

## SALINITY AND WATER DEFICIT IMPACT ON HYDROGEL USE IN LETTUCE CULTIVATION

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**ABSTRACT:** Superabsorbent polymers are soil conditioners capable of improving irrigation water retention for plants. However, semi-arid regions have high evapotranspiration rates, leading to water deficits and salinity issues, which affect the ability of hydrogels to supply water to plants. This study investigated two types of hydrogels under water and salt stress conditions, comparing: a copolymer of acrylamide and potassium acrylate supplemented with CaCO<sub>3</sub>, a copolymer of acrylamide and potassium acrylate and a control treatment. A crop sensitive to the imposed conditions — lettuce (*Lactuca sativa* L.) — was used in a completely randomized design with five levels of irrigation water electrical conductivity (0.0, 0.5, 1.0, 2.0, and 4.0 dS m<sup>-1</sup>). The selected parameters to evaluate treatment responses included soil variables and plant physiological responses. Multiple regression analysis indicated a perfect correlation among the studied variables, explaining treatment responses, group separation related to soil base concentration, and increased lettuce plant biomass.

**KEYWORDS:** optimization, covariance, water-retaining polymer, eggshell

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## **IMPACTO DA SALINIDADE E DÉFICIT HÍDRICO NO USO DE HIDROGÉIS NA CULTURA DA ALFACE**

**RESUMO:** Os polímeros superabsorventes são condicionantes capazes de melhorar a capacidade de retenção da água de irrigação as plantas. Entretanto, regiões semiáridas possuem elevada evapotranspiração que resulta em déficit hídrico e problemas com salinidade, em que esses problemas citados afetam a capacidade dos hidrogeis em disponibilizar água as plantas. Esse trabalho investigou dois tipos de hidrogeis sob condições de estresse hídrico e salino, comparando um co-polímero de acrilamida e acrilato de potássio acrescido com  $\text{CaCO}_3$ , com um co-polímero de acrilamida e acrilato de potássio e um tratamento controle. Foi utilizada uma cultura sensível as condições impostas, no caso a alface (*Lactuca sativa* L.), em delineamento inteiramente aleatorizado com cinco níveis de condutividade elétrica da água de irrigação (0.0, 0.5, 1.0, 2.0 e 4.0  $\text{dS m}^{-1}$ ). Os parâmetros selecionados para avaliar a resposta dos tratamentos envolvem variáveis de solo e de resposta fisiológicas da planta. A generalização da regressão múltipla indica correlação perfeita das variáveis abordadas, capazes de explicar as respostas dos tratamentos, separação de grupos correlacionados com a concentração das bases do solo e aumento das massas das plantas de alface.

**PALAVRAS-CHAVE:** otimização, covariância, hidrotentor, casca de ovo

### **INTRODUCTION**

Arid and semi-arid regions around the world are classified as fragile ecosystems, prone to degradation due to high evapotranspiration and salt accumulation in the soil. These same regions often experience rapid population growth, which in turn increases the demand for food consumption (ETIKALA et al., 2021; GAUR; SQUIRES, 2017). While some crops are tolerant to salinity and water restriction, others are not, such as lettuce (*Lactuca sativa* L.), which is highly sensitive and requires water of good quality and in adequate quantities to avoid production losses (KURUNC, 2021). The limiting conditions of the local climate also affect soil amendments, and hydrogel application is no exception. Alternative hydrogels may be more efficient in improving water availability to plants and reducing stress caused by water scarcity. One example is a co-polymer of polyacrylamide and potassium acrylate synthesized with calcium carbonate, which has properties similar to commercial co-polymers (QUEIRÓS; BEZERRA; FEITOSA, 2017). This study aimed to determine parameters that reflect the

response of an alternative water-retaining co-polymer based on calcium carbonate (produced from eggshells), in comparison with a commercial co-polymer made of acrylamide and potassium acrylate, as well as with no polymer application, under increasing levels of irrigation water salinity in lettuce cultivation.

## MATERIALS AND METHODS

The experiment was conducted in a greenhouse at the Department of Soil Science of the Federal University of Ceará. The geographical coordinates of the site are latitude 3°44'25.4"S and longitude 38°34'31.1"W. The local climate is classified as Aw (tropical savanna), characterized by summer rainfall and a dry winter season (IPECE, 2014). The experiment was established in October 2020 and lasted 90 days.

