawa 1999).

Dry weight of seedling: first, the fresh weight was obtained from 10 previously measured normal seedlings, for four replicates, and then maintained in papers bags in a dryer at a temperature of 60 °C until reaching a constant weight (48 h). Subsequently, the seedlings were weighed on a precision balance (to 0.001 g) and the results were expressed in milligrams (mg) (Nakagawa 1999).

Data analysis: the experimental design was completely randomized in a 2 x 5 factorial scheme respectively with two salts and five saline concentrations. The variables expressed as percentages were converted to arc sen $\sqrt{x/100}$. Data were subjected to analysis of variance and when significant, qualitative variables were compared by the Scott-Knott test at 5% error probability and quantitative variables were submitted to regression analysis using the Sisvar software (Ferreira 2011).

Results and Discussion

Differences in function of the salts and the osmotic potentials of the solutions were observed in the analysis of variance of the germination data of common rue seeds (figures 1 and 2). The highest germination percentages were found in the absence of salts (82%), with a significant linear decrease in the other NaCl concentrations and reaching values below 50% as of -0.1 MPa $CaCl_2$ potential (figure 1 a).

The first germination count evaluated seven days after sowing indicated that seeds presented delayed germination when subjected to saline stress. Gradual decrease in the percentage of normal seedlings was detected with increased NaCl concentration, observing zero germination from seeds as from -0.3 MPa of $CaCl_2$ (figure 1 b). This evidence may be related to the rate of seed water absorption, which decreases with the reduction of water potential, and the excess of salts causes cytotoxicity and dehydration of the cells, reducing the metabolic activity and the synthesis of new tissues in the seeds due to low water availability (Marcos Filho 2015). Therefore, water becomes less accessible and can reduce the percentage of seeds that complete the process or completely inhibit germination (Velázquez-Márquez *et al.* 2015).

Similar results were observed by Stefanello *et al.* (2018) evaluating the influence of different salt concentrations on common thyme seed germination (*Thymus vulgaris* L.). The authors observed a negative effect of NaCl and $CaCl_2$ salts on seed germination and vigor from osmotic potentials respectively of -0.3 and -0.2 MPa. Moreover, Mguis *et al.* (2011) analyzed common rue seed germination (0 to 0.7 MPa NaCl) and concluded that it was significantly inhibited by a NaCl concentration greater than 0.1 MPa. Additionally, Yamashita *et al.* (2009) found a reduction in the germination percentage of common rue seeds as NaCl concentration increased. However, significant reductions were observed only from -0.8 MPa. The lowest germination percentage value was found at osmotic potential of -1.0 MPa (22%).

In another study with four distinct osmotic agents ($CaCl_2$, KCl, $MgCl_2$, and NaCl), Stefanello *et al.* (2019) concluded that chia seeds (*Salvia hispanica* L.) were sensitive to exposure to different salts with osmotic potential between -0.1 and -0.4 MPa, which was observed by decreased germination and vigor. Similarly, Barbieri *et al.* (2019) found that the progressive reduction in osmotic potential induced by NaCl, KCl, $CaCl_2$ and $MgCl_2$ salts negatively affected seed germination and initial growth of quinoa seedlings (*Chenopodium quinoa* Willd.).

Under water deficit conditions, one of the primary effects triggered on plants is cell dehydration, which consequently causes a reduction in pressure potential (turgor) and cell volume. This condition triggers side effects, such as ion concentration in cytosol, becoming cytotoxic because the ions cause protein denaturation and membrane destabilization, which may lead to cell death (Taiz *et al.* 2017). In seeds, the main effects observed are decreased germination percentage, increased average time for germination and reduction of primary root and hypocotyl (Demontiêzo *et al.* 2016, Freire e *et al.* 2018).

