Interactive effect of moisture restriction and salicylic acid on biochemical responses in *Phaseolus coccineus*

Efecto interactivo de la restricción de humedad y ácido salicílico sobre las respuestas bioquímicas de *Phaseolus coccineus*

Efeito interativo da restrição de umidade e ácido salicílico nas respostas bioquímicas em *Phaseolus coccineus*

Jesús Mao Aguilar-Luna[1*]
Juan Manuel Loeza-Corte[2]
Ernesto Díaz-López[3]

Rev. Fac. Agron. (LUZ). 2022, 39(3): e223940
ISSN 2477-9407
DOI: https://doi.org/10.47280/RevFacAgron(LUZ).v39.n3.06

Crop Production

Associate editor: Dr. Jorge Vilchez-Perozo
University of Zulia, Faculty of Agronomy
Bolivarian Republic of Venezuela

[1]Benemerita Autonomous University of Puebla, Agroforest Engineering. 73640. Mexico.

[2]University of Cañada, Agroindustrial Engineering. 68540. Mexico.

[3]Technological University of Tehuacan, Biofood Process Engineering. 75859. Mexico.

Received: 30-06-2022
Accepted: 01-08-2022
Published: 22-08-2022

Keywords:
Carotenoids
Drought
Glycinebetaine
Osmolytes
Photosynthetic pigments

Abstract

The increase in water scarcity leads to consider the understanding of staple crops under these conditions, coupled with this, the positive responses of salicylic acid in different crops, may be an option in bringing to fruition the cultivation of runner bean (*Phaseolus coccineus*). This study evaluated the effect of salicylic acid (SA) on the biochemical responses in *P. coccineus*, with humidity restriction in the periods from January to July 2019 and 2020, at the Benemerita Autonomous University of Puebla, Mexico. The research consisted of three levels of drought: 30, 60 and 100% soil moisture; five levels of SA: 0, 0.5, 1.0, 1.5 and 2.0 mM; and two levels of fertilization: non-fertilizer and fertilizer [(00-60-30) at sowing + (30N) foliar nitrogen at grain filling stage] for the two growing periods. The experimental design was in factorial random blocks with five replications. The results showed that the foliar application with 1.5 mM of SA maintained the highest relative water content in leaves (89.05%), as well as chlorophyll a, b and carotenoids (2.20, 1.11 and 0.90 $\mu g.mL^{-1}$, respectively); of glycinebetaine (24.80 $\mu mol.g^{-1}$ DW) and total soluble sugars (31.15 mg eq.glucose g^{-1} DW), excluding proline. The SA did not increase the protein fractions, even in plants with fertilizer; but the positive effects of SA were greater in plants without hydric stress and with fertilization.

*Corresponding author: mao.aguilar@correo.buap.mx

Resumen

El incremento en la escasez de agua lleva a considerar el entendimiento de los cultivos básicos bajo estas condiciones, aunado a esto, las respuestas positivas del ácido salicílico en diferentes cultivos, puede ser una opción para llevar a buen término el cultivo de frijol ayocote (*Phaseolus coccineus*). En este estudio se evaluó el efecto del ácido salicílico (ÁS) en la respuesta bioquímica de *P. coccineus*, con restricción de humedad en los periodos de enero a julio de 2019 y 2020 en la Benemérita Universidad Autónoma de Puebla, México. La investigación constó de tres niveles de sequía: 30, 60 y 100% de humedad del suelo; cinco niveles de ÁS: 0, 0.5, 1.0, 1.5 y 2.0 mM; y dos niveles de fertilización: sin fertilizante y con fertilizante [(00-60-30) al momento de la siembra + (30N) nitrógeno foliar en la etapa de llenado de grano] para los dos períodos de cultivo. El diseño experimental fue factorial en bloques con cinco repeticiones. Los resultados mostraron que la aplicación foliar con 1.5 mM de ÁS mantuvo el mayor contenido relativo de agua en hojas (89.05%), así como clorofila a, b y carotenoides (2.20, 1.11 y 0.90 $\mu g.mL^{-1}$, respectivamente); de glicinabetaína (24.80 $\mu mol.g^{-1}$ en peso seco) y azúcares solubles totales (31.15 mg eq.glucosa g^{-1} en peso seco), excluyendo prolina. El ÁS no incrementó las fracciones protéicas, incluso en plantas con fertilización; pero los efectos positivos del ÁS fueron mayores en plantas sin estrés hídrico y con fertilización.

Palabras clave: carotenoides, sequía, glicinabetaína, osmolitos, pigmentos fotosintéticos.

