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Study on fruit and seed morphology and morphometry, seed phytochemicals, and germination characterization in three populations of *Echinocereus stramineus* (Cactaceae) at Ciudad Juárez Municipality, Chihuahua, Mexico

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Abstract. In this study, the fruit and seed morphological and morphometric parameters, the seed phytochemical content, and the germination process were analyzed for three populations of *Echinocereus stramineus* located at Juárez Municipality, Chihuahua, Mexico. The morphometric variables were measured for each fruit and seeds obtained from each one. The seeds were subjected to three times of immersion in H_2SO_4 as scarification treatment, allowing the seed development for 21 days for the characterization of the germination process. The germination percentage, mean germination time, germination speed, and mean germination speed were calculated. The seedling morphometry was also measured for all treatments. The concentration of reducing sugars, total phenols, tannins, flavonoids, and antioxidant activity (DPPH and FRAP) were determined for seeds under basal conditions. The fruits from the three populations showed significant differences in length and width, with the Sierra of Ciudad Juárez (SCJ) population having the longest and widest fruits. The SCJ seeds had the highest mass values, while those from the Sierra of Presidio (SP) and SCP populations showed the highest length, length/width ratio, area, and perimeter. Immersion in H_2SO_4 improved the germination percentage in SCJ and SP seeds. This treatment also caused a gradual decrease in the mean germination time and a progressive increase in the mean germination rate and germination speed index across seeds from all three populations as the immersion time increased. Similarly, the seedling stem length and area in SP seeds increased with immersion time. The seeds from the SP population had the highest reducing sugar content, while those from the Sierra of Samalayuca (SS) population showed the highest levels of flavonoids, phenolics, tannins, and antioxidant activity.

Keywords: *Echinocereus, variations, seeds, scarification, germination, seedling, phytochemicals*

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Introduction

In the Desert Chihuahuan, the semiarid and arid climatic regions modulate structural features such as stem size and plant shape and the presence and quantity of the ribs, trichomes, and spines of Cactaceae family plants. In this context, the temperature, the light, and the lack of water affect sexual reproduction in the Cactaceae plants (Bauk *et al*., 2017). However, other factors, including seed maturity and genotype, seed size and weight, and seed age, also influence germination success, seedling development, and survival (Ruíz-Pérez *et al.*, 2021).

Besides, some seeds of the Cactaceae family present dormancy and germinate after an extended period. Some studies *in vitro* conditions have proposed different vias to increase germination rates and percentages such as the chemical scarification using the application of sulfuric acid (Monroy-Vázquez *et al*., 2017; García-González *et al*., 2022), which is a corrosive compound, being a method that simulates the passage of seeds through the digestive tract of animals, increasing the permeability of the coating and accelerating the germination percentages (Monroy-Vázquez *et al.*, 2017; Almeida-Bezerra *et al*., 2020, García-González *et al.*, 2022). At present, the Cactaceae family is also suffering the loss of its natural habitats and its diversity due to anthropogenic pressure, predation of nurse plants, and plant predation, leading to problems of sexual reproduction and lowering its production and establishment levels of seedlings (Pillet *et al*., 2022; Hultine *et al*., 2023).

One of the species of cacti that could suffer from this problem is *Echinocereus stramineus*. This species is distributed in the arid and semi-arid zones of the southern states of New Mexico and Texas in the United States of North America and the northern states of Chihuahua, Coahuila, Tamaulipas, Nuevo León, Durango, Zacatecas, and San Luis Potosí in Mexico (Miller, 1988). The plants of *E. stramineus* are in a group of mono-articulated cacti that show small stems of globose to cylindrical shape with a variation of ten to seventeen slightly tuberculate green ribs that form hemispherical conglomerates of more than 1 m wide and with up to ten stems. Each plant contains one to four stout central spines, straight or curved, straw yellow to whitish, four to nine cm long, with seven to fourteen pinkish to yellowish radial spines of three cm long. The flowers show a diameter of 6 to 12 cm of bright magenta color with a funnel shape (Taylor, 1988).

The north of Chihuahua State, Mexico in the Municipality of Ciudad Juárez has three mountain areas known as Sierra of Ciudad Juárez, Sierra of Samalayuca, and Sierra Presidio, where the cactus *E. stramineus* develops. In these areas, this cactus is ornamentally used due to the shape and beauty of its flowers, and its fruits (pulp and seeds) are consumed by the local population in the production season from June to September and fruits are commonly consumed fresh, but they are also used to make fresh water and jam (Manzano, 2014; González-Fernández *et al*., 2024). Moreover, the stems of this species are used to disintegrate or expel the thorns accidentally stuck in domesticated animals (González-Fernández *et al*., 2024). In the last 7 years, the distribution area of *E. stramineus* in these three mountain ranges has been strongly impacted by land use change, soil loss, and more severe droughts (González-Fernández *et al*., 2024).

Therefore, the need arises to increase knowledge of their ecology and reproduction systems. Currently, the generation of information on morphology, morphometry, and germination of seeds and the morphometry and early growth of seedlings is of great interest to better understand the structure and dynamics of the populations and communities of plants that grow in semi-arid and arid areas of our country (González-Cortés *et al*., 2019). This study focused on three main objectives to characterize three populations of *E. stramineus* at the Municipality of Ciudad Juárez, Chihuahua, Mexico. The first was the variation of morphometric characteristics in fruit and seeds among the different populations; the second was the effect of chemical scarification (H_2SO_4 at three-time immersion) on seed germination and seedling development; and the last was the variation in seed phytochemical composition and antioxidant capacity across the populations.

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Material and Methods

Sampling areas

The samples were collected in three locations at the Municipality of Ciudad Juárez, Chihuahua, Mexico, Sierra of Ciudad Juárez (SCJ) (Latitude 31° 41' 56.2" N, longitude 106° 30' 22.7" W, and altitude of 1350 m.a.s.l.), Sierra of Samalayuca (SS) (latitude 31° 18' 04.0" N, longitude 106° 30' 04.5" W, and altitude of 1340 m.a.s.l.), and Sierra of Presidio (SP) (latitude 31º 22' 50'' N; longitude 106º 24' 02'' W, and altitude of 1317 m.a.s.l.). The fruit sampling was conducted according to the methodology proposed by González-Fernández *et al*. (2024). A total of ten plants with fruits and ten different fruits were randomly selected for each population. After harvesting fruits, the thorns were carefully removed with the help of tweezers to avoid damaging the peel. The fruits were placed in hermetically sealed plastic bags and immediately transported to the laboratory (Figure 1).

