







NaCl effect on germination and seedling fresh weight of *Stenocereus eruca* (Brandege) A.C. Gibson & K.E. Horak

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ABSTRACT

Environmental disruption is particularly determinant for cacti development since they have slow growth and long life cycles. Soil and water salinity are two of the main environmental factors that are considered primordial for the propagation, seedling growth, establishment, and development of coastal cacti. The objective of this study was to evaluate chirimola (*Stenocereus eruca*) seed germination and seedling fresh weight using NaCl as a saline medium. One hundred seeds divided into five replicates of 20 seeds in a completely randomized design, were treated with five salinity levels and distilled water as control (0, 2, 6, 8, and 10 dS m⁻¹). Seeds were picked up from ripe fruits at a wild *Stenocereus eruca* population near San Carlos, Baja California Sur, México. Seedling fresh weight was significantly affected by saline stress, with lower fresh weight at the highest doses of 10 dS m⁻¹, and the greatest fresh weight production was obtained with 0 and 2 dS m⁻¹. However, germination percentage was greater at intermediate levels of 2 and 4 dS m⁻¹ and lowest at 10 dS m⁻¹ using NaCl; however, more studies should be conducted including other growth variables, seed viability, and salinity levels.

Keywords: *endemic species, salinity, endangered species.*

INTRODUCTION

Cacti generally are found in dry and arid desert or semi-desert regions with high average daytime temperatures and cold nights, and high evaporation rates. Cacti distribution range from Canada to Argentina, most of them develop in the warm and arid areas of the continents of both North and South America across a wide range of different habitats like deserts, sandy coastal stretches, scrublands, dry deciduous forests, high alpine desert, and tropical rain forests (Barthlott and Hunt,

1993; Gibson and Nobel, 1986; Nyffeler, 2002). The main diversity centers are Mexico and south-west USA, central Andes, Brazil, Paraguay, Uruguay, and Argentina with Mexico being the richest and most endemic region (Boyle and Anderson, 2002; Ortega-Baes and Godínez-Alvarez, 2006). The family is classified into three subfamilies, *Pereskioideae*, *Opuntioideae*, and *Cactoideae* (Barthlott and Hunt, 1993). The most apparent characteristic of cacti is the presence of areolas, considered as the growing part similar to the axillary buds of other dicotyledons. A main characteristic of buds or areolas is their ability to form reduced leaves, flowers, new stems, thorns, glands, bristles, hairs, and adventitious roots (Bravo-Hollis, 1978). The ethnobotanical importance of cacti has been described extensively in the literature, including their use as food, medicine, among others. In addition to their taxonomic and ecological importance (Godínez-Alvarez et al. 2003), many species (about 45) have been exploited since pre-Hispanic times (Casas, 2002; Luna-Morales, 2004).

Cacti play a very important role in Mexico from a biological and social context. There are about 715 species of cacti, 80 % of them are endemic and nearly all the others are shared with the Southern area of the United States or Central and South America. Many of their fruits and stems are important in Mexicans diet, although they are also used as fodder, ornamentally, or as a source of chemical substances for medical and pharmacological purposes; besides, there is clear evidence pointing out the outstanding ecological role of many cacti maintaining Mexican deserts (Benítez and Dávila, 2002). Additionally, it is also known that this group of plants has been the target of an intense illegal trade (plants and seeds) in the international market, especially in the United States, Japan, and several European countries. This illegal trade has been carried out for several years, and even now, there are signs that it is still practiced. This scenario takes place even though protection measures of wild environments in Mexico are more efficient than in the past. Given the diversity of species and life forms of cacti and the ease with which looters get seeds that can take out of the country covertly and without problems, there is much more that needs to be done in this regard. In Mexico, it is a priority that many of its species remain included in I CITES Appendices (Benítez and Dávila, 2002).

Stenocereus eruca (PNUMA-CMCM, 2011), which is distributed in the Magdalena region of Baja California Sur near the coastal areas (Fig. 1), has thick stalks that can reach up to 2 m long. Stems often form roots on the part where they touch the ground, they grow slowly forward and the oldest part at the end dies; they have unequal thorns grouped between 15 to 21 ribs and flattened yellowish-white spines. The flowers are large, tubular 10 a 12 cm in length, and are pinkish. They bloom around July and August. The fruit occurs in November and it is scarlet red three-spined stickleback and it has a size of a golf ball. *Stenocereus eruca* (chirinola) grows in alluvial sandy soils with low organic matter content (Rebman and Roberts, 2012).