In soil salinity situations, the soil faces water potentials that hinder water absorption required for germination. The imbibition with accumulation mainly of sodium chloride can cause the rupture of the tegument seed layers, causing damage to the embryo and, consequently, the death of the embryos (Freitas *et al.* 2013). Salinity effects are the results of complex interactions among morphological, physiological, and biochemical processes including seed germination, plant growth, and water and nutrient uptake (Akbarimoghaddam *et al.* 2011). Salinity affects almost all aspects of plant development including: germination, vegetative growth and reproductive development (Bano & Fatima 2009).

Regarding the total length of common rue seedlings (figure 2 a) a reduction from 5.1 cm (control) to 1.14 cm was verified when seedlings were exposed to -0.4 MPa NaCl. On the other hand, normal seedlings were not observed from -0.3 MPa $CaCl_2$ salt exposition. Similarly, the dry weight decreased from 0.9 mg to 0 mg when subjected to calcium chloride solution (figure 2 b). In NaCl solution, although there was a reduction in seedlings length, it was not observed significant difference on dry weight (figure 2 b). Unfortunately, as the amount of material was very small (mg) errors (human or from the equipment) may have occurred when weighing the material.

The findings of our research corroborate with those observed by Cavalcante *et al.* (2019) in rice seeds (*Oryza sativa* L.), where root growth was negatively affected by NaCl, especially at higher concentrations (up to 0.6 MPa). This reduction was also observed by Dalchiavon *et al.* (2016), in studies on the effect of saline stress on common bean seeds (*Phaseolus vulgaris* L.), where increased saline concentration caused a reduction in root growth, hindering their normal development. In addition, Oliveira and Silva (2019) verified reduction of shoot and root growth of common bean seedlings and decreased fresh and dry seedling biomass even in small salt concentrations (0.1 MPa), demonstrating that this species has low tolerance to salt stress caused by KCl.

When a plant tissue, especially seed or root, is subjected to higher saline concentrations, salt stress causes reduction in seedling growth (Cavalcante *et al.* 2019), as observed in our study. Cell growth is the most affected process with reduced water absorption. The effect of salinity on the root system is due to the conditions where the roots are in direct contact with the salts of the medium, causing a reduction in the transpiration and growth rate (Guimarães *et al.* 2013). Salinity affects the germination process not only by hindering the kinetics of water absorption but also by facilitating the entry of large amounts of ions that may be toxic to seeds, such as Na^- and Cl^-, and may promote physiological disorders and culminate in plant death (Pereira *et al.* 2014).

Lastly, the results of our study demonstrated that increased saline concentration in the substrate promoted a significant decrease in seed germination and initial growth of common rue seedlings. The calcium chloride ($CaCl_2$) promoted more drastic effects on seedling growth when compared to sodium chloride (NaCl). According to Souza and Cardoso (2000), the chemical differences between these solutions can cause changes in germination and vigor results even at similar water potentials.

These results are of ecological importance because insights on the tolerance of plant species to the presence of salts are essential to define the type of crop that should be cultivated in a particular environment, especially in saline areas, as well as implement appropriate management and irrigation techniques for each environment, avoiding salinity intensification (Pereira *et al.* 2017). The low tolerance to water stress provides the common rue a non-adaptive character and indicates that the species has reduced establishment capacity in locations with water restriction due to the narrow germination limits.

Conclusion

The excess of salt (NaCl and $CaCl_2$) reduces the germination percentage and the initial development of common rue seedlings, regardless of the substance used for stress induction.

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Author Contributions

Henrique Araujo Barichello: Contribution to data collection; Contribution to data analysis and interpretation; Contribution to manuscript preparation.

Raquel Stefanello: Substantial contribution in the concept and design of the study; Contribution to manuscript preparation; Contribution to critical revision, adding intellectual content.

Géssica Gaboardi de Bastiani: Contribution to data collection; Contribution to data analysis and interpretation; Contribution to manuscript preparation.

Luiz Augusto Salles das Neves: Contribution to critical revision, adding intellectual content.

Conflicts of interest

There is no conflict of interest.

