Effect of saline concentrations and humidity percentage on alfalfa varieties (*Medicago sativa* L.) from the Mexicali Valley, Mexico

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ABSTRACT
Objective: The aim of this experiment was to evaluate the resistance of different varieties of alfalfa grown in the Mexicali Valley against abiotic factors such as salt stress and drought stress.
Design/methodology/approach: Four alfalfa (*M. sativa* L.) varieties were used: Cuf-101 C, FD9, Pioneer and Cuf-101 P.
Drought stress resistance was evaluated using four different percentages of commercial peat moss substrate (100%, 50%, 25%, and 15%), and saline stress resistance was evaluated by applying four concentrations of NaCl (0 mM, 50 mM, 100 mM, and 200 Mm.The variables evaluated in each treatment were: plant height, number of leaves and root length. The data obtained were subjected to an analysis of variance using the SAS statistical package version 9.0 with an $\alpha=0.5$.
Results: Alfalfa variety Cuf-101C had a good adaptation to both stresses with an average height of 11.28 cm and an average number of leaves of 3.54. Seed germination of alfalfa varieties subjected to salt stress were affected even at the lowest NaCl concentration. Conclusions: The alfalfa variety with the highest germination percentage in all NaCl concentrations was Cuf-101 C with 91.17%.

Keywords: adaptation, alfalfa, salinity, drought.

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INTRODUCTION

Alfalfa (*Medicago sativa* L.) is one of the most important perennial forage legumes worldwide, due to the qualities it presents in terms of performance, its nutritional value, and the ability to resist various abiotic stress factors (Wang *et al.*, 2021). In the Mexicali Valley, Baja California, it is the third most important crop by established and harvested area and is the most used forage for cattle in the arid and semi-arid regions of Mexico (Martínez-Varela *et al.*, 2015; SADER, 2020; Sánchez-Santillán *et al.*, 2019). Salinity and

drought stress are critical environmental factors limiting plants' growth, development, and agricultural productivity, such as alfalfa (Li *et al.*, 2022; Khodayari, & Abedini, 2022). Studies indicate that plants' responses to different stressors reveal they can detect subtle environmental changes and respond immediately to complex stress conditions, minimizing damage and conserving valuable resources for growth and reproduction (Martínez *et al.*, 2016). There are also reports detailing alfalfa varieties' growth capacity when under various stress conditions, and their ability to develop different resistance mechanisms has been demonstrated (Quan *et al.*, 2016). Further to this, current information reflects the effects of water or saline stress or a combination of both on alfalfa yield. The development, growth, quality, and yield of alfalfa are significantly inhibited and decrease drastically under salt stress conditions (Ling *et al.*, 2022). However, it is necessary to evaluate the physiological response mechanisms exerted by alfalfa when subjected to both factors (Hou *et al.*, 2022). Studies indicate that plants' responses to different stressors reveal they can detect subtle environmental changes and respond immediately to complex stress conditions, minimizing damage and conserving valuable resources for growth and reproduction (Martínez *et al.*, 2016). There are also reports detailing alfalfa varieties' growth capacity when under various stress conditions, and their ability to develop different resistance mechanisms has been demonstrated (Quan *et al.*, 2016). Further to this, current information reflects the effects of water or saline stress or a combination of both on alfalfa yield. The development, growth, quality, and yield of alfalfa are significantly inhibited and decrease drastically under salt stress conditions (Ling *et al.*, 2022). However, it is necessary to evaluate the physiological response mechanisms exerted by alfalfa when subjected to both factors (Hou *et al.*, 2022).

In this sense, it is necessary to understand the physiological responses of plants to different stress conditions to ensure a good harvest in future climatic conditions that may arise. Therefore, this work focuses on identifying the best alfalfa varieties resistant to saline and drought stress in the Mexicali Valley, Mexico.

MATERIAL AND METHODS

Study site

This research was carried out in the Phytopathology Laboratory and in the greenhouse Area of the Instituto de Ciencias Agrícolas (ICA) of the Universidad Autónoma of Baja California (UABC) located in the Mexicali Valley in the extreme northeast of the state of Baja California, between 114° 45' to 115° 40' west longitude and 31° 40' to 32° 40' north latitude.