Resumo

O aumento da escassez de água leva-nos a considerar a compreensão das culturas básicas sob estas condições, juntamente com isto, as respostas positivas do ácido salicílico em diferentes culturas, pode ser uma opção para levar à frutificação o cultivo de feijão corredor (*Phaseolus coccineus*). Este estudo avaliou o efeito do ácido salicílico (ÁS) sobre a resposta bioquímica do *P. coccineus*, com restrição de umidade nos períodos de janeiro a julho de 2019 e 2020, na Universidade Autônoma Benemerita de Puebla, México. A pesquisa consistiu em três níveis de seca: 30, 60 e 100% de umidade do solo; cinco níveis de SA: 0, 0.5, 1.0, 1.5 e 2.0 mM; e dois níveis de adubação: não fertilizante e fertilizante [(00-60-30) na semeadura + (30N) nitrogênio foliar na fase de enchimento de grãos] para os dois períodos de crescimento. O delineamento experimental foi em blocos ao acaso fatorial com cinco repetições. Os resultados mostraram que a aplicação foliar com 1.5 mM de ÁS manteve o maior teor relativo de água nas folhas (89.05%), assim como clorofila a, b e carotenóides (2.20, 1.11 e 0.90 $\mu g.mL^{-1}$, respectivamente); de glicinabetaína (24.80 $\mu mol.g^{-1}$ de peso seco) e açúcares solúveis totais (31.15 mg eq.glicose g^{-1} de peso seco), excluindo prolina. O SA não aumentou as frações protéicas, mesmo em plantas com fertilizante; mas os efeitos positivos do SA foram maiores em plantas sem estresse hídrico e com adubação.

Palavras-chave: carotenóides, seca, glicinabetaína, osmolitos, pigmentos fotossintéticos.

Introduction

The runner bean [*Phaseolus coccineus* subsp. Polyanthus (Grenm.) Maréchal, Mascherpa and Stainer] rank third in importance within the genus *Phaseolus*, after *P. vulgaris* (L.) and *P. lunatus* (L.) (Reyes-Matamoros *et al*., 2014; Morosan *et al*., 2017). *P. coccineus* plants are sensitive to water deficiency, which may be because it is a semi-domesticated plant, and it presents the same sensitivity pattern as *P. vulgaris* (Reyes-Matamoros *et al*., 2014).

When plants are exposed to humidity restriction, their cells protect themselves by synthesizing and accumulating specific osmolytes (betaine, soluble carbohydrates, glycine-betaine, proline, and proteins such as osmotin) (Ozturk *et al*., 2020; Hossain *et al*., 2022); to maintain cellular osmotic balance, which is disrupted under stress conditions (Surabhi and Rout, 2020). Consequently, plants stressed by humidity restriction exhibit a different biochemical response than plants grown under optimal conditions.

To reduce hydric stress in plants, various growth regulators have been used in the past by many researchers, e.g.: mepiquat chloride, polyamines, brassinosteroids, jasmonate, 5-aminolevulinic acid and salicylic acid (SA) (Kordi *et al*., 2013; Hossain *et al*., 2022). SA is one of those that offers the best response, since it is a lipophilic monohydroxybenzoic acid that regulates different physiological processes in plants (Abdelaal *et al*., 2020); e.g.: positive effect on both hypocotyl height and diameter, nutrient absorption, stomatal closure, transpiration, chlorophyll, protein biosynthesis, proline biosynthesis, antioxidative enzymes, plant defense and secondary metabolism (Afshari *et al.*, 2021; Gordillo-Curiel *et al*., 2021; Yan *et al.*, 2022). It improves the transpiratory rate, water use efficiency, stomatal conductance, internal CO_2 concentration, photosynthetic assimilation rate and fixation of enzymes such as RuBisCo (Galon *et al.*, 2022; Muhie, 2022).

The mode of action of SA depends on several factors, such as the species, environmental conditions, concentration, and method of application. Low SA concentrations have shown to exhibit beneficial effects in crops with hydric restriction, e.g.: a 10^{-5} M SA solution in *Brassica juncea* (L.), *Zea mays* (L.), *Glycine max* (L.) and other crops, increased net photosynthesis (Muhie, 2022). A concentration of 10^{-5} M SA in *Cymbopogon flexuosus* (Nees ex Steud.) Watson, improved proline content and production of essential oils (Idrees *et al*., 2010). SA's from 0.01 to 0.05 mM applications in *Coffea arabica* (L.) increased its growth (Gordillo-Curiel *et al*., 2021). A concentration of 1.5 mM of SA in *Ocimum basilicum* (L.) favored the proline content (Kordi *et al*., 2013). Applications of 0.05 M SA in *Mentha arvensis* (L.) increased the content of chlorophylls a and b, and carotenoids (Elhakem, 2019).

Worldwide, the works with SA to mitigate the humidity restriction (hydric stress) in crops are very extensive; however, for *P. coccineus* scarce information is available. Therefore, the objective of the present study was to evaluate the effect of five concentrations of SA in the presence of two levels of fertilization with three levels of humidity restriction on the biochemical responses in *P. coccineus*.

