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# WATER STRESS IN TWO CAPSICUM SPECIES WITH DIFFERENT DOMESTICATION GRADE

# [ESTRES HIDRICO EN DOS ESPECIES DE CAPSICUM CON DIFERENTES GRADOS DE DOMESTICACION]

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## SUMMARY

Seed germination is usually affected by water stress. The aim of this study was to compare the effect of water stress on the final germination percentage, mean germination rate, epicotyl and hypocotyl growth, and root:shoot ratio index of semi-domesticated, *Capsicum frutescens* L., and cultivated, *C. annuum* L., pepper species. Seeds were exposed to eight levels of water potential (Ψ) induced by polyethylene glycol (PEG-8000). *Capsicum annuum* showed final germination percentage of 73%; however, no significant differences (P =0.01) were observed among Ψ treatments between species. Mean germination rate was lower for *C. frutescens* than for *C. annuum*. Variation in epicotyl and hypocotyl growth was observed between treatments, but no clear pattern was observed across Ψ treatments. Root:shoot ratio index was higher for *C. annuum* than for *C. frutescens*. Root:shoot ratio index differences were significant among Ψ treatments in the case of *C. annuum*, while for *C. frutescens* differences were not significant. Root:shoot ratio index was a reliable drought tolerance indicator for both pepper species. An increase in root:shoot ratio as a function of water stress levels suggest a priming effect on *C. annuum* improving seedling growth under water stress. In the case of *C. frutescens*, no decrease effect on root:shoot ratio index was evident suggesting an adaptive characteristic to growth under arid and semiarid climatic conditions in Baja California Sur.

**Key words:** PEG-8000; cultivated; semi-domesticated; germination; mean germination rate; root:shoot ratio index.

## RESUMEN

La germinación en semillas está comúnmente afectada por el estrés hídrico. El objetivo de este estudio fue comparar el efecto del estrés hídrico en el porcentaje final de germinación, tasa media de germinación, y longitud de epicotilo e hipocotilo, índice de radícula:tallo de las especies de chile semi-domesticado *Capsicum frutescens* L. y cultivado *Capsicum annuum* L. Las semillas fueron expuestas a ocho niveles de potencial hídrico (Ψ) inducidas con polietilen glicol (PEG) 8000. *Capsicum annuum* presentó el porcentaje final de germinación de 73%; sin embargo, se presentaron diferencias no significativas (P=0.01) entre tratamientos en cada especie. La tasa media de germinación fue menor en *C. frutescens* que en *C. annuum*. El índice de la relación radícula:tallo fue mayor para *C. annuum* que para *C. frutescens*. Las diferencias entre el índice radícula:tallo fue significativa entre tratamientos en el caso de *C. annuum*, mientras que para *C. frutescens* no. El índice radícula:tallo fue un indicador relevante de tolerancia a la sequía en ambas especies de chile. Un incremento en el índice radícula:tallo como una función de los niveles de estrés hídrico sugiere un efecto promotor en *C. annuum* mejorando el crecimiento de plántula bajo estrés hídrico. En el caso de *C. frutescens*, no se evidenció una disminución en el índice radícula:tallo. Lo cual sugiere una característica adaptativa del crecimiento bajo condiciones climáticas aridas y semiaridas en Baja California Sur.

**Palabras clave:** PEG-8000; cultivado; semi-domesticado; germinación, tasa media de germinación; índice radícula:tallo.

## INTRODUCTION

In some regions of Mexico, including Baja California Sur (BCS), it is common to find relatively large and variable populations of wild, semi-domesticated, and cultivated pepper species. BCS is an arid region where domestic green peppers of *C. annuum* varieties, such as 'California' and 'Ancho', have been important crops, despite adverse environmental conditions that prevail in this region, such as water scarcity, high temperatures, and water and soil salinity (Nieto-Garibay, 1999; Murillo-Amador *et al*. 2001).

Most studies about the adaptation of pepper species to adverse climatic conditions, such as drought and salinity stress, have focused on cultivated species (Chartzoulakis and Klapaki, 2000). Little attention has been given to wild and semi-domesticated species, despite the fact that their adaptive mechanisms to environmental stresses in arid regions have been shaped by natural selection over a long period of time (Lanteri *et al*. 2003).

