# Study of the effect of drought-induced by polyethylene glycol in *Capsicum* frutescens in a hydroponic system

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## — Abstract—

Drought is the most common factor limiting crop development and productivity, severely affecting agriculture. In Mexico, one of the crops of economic and gastronomically important sensitive to water stress is the Capsicum genus. In the present study, the application of proline on Capsicum frutescens plants exposed to drought induced by polyethylene glycol (PEG) in a hydroponic system was evaluated. Using a 22-factorial design, a total of 60 seedlings were evaluated for 120 hours divided into four treatments: PEG (o and 10 %) and Pro (o and 10 mM) as study variables. The results showed that exposure to 10 mM of Pro induced a significant increase in the chlorophyll concentration and endogenous proline (leaf and root) in seedlings in the absence of stress. In addition, 80 % survived of seedlings, and an increase in proline content was observed in those exposed to 10 mM Pro + 10 % PEG. Similarly, there was an increase in chlorophyll content (13  $\mu q \cdot m L^{-1}$ ), relative water content (RWC) in the root (77.6 %), and percentage of electrolytes in the leaf and root (~44 and ~52 % respectively) compared to the treatment of o mM Pro + 10 % PEG. These results suggest that the pre-application of proline has a positive effect on seedlings' survival under PEG-induced drought conditions.

## **Keywords:**

Drought; Capsicum frutescens; tolerance; survival.

rought is considered the main stress that affects the growth and productivity of agricultural crops, and economic losses are estimated to exceed those caused by other types of abiotic stress. Unfortunately, due to climate change, more extreme conditions are expected in the coming years (dos Santos et al., 2022). One of the crops affected of great importance due to the wide variety of culinary and economic uses is chili. The genus Capsicum belongs to the family Solanaceae and is native to tropical and subtropical America. As of 2019, 40 species have been identified, of which five are of great economic relevance (C. annum, C. chinense, C. baccatum, C. frutescens, and C. pubescens) and have been domesticated or semi-domesticated depending on the region in which they are grown. However, it experiences a significant impact on its production due to water stress during the different phenological stages of the crop, which limits its development and productivity (Tripodi & Kumar, 2019; Toppino et al., 2021). The effects of water deficit on plants depend on their stage of development, the time of exposure, the species, and the degree of severity, which leads to metabolic alterations such as the reduction in the synthesis of photosynthetic pigments, loss of turgidity, increase in reactive oxygen species, among others (Jalil & Ansari, 2020; Taiwo et al., 2020). Currently, Mexico is the fourth largest producer of fresh chili in the world and the five main species of economic importance are cultivated (Food and Agriculture Organization [FAOSTAT], 2023). In addition, around 25 wild or semi-domesticated species are present in the territory, one of which is C. frutescens, distributed in the southeast of the country (La Cruz-Lázaro et al., 2017), the gene pool of semi-domesticated species offers the opportunity to use it as unique study models for crop breeding. In recent years, various investigations have been carried out to mitigate the effects of water stress and improve plant tolerance. The exogenous application of biostimulants to increase performance and minimize the effects of environmental stress is one of the most promising strategies (Sahoo et al., 2019). The amino acid proline (Pro) is considered one of the most important signaling molecules, in addition to participating as an osmoprotectant by presenting a positive correlation between proline accumulation and stress tolerance (Elewa et al., 2017; Suekawa et al., 2019). However, the application of exogenous proline in various agricultural crops under drought conditions reduced the effects and increased their tolerance (Elewa et al., 2017; Faroog et al., 2017; Alkahtani et al., 2021). However, plant response varies based on genotypes, severity, and extent of drought. Therefore, the effect of proline application and polyethylene glycol (peg) -induced drought on semidomesticated chili (C. frutescens) seedlings in a hydroponic system was evaluated in this study.

## 2. MATERIALS AND METHODS

#### 2.1 Plant material

The chili seeds were germinated in polystyrene seedbeds with a mixture of peat (Peat moss®) and agrolite (Termolita® Hortiperl) in a ratio of 3:1 v/v. After 30 days of germination, the seedlings were transplanted to a hydroponic system with Hoagland solution (Hoagland & Arnon, 1950) for 3 days under constant aeration for adaptation (de Freitas *et al.*, 2019), with a photoperiod of 16/8 h light/dark, at an average temperature of 25 ± 2°C. Hoagland's solution contains 50  $\mu$ M de CaCl<sub>2</sub> (Sigma Aldrich®), 12.5  $\mu$ M de H<sub>3</sub>BO<sub>3</sub> (Sigma Aldrich®), 1  $\mu$ M de CuSO<sub>4</sub> (Sigma Aldrich®), 0.1  $\mu$ M de CuSO<sub>4</sub> (Sigma Aldrich®), 0.1  $\mu$ M de CuSO<sub>4</sub> (Sigma Aldrich®), 0.1  $\mu$ M fe-EDTA (Sigma Aldrich®), 1.2  $\mu$ M de KNO<sub>3</sub> (Sigma Aldrich®), 0.8  $\mu$ M de Ca(NO<sub>3</sub>)<sub>2</sub> (Sigma Aldrich®), 0.2  $\mu$ M de KNO<sub>3</sub> (Sigma Aldrich®), 0.8  $\mu$ M de Ca(NO<sub>3</sub>)<sub>2</sub> (Sigma Aldrich®), 0.2  $\mu$ M de KH<sub>2</sub>PO<sub>4</sub> (Sigma Aldrich®), and 0.2  $\mu$ M de MgSO<sub>4</sub> (Sigma Aldrich®).