Materials and methods

Study location

The study was carried out on an agricultural plot with a tunnel-type greenhouse at the Benemerita Autonomous University of Puebla (latitude: 19°49'01''N, longitude: 97°47'36''W, altitude: 1,764 m); during January to July 2019 and 2020. The region's climate is temperate subhumid, in the two years, the climatic conditions were similar, therefore, the monthly averages of the two years are shown (figure 1). Experimental soil used had following characteristics, it was Luvisol (FAO, 2015), with pH 6.83, 0.14 $g.cm^{-3}$ in bulk density, 0.50 Cmol(+) kg^{-1} in cation exchange capacity, 0.34% organic matter, 0.19% total N, 0.80 $mg.kg^{-1}$ of P, 0.01 Cmol(+) kg^{-1} of K, 0.03 Cmol(+) kg^{-1} of Ca and 0.03 Cmol(+) kg^{-1} of Mg.

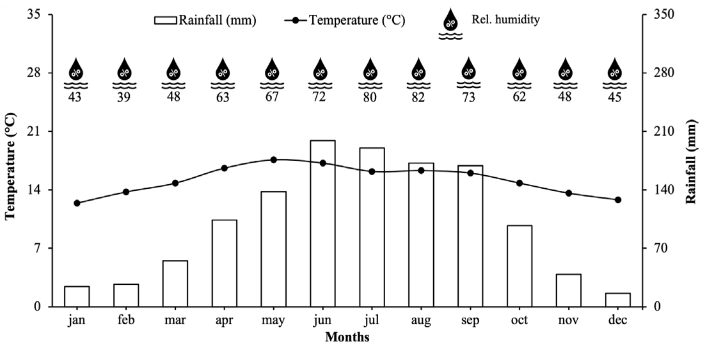

Figure 1. Climatic conditions of the experimental site (monthly averages, 2019 and 2020).

Biological material and treatments

The seeds were acquired from regional producers, washed with running water, and disinfected before sowing with a fungicide (N-Trichloromethylthio-4-cyclohexene-1,2-dicarboximide) at a dose of 1.50 $g.kg^{-1}$ of seed. One seed per bag was sown, a black nursery bag 30 cm high and 23 cm in diameter was used; the same ones that were filled with reconstituted substrate, with soil from the region and peat moss (3:1, $^{v}/_{v}$).

The experiments were developed in the dry season, with controlled humidity conditions. The adaptation of the plants to drought stress began 15 days after sowing and until the end of the experiment. Following the method used by Reyes-Matamoros *et al*. (2014) three humidity levels were established: without stress (100% humidity in the soil), moderate stress (60% humidity in the soil) and severe stress (30% humidity in the soil).

Five levels of SA (0, 0.5, 1.0, 1.5 and 2.0 mM) were evaluated, which were applied to the foliage 15 days after sowing with a hand sprinkler. In total, five applications were made with intervals of 15 days. The SA concentrations were obtained from its molecular weight (138.12 $g.mol^{-1}$), each SA concentration was dissolved in 5% ethanol, then it was filled with distilled water in a one-liter volumetric flask and finally Tween 20 washing buffer was added, 0.5% as a surfactant.

Two levels of fertilization were also used: non fertilizer and fertilizer [(00-60-30) edaphic fertilization at sowing + (30N) foliar nitrogen applied in the grain filling stage]. Two pests appeared during the development of the crop *Liriomyza trifolii* (Burgess) and *Bemicia tabaci* (Gennadius), which were controlled with a systemic insecticide: N-[1-(6-Chloro-pyridin-3-ylmethyl)-imidazolidin-2-ylidene]nitramide. The experiment was presented in a factorial randomized block design with five replications. Each experimental unit consisted of a bag with a plant.

Measurement of experimental variables

Relative water content (RWC). The second leaf of five plants selected at random by treatment was used; the fresh weight (FW) was immediately recorded in them, then the leaves were soaked with distilled water for 16 h in the dark to record the turgid weight (TW), then they were dried in an oven at 70°C for 72 h to estimate the dry weight (DW). The RWC was calculated using the following formula (Chaimala *et al*., 2021):

$$\mathrm{RWC}\ (\%) = \left[\frac{\mathrm{FW}-\mathrm{DW}}{\mathrm{TW}-\mathrm{DW}}\right](100)$$

Photosynthetic pigments. To determine the concentration of pigments: chlorophyll a (CHLa), chlorophyll b (CHLb) and carotenoids (CRT); the methodology of Lichtenthaler & Buschmann (2001) was followed. The absorbance was read with a Perkin Elmer Lambda 25 UV/Visible spectrophotometer, the optical density of the solution was read at 662, 645 and 470 nm (CHLa, CHLb and CRT, respectively). Finally, the concentration of pigments was calculated in µg for each mL of $extract^{-1}$, according to the equations:

$$\mathrm{CHLa} = (((12.77\mathrm{x}\lambda662 - 2.43\mathrm{x}\lambda645)\mathrm{x}10)/\mathrm{M})/1{,}000$$

$$\mathrm{CHLb} = (((22.52\mathrm{x}\lambda645 - 4.82\mathrm{x}\lambda662)\mathrm{x}10)/\mathrm{M})/1{,}000$$

$$\mathrm{CRT} = (((1000\mathrm{x}\lambda470 - 1.97\mathrm{xCHLa} - 65.77\mathrm{xCHLb})/229\,\mathrm{x}10)/\mathrm{M})/1{,}000$$