A completely randomized 3 × 5 factorial design was employed, with four replications, totaling 60 experimental units consisting of 5.0-liter pots. The first factor comprised three treatments: soil without hydrogel (SEM), soil + 2 g kg<sup>-1</sup> of alternative hydrogel (ALT), and soil + 2 g kg<sup>-1</sup> of commercial hydrogel (COM). The second factor included five levels of irrigation water salinity: 0, 0.5, 1.0, 2.0, and 4.0 dS m<sup>-1</sup>. These salinity levels were based on the electrical conductivities of surface and subsurface waters typically found in semi-arid regions (Gebremeskel et al., 2018; Nunes Filho et al., 2000). In the hydrogel treatments, the applied amount corresponded to 0.2% (mass:mass). The properties of the hydrogels used in this study are presented in Table 1.

**Table 1.** Chemical composition of the hydrogels used in the experiment.

| <b>Polymers</b> | <b>C</b>    | <b>H</b> | <b>O</b> | <b>C+H+O</b> |
|-----------------|-------------|----------|----------|--------------|
|                 | -----%----- |          |          |              |
| Commercial      | 31.9        | 5.5      | 6.6      | 44.0         |
| Alternative     | 39.7        | 6.7      | 15.0     | 61.4         |

The irrigation water for observational units was prepared using distilled water supplemented with sodium chloride (NaCl), calcium chloride (CaCl<sub>2</sub>), and magnesium chloride (MgCl<sub>2</sub>) in a 7:2:1 ratio (Rhoades et al., 1992).

Air-dried soil was sieved through a 2-mm mesh. Approximately 3 kg of soil were allocated to each pot. Hydrogel treatments were applied after liming and fertilization. Liming was performed to adjust soil pH from 4.1 to achieve 70% base saturation. Primary macronutrient fertilization followed lettuce fertility requirements (Fernandes et al., 1993), with

basal fertilization applied during experimental setup and top-dressing fertilization at 15 days after transplanting.

Lettuce seedlings were grown in plastic trays containing a carbonized rice husk-compost substrate. At the four-true-leaf stage, one seedling was transplanted per pot, with observational units previously randomized for treatment allocation. Soil moisture was maintained at 60% of field capacity throughout the trial to induce controlled water stress and evaluate hydrogel performance (Martins Melo et al., 2023). At the end of this period, plants and pot soils were carefully separated to determine biometric and biochemical parameters in plant tissue.

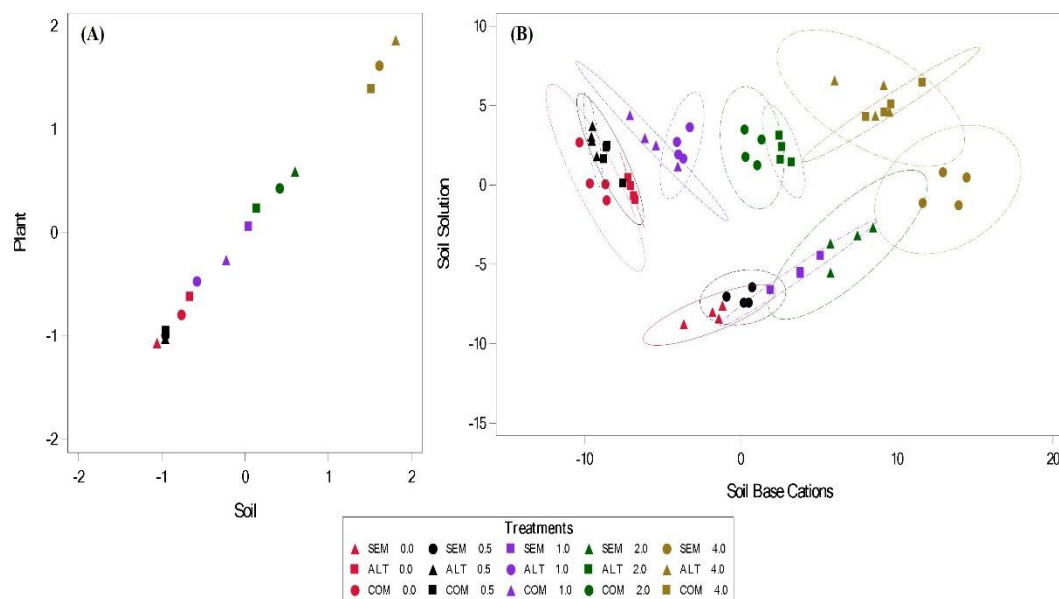
It was evaluated soil parameters P (Phosphorus),  $\text{Ca}^{2+}$  (Calcium),  $\text{Mg}^{2+}$  (Magnesium),  $\text{K}^+$  (Potassium),  $\text{Na}^+$  (Sodium),  $\text{Al}^{3+}$  (Aluminum), H+Al (Potencial acidity), EC (Electrical conductivity), CEC [T] (Total cation exchange capacity), Sum of bases (SB), base saturation percentage (V%), aluminum saturation percentage (m%) and exchangeable sodium percentage (ESP). Plant attributes: Fresh shoot mass (FSM), dry shoot mass (DSM), nutrient accumulation in plant tissue (N, P, K, Na), stress biomarkers (proline, chloride, nitrate), and SPAD index.