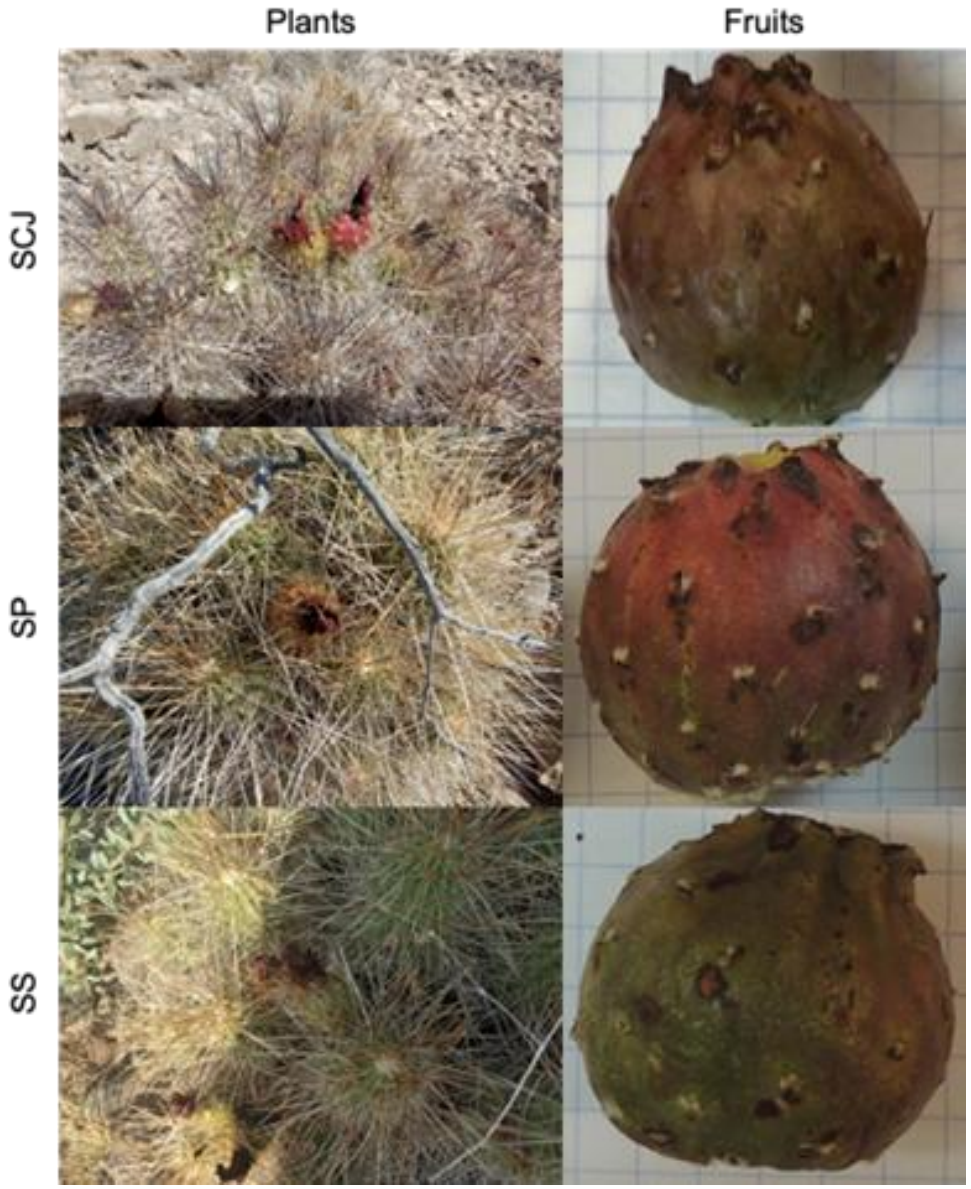

Figure 1. The plants and fruits from the three populations of *E. stramineus* analyzed in this study. Sierra of Ciudad Juárez (SCJ), Sierra of Samalayuca (SS), and Sierra of Presidio (SP).

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Morphological and morphometric analysis of fruits and seeds

In the laboratory, the ten fruits from each population were placed in a beaker to wash with 50 mL of soapy water for 10 min in an orbital shaker (Labnet®, USA), rinsed twice with distilled water under the same conditions as the previous step, and dried on blotting paper at 27 °C for 1 h (González-Fernández *et al*., 2024). Later, each of the ten fruits from each population was weighed individually using an analytical balance (Velab®, USA). Following, a digital photograph (Nikon®, Japan) of all ten fruits by population was taken. Subsequently, fruits were cut lengthwise, and the peel separated from the pulp and seeds. The peel and the pulp plus seeds from each fruit were both weighed individually. Afterward, seeds were separated from the pulp, breaking up manually in a beaker with 100 mL of distilled water, then placed in a sieve and washed with distilled water until the plant and pulp remains were removed. Finally, seeds were put on blotting paper and left to dry at 27 °C for 24 h. Once dried, 50 seeds were placed on graph paper to take a digital photograph. The area, perimeter, length, and width of the fruits and seeds were measured using the ImageJ program (National Institute of Health, USA), according to González-Fernández *et al*. (2024).

Five seeds of each fruit for each population were randomly selected (n= 50 seeds per population) and were individually observed using a stereoscope (VanGuard®, 1275ZP, China) at a magnification of 4X for evaluating the shape, smooth interlacing, type of edge, and color of the seed. In addition, five seeds from the SCJ population were randomly selected to characterize the testa in a Scanning Electron Microscope (SEM) (Hitachi® SU50000 Schottky, Japan). The micrographs were taken at a depth of field of 1 mm up to 500 µm, under working conditions under a vacuum of 30 Pa, using electrons scattered to the ultra-variable-pressure detector (UVD).

Seed germination analysis

The seeds of *E. stramineus* underwent chemical scarification through immersion in H_2SO_4 to enhance the germination process. Before germination, the seeds were disinfected using a 70% (v/v) sodium hypochlorite solution for 3 min and then rinsed with plenty of distilled water for 1 min. Three different immersion times were tested, during which 30 seeds were soaked in 5 mL of H_2SO_4 concentrate for 1, 3, and 5 min in each population (three replicates per population and time of immersion). After the scarification method application, seeds were rinsed with plenty of distilled water. Subsequently, seeds were placed in groups of 10 in sterilized Petri dishes, to which 20 g of pre-sterilized sandy-type soil followed by 13 mL of distilled water were added (Reyes-Corral *et al*., 2022). Finally, plates were placed in a bioclimatic chamber at 25 °C, applying a photo period of 12 h light/darkness. The germination progress of each seed was examined every three days for 21 days (about 3 weeks), counting the number of germinated seeds. Next, the germination percentage (GP), mean germination time (MGT), mean germination rate (MGR), and germination speed index (GRI) were calculated, according to Souza *et al*. (2016). Finally, five seedlings per population were placed on graph paper to take a digital photograph. The digital image was used to measure the length, width, and area of stems and the root length using the ImageJ program (National Institute of Health, USA), according to González-Fernández *et al*. (2024).

Seed phytochemicals and antioxidant activity quantification

A standard extract was obtained according to the method proposed by Reyes-Corral *et al*. (2022) and Álvarez-Parrilla *et al*. (2011). From each population, three replicates of a pool of 30 mg of seeds for each population were manually ground in a mortar using a pestle until a fine powder. Then, 500 µL of

80% (v/v) methanol (JT Baker®, USA) solution was added and blended with the help of the same pestle. Next, the homogenate was transferred to a tube, stirred at 500 rpm for 10 min in darkness, and sonicated for 30 min at 4 °C in darkness. Afterward, the extract was centrifuged (Eppendorf®, USA) at 3,500 rpm for 15 min at 4 °C, and the supernatant was collected into a new tube. This methodology was repeated twice, and the two supernatants were mixed and brought to a final volume of 2 mL. The samples were stored at -20 °C until further analysis.

The reducing sugar content was measured by the spectrophotometric approach described by Ávila Núñez *et al*. (2012). Briefly, 100 µL of each standard extract was mixed individually with 300 µL of the DNS (3,5-dinitrosalicylic acid) reagent in an assay tube. Then, samples were incubated at 95 °C in a dry bath for 5 min, and immediately later, the mixture was cooled in an ice bath for 5 min. Next, 250 µL of each sample was taken and individually placed in a 96-well microplate, and the absorbance was measured at 540 nm. The calibration curve was performed using glucose as the standard, and results were expressed as mg of glucose equivalents (GE) per g of seed dry weight (DW) (mg GE·g^{-1} DW).