This endemic species is considered an endangered organism according to the Mexican standard (NOM-059-SEMARNAT-2001). Illegal collection and habitat damage due to farming practices are the main dangers to its survival (Cancino et al., 1995). Reduced chirinola seedling recruitment could have a unique mechanism, which is a distinctive characteristic of clonal plants (Del Mandujano et al., 1996; Eriksson, 1997). The evidence on clonal diversity indicates that *S. eruca* and *S. gummosus* combine clonal growth and sexual recruitment as mechanisms of regeneration

(Molina-Freaner and Clark-Tapia, 2005). It has been observed that *Stenocereus eruca* has low pollination; therefore, its seed production is low (Bierzuchudek, 1981). Moreover, although seed input is low, seeds are available for germination every year. Thus, low seedling recruitment in *S. eruca* is probably due to a lack of favorable conditions for seed germination and establishment (Clark-Tapia and Molina-Freaner, 2004). Additionally, seedling recruitment could be diminished due to the absence of microsites with favorable environments for seed germination and setting (Del Mandujano et al., 1996, 1998). Arce-Amezquita et al. (2017) evaluated two different solvents for chirimola seed germination; seeds with acetone increased total germination almost double (62 %) as compared to the control (32 %). On the other hand, pretreatment of the seeds with dichloromethane reduced total germination to 0 %. The use of these two solvents for pretreatment of the seeds had different effects on germination, being acetone a suitable solvent to increase total germination of chirimola seeds.

Salinity is one of the most environmental factors limiting the productivity of crop plants because most are sensitive to salinity caused by high concentrations of salts in the soil, and the area of land affected by it gradually increases (Shrivastava and Kumar 2015). A concept of saline agriculture was proposed for the rehabilitation of saline and degraded lands (Nikalje et al., 2017). Salinity effects are the results of complex interactions among morphological, physiological, and biochemical processes (Akbarimoghaddam et al., 2011). Mahajan and Tuteja (2005) mentioned that salinity negatively influences germination and plant growth, as well as physiological processes (photosynthesis, respiration, and transpiration), nutrient balance, membrane properties, and cellular homeostasis, enzymatic and metabolic activities. Sun et al. (2016) found that salinity decreased photosynthetic carbon assimilation, stomatal conductance, and lowered photosynthetic electron transport efficiency. Ion accumulations of Na^+ and Cl^- cause stress and the greatest destructive effects on plant tissues.

Around the world, more than 75 countries are fighting against issues related to salinity (Qadir et al., 2007; Alaghmand et al., 2016). Approximately, 20 % of the irrigated lands around the world also have issues with salinity (Qadir et al., 2008).

Plants exposed to stressful environments produce low amounts of dry matter, leaf area, and low yield (Amirjani, 2011). Furthermore, it has been described that high amounts of salt induce important morphological changes in the plant (Zadeh and Naeini, 2007). The influence of saline stressful conditions has been linked to the modification of some physiological and morphological characteristics like fresh/dry weight and seed germination (Chartzoulakis and Klapaki, 2000).