Quantification of osmolytes. The samples for these analyze were obtained during the grain filling stage (approximately 100 days after sowing), five samples were used per treatment, the data were obtained, and the averages were calculated for:

Free Proline (PRO). It was determined according to the method of Bates *et al*. (1973); the absorbance of the organic phase was read at 520 nm. The concentration of PRO was expressed as *µmol*.g^{-1} of DW.

Glycine betaine (GB). It was determined following the method of Grieve & Grattan (1983); the absorbance of the solution was read at 365 nm. The concentration of GB was expressed as *µmol*.g^{-1} of DW.

Total soluble sugars (TSS). They were quantified according to the method of Dubois *et al*. (1956); the absorbance of the solution was read at 490 nm. The TSS concentration was expressed as 'mg glucose equivalent' g^{-1} of DW.

Protein fractions. The extraction of the fractions: albumin (ALB) and globulins (GLB) was carried out using the method described by Barba de la Rosa *et al*. (1992), using 50 g of dry seed flour. For the extraction of glutelins (GLT), the residue from the extraction of prolamins (PRL) was dispersed in a 1M NaOH solution at a 1:15 solute/solvent ratio. The percentage of protein extracted in each fraction was calculated, using the equation:

$$\mathrm{Protein}\ (\%) = \left[\frac{\text{protein gram extracted in the fraction}}{\text{protein gram in the flour}}\right](100)$$

The protein content was calculated by the Kjeldahl method according to the AOAC methodology (Latimer, 2012), using 6.25 as the nitrogen to protein conversion factor. Protein quantification was carried out by the method of Morr *et al*. (1985) with a kit (Sigma-Aldrich-L1013). A standard curve was made with bovine serum albumin (Sigma-Aldrich-A8531) at a concentration of 0.40 $mg.mL^{-1}$; the absorbance was measured at 600 nm.

Statistical procedures

All the experiments were conducted using a factorial randomized block design, with five replications. The assignment of treatments to the experimental units, as well as the data, were processed for a regression analysis and multivariate statistical (correlations), using Rstudio version 1.4.1717 (R Core Team, 2022).

Results and discussion

Relative water content

The application with 1.5 mM of SA, without fertilizer and without stress (100% humidity in the soil), maintained the highest RWC (89.05%) in the leaves; the lowest (25.36%) was in plants with severe stress (30% humidity in the soil), with fertilizer and with SA (0.5 mM) (figure 2). The RWC increased as the intensity of drought decreased; in plants with fertilizer, with SA (1.5 mM) and without stress, the RWC improved by 49.79%, with respect to plants without fertilizer, with severe stress and without SA (0 mM).

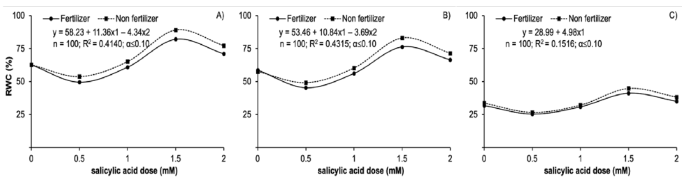

Figure 2. Relative water content in *P. coccineus* under different doses of salicylic acid and humidity restriction. A) without stress, B) moderate stress, and C) severe stress.

The foliar application of SA can reduce the loss of water by transpiration when there is hydric stress, since it regulates the opening and closing of the stomata (Rao *et al*., 2012). In this research, the foliar application of 1.5 mM of SA in *P. coccineus* plants, improved the RWC. Similar results were obtained in *Lippia citriodora* (L.) (Dianat *et al*., 2016), *Z. mays* (Farouk *et al*., 2018) and in *Calendula officinalis* (L.) (Gholinezhad, 2020).

Photosynthetic pigments

The highest contents of CHLa, CHLb and CRT pigments (2.20, 1.11 and 0.90 µg.mL^{-1}, respectively) were presented in plants without stress (100% soil humidity), with fertilizer and with SA (1.5 mM); which contributed to the increase in chlorophyll contents by 47.27, 49.54 and 53.33%, with respect to plants without fertilizer, with severe stress (30% of soil humidity) and without SA (0 mM) (figure 3).

Photosynthetic efficiency depends on pigments (CHLa, CHLb and CRT), since they are fundamental in the photochemical stage of photosynthesis (Muhie, 2022). The foliar application of 1.5 mM of SA in *P. coccineus*, improved the chlorophyll contents. Similar results were reported in *M. arvensis* (Elhakem, 2019), in *Helianthus annuus* (L.) (Rehman *et al*., 2019) and in *H. vulgare* (Abdelaal *et al*., 2020). This is because SA reacts as an antioxidant substance by eliminating reactive oxygen species (Rehman *et al*., 2019).