The total phenolics compounds were determined by a spectrophotometric method, according to Georgé *et al*. (2005). Briefly, 25 µL of standard extract, 125 µL of the Folin-Ciocalteu reagent (10% v/v), and 100 µL of Na_2CO_3 were placed into a well of 96-well microplate, and the mixture was incubated at 25 ºC for 15 min in darkness. Finally, the absorbance was measured at 740 nm. The calibration curve was performed using gallic acid as standard, and data were expressed as mg gallic acid equivalents (GAE) per g of seed (mg GAE·g^{-1} DW).

The total flavonoids were determined by a spectrophotometric method defined by Georgé *et al*. (2005). Briefly, 62.5 µL of standard extract, 46.5 µL of $NaNO_2$ (5%), 46.5 µL of $AlCl_3$ (10%), and 62.5 µL of 0.5 M NaOH were placed into a well of 96-well microplate, and the mixture was incubated for 30 min at 25 ºC in darkness. Finally, the absorbance was measured at 510 nm. The calibration curve was performed using catechin reactive as standard, and data were expressed as mg catechin equivalents (CE) per g of seed (mg CE·g^{-1} DW) (Guillén-Enríquez *et al.,* 2022).

The total condensed tannins were determined by the colorimetric method using the 4-(dimethylamino) cinnamaldehyde (DMAC) assay (Reyes-Corral *et al*., 2022). In brief, 50 µL of each standard extract was mixed individually with 200 µL of 0.1% DMAC reactive in a 96-well microplate, and the reaction was allowed to proceed for 5 min at 25 ºC in darkness. The absorbance was measured at 640 nm. The calibration curve was performed using catechin reactive as the standard, and data were presented as mg catechin equivalents (CE) per g of seed (mg CE·g^{-1} DW).

The antioxidant activity was analyzed using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) method, according to the methodology proposed by Moreno-Escamilla *et al*. (2017). In a well of a 96-well microplate, 25 µL of the standard extract and 200 µL of DPPH reagent (190 mM DPPH (Sigma-Aldrich®, México) in 100% (v/v) methanol) were added. Then, the mixture was incubated for 30 min at 26 ºC in darkness, and absorbance was read at 517 nm. The antioxidant activity was also analyzed using the ferric-reducing antioxidant power (FRAP) method, according to the methodology proposed by Moreno-Escamilla *et al*. (2017). In a well of a 96-well microplate, 24 µL of the standard extract and 180 µL of FRAP reagent (10 mM 2,4,6-tri[pyridil]-s-triazine (TPTZ) (Thermo-Fisher®, México); 300 mM $C_2H_3O_2Na$ (Thermo Fisher®, México); 20 mM $FeCl_3$ (Thermo-Fisher®, México)) were mixed. Then, the

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mixture was incubated for 30 min at 37 °C, and the absorbance was read at 595 nm. For both methods, the calibration curve was performed using TROLOX (Sigma-Aldrich®, México) as standard, and results were expressed as µM of TROLOX equivalents per g^{-1} of dry weight (mg TE·g^{-1} DW).

All absorbances were measured in a BioRad xMarkTM Plus Microplate Absorbance Spectrophotometer (Hercules®, USA), and data were acquired using the Microplate Manager 6.0 (Tokyo, Japan) computer software. All quantifications were carried out in triplicates (analytical replicates) per population.

Extraction and quantification of seed proteins

The proteins were extracted from 0.03 g of a pool of seeds for each population by the TCA/acetone-phenol method (Valero-Galvan *et al*., 2014). The final pellet of proteins was solubilized in 50 µL of a solution of 7 M urea (Jalmek-Scientific®, México). The insoluble material was removed by centrifugation. The proteins were quantified using the Bradford method, with BSA as the standard (Merck®, México) (Ramagli and Rodriguez, 1985). All seed protein extractions were carried out in triplicates per population.

Statistical analysis

A Shapiro-Wilk normality test was conducted to verify the normality of the data. Once normality was confirmed, the fruits and seeds morphometric, seed phytochemical, seed germination, and seedling morphometric data were analyzed using a one-way ANOVA. The Tukey means test was performed to determine significant differences between the groups when the analyzed variables showed statistical differences, using a 95% confidence level. In addition, the morphometric data of fruits and seeds and phytochemical seeds were subjected to canonical discriminant analysis (CDA). All data were analyzed using the IBM SPSS® Statistics Base 22.0 software.

Results and Discussion

Fruit and seed morphological and morphometrical analysis

The fruits and seeds of *E. stramineus* from the three populations showed similar morphology. Fruits were fleshy, globe-style, and red, with a thin pericarp and numerous spiny areoles that become deciduous when ripening, leaving a smooth surface on the fruit (Figures 2a, b, and c). Seeds had a small size and a black and shiny appearance in the stereoscopic image (Figures 2d, e, and f).

At the microscopic, the seed shape was reniform (Figure 3a) and show a broad basal hilum (Figure 3 b). The testa was reticulated or tuberculated (Figure 3c and d), with the cells near the hilum becoming less pronounced (Figure 3a and b).

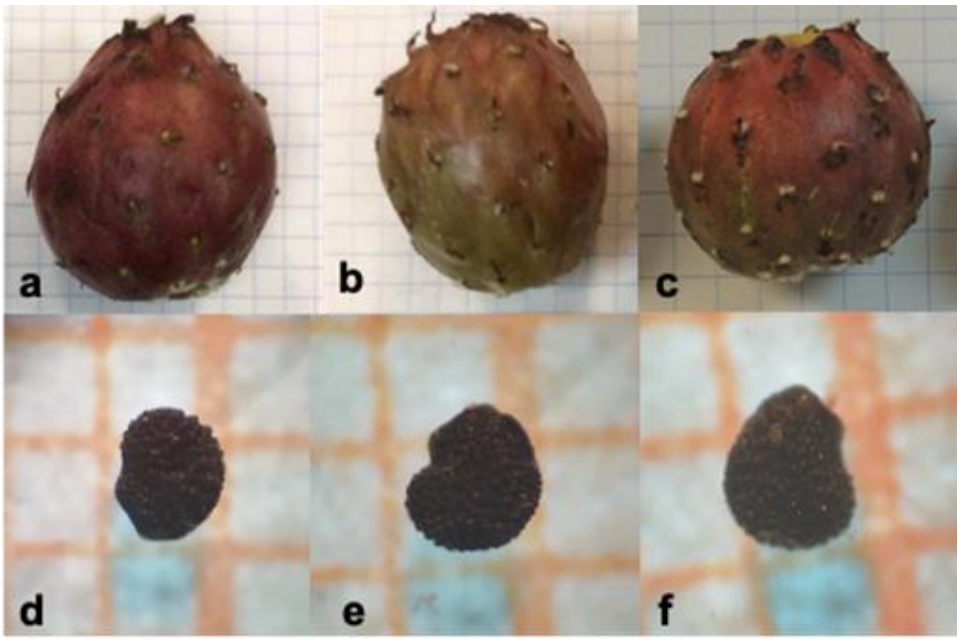

Figure 2. The fruits and seeds from the three *E. stramineus* populations in the Ciudad Juárez Municipality. a) and d) Sierra of Ciudad Juárez (SCJ); b) and e) Sierra of Samalayuca (SS); and c) and f) Sierra of Presidio (SP).