## 2.2 Water stress induced by polyethylene glycol

The experiment was performed using a 2²-factorial design to analyze the effect of proline and polyethylene glycol (PEG-8000 Sigma Aldrich®) -induced drought on chili seedlings. A total of 60 seedlings distributed in four treatments were evaluated. After acclimatization of the chili plants, they were treated with proline for 48 h, which was done by supplementing Hoagland's solution in the hydroponic culture, using concentrations of 0 and 10 mM proline (Sigma Aldrich®).

Subsequently, the seedlings were exposed to o and 10 peg for 120 h.

At the end of the exposure to drought stress, the percentage of survival, relative water content (RWC), electrolyte quantification, chlorophyll content, and endogenous proline were evaluated for each of the treatments.

#### 2.3. Survival rate

The survival rate of chili seedlings was determined by calculating the percentage of live plants at the end of the experimental phase concerning the number of live plants at the beginning, using equation (2), proposed by Linares (as cited in Peñalba, 2022).

Survival % = 
$$\frac{P_V}{P_v + P_m} * 100$$
 (2)



Where:

 $P_v$  = live plants

 $P_m = dead plants$ 

# 2.4. Relative Water Content (RWC)

To evaluate the CRA of leaves and roots, the fresh weight  $(P_F)$  of the seedlings was determined after collection. Subsequently, they were dried at 65 °C in a Hamilton Beach® hot air desiccator for 48 h to obtain the constant dry weight  $(P_S)$ . Equation (1) described by Jothimani & Arulbalachandran, (2020) was used.

$$\% CRA = \frac{P_F - P_S}{P_F} * 100$$
 (1)

Where:

% CRA = Relative water content

 $P_{E}$  = Fresh weight of plant tissue

 $P_s$  = Dry weight of plant tissue

# 2.5. Electrolyte Quantification

To evaluate the percentage of electrolyte leakage, the methodology described by Restrepo *et al.* (2013) was followed with some modifications. Discs of the fresh plant material were placed in test tubes with tri-distilled water. Initial conductivity ( $CE_1$ ) was measured, using a CON-BTA (Vernier®) conductivity probe, after 2 hours of incubation at room temperature (30±2 °C). Subsequently, the samples were incubated at 120°C for 20 min, and the final conductivity ( $CE_2$ ) was measured. The percentage of electrolytes released was calculated using equation (3).

Electrolytes % = 
$$\frac{CE_1}{CE_2} * 100$$
 (3)

#### 2.6. Chlorophyll content

The total chlorophyll content was determined, using the method of Inskeep & Bloom (1985) with modifications. 50 mg of fresh leaf was macerated with 80 % acetone (MEYER®) and incubated at 4°C for 60 min, then centrifuged at 10,000 rpm for 5 min. The quantification of total chlorophyll was per-



formed by the UV-visible spectrophotometry technique, using a HACH® brand spectrophotometer, DR model 5000, at wavelengths ( $\lambda$ ) of 664 and 647 nm, using equation (4).

Total chlorophyll 
$$(\mu g \cdot mL^{-1}) = 17.95A_{647} + 7.90A_{664}$$
 (4)

## 2.7. Determination of endogenous proline content in leaf and root

The Escalante-Magaña protocol (2020) was used to extract and quantify endogenous proline in leaf and root samples. The reaction was carried out with acid ninhydrin (Sigma Aldrich®) and glacial acetic acid (MEYER®), and the samples were incubated at 96°C for 60 min. Subsequently, the organic phase was extracted with toluene (MEYER®) and the amount of proline was quantified by spectrophotometry at  $\lambda$  of 520 nm, using equation (5).

$$\mu mol \ proline * g^{-1} = \left(\frac{(\mu g \ proline * mL^{-1})(mL \ toluene)}{115.5 \ \mu g * \mu mol^{-1}} \right)$$
(5)

## 2.8. Statistical Analysis

The data obtained were evaluated using one-way analysis of variance (ANOVA) and the comparison of means was performed by LSD test ( $P \le 0.05$ ), through Statgraphics Centurion XIX® software (Statgraphics Technologies, Inc., Madrid, Spain).