*Capsicum frutescens* is a semi-domesticated pepper species in Mexico that is present in BCS. 'Tabasco' and 'Cayenne' are examples of this pepper species that are cultivated as a family garden hot pepper species. This pepper species becomes an important study case, particularly during seed germination, when it encounters soil water stress conditions (Nieto-Garibay, 2001; Hernández-Verdugo *et al*. 2001; Portis *et al*. 2004; Nieto-Garibay *et al*. 2009). Semi-domesticated and wild hot pepper species show seed dormancy and low germination percentages which limits commercial production (Hernández-Verdugo *et al*. 2001).

It is important to understand the effects of water stress on germination in semi-domesticated and wild hot pepper species in order to improve germination rate for commercial production. Polyethylene glycol (PEG) has been successfully used to induce water stress in some horticultural and pepper species to study the effects on the germination (Emmerich and Hardegree, 1991; Santos-Díaz and Ochoa-Alejo, 1994). In previous works, PEG was used to induce osmotic stress in seeds of *C. annuum* to evaluate germination performance and seedling vigor (Sánchez *et al*. 1999; Trawatha *et al*. 1990; Zgallai *et al*. 2005).

To date, the available information on the response of *C. frutescens* to osmotic stress is scarce. Here, we compare the germination capability and morphological differences in seedlings of *C. frutescens* and *C. annuum* exposed to an artificial gradient of water potential (Ψ) treatments induced by PEG as a hypertonic osmoregulator. We hypothesized that, as a consequence of the greater genetic variation in semi-domesticated species, the seeds will show greater variability in response to water stress during seed germination and seedling development as compared to domesticated species. This variability, as an important component of the domestication process, is being evaluated to determine important seed germination and seedling development traits, useful for incorporating native BCS *C. frutescens* varieties into breeding programs.

## MATERIAL AND METHODS

Semi-domesticated hot pepper (*C. frutescens*) seeds were obtained from plants established at the Experimental Station of Centro de Investigaciones Biologicas del Noroeste (CIBNOR) 17 km NW of La Paz, Baja California Sur, Mexico (24°08´N. 110°24´W). These seeds were originally collected in the nearby semi-rural community of El Centenario, BCS. Before starting the water potential (Ψ) treatments, seeds were disinfected in a CaHCl solution, containing 5% active chlorine, for 5 min, and then washed three times with sterilized distilled water. Commercial seeds of *C. annuum* cv. California Joe Parker (FAX, lot. MVF-CT-02, 88% germination) were used for comparison. It was not necessary to disinfect seeds because commercial seeds were treated with fungicide. Water deficit simulated treatments were established using an aqueous solution of polyethylene glycol (PEG-8000, Spectrum Chemical Mfg. Corp., Gardena, CA 90248, New Brunswick, NJ 08901), as described by Emerich and Hardegree (1991). Water potential values were validated using the calibration curves developed by Michel (1983). PEG-8000 was used because it does not cause root damage (Michel, 1983; Michel *et al*. 1983). Control groups were not treated with PEG-8000. Water potential treatments were applied at 0.0, -0.4, -0.8, -1.2, -1.6, -2.0, -2.4, -2.8, and -3.2 MPa. Seeds of *C. annuum* and *C. frutescens* were placed in Petri dishes and exposed to one of eight concentrations of PEG-8000 for 72 h at 25°C. Thereafter, seeds were rinsed with distilled water and air dried. Seeds were sown in 9-cm Petri dishes containing 20 ml distilled water as a substrate.

The experiment was conducted in a germination chamber (Model MB-60B, Percival Manufacturing Company, city, state) providing a 12 h photoperiod with white fluorescent light, luminosity from 120 to 130 μ mol $m^{-2}$ $s^{-1}$. Temperature varied from 20 to 30°C with relative humidity ranging from 80 to 90%. The experimental design was a factorial with the two pepper species and eight Ψ levels arranged in a completely randomized design (Zar, 1999) using three replications of 20 seeds per treatment unit. The germination experiment was conducted according to International Seed Test Association (ISTA, 1985). Germinated seeds were counted daily during the experimental period. Final germination was calculated as an absolute percentage (FGP), considering the total number of seeds included in each treatment (Maguire, 1962). A seed was considered as germinated when the epicotyl tip had elongated to 2 mm from the seed coat. The mean germination rate (MGR) was calculated according to Maguire's equation (Maguire, 1962):

$$M = n_1/t_1 \ldots n_{10}/t_{10}$$

Where:
$n_1, n_2, \ldots, n_{10}$ are the number of germinated seeds at time $t_1, t_2, \ldots t_{10}$ (in days). Total germination was recorded up to 13 days after sowing, at which time germination no longer occurs.