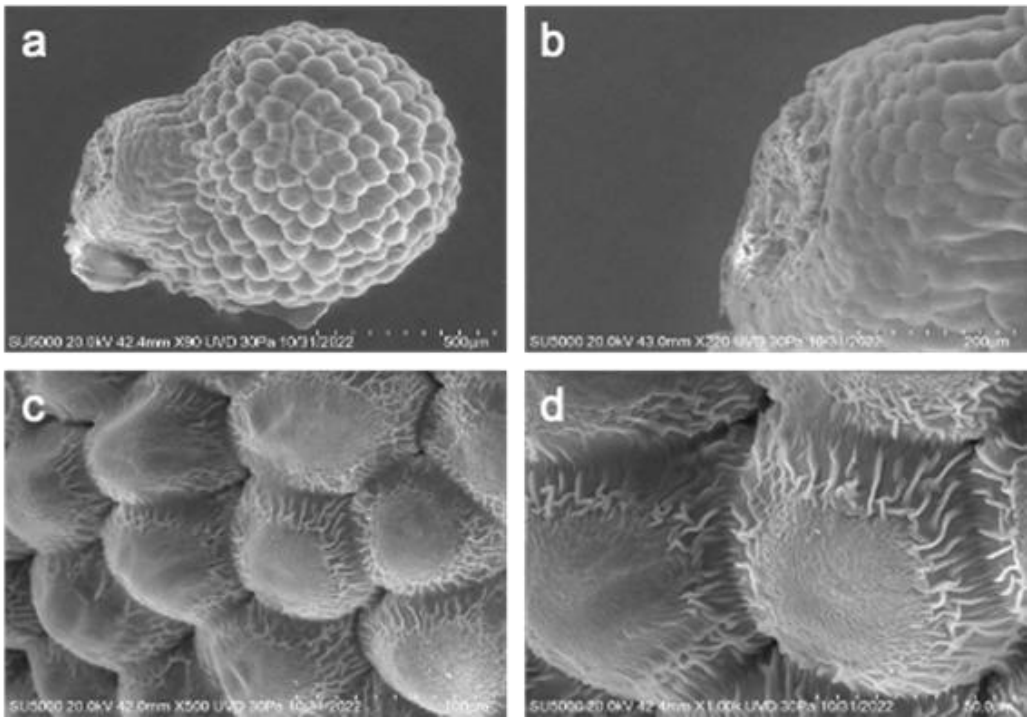

Figure 3. The SEM micrographs of *E. stramineus* seeds from the SCJ population. a) Complete seed view to 90x. b) Apical region of hilum seed view to 220x. c) Testa cell view to 500x and d) 1000x. Concerning fruit morphometric parameters, only the length and width showed significant differences ($p \leq 0.05$) (Table 1). The plants from the SCJ population had the longest and widest fruits, while those from the SP population presented the smallest ones (Table 1).

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Regarding seed morphometric parameters, values of the weight, length, length/width ratio, area, and perimeter showed significant differences ($p \leq 0.05$) (Table 1). Fruits collected from plants at the SCJ population had the highest values of seed mass, those from the SS population showed the most elevated values of seed length, and those from the SP population exhibited the most increased values of seed length/width ratio, area, and perimeter (Table 1).

Table 1. The morphometric variables of fruits and seeds from the three *E. stramineus* populations in the Ciudad Juarez Municipality, Chihuahua, México.

	Populations		
	SCJ	**SS**	**SP**
Fruit variables			
Total weight (g)	18.28±4.18^{a*}	19.70±2.45^{a}	17.54±4.23^{a}
Pulp plus seed weight (g)	12.54±3.50^{a}	12.81±1.56^{a}	11.86±4.40^{a}
Peel weight (g)	05.74±1.70^{a}	06.89±1.74^{a}	05.68±0.68^{a}
PSPTW† (%)	68.14±8.77^{a}	65.32±6.21^{a}	65.91±9.15^{a}
PPTW‡ (%)	31.86±8.77^{a}	34.69±6.21^{a}	34.09±9.15^{a}
Length (cm)	03.74±0.49^{c}	03.28±0.25^{b}	02.87±0.18^{a}
Width (cm)	03.57±0.33^{b}	03.16±0.21^{a}	03.03±0.23^{a}
Length/width ratio	01.05±0.09^{a}	01.05±0.12^{a}	00.96±0.09^{a}
Area (cm^2)	10.77±2.02^{a}	12.13±1.11^{a}	11.38±1.25^{a}
Perimeter (cm)	11.89±1.14^{a}	12.58±0.57^{a}	12.28±0.65^{a}
Seed variables			
Total weigh (mg)	0.30±0.00^{b}	0.20±0.00^{a}	0.20±0.00^{a}
Length (mm)	1.27±0.05^{b}	1.20±0.07^{a}	1.31±0.09^{b}
Width (mm)	0.94±0.05^{a}	0.96±0.06^{a}	0.91±0.07^{a}
Length/width ratio	1.36±0.09^{b}	1.25±0.07^{a}	1.44±0.11^{b}
Area (mm^2)	0.99±0.10^{ab}	0.92±0.12^{a}	1.04±0.12^{b}
Perimeter (mm)	3.74±0.22^{ab}	3.59±0.24^{a}	3.83±0.22^{b}

The data are expressed as the mean ± standard deviation (n= 10 for fruits and n= 50 for seeds) and were analyzed using a one-way ANOVA ($p \leq 0.05$). *Values in rows with different letters differ significantly (Tukey, $p \leq 0.05$). †PSPTW: the pulp and seed proportion with respect to the total weight of the fruit; ‡PPTW: the peel proportion with respect to the total weight of the fruit.

The canonical discriminant analysis (CDA) performed on fruit and seed morphometric data resulted in two functions. The function 1 explicated 92.5% of the total variance, and this function had a high loading for the seed weight (0.362), seed length (0.263), and fruit width (0.229) with a positive association. The function 2 accounted for 7.5% of the total variance, and this function had a high loading for the seed length/width ratio (-0.648) and seed length (-0.547) with a negative association. The scatterplot of the two functions separated individuals from each population, where the SS and SP populations were closely related; in contrast, individuals from SCJ were clustered separately (Figure 4).