#### 3. RESULTS

To determine the effect of proline application on chili seedlings (*C. frutescens*) 35 days after germination were subjected to water stress induced by -8000 PEG 10% for 120 h. The results indicate that exposure to 10 mM Pro significantly improved survivability reaching a value of 80% in seedlings pre-treated with proline (Table 1), compared to 40% in untreated seedlings.

#### 3.1. Chlorophyll content

The chili seedlings previously treated with proline had a higher chlorophyll content, compared to the control treatment in the absence of stress (Table 2). On the other hand, when exposing seedlings to PEG-induced drought stress, it was observed that treatment with 10 mM Pro + 10% PEG had a



higher total chlorophyll content than treatment with 0 mM Pro + 10% PEG, which showed a significant decrease due to the effects of drought.

# 3.2. Relative Water Content (RWC)

It was observed that the RWC, both in the aerial part and the root system of the chili seedlings significantly decreased under drought conditions (Table 3), compared to the control (no PEG). However, seedlings previously treated with proline and exposed to PEG had a significantly higher RWC in the root system, compared to seedlings without prior proline treatment. These results suggest that the application of proline could have helped maintain hydration in the roots during drought.

## 3.3. Electrolyte leakage percentage

PEG-induced drought exposure significantly increased electrolyte leakage into leaf and root tissues in chili (*C. frutescens*) seedlings (Table 4), relative to control. However, pre-treatment with proline reduced electrolyte loss in both tissues, compared to 0 mM Pro + 10% PEG treatment.

# 3.4. Endogenous proline content

The values obtained indicate that the endogenous proline content in the leaves and roots of *C. frutescens* increased significantly in response to treatment with 10% PEG, compared to treatments in the absence of stress (Table 5). This suggests that plants exposed to PEG-induced drought increased proline synthesis as a defense response to water stress. In addition, a significant difference in endogenous proline concentration was observed in the leaves and roots of *C. frutescens* treated with exogenous proline. It is important to note that the seedlings previously treated with exogenous proline and subsequently exposed to stress, had the highest content of endogenous proline.

## 4. DISCUSSION

Drought stress reduced the survival rate of chili seedlings, the relative water content in leaf and root tissues, and total chlorophyll, as well as an increase in the percentage of electrolyte leakage and endogenous proline content in both tissues. This is because plant water potential and turgidity are significantly reduced, which can interfere with plant metabolic functions (Pandey *et al.*, 2019; Abobatta, 2020).



Jothimani and Arulbalachandran (2020) reported that the water deficit induced by 20% PEG in tomato cultivation critically affects photosynthesis, due to the increase in reactive oxygen species (ROS), altering the total chlorophyll content, and damage to cell membranes. While Restrepo *et al.* (2013) demonstrated that electrolyte loss can indicate the integrity of cell membranes and that the impairments are mainly due to lipid peroxidation under stress conditions, due to ROS acting on the free radicals of the lipids that make up cell membranes in corn plants exposed to abiotic stress.

Even though the effects of drought-induced by 10% PEG in *C. frutescens* were negative, in the seedlings that were pre-treated with proline at the concentration of 10 mM, a decrease in the negative impact of stress was observed. This led to a significant increase in the survival rate, the RWC of the root system, and a decrease in the percentage of electrolyte leakage, suggesting that the integrity of the cell membrane was maintained, and the efficiency of photosynthesis by presenting an increase in the total chlorophyll content, compared to untreated plants.

Proline is considered a multifunctional amino acid under stressful conditions. Its accumulation in high concentrations is related to the ability to provide protection and participate in various cellular processes, such as osmotic regulation, energy, nutrient availability, and changes in redox balance. The accumulation of this amino acid under dehydration conditions, such as in seedlings exposed to PEG, is because the anabolism of Pro, mainly in the leaves, is activated and catabolism is repressed (Abobatta, 2020; Alvarez *et al.*, 2022; Hosseinifard *et al.*, 2022).

Previous application of exogenous proline to drought-exposed chili seedlings may likely have activated physiological and biochemical mechanisms that enhanced their ability to tolerate PEG-induced stress, which enabled an increase in chlorophyll content, reduced electrolyte leakage, and increased survival rate. Semida  $et\ al.\ (2020)$  report an increase in chlorophyll in onion plants treated with proline  $(1-2\ mM)$  in a foliar manner, indicating that one of the mechanisms that may be related to the increase is due to the protection of the structure and function of photosystems. In addition, proline can also act as an antioxidant, protecting cells from reactive oxygen species by reducing chloroplast cell membrane damage and chlorophyll degradation (Ashraf  $et\ al.\ 2018$ ; Merwad  $et\ al.\ 2018$ ; Cha-um  $et\ al.\ 2019$ ).