These parameters were obtained for each repetition of each treatment and pepper species by performing a standardized non-linear curve-fitting procedure using the logistic model for 4 parameters of the SIGMAPLOT computer program. Values of germination were transformed to arcsine (√ %), where data of germination are expressed as a percentage.

Normal and abnormal germinated seeds were determined according to the criteria set by the ISTA (1985). Growth characteristics of seedlings, such as hypocotyl and epicotyl length, root:shoot length ratio index, dry and fresh total weight, were obtained from the seedlings of each treatment at the end of experimental time. Hypocotyl and epicotyl length were measured with a digital caliper (General, No. 143, General Tools, Manufacturing Co. Inc, New York, NY) and were used to calculate root:shoot length ratio. Seedlings were weighted on an analytical balance (Mettler Toledo, AG204, Mettler Toledo, Inc. Switzerland) to obtain total fresh weight. The dry weights were determined after drying the seedlings in a force-air dryer (Blue M, OV-490A, Sheldon Manufacturing, Cornelius, OR) at 80°C until they reached constant weight, and were used to calculate dry biomass production per plant and dry total weight.

Data were analyzed by performing a factorial ANOVA. Means were separated by Fisher LSD test ($P<0.01$) using STATISTICA v. 6 (StatSoft, Inc., Tulsa, OK). Figures were drawn using SIGMAPLOT v. 8 (Systat Software Inc. San José, California)

## RESULTS

No differences among Ψ treatments for each pepper species were found for final germination percentage (Fig 1). Data for both pepper species were analyzed together in order to evaluate differences between species. The final germination percentage was significantly ($P<0.01$) higher for *C. annuum* (73%) than for *C. frutescens* (62%) (Fig.1).

When the statistical analysis was applied to mean germination rate, no differences among Ψ treatments was observed and the data was adjusted with linear and non-linear regression models (Fig. 2). Mean germination rate showed a non-linear tendency for *C. annuum* with better results in polynomial tendency. For *C. frutescens*, a linear regression model was observed, *C. frutescens* showed a lower mean germination rate than *C. annuum*.

In order to evaluate differences between species, data for *C. frutescens* and *C. annuum* were combined for analysis, which showed that mean germination rate appeared later for *C. frutescens* than for *C. annuum*. Growth measurements of seedlings showed little differences in the length of hypocotyl among Ψ treatments for both pepper species (Table 1). *Capsicum frutescens* tended to show shorter hypocotyl growth. Differences in *C. annuum* were more evident than in *C. frutescens* among Ψ treatments. For *C. annuum*, the difference between the treatment with the shortest hypocotyl growth (-2.8 MPa) was 35% less than the treatment with the longer hypocotyls growth (-2.0 MPa). This difference in hypocotyl growth was 24% for *C. frutescens*. Epicotyl growth showed statistical differences among Ψ treatments with a positive effect for all Ψ treatments for *C. annuum*. It is clear that *C. frutescens* had less epicotyl growth that *C. annuum*. Results of root:shoot length ratio showed larger ratios for *C. annuum* than for *C. frutescens* (Table 1). Differences among Ψ treatments for root:shoot length ratio were present for *C. annuum*; whereas, *C. frutescens* showed non-significant differences between Ψ treatments.

Water potential treatments did not cause any effect on seedling fresh weight, total dry weight (Table 2), or biomass per plant (Table 3). However, the results showed differences between both species. *Capsicum annuum* showed larger fresh weight, total dry weight, and total biomass per seedling (Table 4). Data for abnormal seedlings percentage revealed no differences between species and treatments, with a low occurrence of abnormal seedlings for both pepper species, and mean values of 11.8% for *C. frutescens* and 13.8% for *C. annuum*.