Osmolyte accumulation

The highest content of PRO (31.77 µmol.g^{-1} DW) was found in plants without fertilizer, with severe stress and without SA (0 mM). The lowest content of this (3.24 µmol.g^{-1} DW) was in plants with fertilizer, without stress (100% humidity in the soil) and 1.5 mM of SA (best dose for RWC and photosynthetic pigments) (figure 4). PRO increased with drought intensity by 8.8 times in plants without fertilizer, without SA and severe stress; with respect to those plants with fertilizer, with SA and without stress.

The accumulation of osmolytes (PRO, GB, TSS) in the cytoplasm helps to maintain cellular osmotic balance in response to abiotic stress (Ozturk *et al*., 2020). But with hydric stress, the plant consumes many photosynthates to produce osmotic regulators and reduce stress (Gholinezhad, 2020).

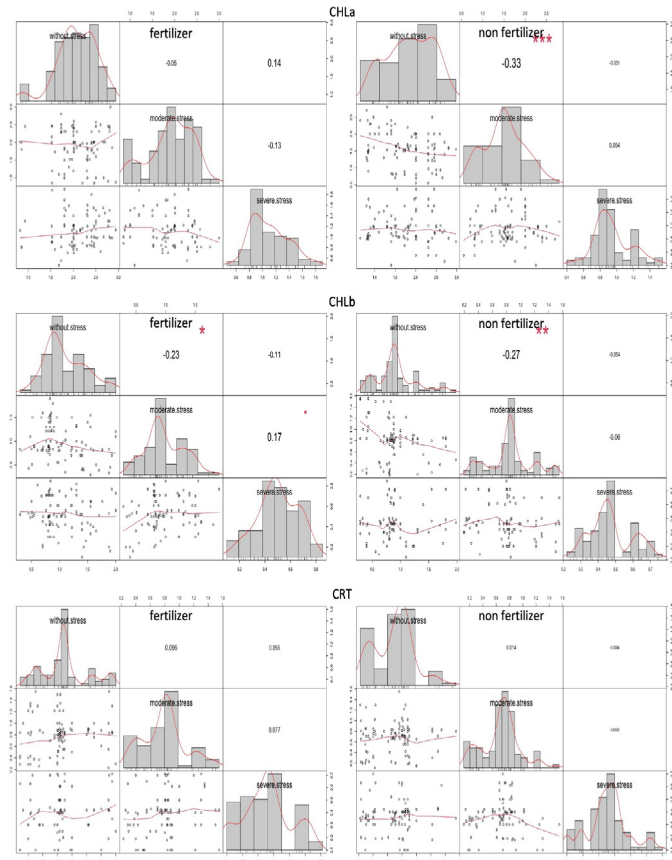

Figure 3. Concentration of chlorophyll a (CHLa, µg.mL^{-1}), chlorophyll b (CHLb, µg.mL^{-1}) and carotenoids (CRT, µg.mL^{-1}); in *P. coccineus* under different doses of salicylic acid and humidity restriction. Asterisks indicate significant differences in each level of water stress, in plants with fertilizer and without fertilizer. *, $\alpha<0.05$; **, $\alpha<0.01$; ***, $\alpha<0.001$.

In this research, PRO increased with the application of SA (1.5 mM) in a condition of greater hydric stress, which was similar to that obtained in *C. flexuosus* (Idrees *et al*., 2010), in *P. vulgaris* (Ghanbari *et al*., 2013) and in *H. vulgare* (Abdelaal *et al*., 2020).

The highest content of GB (24.80 µmol.g^{-1} DW) was found in plants with fertilizer, without stress and with SA (1.5 mM); the lowest content of this (19.50 µmol.g^{-1} DW) was obtained in plants without fertilizer, severe stress and without SA. The GB increased by decreasing the drought intensity by 1.2 times in the plants with fertilizer, without stress and with SA; this with respect to those plants without fertilizer, without SA and severe stress. In this research, the GB was increased with the application of SA (1.5 mM) in plants without water stress. Its presence under stress conditions has been favorable in *P. coccineus* but not in *P. vulgaris* (Morosan *et al*., 2017). Surabhi & Rout (2020) mentioned that high concentrations of GB increase the permeability of the membrane, the efficiency of water use in conditions of drought stress and the photosynthetic efficiency.