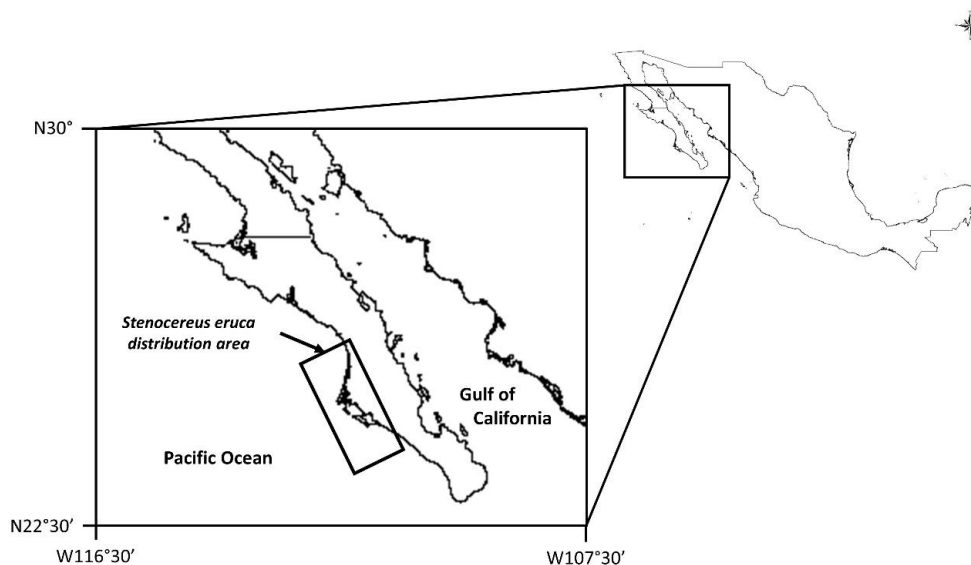


Figure 1. Map of *Stenocereus eruca* distribution in the Magdalena region of Baja California Sur, México (Arce-Amezquita et al., 2017).

Naturally, seed germination is strongly affected by salinity, which in turn reduces seedling development of many species (Debez et al., 2004). One of the most important stages of a plant cycle is germination, particularly for those species that develop in saline conditions (Song et al., 2005). Even though soil contains many ionic compounds with different impacts on development and seed germination (Tobe et al., 2002), most of the studies are usually carried out using sodium chloride (Ungar, 1996; Song et al., 2005; Nichols et al., 2008).

Studies with cacti have demonstrated that increasing salt concentration impacts their germination percentage and seedling biomass production (Beltrán-Morales et al., 2015; Orozco et al., 2017). The effects of salinity on growth and production of young cladodes "nopalitos" (cladode sprouts) of *Opuntia ficus-indica* (L.) Mill., were investigated by Murillo-Amador et al. (2001) using irrigation levels of NaCl with electrical conductivities of 2, 5, 10, 13, 18, and 21 dS m⁻¹. In general, all cladode variables (stem area, number of young cladodes, length and width of cladodes, fresh and dry weight) decreased with increasing salinity. The root-to-stem ratio and young cladode water content decreased significantly as salinity increased. For rooted cladodes, increased salinity decreases fresh weight and succulence and root fresh weight, dry weight, and length. The effect of salinity on *Pachycereus pringlei* seed germination was observed. When the concentration of NaCl was increased, the percentage of germination decreased (Nolasco-Soria et al., 1996). Seeds of *Cereus jamacaru* were submitted to saline stress. The saline treatments showed a significant reduction in germinability as the saline solution concentration increased (Meiado et al., 2010). Podda et al. (2017) mentioned that *Opuntia ficus-indica* seeds germinated with NaCl in the substrate up to 50 dS m⁻¹ of concentration, although lower germination percentages were observed in saline substrates, in comparison with those detected under control condition (0 mM NaCl). *Pachycereus pecten aboriginum* seeds

germinated under different salinities decreased their germination percentage when salinity increased (Vega-Villasante *et al.*, 1996).

However, there is not enough information about seed germination of *Stenocereus eruca* submitted to NaCl-stress and, more significantly, this would be the first report of seedling fresh weight of this species under salt-stress. The objective of this study was to analyze chirimola (*Stenocereus eruca*) seed germination and seedling fresh weight under saline stressful conditions caused by NaCl at different levels of electrical conductivity.

MATERIALS AND METHODS

Study area

Stenocereus eruca seeds were gathered in November 2018 from established wild mature plants (Figs. 2 and 3) in San Carlos located at 24°49'35" LN and 112°05' 09" LW, 10 m.a.s.l., 250 km north of La Paz city in Baja California Sur (BCS), México. This area is situated near Magdalena Bay, in the Sonoran Desert of Baja California Sur, México. This region has dry weather with 100 mm of annual rainfall, most of this rainfall (around 80 %) takes place around July and September (1988-2001, data provided by the Comisión Nacional del Agua, La Paz, B.C.S.).

In the coastal area of Sonora and Baja California, as well as in the lowland frequently flooded by marine water, there is a prevalence of saline soil; sodium-salt soils predominate, with sodium chloride or common salt high content. Alternatively, there may be an accumulation of calcium carbonate (Cervantes-Ramirez, 2002).