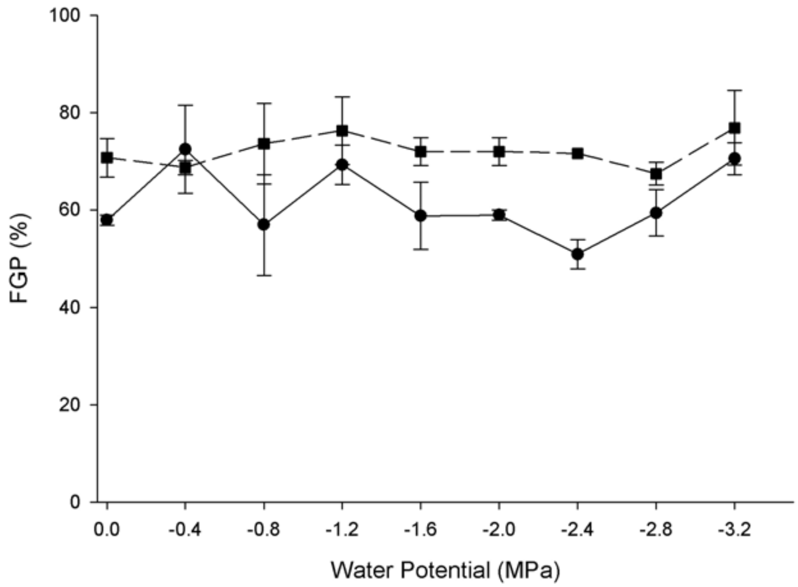


Fig. 1 Final germination percentage (FGP) for *C. frutescens* -●- and *C. annuum* pepper species --■-- for the nine water potential (Ψ) treatments. Each point is the mean of three replications and vertical bars are the ±SE of the mean.

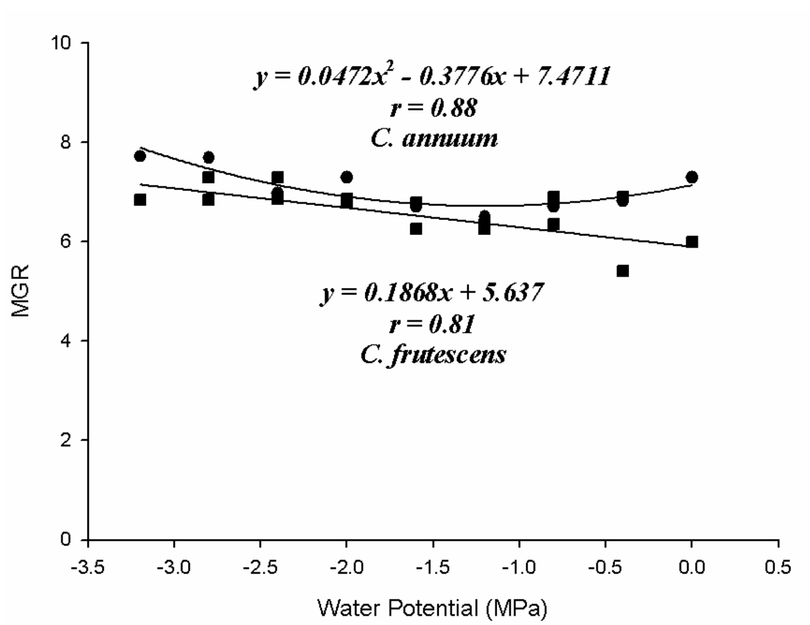


Fig. 2 Tendency regression analysis of different water potential (Ψ) treatments on mean germination rate (MGR) for *C. annum* -●- and *C. frutescens*-■- pepper species.

Table 1. Effect of different levels of water stress (Ψ) using PEG-8000 for osmotic regulation on hypocotyl, and epicotyl length, and ratio root:shoot ratio for *C. annuum* (cultivated) and *C. frutescens* (semi-domesticated) pepper species.