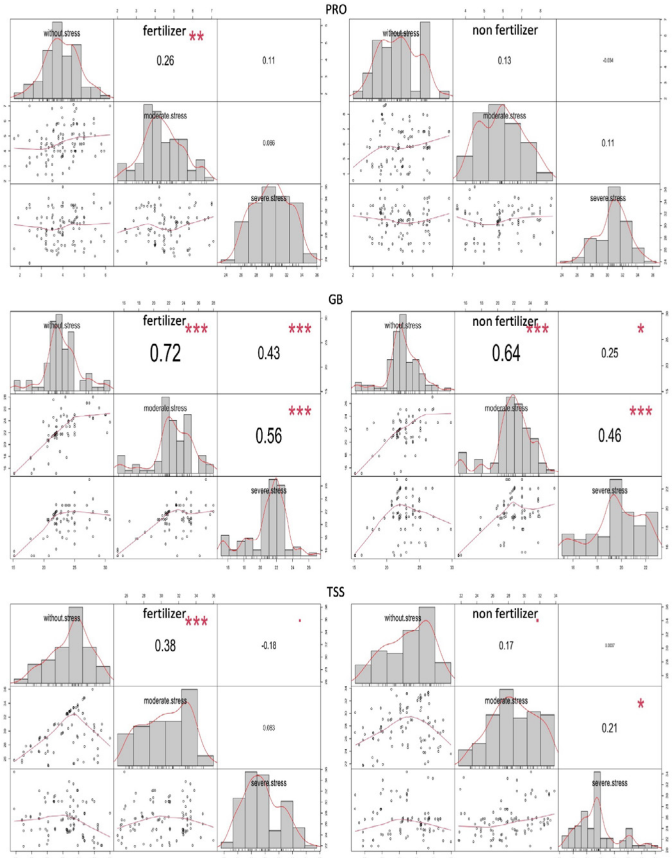

Figure 4. Osmolyte levels: proline (PRO, µmol.g^{-1}), glycine betaine (GB, µmol.g^{-1}) and total soluble sugars (TSS, mg.g^{-1}); in *P. coccineus* under different doses of salicylic acid and humidity restriction. Asterisks indicate significant differences in each level of water stress, in plants with fertilizer and without fertilizer. *, α<0.05; **, α<0.01; ***, α<0.001.

The highest content of TSS (31.15 mg eq.glucose g^{-1} DW) was obtained in plants with fertilizer, without stress and with SA (1.5 mM); the lowest content of this (25.11 mg eq.glucose g^{-1} DW) was in plants without fertilizer, severe stress and without SA. TSS increased by decreasing stress by 1.2 times in plants with fertilizer, without stress and with SA; the above with respect to plants without fertilizer, without SA and severe stress.

TSS increased when there was less hydric stress, which differs from that obtained in *Ziziphus mauritiania* (Lam) (Gadi & Laxmi, 2012) and *Saposhnikovia divaricata* (Turcz.) Schischk. (Men *et al*., 2018). In *P. coccineus*, Morosan *et al*. (2017) found no differences in the TSS of plants subjected to different stress; but a sugar content of 9.45 to 11.60% was associated with a sweeter taste (Jacinto-Hernández *et al*., 2019).

Protein fraction

In plants without stress, with fertilizer and without SA (0 mM), higher values of ALB, GLB, PRL and GLT were obtained (23.54, 11.28, 0.54 and 0.61%, respectively). The SA did not increase the percentage of protein fractions, even in plants with fertilizer (figure 5). On the contrary, 2.0 mM of SA and severe stress (30% of soil humidity), caused a decrease of 19.71, 35.06, 25.64 and 20.83% in the protein fractions of plants without fertilizer; and 17.71, 25.88, 20.37 and 18.03% in plants with fertilizer; all this with respect to plants without SA and without stress.

The significant accumulation of PRO with a decrease in protein fractions (ALB, GLB, PRL and GLT) in a severe stress condition, suggests that the content of PRO may be related to the hydrolysis of proteins (Goswami *et al*., 2020). In this research, the protein fractions were affected by hydric stress because this type of stress reduces the soluble carbohydrates of the leaves, amino acids, and total proteins (Dianat *et al*., 2016). While the application of SA did not increase the percentage of protein fractions. Regarding *P. coccineus*, Teniente-Martínez *et al*. (2016) reported a protein content of 21.9% for the species, while Jacinto-Hernández *et al*. (2019) indicated that with a larger grain size there is also lower protein content, with an average of 22.70%.

Conclusions

The humidity restriction in *P. coccineus* had negative effects on the biochemistry of the crop. The foliar application of salicylic acid improved the tolerance of the crop towards humidity restriction, favoring physiological processes such as relative water content, photosynthetic pigments, glycine betaine and total soluble sugars. The positive effects of salicylic acid must be considered in crop production, due to the erratic behavior of the climate worldwide.

