Colegio de Postgraduados

Sodium Chloride in the Germination and Initial Growth of Three Huauzontle (*Chenopodium berlandieri* subsp. *nuttalliae*) Cultivars

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ABSTRACT

Objective: To determine the effect of sodium chloride (NaCl) and cultivar on germination and initial growth of huauzontle (*Chenopodium berlandieri* subsp. *nuttalliae*).

Design/methodology/approach: A 3×5 factorial experiment in a completely randomized design was conducted. Three huauzontle cultivars (Mexico City, Puebla, and Tlaxcala) and five doses of NaCl (0, 75, 150, 225, and 300 mM) were evaluated. Each treatment had five replicates. The germination percentage was evaluated 24 h after sowing, plant height of the seedlings for 11 d, and the weights of fresh and dry biomass were determined at 11 d after germination. The data were analyzed using ANOVA, Tukey's test ($p \leq 0.05$), and a regression model was generated for the variable seedling height as a function of the time of exposure to NaCl.

Results: Seed germination began 24 h after sowing in the three cultivars at all NaCl concentrations evaluated. The germination percentage was reduced by 17.6% at 300 mM compared to the control without salinity. Seedling height was higher in the Mexico City and Tlaxcala cultivars at 75 and 150 mM compared to the control; in these treatments, growth was only inhibited with 300 mM NaCl. On the contrary, the Puebla cultivar showed a reduction in growth starting at 225 mM NaCl. The weights of fresh and dry biomass were only affected by the cultivar study factor, where Mexico City followed by Puebla had the highest means; both higher than Tlaxcala.

Limitations of the study/implications: This study only considers the germination and initial growth phases in the three huauzontle cultivars. Therefore, it is necessary to evaluate the effects of salinity in later phenological stages.

Findings/conclusions: The responses observed in huauzontle in germination and initial growth depend on the NaCl concentration and the cultivar. The germination percentage decreased only with the 300 mM NaCl dose with respect to the control. NaCl at doses of 75 and 150 mM NaCl promoted growth in the Mexico City and Tlaxcala cultivars. The Mexico City cultivar had the highest fresh and dry biomass weights, followed by Puebla and Tlaxcala.

Keywords: Amaranthaceae, Chenopodiaceae, underutilized crops, abiotic stress, salinity.

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INTRODUCTION

Huauzontle (*Chenopodium berlandieri* subsp. *nuttalliae*) is a pseudocereal native to Mexico belonging to the Amaranthaceae family, Chenopodiaceae subfamily, which has been consumed since pre-Hispanic times until today as a leaf vegetable [1,2]. It contains dietary fiber, proteins, essential amino acids, fatty acids, minerals, and phenolic compounds [3-5]. In Mexico, it is grown in Puebla, Tlaxcala, Guerrero, and Morelos, with average yields of 2 to 11 Mg ha^{-1} [6].

Huauzontle is an annual, dicotyledonous plant, adapted to adverse climatic conditions [7]. It is found in arid and semi-arid areas of Mexico; it has a taproot, stems with sympodial branches, alternate leaves with serrated edges, and seeds of various colors, from orange to red [8]. Its inflorescences are green and are consumed in their mature state [2].

Like many cultivated plants, huauzontle is exposed to various stressors during its growth and development [9]. Soil salinity stands out as one of these factors, characterized by the presence of dissolved salts such as NaCl, Na_2SO_4, $MgSO_4$, $CaSO_4$, $MgCl_2$, KCl, and Na_2CO_3 [10,11].

The dominant ions in saline environments are Na^+ and Cl^-, and their high concentrations are toxic to seeds [12], so salinity represents a risk for plants, mainly in germination and in the seedling phase because it causes three types of stress: oxidative, ionic, and osmotic [13]. Osmotic stress affects germination, reduces the water potential of the medium, and influences water absorption, the development of embryonic organs, and the vigor of seedlings [14,15]. The effect of salinity on germination and emergence has been studied in cereals and pseudocereals. In quinoa (*Chenopodium quinoa* Willd.), germination was reduced to 50% with 50 mM Na_2CO_3 [16]. In sorghum [*Sorghum bicolor* (L.) Moench], the dose of 200 mM NaCl decreased germination to 57% [17].