Obtaining seeds and sowing

Four varieties of seeds were used, three provided by the seed marketer Stell: Cuf-101 C, FD9, Pioneer, and one donated by an alfalfa farmer from the Mexicali Valley, produced by himself Cuf-101 P. Three alfalfa seeds were sown per experimental unit using Styrofoam cups with a capacity of 1 L. Peat moss was used as the substrate, which was previously sterilized and had its pH adjusted to 7.17. The glasses were placed in the ICA greenhouse. Fertilization was based on Miracle-Gro Brand granulated inorganic fertilizer and following the manufacturer's instructions (Echeverria *et al.*, 2021).

To evaluate the effect on germination, a completely randomized design with a factorial arrangement of two factors at four levels each was used: Factor A: variety (Cuf-101 P, Cuf-101 C, FD9 and Pioneer).

Height (H), number of leaves (LN), and root length were evaluated for both cases. Data for H and LN was taken once a week, and root length was taken at the end of the experiment.

Treatments salinity and drought *in vivo*

NaCl concentrations, and in the case of drought stress, the different percentages of substrate humidity were recorded.

In the case of salt stress, 48 experimental units were evaluated (a Styrofoam glass with two alfalfa plants of one of the varieties. Four concentrations of NaCl were applied: 0 mM, 50 mM, 100 mM, and 200 mM, for 16 treatments with three repetitions (Lastiri-Hernández *et al.*, 2017; Pacheco *et al.*, 2022). Each experimental unit (a 9 cm diameter petri dish) with 10 seeds of each of the varieties had four percentage of humidity (100, 50, 25, and 15%) applied, for a total of 16 treatments, and each treatment had three repetitions, for a final total of 48 experimental units. Each experimental unit was a Styrofoam cup, which contained two alfalfa plants of one of the varieties, and was subjected to four different irrigation distributions: 1 day of irrigation and 2 days without irrigation (1R/2SR), 1 day of irrigation and 5 days without irrigation (1R/5SR), 1 day of irrigation and 10 days without irrigation (1R/10SR), and 1 day of irrigation and 15 days without irrigation (1R/15SR), for a total of 16 treatments with three repetitions, for a final total of 48 experimental units (Hou *et al.*, 2022).

Germination tests for salinity and drought

A standard "between paper" germination test was performed for both effects. 25 seeds were sown and distributed in five columns and five rows, in two towels previously moistened with the corresponding treatment on a flat surface and subsequently covered with two other wet towels to then be then rolled into the shape of a "taco", which were introduced into a VWR Scientific Inc. brand incubator Model VWR 1550 C at ±20 °C. The initial (GP %) data was taken on the third day, and the final percentage was taken on the seventh day (Martínez-Solis *et al.*, 2010). The seed was germinated when the radicle was 5 mm long (Abril-Saltos *et al.*, 2017). The germination test moistened the substrate at four different percentages (100, 50, 25 and 15%). A Lutron humidity sensor model, PMS 714, was used to measure the percentage of humidity. Once the humidity was adjusted, petri dishes were filled, and 10 seeds per box were sown. This procedure was the same for all treatments.

Treatments salinity and drought

The treatments were applied when the plants reached an approximate height of 10 cm. Watering was applied manually three times a week. Once the approximate height of 10 cm was obtained, each experimental unit was brought to field capacity and placed in two environmental chambers (Lab-Line Instruments Inc. Biotronette Mark III Model) at ±40

°C to begin the application of the treatments. The variables evaluated in each treatment were: H, LN, and root length. Data for H and LN were taken once a week and root length was determined at the end of the experiment.

Statistical analysis

The data obtained was subjected to an analysis of variance using the SAS statistical package version 9.0 with an $\alpha = 0.5$

RESULTS AND DISCUSSION

Germination percentage (GP %) It was observed during the two data collection dates that the interaction between the factors did not influence the (GP %). However, their influence was observed among the four varieties analyzed (Table 1). These results coincide with what Castroluna and collaborators (2014) reported, who evaluated three varieties of alfalfa, which were affected by the incidence of both factors. Concerning the factor humidity percentage, a highly significant influence on the germination percentage (%) was observed. On the first date, there was very significant evidence of the influence of the factors separately on germination, and on the second date, there was much notable evidence due to the variety factor, and highly significant due to the NaCl concentration, both on germination. On both dates, the best variety was CUF-101 C at a concentration of 0 mM NaCl. In Table 1, it is observed in data collection 1 that the interaction between the factors had no influence on the germination percentage with stress in alfalfa due to drought; however, on both dates, the best percentage of substrate humidity was 15%, with an average germination of 98.33%, which was obtained from the initial germination data collection and was maintained up to the final one.