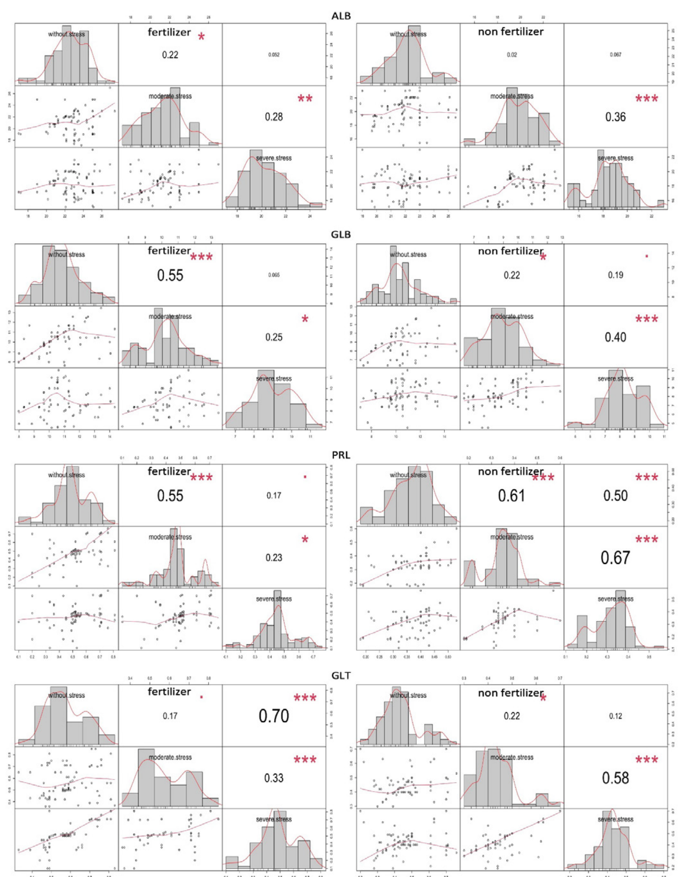

Figure 5. Extracted protein (%) in each protein fraction: albumins (ALB), globulins (GLB), prolamines (PRL) and glutelins (GLT); in *P. coccineus* under different doses of salicylic acid and humidity restriction. Asterisks indicate significant differences in each level of water stress, in plants with fertilizer and without fertilizer. *, α<0.05; **, α<0.01; ***, α<0.001.

Literature cited

Abdelaal, K.A.A., Attia, K.A., Alamery, S.F., El-Afry, M.M., Ghazy, A.I., Tantawy, D.S., & Hafez, Y.M. (2020). Exogenous application of proline and salicylic acid can mitigate the injurious impacts of drought stress on barley plants associated with physiological and histological characters. *Sustainability*, 12:1-15. http://dx.doi.org/10.3390/su12051736

Afshari, F., Nakhaei, F., Mosavi, S., & Seghatoleslami, M. (2022). Physiological and biochemical responses of *Stevia rebaudiana* Bertoni to nutri-priming and foliar nutrition under water supply restrictions. *Industrial Crops and Products*, 176: 114399. https://doi.org/10.1016/j.indcrop.2021.114399

Barba de la Rosa, A.P., Gueguen, J., Paredes-López, O., & Viroben, G. (1992). Fractionation procedures, electrophoretic characterization, and amino acid composition of amaranth seed proteins. *Journal of Agricultural and Food Chemistry*, 40:931-936. https://doi.org/10.1021/jf00018a002

Bates, L.S., Waldren, R.P., Teare, I.D., Bates, L.S., Waldern, R.P., & Teare, I. (1973). Rapid determination of free proline for water-stress studies. *Plant Soil*, 39:205-207. https://doi.org/10.1007/BF00018060

Chaimala, A., Jogloy, S., Vorasoot, N., Holbrook, C.C., Kvien, C.K., & Laohasiriwong, S. (2021). The variation of relative water content, SPAD chlorophyll meter reading, stomatal conductance, leaf area, and specific leaf area of Jerusalem artichoke genotypes under different durations of terminal drought in tropical region. *Journal of Agronomy and Crop Science*, 00:1-15. https://doi.org/10.1111/jac.12561

Dianat, M., Saharkhiz, M.J., & Tavassolian, I. (2016). Salicylic acid mitigates drought stress in *Lippia citriodora* L.: Effects on biochemical traits and essential oil yield. *Biocatalysis and Agricultural Biotechnology*, 8:286-293. https://doi.org/10.1016/j.bcab.2016.10.010

Dubois, M., Gilles, K.A., Hamilton, J.K., Rebers, P.A., & Smith, F. (1956). Colorimetric method for determination of sugars and related substances. *Analytical Chemistry*, 28:350-356. https://doi.org/10.1021/ac60111a017

Elhakem, A.H. (2019). Impact of salicylic acid application on growth, photosynthetic pigments and organic osmolytes response in *Mentha arvensis* under drought stress. *Journal of Biological Sciences*, 19:372-380. https://doi.org/10.3923/jbs.2019.372.380

FAO. (2015). Base referencial mundial del recurso suelo. Organización de la Naciones Unidas para la Alimentación y la Agricultura (FAO). Rome, Italy. https://www.fao.org/soils-portal/soil-survey/clasificacion-de-suelos/base-referencial-mundial/es/