The emergence of seedlings in a saline environment provides information to investigate the degree of sensitivity of seeds to this type of stress [18]. In the specific case of huauzontle, this aspect has not been studied and its level of tolerance to salinity in germination and in the initial growth phase is unknown. Therefore, the objective was to study the effect of the application of NaCl on the germination and initial growth of three cultivars of huauzontle.

MATERIALS AND METHODS

Plant material

Three cultivars of huauzontle (*Chenopodium berlandieri* subsp. *nuttalliae*) were collected from (i) Mexico City, in the municipality of Iztapalapa; (ii) Puebla, municipality of Texmelucan; and (iii) Tlaxcala, municipality of Panotla.

Treatment design and experimental design

Fifteen treatments were evaluated from a 3×5 factorial experiment. The first factor was the huauzontle cultivar with three levels: Mexico City, Puebla, and Tlaxcala. The second factor was the NaCl concentration: 0, 75, 150, 225, and 300 mM. The experimental units consisted of hinge-type plastic trays with 30 seeds each using peat as a substrate. Each treatment had five replicates, which were distributed in a completely randomized design.

Application of treatments

The seeds of each cultivar were immersed for 5 min in each of the NaCl concentrations. Thirty seeds were then sown in a tray with peat (experimental unit described above), a substrate that was previously saturated with 25 mL of each NaCl solution. In the following days of evaluation, 15 mL irrigations were applied with the corresponding NaCl solution. The minimum and maximum temperatures during the test ranged between 15 and 27 °C, with a relative humidity of 62.7%.

Variables evaluated

To obtain the germination percentage, the germinated seeds were counted (2 mm radicle emergence). The height of the seedling was measured with a graduated ruler, from the moment of germination until they had two true leaves (11 d). The fresh biomass of each seedling was determined on an analytical scale, and the dry biomass was obtained after drying the sample in a forced air circulation oven for 24 h at 70 °C.

Statistical analysis of the data

The results of germination, seedling height, and fresh and dry seedling biomass were analyzed for variance and the means were compared with the Tukey test ($p \leq 0.05$), using the statistical program Statistical Analysis Systems (SAS) 9.1®. In addition, regression models were performed using the method proposed by Volke [19], starting from an initial model based on the graphical relationship between the response variables and the study factors that showed a response trend until obtaining a model with the lowest mean square error (MSE).

RESULTADOS Y DISCUSIÓN

Germination

The germination of the seeds of the three evaluated cultivars of huauzontle began 24 h after sowing in all NaCl concentrations; although, in general, the germination time has a negative relationship with the level of salinity present in the medium [20,21]. This time is greater than that reported in quinoa seeds cv. Sajama, where germination was recorded at 6 h with 250 mM NaCl, the maximum germination (80%) was observed at 14 h [22]. On the contrary, in amaranth seeds (*Amaranthus caudatus* L.) Rojo genotype, germination began at 72, 96, 100, and 72 h with treatments with 0, 50, 75, and 150 mM NaCl; while in the Verde genotype, germination was recorded after 150, 168, 170, and 180 h for the same NaCl doses tested [23].

Figure 1 shows the appearance of seed germination under the different NaCl concentrations. Only the NaCl dose had significant effects on this variable; that is, there was no effect of cultivar or cultivar × NaCl interaction. Although there is no cultivar effect, it is pertinent to indicate that the Mexico City and Puebla cultivars reached 100% germination in the control and this decreased as salt stress increased, with values of 85.3 and 79.3% respectively with 300 mM NaCl. The Tlaxcala cultivar presented 100% germination with 75 mM NaCl and 93.3% in the control, while with 300 mM NaCl it recorded 76% germination.

The germination percentage decreased with increasing salinity; with 300 mM NaCl it was reduced by 17.6% with respect to the control (Figure 2). The ability of plants to survive germination and emergence is recognized as the main indicator of their tolerance