For date one, in the variables H and LN highly significant evidence was observed with respect to the variety factor. The best variety in both cases was the commercial seed variety Cuf-101 C, with an average height of 11.28 cm and an average number of leaves of 3.54. In week two, the data for the variables H and LN showed significant and highly significant evidence due to the variety factor. The best variety was the Cuf-101 C, with an average height of 12.72 cm and an average number of leaves of 4.42. In week three, the data for the H variable showed highly significant evidence due to the variety factor. Significant evidence was found in the LN variable due to an interaction between the factors. The variety that presented the most significant height was Cuf-101 C with 18.81 cm. In the case of the variable number of leaves, the best treatment was that corresponding to the variety Cuf-101 C seed with a concentration of 100 mM NaCl, with 8.17 average leaves. In week four, significant evidence was found in the height variable due to the variety factor. In the case of the LN it was found that the interaction between the factors influenced said variable the best variety corresponds to the variety Cuf-101 C seed with an average height of 18.81 cm. In the case of the LN, the best treatment corresponded to the variety Cuf-101 C seed with a concentration of 100 mM, with an average Cuf-101 C of 12.50. In week five, highly significant evidence was found for the H variable due to the variety factor. In the case of the LN, very highly significant evidence was found due to separate factors. The variety with the highest height was the

Table 1. Percentage of initial and final germination of alfalfa at different concentrations of NaCl.

Date	Variety	[NaCl]	Germination (%)
1	1 ('Cuf 101' P)	1	91.33
		2	91
		3	87
		4	78
	2 ('Cuf 101' C)	1	91.67
		2	89.33
		3	92
		4	84.33
	3 (FD9)	1	84.67
		2	80.33
		3	85
		4	72.33
	4 (Pioneer)	1	84.67
		2	85.33
		3	80.33
2	1 ('Cuf 101' P)	1	93.33
		2	92.67
		3	88
		4	88.33
	2 ('Cuf 101' C)	1	93
		2	90.67
		3	92.67
		4	88.33
	3 (FD9)	1	85.33
		2	81.33
		3	85.33
		4	76
	4 (Pioneer)	1	86.33
		2	89.67
	1 ('Cuf 101' P)	3	81.33
		4	81.67
		Variety	***
		NaCl	**
		Variety*NaCl	NS

* ≤0.05 Significant.
** ≤0.01 Highly significant.
*** ≤0.001 Very highly significant.

Cuf-101 C with an average height of 18.27 cm, and the variety with the highest LN with an average of 13.10. The NaCl concentration, was the best, corresponding to 50 mM, with an average of 13.05 leaves. The experimental data did not show significant

evidence that the interaction of the factors, or the factors separately, affected the root length variable. All treatments were the same (Figure 1).

Effect of salt stress on alfalfa development

For date one, highly significant evidence was observed in the variables H and LN due to the variety factor. The best variety in both cases was the Cuf-101 C, with an average H of 11.28 cm and an average LN of 3.54. In week two, the data for the variables H and LN showed significant and very highly significant evidence due to the variety factor. The best variety was the Cuf-101 C with an average height of 12.72 cm and an average LN of 4.42. Some studies show that alfalfa has salinity tolerance at all growth stages (Pacheco *et al.*, 2022). In week three, the data for the height variable showed highly significant evidence due to the variety factor, and in the case of the number of leaves variable, significant evidence was found due to the interaction between the factors. The variety that presented the most significant height was the Cuf-101 C with 18.81 cm. In the case of the variable number of leaves, the best treatment was that corresponding to the Cuf-101 C with a concentration of 100 mM NaCl, with 8.17 average leaves. In week four, significant evidence was found in the height variable due to the variety factor. In the case of the LN, it was found that the interaction between the factors had the more significant influence. In the case of height, the best variety was the one corresponding to the Cuf-101 C with an average height of 18.81 cm, and in the case of the LN, the best treatment was the one corresponding to the Cuf-101 C with a concentration of 100 mM, with an average LN of 12.50. In week five, significant evidence was found for the height variable due to the variety factor. In the case of the LN, highly significant evidence was found with the factors separately. The variety with the highest height was the Cuf-101 C with an average height of 18.27 cm, and the variety with the highest LN with an average of 13.10. The best concentration for the NaCl concentration was 50 mM, with an average of 13.05 leaves. The experimental data did not show significant evidence that the interaction of the factors, or the factors separately, affected the root length variable. All treatments were the same.