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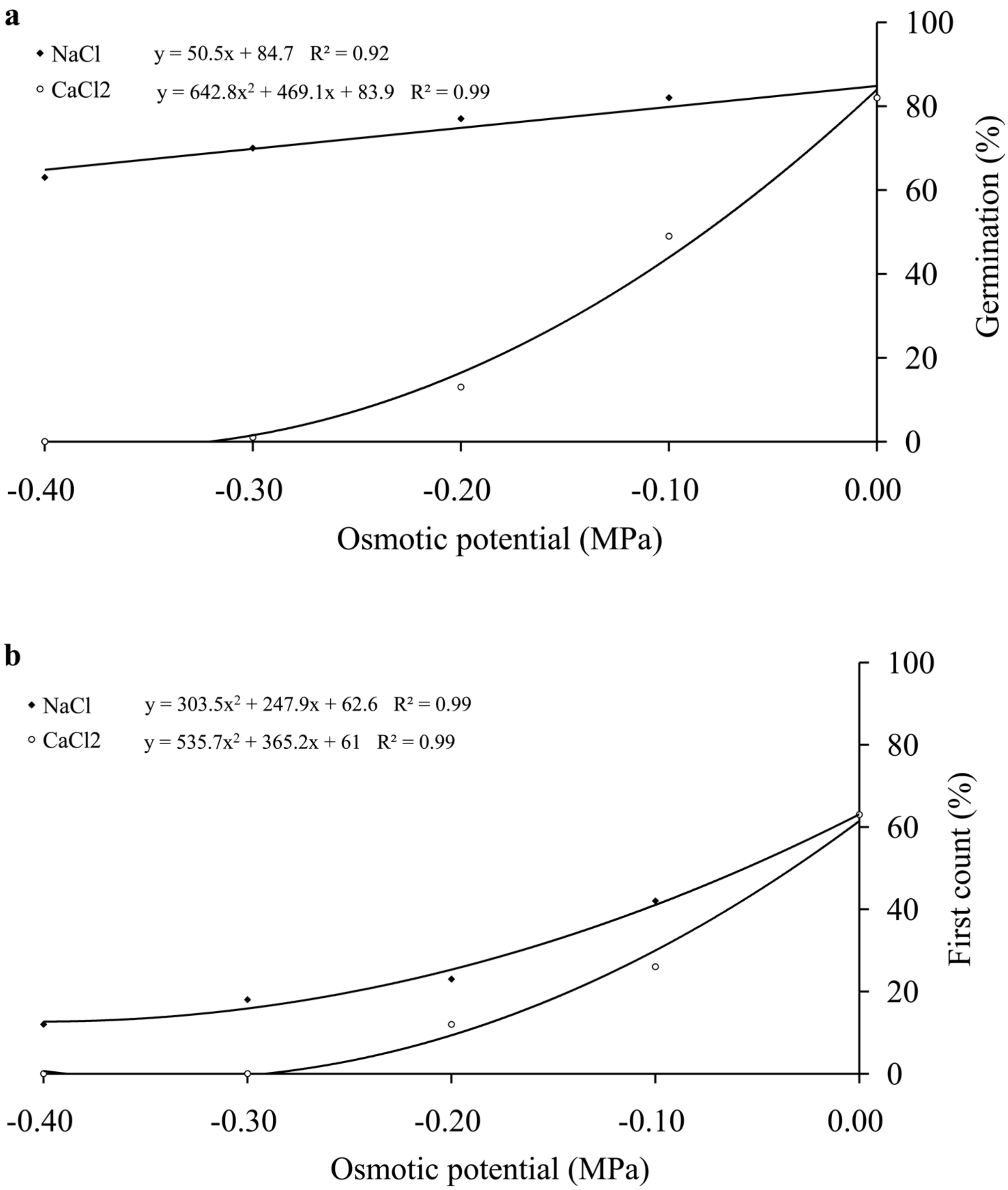

Figure 1. *Ruta graveolens* L. seeds exposed to different osmotic potentials in solution of NaCl and CaCl2. a. germination. b. first germination count.