| Ψ (MPa) Treatment | Pepper species | Hypocotyl (cm) | Epicotyl (cm) | Ratio root:shoot |
|---|---|---|---|---|
| 0 | *C. frutescens* | 1.23cdef | 2.8bcd | 0.43a |
| -0.4 | *C. frutescens* | 1.39f | 3.06cde | 0.47a |
| -0.8 | *C. frutescens* | 1.20cde | 2.61abcd | 0.42a |
| -1.2 | *C. frutescens* | 1.21cdef | 2.05a | 0.80a |
| -1.6 | *C. frutescens* | 1.33ef | 3.22def | 0.44a |
| -2.0 | *C. frutescens* | 1.08abc | 2.30ab | 0.50a |
| -2.4 | *C. frutescens* | 1.12bcd | 2.46abc | 0.48a |
| -2.8 | *C. frutescens* | 1.20cde | 2.41ab | 0.53a |
| -3.2 | *C. frutescens* | 1.06abc | 2.27ab | 0.49a |
| 0 | *C annuum* | 0.98ab | 3.60efg | 3.7cd |
| -0.4 | *C annuum* | 1.05abc | 4.82hi | 4.14de |
| -0.8 | *C annuum* | 0.99ab | 5.05i | 5.7f |
| -1.2 | *C annuum* | 1.20cdef | 5.0i | 4.25de |
| -1.6 | *C annuum* | 1.28def | 5.04i | 3.98cd |
| -2.0 | *C annuum* | 1.39f | 3.86fg | 2.92b |
| -2.4 | *C annuum* | 1.28def | 4.23gh | 3.42bc |
| -2.8 | *C annuum* | 0.91a | 4.18gh | 4.68e |
| -3.2 | *C annuum* | 1.15bcde | 4.62hi | 4.14de |
| LSD0.01 | | 0.8 | 0.94 | 0.88 |

Means followed by different letters in a column are significantly different ($P < 0.01$).

Table 2. Means of total seedling fresh and dry weight for *C. annuum* and *C. frutescens* without differences among water potential (Ψ) treatments induced by PEG-8000.

| Treatment | Total fresh weight (g) | | Total dry weight (g) | |
|---|---|---|---|---|
| Water Potential Ψ (MPa) | *C. frutescens* | *C. annuum* | *C. frutescens* | *C. annuum* |
| 0.0 | 0.23 | 0.50 | 0.025 | 0.056 |
| -0.4 | 0.22 | 0.51 | 0.025 | 0.056 |
| -0.8 | 0.17 | 0.52 | 0.021 | 0.055 |
| -1.2 | 0.20 | 0.53 | 0.024 | 0.056 |
| -1.6 | 0.22 | 0.52 | 0.025 | 0.057 |
| -2.0 | 0.18 | 0.53 | 0.023 | 0.057 |
| -2.4 | 0.20 | 0.53 | 0.022 | 0.054 |
| -2.8 | 0.19 | 0.52 | 0.023 | 0.054 |
| -3.2 | 0.16 | 0.53 | 0.022 | 0.054 |

## DISCUSSION

When evaluating wild peppers, the requirements for germination are considered as an adaptive trait (Hernández-Verdugo *et al*. 1998). Commonly, wild pepper seed shows dark dormancy requiring auxiliary chemicals and special light conditions to induce germination (Ramírez-Meraz *et al*. 2002; Hernández-Verdugo, 1998). Usually, seeds of *C. frutescens* have been exposed to cultural practices by rural house keepers in their gardens that enhance germination; which suggests that *C. frutescens* has been subjected to constant empirical breeding, with positive effects on its germination capability and a reduction in seed dormancy. In comparison *C. annuum* shows no inhibition of seed germination, which is a common property of domesticated species. As Ladizinsky (1985) mentioned, one of the physiological traits modified by domestication by seed propagation is the seed dormancy period. This widely explains why semi-domesticated seeds of *C. frutescens* rendered a lower percentage of germination than seeds of cultivated *C. annuum*. Similar results were found during seedling emergence when *C. frutescens* and *C. annuum* were grown under soil water stress. *C. frutescens* did not require auxiliary chemicals for seedling emergence but had lower seed germination than *C. annuum* (Nieto-Garibay *et al*. 2009).

Water potential treatments applied in this study did not cause any significant effect on final germination percentage. Seed germination for pepper differed from similar studies carried out for winter wheat and tomato genotypes by Main and Nafziger (1994) and Cheng and Bradford (1999). They found that decreasing Ψ reduced final germination. However, the presence of drought tolerance mechanisms in the two pepper species could explain the observed difference. Santos-Díaz and Ochoa-Alejo (1994) successfully screened drought-tolerant pepper cell clones, using PEG as a drought simulator and suggested that osmotic adjustment is involved in the tolerance to PEG. Additionally, in previous studies with *C. frutescens*, an osmotic adjustment mechanism was observed under drought treatment where partial turgor pressure is maintained at low water potentials (Nieto-Garibay *et al.,* 2001).