Regarding the decrease in the percentage of electrolyte leakage and the increase in the concentration of endogenous proline, Farooq *et al.* (2017) reported that the foliar application of osmoprotectants (proline, gamma-aminobutyric acid) in wheat reduced lipid peroxidation of cell membranes, allowing greater stability in the integrity of cell membranes; and that proline, applied exogenously, improved the endogenous proline content, under

conditions of drought stress could be related to an increase in the content of proline precursors (ornithine, glutamic acid, and arginine).

## **CONCLUSION**

Previous application of exogenous proline at a concentration of 10 *mM* has a positive effect on percent survival, electrolytes, relative water content, chlorophyll, and proline in chili plants *C. frutescens* exposed to 10% PEG under *in vitro* conditions. These findings could lay the groundwork for investigating different concentrations of proline and establishing the optimal concentration to improve percent survival in hydroponic cultivation under drought-stress conditions.

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**Table 1** *Effect of proline application on Capsicum frutescens under PEG drought* 

Treatments		
		Survival %
% PEG	[mM Pro]	
0	0	100 <sup>Aa</sup>
Ü	10	100 <sup>Aa</sup>
10	0	40 <sup>Bc</sup>
	10	80 <sup>Ab</sup>

Note. Different capital letters indicate a statistically significant within-treatment difference (% PEG); whereas, different lowercase letters indicate a statistically significant difference between LSD treatments ( $P \le 0.05$ ).

**Table 2**Total chlorophyll content in drought-exposed Capsicum frutescens

Treati	ments	
		Chlorophyll (µg/mL)
% PEG	[mM Pro]	
0	0	19.03 <sup>Bb</sup> ±1.4
U	10	28.96 <sup>Aa</sup> ±2.1
10	0	$6.05^{Bd} \pm 1.7$
	10	13.14 <sup>Ac</sup> ±2.9

Note. Different capital letters indicate a statistically significant within-treatment difference (% PEG); whereas different lowercase letters indicate a statistically significant difference between LSD treatments ( $P \le 0.05$ ).

**Table 3** *Relative water content (RWC) in Capsicum frutescens seedlings under drought* 

Treat	ments		
		Aerial part %	Root system %
% PEG	[mM Pro]		
0	0	86.80 <sup>Aa</sup> ±1.3	85.32 <sup>Aa</sup> ±1.2
	10	85.95 <sup>Aa</sup> ±0.9	85.34 <sup>Aa</sup> ±1.6
10	0	80.46 <sup>Ab</sup> ±5.2	55.90 <sup>Bc</sup> ±2.6
	10	82.44 <sup>Ab</sup> ±1.4	77.63 <sup>Ab</sup> ±2.6

Note. Different capital letters indicate a statistically significant within-treatment difference (% PEG); whereas different lowercase letters indicate a statistically significant difference between LSD treatments ( $P \le 0.05$ ).



**Table 4** *Percentage of electrolyte leakage in drought-exposed Capsicum frutescens seedlings* 

Treat	ments		
		Aerial part %	Root system %
% PEG	[mM Pro]		
0	0	14.87 <sup>Ac</sup> ±2.6	31.16 <sup>Ac</sup> ±2.4
O	10	12.31 <sup>Ac</sup> ±1.1	30.88 <sup>Ac</sup> ±1.1
10	0	58.47 <sup>Aa</sup> ±0.9	72.96 <sup>Aa</sup> ±3.9
	10	43.91 <sup>Bb</sup> ±3.0	51.81 <sup>Bb</sup> ±3.6

Note. Different capital letters indicate a statistically significant within-treatment difference (% PEG); whereas different lowercase letters indicate a statistically significant difference between LSD treatments ( $P \le 0.05$ ).

**Table 5** *Endogenous proline content in drought-exposed Capsicum frutescens seedlings* 

Treat	ments		
		Leaf (µmoles Pro·gPF/mL)	Root (µmoles Pro∙gPF/mL)
% PEG	[mM Pro]		
0	0	33.52 <sup>Bd</sup> ±1.4	29.77 <sup>Bd</sup> ±4.9
U	10	57.06 <sup>Ac</sup> ±3.2	37.16 <sup>Ac</sup> ±2.6
10	0	168.87 <sup>Bb</sup> ±2.3	99.17 <sup>Bb</sup> ±3.1
	10	212.38 <sup>Aa</sup> ±11.8	129.03 <sup>Aa</sup> ±4.5

Note. Different capital letters indicate a statistically significant within-treatment difference (% PEG); whereas different lowercase letters indicate a statistically significant difference between LSD treatments ( $P \le 0.05$ ).