The assumptions for analysis of variance were tested using Levene's test for homogeneity, the Shapiro-Wilk test for normality, the Student's t-test for outliers, and the Durbin-Watson test for residual independence. Subsequently, the data were standardized ( $\mu = 0$ ;  $\sigma^2 = 1$ ) to avoid the dominance of one variable over another. After this procedure, canonical correlation analysis, discriminant function analysis and principal component analysis were performed using the SAS OnDemand for Academics statistical software (SAS, 2023).

## RESULTS AND DISCUSSION

The results from the Canonical Correlation and Discriminant Function Analyses demonstrate a strong correlation, as evidenced by data dispersion along an ascending line (Figure 1). The proximity of observations to the line indicates a strong relationship between soil treatments and plant physiological responses. As a generalization of multiple regression, canonical correlation analysis confirmed a strong association between soil cations and biochemical stress indicators (Figure 1A). For canonical 1 equation  $0.062(\text{P}) + 0.533(\text{Ca}) + 0.181(\text{K}) + 0.280(\text{Na}) + 0.238(\text{H+Al}) + 0.011(\text{pH})$ , Calcium had the highest weight in the "Soil" canonical equation; while for canonical 2 equation  $0.011(\text{SPAD}) + 0.558(\text{Nap}) + 0.143(\text{NO}_3) + 1(\text{proline}) - 0.605(\text{AFM}) - 0.272(\text{Cl-})$  proline showed the highest weight in the "Plant" equation. The positive and negative signs of these dominant variables suggest that the increase

in base cation concentrations in the soil—added via irrigation water salts, especially  $\text{Ca}^{2+}$ , leads to increased proline concentrations in lettuce leaves. This reflects a physiological response to the water and salt stress, as proline is an amino acid involved in osmoprotection under drought and salinity conditions, protecting plant tissues without causing ion-related injury (FRANZONI; COCETTA; FERRANTE, 2021). The protective role of hydrogels regarding water availability did not meet the minimum conditions necessary to minimize stress in lettuce plants. Salt concentrations in the soil increased as a consequence of the rising salinity of the water used for irrigation in the treatments. In response to this stress, the plants increased their proline production.



**Figure 1.** Canonical Correlation and Discriminant Function Analysis of the response variables from the applied treatments.

Discriminant function analysis was used to maximize the separation of treatments based on their similarities and dissimilarities. In Figure 1B, the treatments are represented by confidence ellipses, with their size indicating the variance of the original data. The variables that define the canonical functions were selected from the canonical structure matrix. Canonical 1 is defined by the variables Ca, Mg, Na, CEC, SB, V%, ESP, EC, chloride, and proline, which are positively correlated, and by m%, which is negatively correlated. This canonical function was designated “Soil Base Cations.” Canonical 2 is defined by the variables water content and pH, both positively correlated, and was designated “Soil Solution.”