Ethics statement

The study carried out herein neither involves experimentation with humans or animals. The collection area is unprotected; though, *Stenocereus eruca*, is an endangered species hence permission was requested and granted by the deputy office of Federal Officer of SEMARNAT (Secretaría del Medio Ambiente y Recursos Naturales) in BCS.



Figure 2. An adult plant of *Stenocereus eruca* showing ripe and immature fruits. Plant located in San Carlos, B.C.S, México (photograph by Beltrán-Morales F.A., November 19, 2018).

Quantification of evaporated water

Before the application of the NaCl-treatments, an experiment was performed to quantify the evaporation rate at each Petri dish. Also, this test was used to identify if salt concentration was raised after replenishing the evaporated water with saline solutions at similar concentrations instead of distilled water, following Beltrán-Morales *et al.* (2015) method.



Figure 3. Adult plants of *Stenocereus eruca* associated with wild sarcocauliscent and scrubland vegetation (photograph by Beltrán-Morales F.A. November 19, 2018).

Seed acquisition

Ripe fruits were picked-up from healthy chirinola plants (NOM-007-SEMARNAT-1997). Seeds were separated from the pulp of the fruit and then sterilized by soaking them in a 5 % calcium hypochlorite solution for 5 min. The seeds were rinsed three times with sterilized distilled water and finally dried at 25 °C.

Saline solutions

The salinity treatments were 0 (distilled water as control), 2, 4, 6, 8, and 10 dS m⁻¹ prepared with NaCl to evaluate seed germination and fresh weight accumulation by *Stenocereus eruca* seedlings.

Germination conditions

The experiment was developed under laboratory conditions at the Universidad Autónoma de Baja California Sur located at 24° 08' 32" LN and 110° 18' 32" LW (INEGI, 2017). *Stenocereus eruca* seeds were treated with different salinity levels or electrical conductivities (0, 2, 4, 6, 8, and 10 dS m⁻¹) following the same method carried out by Beltrán-Morales *et al.* (2015).

The germination percentage was registered daily, and total germination was registered after 7 days. The germination rate was quantified using Maguire's equation: $M = n_1/t_1 + n_2/t_2 + \dots + n_7/t_7$;

where n_1, n_2, \dots, n_7 are the germinated seeds at times t_1, t_2, \dots, t_7 (in days). Germination was considered when a 1 mm long radicle was observed (Fig. 4).

Experimental design

Five replications of 20 seeds, arranged in a completely randomized design were submitted to six saline levels or electrical conductivities (0, 2, 4, 6, 8, and 10 dS m⁻¹) using NaCl as the main source of salt. A limited number of seeds per replication (twenty seeds only) were used due to a restricted number of ripe fruits available. The collecting permission granted let us collect only a limited number of fruits of *Stenocereus eruca* based on the standards of protection and management of this species (NOM-007-SEMARNAT-1997).



Figure 4. Seed of *Stenocereus eruca* germinated under 0 dS m⁻¹ (photographed by Beltrán-Morales F.A. January 30, 2019).

Statistical analysis

Variance homogeneity was assessed by Bartlett's test. Data were analyzed using a one-way ANOVA where salinity levels (0, 2, 4, 6, 8, and 10 dS m⁻¹) were used as a fixed factor. Significant differences between all variables among treatments were considered at $p \leq 0.05$. Tukey's HSD multiple range test at $p \leq 0.05$ was used to determine differences of all variables between means of each treatment. A simple correlation analysis (Pearson) was performed. Statistical analyses were carried out with Statistica v. 13.5 (TIBCO Software Inc., 2018). Germination percentage data were transformed using arcsine function before the ANOVA analysis (Sokal and Rohlf, 1995).

RESULTS

Germination rate

Germination rate of *Stenocereus eruca* was influenced by level of salinity-stress ($p \leq 0.01$). The germination rate decreased as salinity increased ($r = -0.60$; $p \leq 0.01$; $n = 30$). Germination rate was highest at 2 dS m⁻¹, followed by 0, 4, 8, and 6 dS m⁻¹, while germination rate at 10 dS m⁻¹ decreased significantly (Table 1).