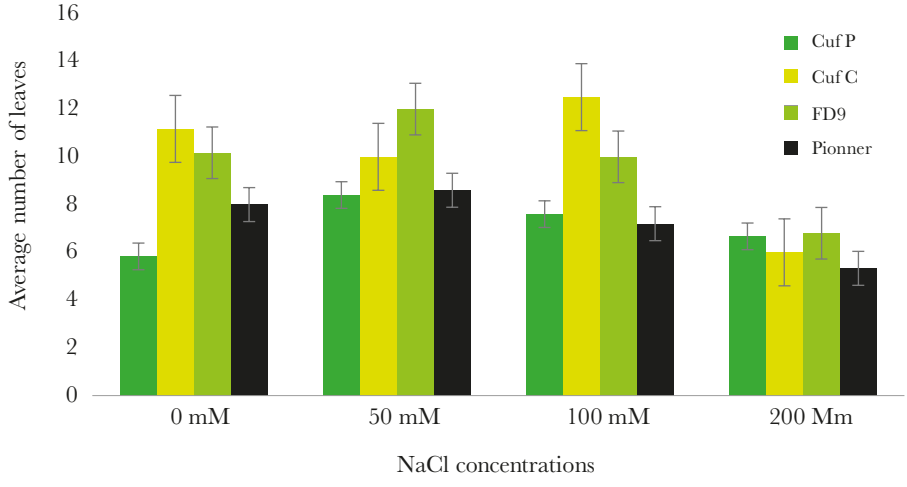

Figure 1. Average number of leaves of the different treatments in week four.

Effect of drought stress on alfalfa development

For date one, it was found that the variables H and LN were influenced by the factors separately. In the case of height, there was significant evidence for the variety factor and very highly significant evidence for the irrigation distribution factor. In the case of the LN, there was significant evidence for the variety factor and highly significant evidence for the irrigation distribution factor. The best variety according to height was the Cuf-101 C, with an average height of 9.25 cm, and the highest LN, with an average of 2.96 leaves. The best irrigation distributions corresponded to (1R/5SR) for both variables, and (1R/15SR). For date two, the experimental data did not show significant evidence that the interaction of the factors or the factors separately influenced the height variable. All treatments were the same. For the variable LN, the data showed significant evidence that irrigation distribution influenced said variable. The best irrigation distribution corresponds to (1R/5SR) with an average LN of 4.88. For date three, the experimental data showed significant evidence due to the interaction between the factors on the H variable and significant evidence due to the irrigation distribution factor for the LN, variable. The best treatment was the one corresponding to the Pioneer variety with an irrigation distribution of (1R/5SR) with an average height of 23.80 cm. For the LN variable, the best irrigation distribution corresponded to (1R/5SR) with an average LN of 6.42. For date four, the experiment data showed highly significant evidence due to the interaction between the factors on the height variable. In the case of the leaf number variable, the data show highly significant evidence due to the irrigation factor. The best treatment was the one corresponding to the Pioneer variety with an irrigation distribution of (1R/5SR) with an average height of 29.22 cm. For the variable LN, the best irrigation distribution was that of (1R/5SR), with an average of 13.35 leaves.

For date five, the experiment data on the height variable did not show significant evidence due to the interaction of the factors or the factors separately. All treatments were the same. In the case of the variable LN significant evidence was found due to the irrigation distribution factor. The best irrigation distribution was that corresponding to (1R/5SR), with an average of 13.24 leaves. The experimental data did not show significant evidence that the interaction of the factors, or the factors separately, affected the root length variable. All treatments were the same. The dry weight of the shoots of five alfalfa cultivars was significantly reduced by water deficit and showed a significant difference between the cultivars. Similar results were also observed in previous studies on drought response of alfalfa (Guo *et al.*, 2019). However, drought itself is a stress for plants. The positive influence of drought on subsequent salt stress can be counteracted by the negative effect of drought itself. Therefore, the ameliorative effect of light drought was more pronounced than severe drought's. In contrast, if drought occurred simultaneously with salinity, it reinforced salinity-induced damage, according to multiple studies.

CONCLUSIONS

All alfalfa varieties (*M. sativa* L.) subjected to salt stress were affected in their germination even at the lowest NaCl concentration. The highest average total (GP %) variety in all NaCl concentrations was Cuf-101 C with 91.17%. The (GP %) in the case of drought stress was

higher when the substrate had 15% humidity. All varieties had a good (GP %), but this is conditioned by the moisture content in the seed. The variety presented a better response to salt stress was the Cuf-101 C variety because it presented greater height and average total number of leaves even at concentrations of 100 mM NaCl.

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