Discriminant function analysis is based on differences among treatments, which are represented by ellipses. Therefore, when one ellipse overlaps another, the corresponding treatments are considered similar in terms of mean vectors. The larger the ellipse, the greater

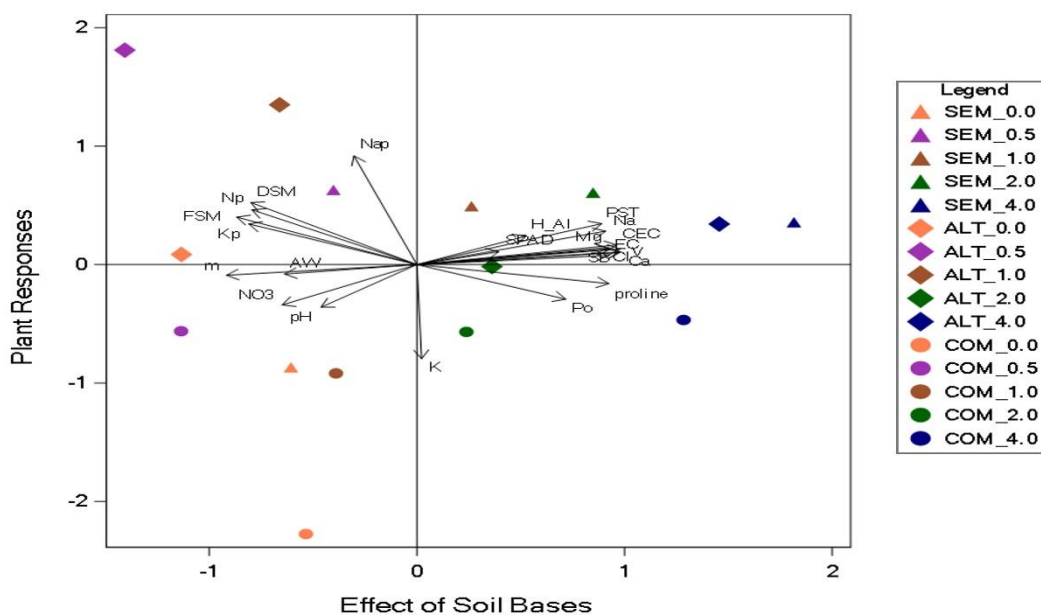
the variability of the data associated with those treatments. The treatment without hydrogel (SEM), across all levels of electrical conductivity (EC) of the irrigation water, formed groups whose confidence ellipses overlapped, indicating that the control treatment differs from the other treatments, ALT and COM. The ALT and COM treatments formed groups with similar characteristics within each level of electrical conductivity. The two hydrogels, within each EC level, were grouped similarly, with their respective ellipses overlapping at each conductivity level. Only at the highest level of electrical conductivity in the irrigation water did the ALT treatment show similarity to both COM and SEM. In summary, the hydrogels used in this experiment presented similar responses regarding the increase of soil cations—mainly Ca, Mg, and Na—as well as the influence of these chemical elements on the calculations of CEC, SB, V%, T, m%, and ESP. They also contributed to increased water availability and a rise in soil pH.

The quality of the irrigation water and the amount of salts present in the soil were important factors in distinguishing the treatment groups. The responses of the ALT and COM treatments, when subjected to the same conditions of water limitation and excess soil salts, demonstrated a reduced capacity for water absorption. This is primarily due to the presence of divalent cations in the solution, such as  $\text{Ca}^{2+}$  and  $\text{Mg}^{2+}$ , which reduce the swelling of hydrogels by binding to functional groups in the polymer structure and forming complexes (NASCIMENTO et al., 2021).

Based on the objective of characterizing the treatments, principal component analysis (PCA) was performed (Figure 2). The first principal component was composed of positively correlated response variables  $\text{Ca}(0.258) + \text{Mg}(0.244) + \text{Na}(0.239) + \text{CEC}(0.253) + \text{SB}(0.257) + \text{V}\%(0.257) + \text{ESP}(0.234) + \text{EC}(0.242) + \text{Cl}^-(0.241) + \text{proline}(0.243) - \text{m}\%(0.241)$ . Based on these variables, this component was designated as the “Effect of Soil Bases.” The second principal component was defined by  $\text{SFM}(0.231) + \text{SDM}(0.303) + \text{Na}_p(0.530) + \text{N}_p(0.267) - \text{K}(0.459)$ . This second component was designated as the “Plant Response.”