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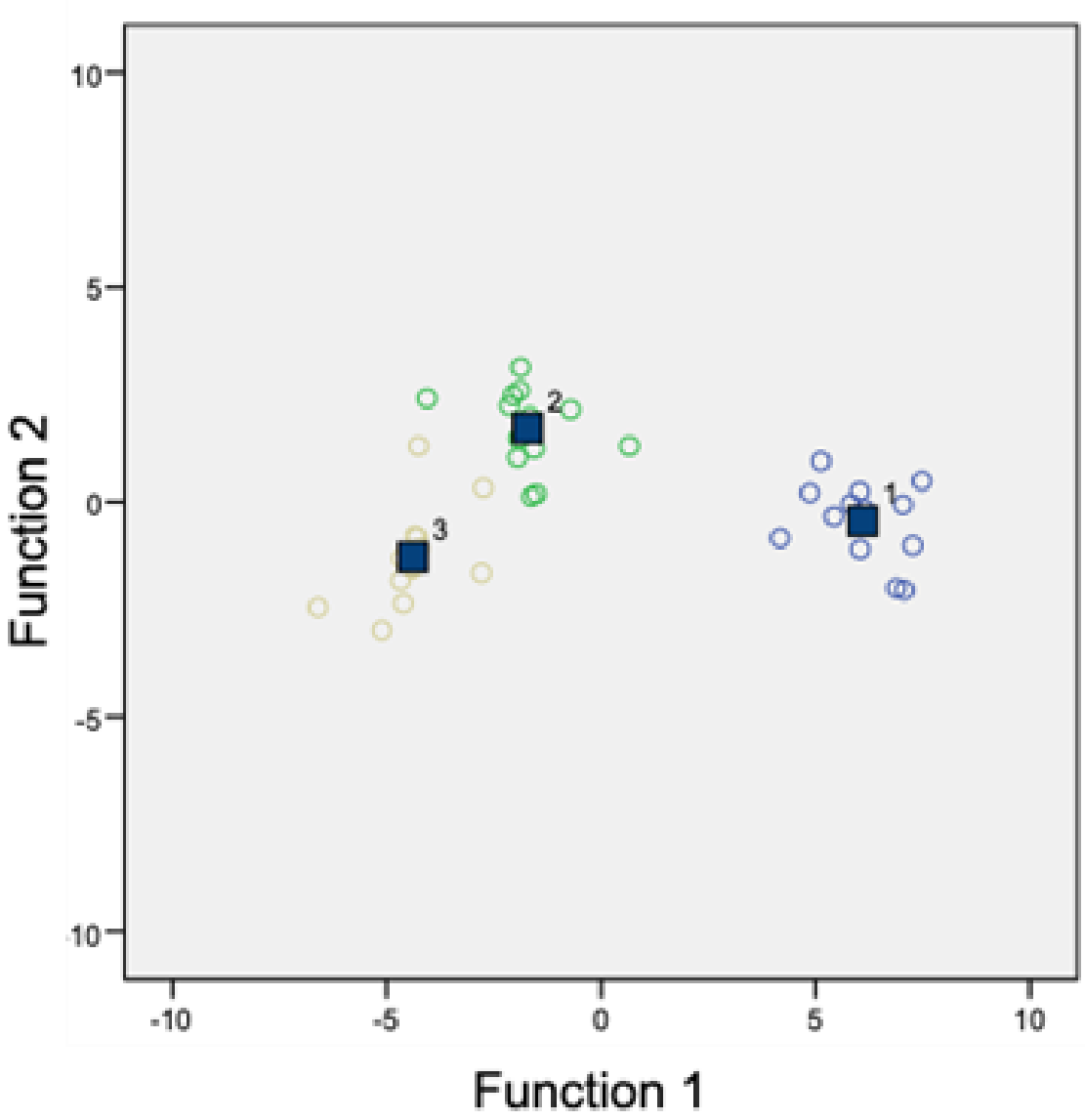

Figure 4. The scatterplot showing the CDA performed on fruit and seed morphometric characteristics from the three *E. stramineus* populations in the Ciudad Juarez Municipality, Chihuahua, México. (1) SCJ population; (2) SS population; and (3) SP population.

The results of the present study for the total weight of the fruit, pulp plus seeds, and peel (Table 1) suggest that fruits were at a ripening stage between EII and EIII, based on previously reported data for *E. stramineus* fruits, which showed weights of 9.9 to 29.0 g for total weight, 6.6 to 21.2 g for pulp plus seeds, and 3.3 to 6.6 g for peel in these two stages (González-Fernández *et al*., 2024). The proportion of pulp and seeds relative to the total fruit weight (PSPTW) calculated in this study was between 65.3 and 68.1% (Table 1), being lower than the previously reported 73.1% for *E. stramineus* fruits at the EII ripening stage; meanwhile, the peel proportion relative to the total fruit weight (PPTW) ranged from 31.8 to 34.6**%** (Table 1)**,** which was similar to the previous value of 33.3% for the same ripening stage (González-Fernández *et al*., 2024). Regarding fruit dimensions, their length was between 2.87 and 3.74 cm (Table 1), placing fruits at an intermediate point between the EIII and EIV

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stages, which ranged from 2.9 to 3.7 cm. The width varied from 3.03 to 3.57 cm (Table 1), aligning with the measures from the EI and EII stages, which ranged from 2.7 to 3.3 cm. However, the length/width ratio (0.96 to 1.05) (Table 1) was lower than the ranges observed for all four ripening stages (1.21 to 1.39) (González-Fernández *et al*., 2024). Additionally, the area (10.77 to 12.13 cm²) and perimeter (11.89 to 12.58 cm) (Table 1) observed in this study were higher than the values reported for all four ripening stages (González-Fernández *et al*., 2024). Concerning the findings on seed parameters reported in this study, the weight was lower than those found in previous studies on seeds from fruits during the four ripening stages of *E. stramineus*; nonetheless, the length, width, area, and perimeter were higher, although the length/width ratio was comparable to those observed in seeds during the four fruit ripening stages (González-Fernández *et al*., 2024).

Seed germination

The seeds of *E. stramineus* underwent to a chemical scarification process, which consisted of immersing them in H_2SO_4 for varying durations to enhance germination. In most cases, the acid scarification improved the germination process. For seeds from the SCJ population, the germination percentage (GP) increased by 20% (from 80% in the control group to 95% in seeds submerged for 5 min in acid); similarly, for those from the SP population, the GP improved by 15% (from 78% in the control group to 93% in seeds submerged for 5 min in acid) (Figure 5A). Concerning other germination indexes, the mean germination time (MGT) decreased (Figure 5B) and the MGR and GRI gradually increased (Figures 5C and 5D) as immersion time in H_2SO_4 increased for the seeds from the three populations.