Mean germination rate for *C. frutescens* showed an increase when exposed to low water potential treatments. For *C. annuum*, osmotic stress appeared to enhance germination rate between -0.4 to -1.6 Ψ treatments. Sánchez *et al*. (1999) observed an increase in germination rate for bell pepper seeds exposed to osmotic stress ranging from -0.60 to -0.81 MPa. In this study, low Ψ treatments also caused increases in length of time for germination for *C. frutescens*, but showed a shorter MGR than *C. annuum*. Mean germination rate for both pepper species suggests an improvement in germination, which is a trait useful for searching drought tolerance characteristics.

Water potential treatments had little effect hypocotyl and epicotyl growth, and biomass production for both species. Epicotyl growth showed clear differences among pepper species with a lower epicotyl growth for *C. frutescens*. Root:shoot ratio is regularly used as a criterion for evaluating the response to increasing salinity (Murillo-Amador *et al*. 2001) and has been used as a water deficit tolerance criterion (Kramer, 1989). In this study, *C. frutescens* showed no differences among treatments in root:shoot ratio. Similar results were reported for *C. frutescens* under soil water stress treatments (Nieto-Garibay *et al.* 2009). Murillo-Amador *et al*. (2001) concluded that the lack of negative effects on growth under low Ψ was an indicator of drought tolerance. In the case of *C. annuum*, root:shoot ratio increased in almost all treatments indicating water stress had a positive effect enhancing drought stress.

Table 3. Means of fresh and dry biomass per seedling for *C. annuum* and *C. frutescens* without differences among water potential (Ψ) treatments induced by PEG-8000.

| | Fresh biomass (g plant$^{-1}$) | | Dry biomass weight (g plant$^{-1}$) | |
|---|---|---|---|---|
| Water Potential Ψ (MPa) | *C. frutescens* | *C. annuum* | *C. frutescens* | *C. annuum* |
| 0.0 | 0.0116 | 0.028 | 0.00128 | 0.0028 |
| -0.4 | 0.0112 | 0.025 | 0.00129 | 0.0028 |
| -0.8 | 0.0086 | 0.026 | 0.00107 | 0.0027 |
| -1.2 | 0.0103 | 0.026 | 0.00120 | 0.0028 |
| -1.6 | 0.0114 | 0.026 | 0.00120 | 0.0028 |
| -2.0 | 0.0093 | 0.026 | 0.00115 | 0.0028 |
| -2.4 | 0.0102 | 0.026 | 0.00112 | 0.0027 |
| -2.8 | 0.0097 | 0.026 | 0.00115 | 0.0027 |
| -3.2 | 0.0081 | 0.026 | 0.00113 | 0.0027 |

Table 4. Differences in total seedling fresh weight, dry weight, fresh biomass per seedling, and dry biomass per seedling observed between *C. annuum* and *C. frutescens*.

| | Total fresh weight (g) | Total dry weight (g) | Fresh biomass (g plant) | Dry biomass (g plant) |
|---|---|---|---|---|
| *C. frutescens* | 0.202 ± 0.006$^{b}$ | 0.023 ± 0.0005$^{b}$ | 0.00119 ± 0.0003$^{b}$ | 0.00119 ± 0.00002$^{b}$ |
| *C. annuum* | 0.532 ± 0.007$^{a}$ | 0.056 ± 0.0006$^{a}$ | 0.00280 ± 0.0003$^{a}$ | 0.00121 ± 0.00003$^{a}$ |
| LSD0.01 | 0.0013 | 0.00001 | 0.0000 | 0.0000 |

Means and differences amongst water potential treatments obtained by Fisher LSD test. Means followed by different letters in a column are significantly different ($P < 0.05$).

## CONCLUSION

Both pepper species, *C. frutescens* and *C. annuum*, showed adaptive characteristics for low Ψ in the germination stage. This is an important consideration for future genetic studies. Promotion of studies to assay and evaluate physiological tolerance to arid and regional environmental stresses has a potential to identify alternative crops for sustainable agriculture. Results of this study identified *C. annuum* cv. California Joe Parker as a suitable cultivated pepper variety for production in arid and semiarid region of Baja California Sur, and that *C. frutescens* represents a potential economic new crop for this region.

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