Germination percentage

Germination percentage of *Stenocereus eruca* was influenced by salinity-stress ($p \leq 0.01$). The germination percentage decreased as salinity increased ($r = -0.51$; $p \leq 0.01$; $n = 30$). Germination percentage was higher at 4 and 2 dS m⁻¹, followed 0, 8, and 6 dS m⁻¹, while at 10 dS m⁻¹ germination decreased reaching down to 14 % (Table 1).

Seedling fresh weight

Seedling fresh weight was significantly affected by saline stress ($p \leq 0.01$). Seedling fresh weight decreased as salinity increased ($r = -0.77$; $p \leq 0.01$; $n = 30$). Seedling fresh weight was highest at 0 and 2 dS m⁻¹, followed by 4 dS m⁻¹. Fresh weight decreased significantly at 10 dS m⁻¹ (Table 1).

Table 1. Effects of salinity-stress by NaCl on seeds germination rate, germination percentage, and seedling fresh-weight of *Stenocereus eruca*.

Salinity (dS m ⁻¹)	Germination rate	Germination (%)	Seedling fresh-weight (mg)
0	2.15 ± 0.22 ab*	31.00 ± 5.33 ab	211.78 ± 25.26 a
2	2.37 ± 0.90 a	39.00 ± 7.14 a	158.74 ± 49.05 a
4	1.42 ± 0.57 ab	42.00 ± 6.81 a	119.70 ± 19.78 ab
6	0.46 ± 0.12 ab	20.00 ± 6.12 ab	38.62 ± 9.24 b
8	0.68 ± 0.14 ab	22.00 ± 4.35 ab	47.38 ± 12.91 b
10	0.37 ± 0.09 b	14.00 ± 1.87 b	25.54 ± 3.85 b
LSD	1.98	24.34	108.64

*Values with the same letter within each column are not significantly different at $p \leq 0.05$ (Tukey's HSD multiple range test). LSD=Least significant difference. N=5 replications of 20 seeds each.

DISCUSSION

Stenocereus eruca seedling fresh-weight accumulation and seed germination were significantly influenced by salinity stress. This response has been previously reported in other cacti species. For example, fishhook barrel cactus (*Ferocactus peninsulae*) (Romero-Schmidt et al., 1992) and Mexican giant cardon (*Pachycereus pringlei*) seeds can germinate at salinities of 2.5 and 5 dS m⁻¹ (Nolasco-Soria et al., 1996). According to Khajeh-Hosseini et al. (2003), salinity may influence the germination of seeds, either by creating an osmotic potential external to the seed preventing water uptake, or by the toxic effects of Na⁺ and Cl⁻ ions on germinating seed. Deleterious effect of high salinity levels on germination of *Stenocereus eruca* seeds agrees with Vega-Villasante et al. (1996) who reported that high salinity levels, 10 and 20 dS m⁻¹, reduced *Pachycereus pringlei* seed germination and was completely inhibited at 30 dS m⁻¹. Likewise, *Opuntia* species, resistant to droughts and large temperature fluctuations, are affected by some concentrations of salt contained in the soil or irrigation water (Nieto-Garibay et al., 2011). Using a fraction of seawater (1/5) for irrigation has been mentioned to harm the root system development of agaves and cacti (Nobel, 1998). *Opuntia quimilo* and *Cereus validus* from Argentina have a higher capacity to survive in the presence of salt than other

Opuntia species. *Opuntia ficus-indica* decreases its growth by half when exposed to certain amounts of seawater (Nobel, 1998).

Other plant species respond more negatively to high salinity levels during seedling development and early growth stages as compared to sprouting, *i.e.* *Cucumis melo* L. is a clear case of this response (Botia et al., 2005). Saline stress could affect via different mechanisms during *Stenocereus eruca* seed germination; for example, can cause an inadequate water uptake or can create a toxic environment for the embryo (Azza et al., 2007). Munns (2002) described that increasing the amount of salts in the substrate induces osmotic changes in plants or even toxic conditions that affect germination. Reduced *Stenocereus eruca* seed germination at high levels of salinity correlates with other literature reports (Abdul et al., 1992; Breen et al., 1977; Abbad et al., 2004; El-Tayeb, 2005).

Sodium and chlorine ions influence protein functioning within cells due to changes in ionic strength, which in turn could affect protein structure (Waisel, 1972). Therefore, the presence of these ions could affect enzymes directly associated with germination processes (Flowers, 1972).