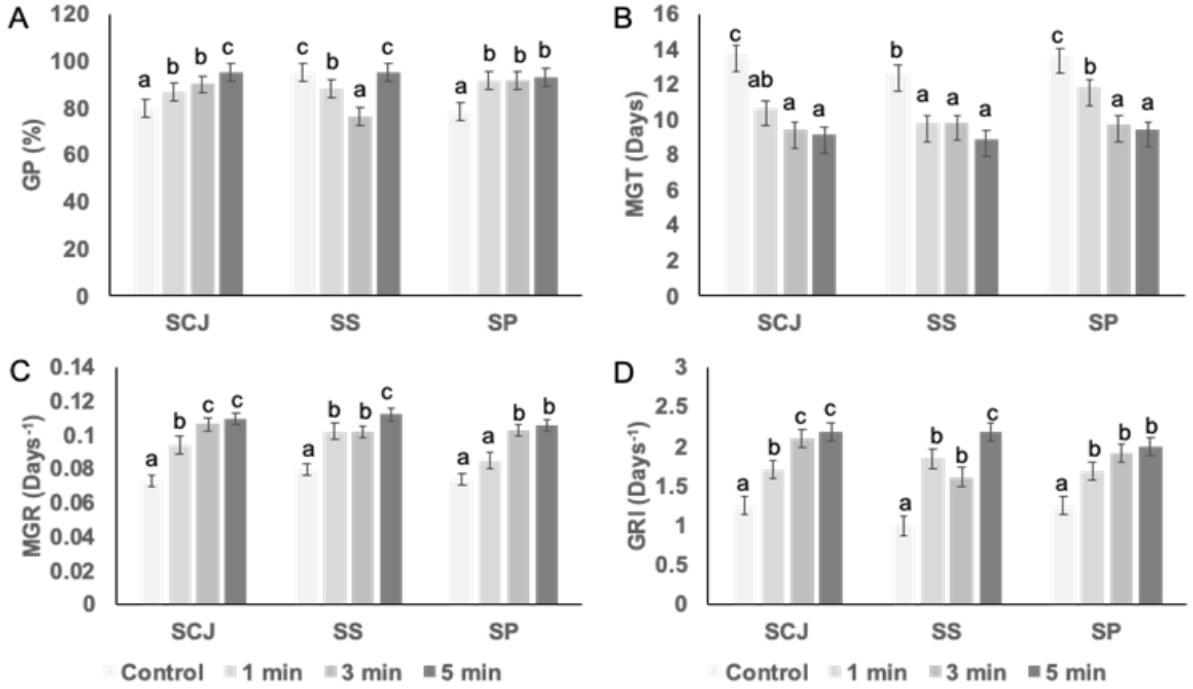

Figure 5. Effect of immersion time in H_2SO_4 on seed germination from the three *E. stramineus* populations in the Ciudad Juarez Municipality, Chihuahua, México. Germination percentage (GP) (A), mean germination time (MGT) (B), mean germination rate (MGR) (C), and germination speed index (GRI) (D). Data are expressed as the mean ± standard deviation and were analyzed using a one-way ANOVA ($p \leq 0.05$). Values in rows with different letters differ significantly (Tukey, $p \leq 0.05$).

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Furthermore, the stem length, width, area, and the root length were measured to evaluate the effect of chemical scarification on seedling morphometry. In general, results showed no differences and marked variability among individuals within the same group (Figure 6). Significant differences were found for the stem length and area of seedlings from the SP population, increasing as the seed immersion time in H_2SO_4 extended (Figures 6A and 6D).

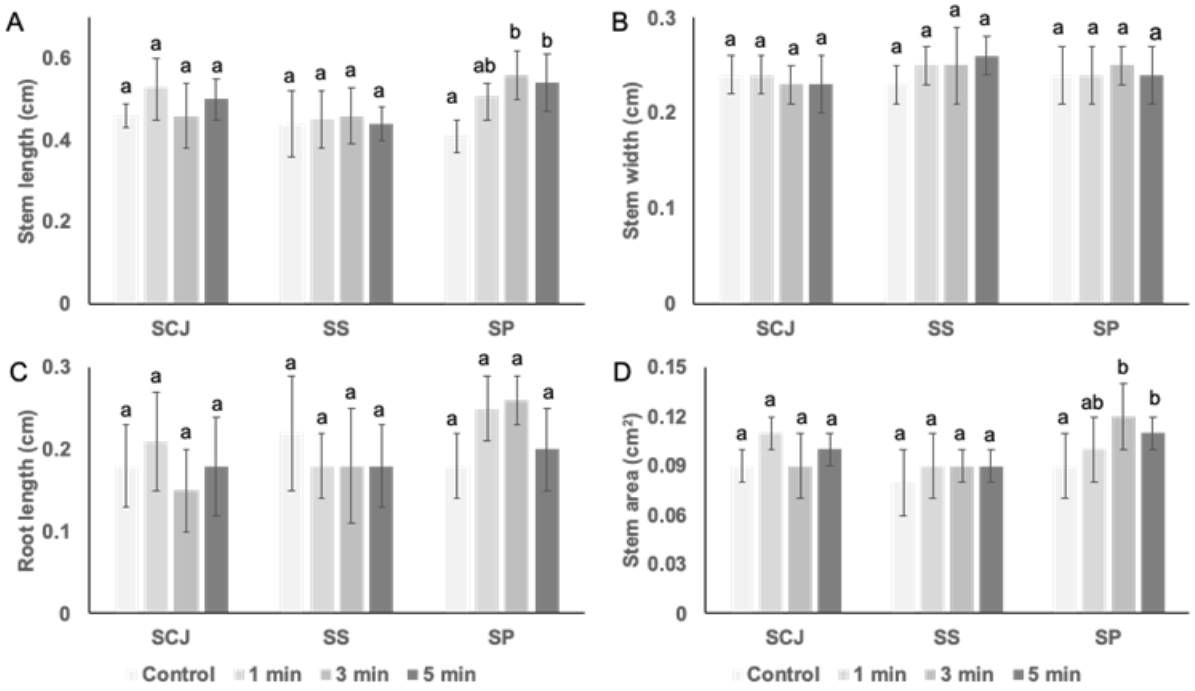

Figure 6. The effect of immersion time in H_2SO_4 on seedling morphometry from the three *E. stramineus populations* in the Ciudad Juarez Municipality, Chihuahua, México. Effect on the stem length (A), stem width (B), root length (C), and stem area (D). The data are expressed as the mean ± standard deviation and were analyzed using a one-way ANOVA ($p \leq 0.05$). Values in rows with different letters differ significantly (Tukey, $p \leq 0.05$).

Several studies have shown that the seeds of some species belonging to the Cactaceae family have low germination percentages mainly because they present dormancy (Barrios *et al*., 2020). The seed dormancy for most plants could be an advantage of adaptation to natural environmental conditions (Penfield, 2017); however, for some cactus species, it is an obstacle to germination under *in vitro* conditions (Monroy-Vázquez *et al*., 2017; Aragón-Gastélum *et al*., 2018; Barrios *et al*., 2020; Rojas-Aréchiga and García-Morales, 2022). The chemical scarification with acids is one of the strategies used to break seed dormancy periods in Cactaceae (Monroy-Vázquez *et al*., 2017; García-González *et al*., 2022). In the present study, the 5-minute immersion treatment in concentrated H_2SO_4 improved the germination percentage of seeds from SCJ and SS populations by 15 to 20 %, respectively. Although there are no comparative results on the use of H_2SO_4 scarification in *E. stramineus* seeds, in other cactus species, this scarification method significantly improved the germination percentage of *Stenocereus griseus* Buxbaum and *Escondia chiotilla* seeds (Martínez-Cárdenas *et al.*, 2006). Similarly, in plants from the *Acacias* genus, the germination percentage of seeds increased as the immersion times at H_2SO_4 expanded (Kheloufi *et al*., 2017). However, this acid scarification method

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had no improvement in germination of seeds from *Mammillaria magnimamma*, *Echinocereus pectinatus*, and *Astrophytum myriostigma* (Sánchez-Salas *et al*., 2006; Ruedas *et al*., 2000; Díaz-Baca *et al*., 2020). Similarly, the germination percentage of *Opuntia ficus-indica* seeds decreased as the immersion time in H_2SO_4 increased (Altare *et al*., 2006). The evaluation of germination capacity depends on the germination percentage and its speed and evolution over time. In the present study, the mean germination time (MGT) decreased, and the mean germination rate (MGR) and germination rate index (GRI) increased as the time of exposure to H_2SO_4 increased in the three populations evaluated. Similar findings showed a reduction in MGT and an increase in GRI in *Acacia cyanophylla* and *A. farnesiana* seeds when subjected to scarification at various immersion times and concentrations of H_2SO_4 (Kheloufi *et al*., 2017).

Phytochemicals and antioxidants activity of seeds

The phytochemical quantification significant differences ($p \leq 0.05$) appeared in the content of tannins, flavonoids, and reducing sugars (Table 2). The seeds had the highest levels of phenols, flavonoids, tannins, and reducing sugars in the SS population. In contrast, seeds collected at the SP location exhibited the lowest flavonoid content, while those from the SCJ population had the lowest tannin content (Table 2). The quantification of antioxidant activity determined by FRAP also revealed significant differences among the three populations (Table 2), with seeds from the SS population exhibiting the highest value and those from SCJ showing the lowest.