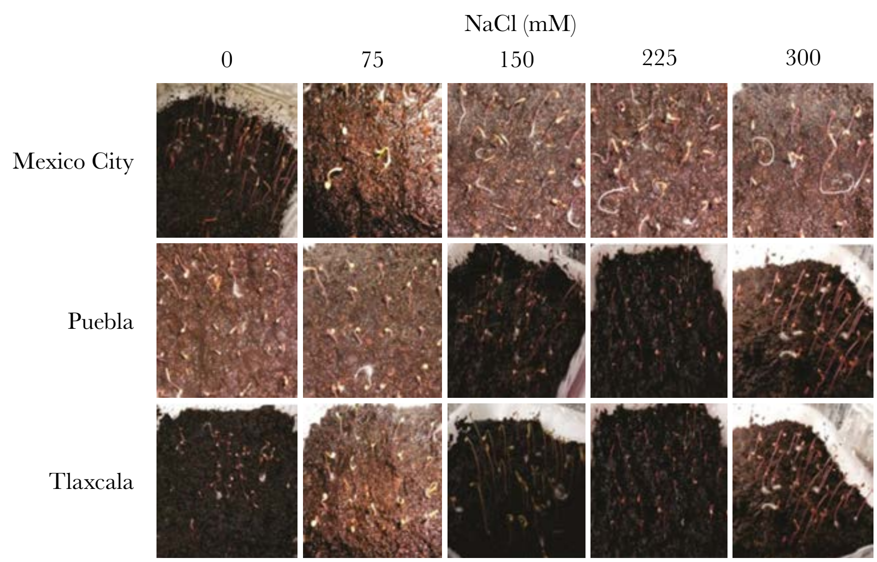

Figure 1. Germination in three cultivars of huauzontle (*Chenopodium berlandieri* subsp. *nuttalliae*) depending on treatment with different concentrations of sodium chloride (NaCl).

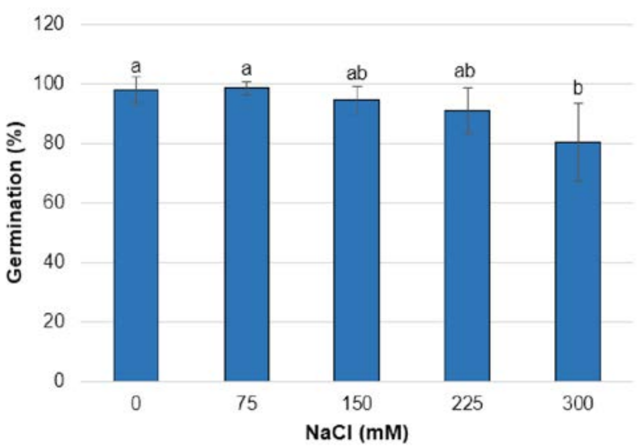

Figure 2. Germination percentage of huauzontle (*Chenopodium berlandieri* subsp. *nuttalliae*) seeds treated with different concentrations of sodium chloride (NaCl). Means ± SD with different letters indicate statistical differences (Tukey, $p \leq 0.05$).

to salinity [24,25]. Salt stress affects the water absorption capacity of the seed and hinders hydration and germination, which causes physiological stress, similar to that of drought [26,27]. Furthermore, if the amount of salt exceeds the tolerance level of the species, the germination process can be completely inhibited [28].

Seedling height

The presence of NaCl in the soil or substrate negatively affects plant growth. The magnitude of this effect depends on several factors, such as the species, stage of development, and NaCl concentration [29]. Likewise, growth delay is an adaptive mechanism for survival, which allows plants to combat salinity stress and depends on time [30]. In the evaluations of seedling height carried out every 24 h between 2 and 11 d, statistical significance was observed both for the main effects of the study factors and for the interaction effects. Figure 3 shows the results of seedling height at 10 d. It can be observed that, without salinity, the Puebla cultivar had the greatest height, followed by Mexico City and Tlaxcala. On the other hand, it is observed that the cultivars have differential responses to the salinity gradient. In the Mexico City and Tlaxcala cultivars, seedling height was increased with doses of 75 and 150 mM NaCl, compared to 0 mM NaCl. In contrast, the Puebla cultivar showed no differences in height in the range of 0 to 150 mM NaCl; doses equal to or greater than 225 mM NaCl inhibited growth.

To explain the behavior of the seedling height of the three cultivars as a function of NaCl concentration and time, the following regression model was used:

$$At = 0.78 + 0.248H + 1.107T - 0.05668T^2$$

PrF: 0.0001; CV: 19.69 %; R^2: 0.854

Where: *At*: seedling height in cm, $H=0$ Mexico City and Tlaxcala; $H=1$ Puebla; $T=$time (evaluation days).