Moreover, high concentrations of salts decrease water uptake reducing water potential of seeds and at the same time affecting germination (Rhoades et al., 1992). Apparently, metabolic processes induced by saline stress lead to increased amounts of phenolic compounds which, in turn, affect germination process as well (Rehman et al., 1997).

Stenocereus eruca seedlings reduced freshweight accumulation at high levels of salt is a typical response observed for other plant species (Gupta and Sharma, 1990; Läuchli and Epstein, 1990; Ebert et al., 1999; Al-Thabet et al., 2004).

Previous studies (Gupta and Srivastava, 1989; Saboora and Kiarostami, 2006) reported that root and shoot biomass were significantly affected when NaCl concentrations increases, a similar response was observed in *Stenocereus eruca* seedlings. The decrease of fresh weight of *Stenocereus eruca* seedlings could be attributed to dehydrated cells. This cellular condition is reversible; however, it affects growth rate hampering cell division and elongation (Munns, 2002). Moreover, salinity may injure sensitive tissues at cotyledons and hypocotyl, thus delaying germination and seedling emergence (Miyamoto et al., 1985; Esechie et al., 2002).

Mineral analysis was not carried out because of the limited amount of fresh weight produced by the seedlings. However, ionic imbalances, triggered by excessive amounts of sodium and chloride ions, are suggested as one of the causes of fresh weight reduction. This circumstance generates toxicity absorption problems of other minerals necessary for plant growth (Yokoi et al., 2002). The presence of high concentrations of sodium has an antagonistic effect on potassium and calcium absorption in roots and has also had a negative impact on other absorption processes (Hu and Schmidhalter, 2005). A high concentration of sodium, besides interfering with absorption processes, inhibits root growth reducing seedling biomass accumulation (Tester and Davenport, 2003).

The present study is relevant since *Stenocereus eruca* is an endangered species endemic to an area where water with a high concentration of salt is regularly used to irrigate crops because of the lack of fresh water. This is especially confirmed in Baja California Sur since it has no running rivers, hence underground water is used in agriculture. Salts presence in Baja California Sur aquifer has contributed to structure and fertility soils degradation (Álvarez-Morales et al., 2014). Large extensions of agricultural lands worldwide have excess of salt and represent a problem in crops production (El-Saidi, 1997). A high concentration of salts is one of the greatest problems that limit plant growth. Many studies about the effect of salts in many different plants have been carried out; however, salinity resistance mechanisms are not completely understood (Mansour, 1997).

Average chlorine amount in plants are between 2-20 mg per gram of dry weight; however, optimal plant growth requires 0.2-0.4 mg of chlorine per gram of dry weight (Navarro and Navarro, 2013). Furthermore, chlorine is an important element since chloride ions binding to photosystem II are essential for water oxidation. Also, it is part of more than 130 important molecules, for example, the auxin 4-chloroindoleacetic acid (Salisbury and Ross, 2000). To our knowledge, this is the first study regarding salinity effect on *Stenocereus eruca* seed germination and seedling fresh weight accumulation. This study could help to identify chirinola genetic ecotypes, resistant to saline-stress, which in turn could help to recover this threatened species.

CONCLUSIONS

Seedling fresh-weight of *Stenocereus eruca* was higher at 0 and 2, followed by 4 dS m⁻¹, and decreased at 6, 8 and 10 dS m⁻¹. Germination rate was higher at 2 dS m⁻¹ and lower at 10 dS m⁻¹, while germination percentage was higher at 4 and 2 dS m⁻¹ and lower at 10 dS m⁻¹. More studies must be done, including other growth variables and salinity levels.

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CONFLICT OF INTEREST STATEMENT

The research has no financial or commercial purpose that must be interpreted as a potential conflict of interest in the future.

AUTHORS' CONTRIBUTIONS

FABM, PMAA, and BMA generated the idea and designed the research. FHRE, VBV, FABM, and PMAA developed the research. BMA and LGHM analyzed the data. VBV and LGHM

contributed materials, reagents, and analysis tools. VBV, FABM, BMA, and LGHM wrote, revised, and edited the paper. FABM, PMAA, FHRE, VBV, LGHM, and BMA approved the latest paper version.

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