Table 2. Phytochemical content and antioxidant activity of seeds from the three *E. stramineus* populations in the Ciudad Juarez Municipality, Chihuahua, México

Variables	Populations		
	SCJ	SS	SP
Phenols (mg GAE·g^{-1})*	13.31±2.24^{a‡}	22.29±0.31^{c}	17.01±1.22^{b}
Tannins (mg CE·g^{-1})**	0.002±0.001^{a}	0.051±0.003^{c}	0.031±0.009^{b}
Flavonoids (mg CE·g^{-1})**	53.51±10.66^{a}	71.81±6.00^{b}	48.91±3.70^{a}
Reducing sugars (mg GE·g^{-1}) ***	26.05±0.60^{a}	111.23±2.32^{b}	27.48±2.98^{a}
Proteins (mg·g^{-1})	01.60±0.10^{a}	01.55±0.10^{a}	01.57±0.09^{a}
DPPH (mM TE·g^{-1})****	31.34±0.32^{a}	33.21±0.55^{a}	31.29±1.39^{a}
FRAP (mM TE·g^{-1})****	26.61±4.63^{a}	112.64±0.00^{c}	94.33±8.59^{b}

The data are expressed as the mean ± standard deviation (n= 5) and were analyzed using a one-way ANOVA ($p \leq 0.05$). ‡Values in rows with different letters differ significantly (Tukey, $p \leq 0.05$). *GAE: gallic acid equivalents, **CE: catechin equivalents, ***GE: glucose equivalents, and ****TE: Trolox equivalents.

The phenols have been widely studied and confirmed to possess diverse bioactivities that could benefit human health. The benefits of many of these conditions come in part through the antioxidant characteristics of phenols; therefore, it is essential to quantify, identify, and evaluate their antioxidant activities. Comparative seed analysis across three populations revealed significant phytochemical variations; while seed coat color influences phenolic content, genetic and environmental factors are primary determinants (Oomah *et al*., 2005; Dinelli *et al*., 2006; Espinosa-Alonso *et al.*, 2006; Chen *et al*., 2015). In this study, the SS population exhibited a distinct profile characterized by elevated levels of phenolic compounds (22.29 mg GAE·g^{-1}), tannins (0.05 mg CE·g^{-1}), and flavonoids (71.81 mg GAE·g^{-1}), suggesting enhanced oxidative stress protection potentially driven by genetic or environmental adaptations (Table 2). Consequently, the seeds of this population represent a promising source of antioxidant compounds, underscoring their significance in phytochemical, pharmacological,

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and nutritional research aimed at identifying novel natural sources of bioactive compounds. Furthermore, the presence and variation of phenolic, condensed tannins, and flavonoid compounds in cactus seeds is a well-documented phenomenon in the scientific literature (Núñez-Gastélum *et al*., 2018; Reyes-Corral *et al*., 2022; González-Fernández *et al*., 2024). The observed phenolic levels, ranging from 13.31 to 22.29 mg GAE·g^{-1}, surpass those of *E. stramineus, Opuntia* spp., and *Cylindropuntia* spp., leguminous, and grape seeds (Negro *et al*., 2003; Tounsi *et al*., 2011; Chahdoura *et al*., 2015; Grela *et al*., 2017; Amrane-Abider *et al*., 2018; Núñez-Gastélum *et al*., 2018; Reyes-Corral *et al*., 2022; González-Fernández *et al*., 2024), but are lower than *O. joconostle* and *O. matudae* seeds (Morales *et al*., 2012). Condensed tannin content (0.002-0.051 mg CE·g^{-1}) was lower than *E. stramineus* and *O. ficus-indica* seeds (Cardador-Martínez *et al*., 2011; Tounsi *et al*., 2011; González-Fernández *et al*., 2024). Conversely, flavonoid levels (48.91-71.81 mg GAE·g^{-1}) exceeded *E. stramineus* but were lower than *O. ficus-indica* and grape seeds (Negro *et al*., 2003; Cardador-Martínez *et al*., 2011; Tounsi *et al*., 2011 González-Fernández *et al*., 2024). These results underscore the complex interplay of genetic and environmental factors in shaping the phytochemical profiles of cactus seeds.

The seed carbohydrates play pivotal roles in plant stress responses, accumulating during cold acclimation, desiccation, and during the stages of seed maturation (Obendorf, 1997; Gilmour *et al*., 2000; González-Fernández *et al*., 2024). They also scavenge hydroxyl radicals, mitigating oxidative damage from environmental stressors (Tahir *et al*., 2011; Alves *et al*., 2024). During germination, carbohydrates are energy sources and substrates for seedling development (Peterbauer and Richter, 2001; Blochl *et al*., 2007). In this study, the SS population exhibited a significantly elevated reducing sugar content (111.2 mg GE·g^{-1}), suggesting enhanced stress tolerance (Table 2). Across all populations, reducing sugar levels ranged from 26.0 to 111.2 mg GE·g^{-1}, aligning with levels found in *Cylindropuntia* seeds (Reyes-Corral *et al*., 2022) but falling below those of *E. stramineus* seeds (González-Fernández *et al*., 2024). These findings indicate that stress-induced changes, including increased carbohydrate content, are associated with plant adaptation, potentially driven by genetic or environmental factors, and contribute to enhanced oxidative stress protection (Tahir *et al*., 2011).

The mature seed proteins serve both metabolic and structural functions, with storage proteins providing essential amino acids for germination and seedling growth (Rasheed *et al*., 2020; Yang *et al*., 2023). These storage proteins are critical determinants of seed protein content and quality for various applications. In this study, seed protein content showed no significant difference among the three populations, ranging from 1.55 to 1.60 mg·g^{-1} (Table 2). These levels were comparable to *E. stramineus* seeds (González-Fernández *et al*., 2024). However, the protein content was higher than *O. joconostle* and *O. matudae* (Morales *et al*., 2012) but lower than *C. spinosior, C. imbricata, O. phaeacantha, O. macrocentra, O. polyacantha,* and *O. engelmannii* (Núñez-Gastélum *et al.*, 2018; Reyes-Corral *et al.*, 2022). This result indicates a consistent protein storage strategy among these populations, with variations compared to other cactus species.

The comparative analysis of antioxidant activity in the three populations revealed consistent DPPH radical scavenging (31.29-33.21 mg TE·g^{-1}) yet significant variation in FRAP activity (26.61-112.64 mg TE·g^{-1}) (Table 2). The SS population, characterized by the highest concentrations of total phenols, tannins, and flavonoids, exhibited the most elevated FRAP activity, indicating a strong correlation between these phytochemicals and ferric ion reduction capacity. Conversely, the SCJ population, with

lower tannin levels, displayed the lowest FRAP activity. These results align with FRAP's established sensitivity to reducing agents like phenolic hydroxyl groups (Benzie and Strain, 1996; Rice-Evans *et al*., 1996). The consistent DPPH results, which surpass those values found in *E. stramineus, Cylindropuntia* spp., *Opuntia* spp., and leguminous seeds (Grela *et al*., 2017; Núñez-Gastélum *et al*., 2018; Reyes-Corral *et al*., 2022; González-Fernández *et al.*, 2024), but are lower than those determined in grape seeds (Poudel *et al*., 2008), likely stem from its distinct mechanism of electron or hydrogen donation. In contrast, the FRAP values were comparable to *E. stramineus* and *Cylindropuntia* species (Reyes-Corral *et al*., 2022; González-Fernández *et al*., 2024). This difference highlights the necessity of employing multiple antioxidant assays due to their varying chemical mechanisms (Sanchez-Moreno, 2002; Prior *et al*., 2005). The greater sensitivity of FRAP to specific phytochemicals, as supported by assay chemistry and comparative plant studies (Huang *et al*., 2005), suggests it is a more sensitive indicator of antioxidant activity related to these compounds in cactus seeds. Also, the variation of the antioxidant activity amongst seed plant species depends on the degree of maturity, the environmental conditions, and the season (Brahmi *et al.*, 2022). Moreover, differences in the reported values are affected by the extraction solvents and methodologies applied (Brahmi *et al*., 2024).