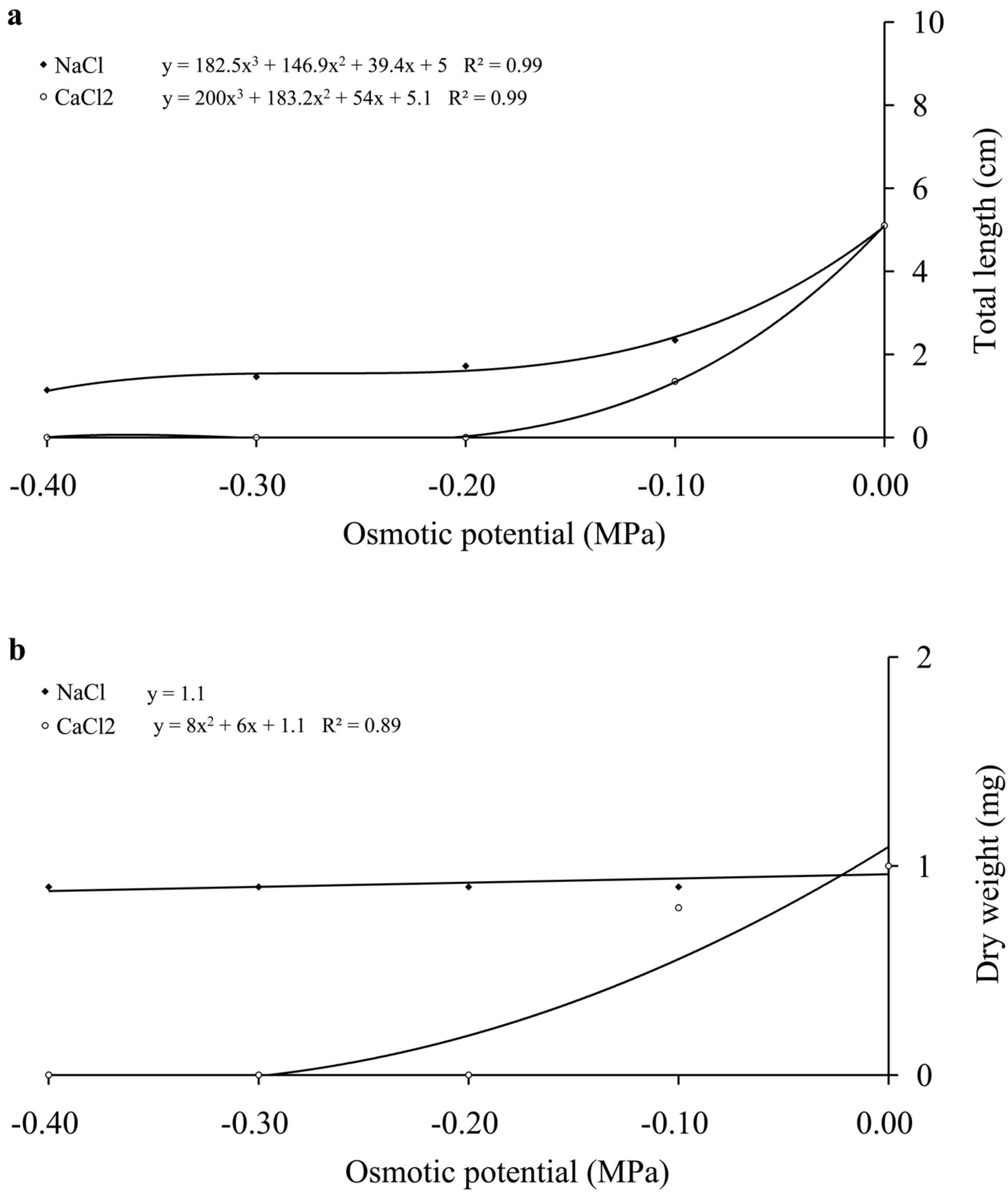

Figure 2. *Ruta graveolens* L. seedlings exposed to different osmotic potentials in solution of NaCl and CaCl2. a. total length. b. dry weight.

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São Paulo, 10 de maio de 2021.

Raquel Stefanello

Raquel Stefanello