In the first quadrant, which is positively correlated with the “Effect of Soil Bases,” the mean vectors of Ca, Mg, CEC, V%, SB, and chloride are highly correlated, showing high values. The proximity of these vectors to the axis of the first principal component indicates a strong correlation. The response variables Na, SPAD, ESP, and potential acidity are also well correlated; however, the mean vectors of SPAD and potential acidity indicate lower values. The variables located in quadrant I (potential acidity, ESP, Na, SPAD, chloride, Mg, Ca, CEC, SB, V%, and EC) show a strong negative correlation with those located in quadrant III (available water, m%, nitrate, and pH), which is diametrically opposite. In the second quadrant, the

response variables  $N_{ap}$ ,  $SDM$ ,  $N_p$ ,  $SFM$ , and  $K_p$  present high values, with the last four being highly correlated with one another.  $N_{ap}$  also shows a strong correlation with the “Plant Response.” The variables in quadrant II ( $N_{ap}$ ,  $SDM$ ,  $N_p$ ,  $SFM$ , and  $K_p$ ) are strongly negatively correlated with the variables in quadrant IV (proline, phosphorus, and potassium), which lies in the opposite direction. In the third quadrant, the variables  $m\%$  and available water are strongly correlated with each other and with component 1, while nitrate ( $NO_3^-$ ) is correlated with soil pH. In the fourth quadrant, proline, phosphorus (P), and potassium (K) exhibit high values, although only soil potassium shows a strong correlation with component 2.



**Figure 2.** Principal Component Analysis (PCA) plots of response variables and treatment factors.

The treatments located in the first quadrant show low variability. The strongest correlations in this quadrant are observed for SEM\_1.0 with “Plant Response” and for ALT\_2.0 with Effect of “Soil Bases”. The variables in quadrant I are strongly negatively correlated with those in quadrant III, which lies directly opposite. In the second quadrant, the greatest variability is observed in ALT treatments (0.5 and 1.0), although the strongest correlations are found for ALT\_0.0 with “Effect of Soil Bases” and SEM\_0.5 with “Plant Response”. The variables in quadrant II are strongly negatively correlated with those in quadrant IV, which is diametrically opposed. In the third quadrant, treatment COM\_0.0 shows the greatest variability and shares the same correlation with “Plant Response” as COM\_1.0, while the other treatments display lower variability. In the fourth quadrant, the treatments exhibit low variability but show strong correlations with both “Plant Response” and “Effect of Soil Bases”, particularly treatments COM\_2.0 and COM\_4.0, respectively. It is worth noting that the principal component analysis effectively distinguished the treatments based on their characteristics. ALT

treatments were positively correlated with both principal components 1 and 2, whereas COM treatments were negatively correlated with both components.

Treatments in the first quadrant are correlated with soil bases and their related calculations. In these treatments, which received irrigation water with  $EC = 4 \text{ dS m}^{-1}$ , there was a higher concentration of soil bases due to the quality of the irrigation water. In the third quadrant, COM treatments (0, 0.5, and  $1.0 \text{ dS m}^{-1}$ ) and SEM ( $0 \text{ dS m}^{-1}$ ) are associated with decreased soil pH, increased aluminum saturation, and elevated nitrate content in the plants. In the second quadrant, the alternative hydrogel under ECs of 0.5 and  $1.0 \text{ dS m}^{-1}$  resulted in greater sodium accumulation in plant tissues and higher potassium levels in the soil. Meanwhile, the alternative hydrogel and the control treatment under lower salinities (0 and  $0.5 \text{ dS m}^{-1}$ ) led to higher SFM, SDM, K, and  $N_p$ , due to better water quality. This resulted in lower proline and phosphorus concentrations in the soil, as the capacity for water availability was greater than in other treatments. Nevertheless, water stress was still present, affecting the biogeochemical cycle of phosphorus and reducing its mineralization (MAZLOOM et al., 2020). In the fourth quadrant, diametrically opposite to the second quadrant, COM treatments under the two highest salinity levels showed strong correlations with increased proline concentration in plants, and elevated phosphorus and potassium in the soil. This is due to the potassium from the structure of the commercial hydrogel being released into the soil as salinity increased. Consequently, the available phosphorus remained in the soil due to the negative interaction between water and salt stress, which reduces phosphorus availability from mineral fertilizers (DING et al., 2020; MARTINS MELO et al., 2023).

## CONCLUSIONS

The key parameter to ensure that superabsorbent copolymers can retain water and swell is the salt concentration in the soil solution. Therefore, soil and irrigation water electrical conductivity are essential parameters to be monitored in semi-arid regions. The alternative copolymer composed of polyacrylamide and potassium acrylate with the addition of  $\text{CaCO}_3$  is capable of increasing lettuce plant biomass and reducing stress caused by drought and salinity under conditions where the irrigation water has low electrical conductivity.

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