This model indicates a negative effect of NaCl on the three cultivars (Figure 4); while, due to time, an increasing behavior was observed in the first six days of evaluation; subsequently, the growth rate decreased and stabilized at 10 d after germination. As previously indicated, it was observed that the growth of huauzontle seedlings during 11 d is different between cultivars; Mexico City and Tlaxcala behaved similarly (Figures 3 and 4a) compared to Puebla, which had greater growth inhibition due to salinity (Figures 3 and 4b).

The growth inhibition induced by NaCl is a commonly observed effect in several crops such as *Amaranthus caudatus* (L.), which showed a decrease in growth from 54 to 27% with 100 mM NaCl [23]; in sorghum, initial growth was affected with 200 mM NaCl [31].

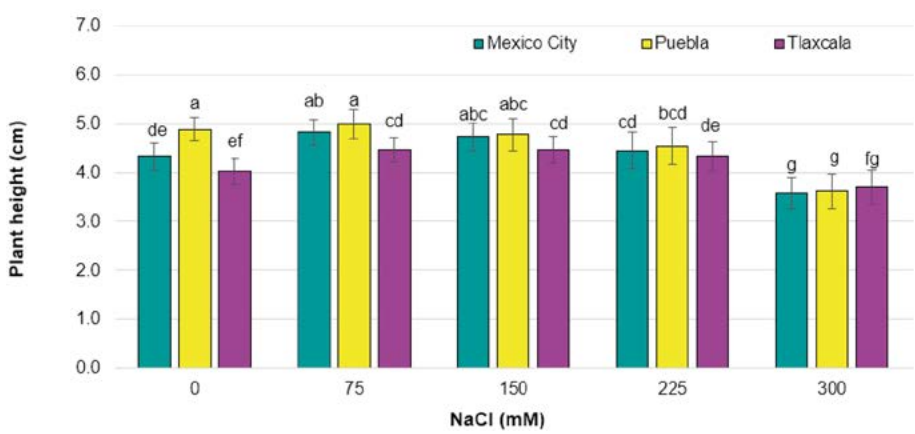

Figure 3. Seedling height of three cultivars of huauzontle (*Chenopodium berlandieri* subsp. *nuttalliae*) treated with different concentrations of sodium chloride (NaCl). Means ± SD with different letters indicate statistical differences (Tukey, $p \leq 0.05$).

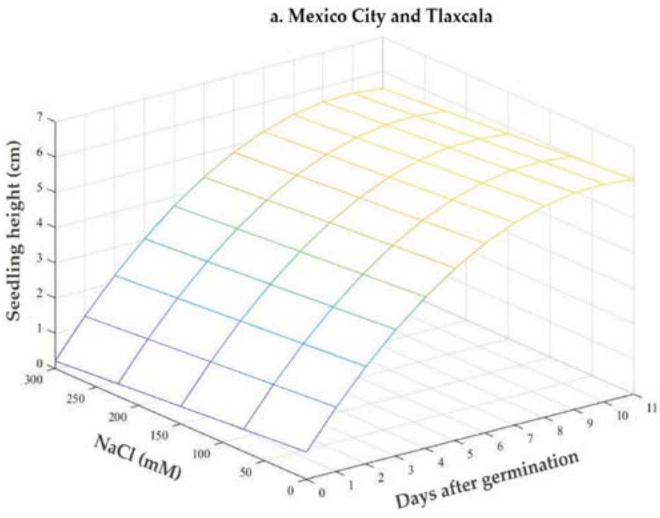

Figure 4. Effect of sodium chloride (NaCl) over time on seedling height in the cultivars Mexico City and Tlaxcala (a), and Puebla (b) of huauzontle (*Chenopodium berlandieri* subsp. *nuttalliae*).

Fresh and dry seedling biomass. The weights of fresh and dry seedling biomass were only influenced by the cultivar study factor (Figure 5). The cultivars Mexico City and Puebla exceeded Tlaxcala by 10.9 and 9.7% in fresh seedling biomass (Figure 5a); as well as by 21 and 18.6% in dry seedling biomass (Figure 5b).

On the other hand, although there was no effect of the NaCl factor, it is pertinent to mention that the values of fresh biomass ranged from 0.854 to 0.795 g seedling^{-1} and corresponded to the concentrations of 75 and 300 mM NaCl, respectively. Meanwhile, with the doses of 75 and 0 mM NaCl, the highest and lowest dry biomass weights were obtained (0.032 and 0.027 g seedling^{-1}), respectively.