The canonical discriminant analysis (CDA) performed on phytochemical content and antioxidant activity data resulted in two functions. Function 1 explicated 99.1% of the total variance, and this function had a high loading for the protein content (-0.636) with a negative association and tannins content (0.102) with a positive association. The function 2 accounted for 0.1% of the total variance, having this function with a high loading for reducing sugar (-0.660), and antioxidant activity determined by DPPH (-0.185) with a negative association, and for flavonoids (0.704), antioxidant activity determined by FRAP (0.539), and phenols (0.095) with a positive association. Considering these data, the SCJ and SP populations were closely related, and the SS population was clustered separately (Figure 7).

When comparing the two CDA, the SS and SP populations showed closer relationships concerning the fruit and seed morphological and morphometric characteristics, separating the SCJ population from them. However, considering seed phytochemicals and antioxidant activity, the SCJ and SS populations showed more similar levels than the SP population.

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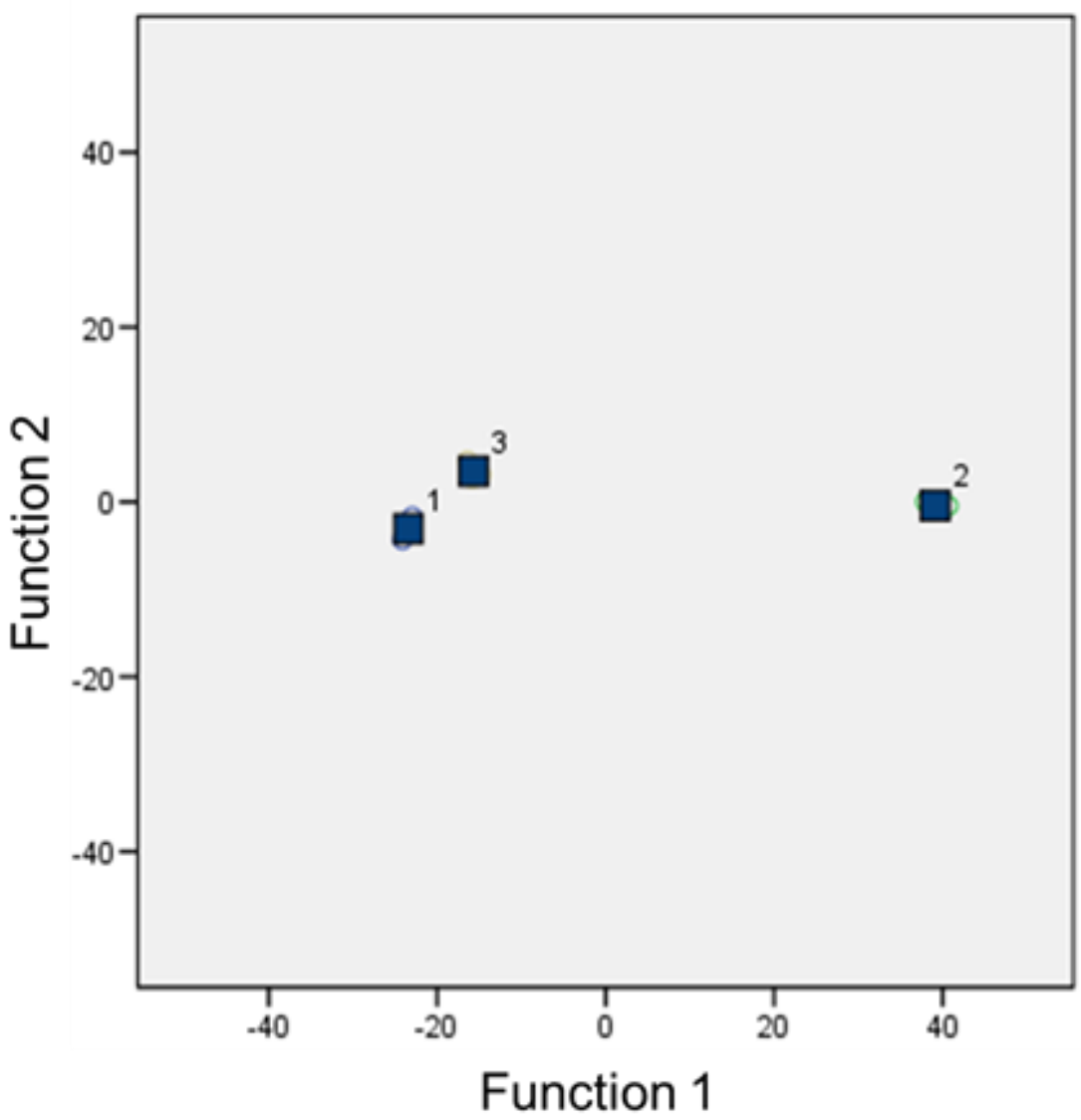

Figure 7. The scatterplot showing the CDA performed on the phytochemical content and antioxidant activity of seeds from the three *E. stramineus* populations in the Ciudad Juarez Municipality, Chihuahua, México. (1) SCJ population; (2) SS population; and (3) SP population.

Conclusions

This study revealed significant interpopulation variability in fruit and seed morphology, phytochemical composition, antioxidant activity, and germination characteristics. The morphological and morphometric analyses demonstrated that the SCJ population exhibited the largest fruits and seeds. However, the phytochemical analysis revealed that the SS population possessed the highest levels of phenols, condensed tannins, flavonoids, and reducing sugar, correlating strongly with increased ferric reducing antioxidant power (FRAP). The variation in FRAP suggested differential contributions of phenolic compounds to antioxidant capacity. Notably, acid scarification improved germination percentages in the SCJ and SP populations, with increasing acid exposure time, reducing mean germination time and enhancing germination rates and seedling growth, particularly in the SP population. These multifaceted findings underscore the potential of *E. stramineus* as a source of

diverse bioactive compounds and highlight the influence of population-specific morphological and physiological traits on seed germination and antioxidant potential, warranting further research into their nutraceutical, pharmacological, and ecological applications.

ETHICS STATEMENT

Not applicable.

CONSENT FOR PUBLICATION

Not applicable.

AVAILABILITY OF SUPPORTING DATA

Not applicable

COMPETING INTERESTS

The authors declare that they have no competing interests.

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AUTHOR CONTRIBUTIONS

Conceptualization: R.G-F. and J.V-G. Methodology: R.G-F., M.Q-M., P.O-A., and J.V-G. Validation: J.V-G. Formal analysis: J.V-G., R.G-F., P.O-A., and M.Q-M. Research: R.G-F., M.Q-M., P.O-A., and J.V-G. Resources: J.V- G., R.G-F., P.O-A., and M.Q-M. Visualization: J.V-G. Literature review: J.V-G. Supervision: R.G-F., J.V-G., P.O-A., and M.Q-M. Review of the final version and approval of the manuscript before sending it: R.G-F., M.Q-M., P.O-A., and J.V-G.

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