Regarding the interactions of the study factors, although they did not cause significant effects either, it is appropriate to indicate that the highest fresh biomass averages in the Mexico City cultivar were with 75 mM NaCl (0.934 g seedling^{-1}), in Puebla with 150

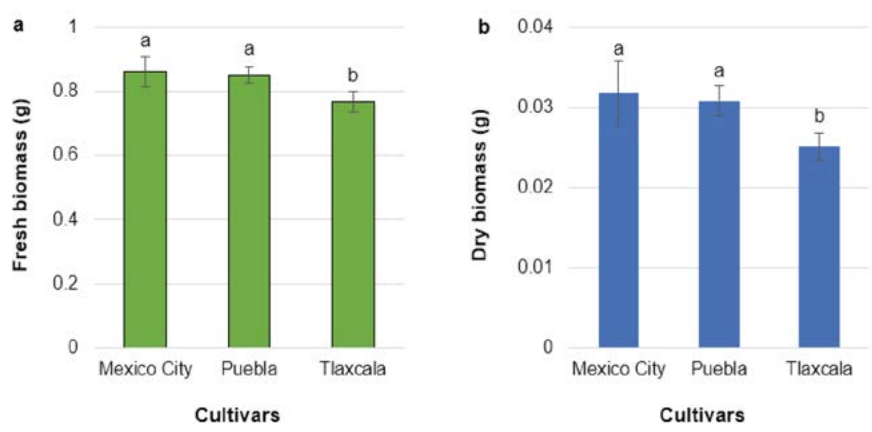

Figure 5. Fresh (a) and dry (b) biomass of seedlings of three genotypes of huauzontle (*Chenopodium berlandieri* subsp. *nuttalliae*). Means ± SD with a different letter in each subfigure indicate statistical differences (Tukey, $p \leq 0.05$).

mM NaCl (0.878 g $seedling^{-1}$), and in Tlaxcala with 225 mM NaCl (0.814 g $seedling^{-1}$). Likewise, the lowest means in the Mexico City cultivar were with 300 mM NaCl (0.792 g $seedling^{-1}$), in Puebla and in Tlaxcala in the treatment without salinity (0.802 and 0.744 g $seedling^{-1}$, respectively). On the other hand, the highest dry biomass in the Mexico City cultivar was with 75 mM NaCl (0.0396 g $seedling^{-1}$); while, in Puebla and Tlaxcala it was obtained with 300 mM with values of 0.0327 and 0.0284 g $seedling^{-1}$, respectively. Similarly, the Mexico City, Puebla, and Tlaxcala cultivars presented the lowest seedling weights with 300, 150, and 0 mM NaCl, respectively, with means of 0.0281, 0.0292, and 0.0217 g.

The decrease in plant dry weight due to increasing salinity may be caused by the interaction of osmotic effects and the presence of specific Cl^{-} and Na^{+} ions, which reduce the photosynthetic activity of young leaves [32]. Moreover, the reduction in biomass with increasing salinity may be attributed to the negative effects of the increased energy needed to produce osmolytes during osmotic adjustment as the plant grows [33]. In *Amaranthus cruentus* (L.), the dry biomass weight of leaves decreased 27% with 50 mM NaCl, compared to the treatment without NaCl [34]. In *Amaranthus tricolor* (L.) with 50 and 100 mM NaCl the dry biomass weight decreased significantly [35]. In quinoa, the dry fresh biomass decreased 1.7 times with 300 mM NaCl [36].

As plants mature, they may develop greater tolerance to salinity, depending on the crop in question. Tolerance is not uniform across species or at all growth stages [30]. However, at vegetative and reproductive growth stages, salinity can affect growth the most [29].

CONCLUSIONS

The responses observed in huauzontle in germination and initial growth depend on the NaCl concentration and the cultivar. The germination percentage decreased only with the 300 mM NaCl dose with respect to the control. Seedling height increased by 75 and 150 mM NaCl in the Mexico City and Tlaxcala cultivars; on the contrary, Puebla shows significant inhibition in seedling growth starting at 225 mM NaCl. The Mexico City cultivar had the highest fresh and dry biomass weights, followed by Puebla and Tlaxcala.

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