Farouk, S., Arafa, S.A., & Nassar, R.M.A. (2018). Improving drought tolerance in corn (*Zea mays* L.) by foliar application with salicylic acid. *International Journal of Environment*, 7:104-123. http://www.curresweb.com/ije/ije/2018/104-123.pdf

Gadi, B.R., and Laxmi, V. (2012). Effect of salicylic acid and moisture stress on sugar content and sucrose synthase activity in Ziziphus seedlings. *Biochemical and Cellular Archives*, 12:21-23. http://www.connectjournals.com/achivestoc2.php?fulltext=1378201H_2.pdf&&bookmark=CJ-033216&&issue_id=01&&yaer=2012

Galon, L., Pawelkiewicz, R., Müller, C., da Silva, M.D., Orestes, S.C., Barretta, F.M., de Oliveira R.E.R., Agazzi, L.R., Aspiazú, I., & Forte, C.T. (2022). Morphophysiological changes in clearfield oilseed rape as a result of the application of ALS-herbicides and weed competition. *Journal of Plant Diseases and Protection*, 129:993-1003. https://doi.org/10.1007/s41348-022-00607-6

Ghanbari, A.A., Mousavi, S.H., Gorji, A.M., & Rao, I. (2013). Effects of water stress on leaves and seeds of bean (*Phaseolus vulgaris* L.). *Turkish Journal of Field Crops*, 18:73-77. http://www.field-crops.org/assets/pdf/product5211c60894006.pdf

Gholinezhad, E. (2020). Impact of drought stress and stress modifiers on water use efficiency, membrane lipidation indices, and water relationship indices of pot marigold (*Calendula officinalis* L.). *Brazilian Journal of Botany*, 43:747-759. https://doi.org/10.1007/s40415-020-00651-2

Gordillo-Curiel, A., Rodríguez-Larramendi, L.A., Salas-Marina, M.Á., & Rosales-Esquinca, M.Á. (2021). Effect of salicylic acid on the germination and initial growth of coffee (*Coffea arabica* L. var. Costa Rica 95). *Revista de la Facultad de Agronomía de la Universidad del Zulia*, 38(1):43-59. https://doi.org/10.47280/RevFacAgron(LUZ).v38.n1.03

Goswami, B., Rankawat, R., & Gadi, B.R. (2020). Physiological and antioxidative responses associated with drought tolerance of *Lasiurus sindicus* Henr. endemic to Thar desert, India. *Brazilian Journal of Botany*, 43:761-773. https://doi.org/10.1007/s40415-020-00666-9

Grieve, C.M., and Grattan, S.R. (1983). Rapid assay for the determination of water soluble quaternary ammonium compounds. *Plant and Soil*, 70:303-307. https://doi.org/10.1007/BF02374789

Hossain, A., Pamanick, B., Venugopalan, V.K., Ibrahimova, U., Rahman, M.A., Siyal, A.L., Maitra, S., Chatterjee, S., & Aftab, T. (2022). Emerging roles of plant growth regulators for plants adaptation to abiotic stress-induced oxidative stress. *In*: Aftab, T., Naeem, M. (Eds). Emerging Plant Growth Regulators in Agriculture. Academic Press, UK. pp: 1-72. https://doi.org/10.1016/B978-0-323-91005-7.00010-2

Idrees, M., Khan, M.M.A., Aftab, T., Naeem, M., & Hashmi, N. (2010). Salicylic acid-induced physiological and biochemical changes in lemongrass varieties under water stress. *Journal of Plant Interactions*, 5:293-303. https://doi.org/10.1080/17429145.2010.508566

Jacinto-Hernández, C., Coria-Peña, M., Contreras-Santos, G., Martínez-López, L., Zapata-Martelo, E., y Ayala-Carrillo, M.R. (2019). Azúcares totales y proteína en frijol nativo de la región Triqui Alta, Oaxaca. *Revista Mexicana de Ciencias Agrícolas*, 10:1667-1674. https://doi.org/10.29312/remexca.v10i7.2114

Kordi, S., Saidi, M., & Ghanbari, F. (2013). Induction of drought tolerance in sweet basil (*Ocimum basilicum* L) by salicylic acid. International *Journal of Agricultural and Food Research*, 2:18-26. https://doi.org/10.24102/ijafr.v2i2.149

Latimer, G.W. (2012). Official methods of analysis of AOAC international. 19th ed. Gaithersburg, Maryland, USA.

Lichtenthaler, H.K., and Buschmann, C. (2001). Chlorophylls and carotenoids: measurement and characterization by UV-Vis spectroscopy. In: Wrolstad, R.E., Acree, T.E., An, H., Decker, E.A., Penner, M.H., Reid, D.S., Schwartz, S.J., Shoemaker, C.F., Sporns, P. (eds). Current protocols in food analytical chemistry. John Wiley and Sons, New York, USA. Unit F4.3. https://doi.org/10.1002/0471142913.faf0403s01

Men, Y., Wang, D., Li, B., Su, Y., & Chen, G. (2018). Effects of drought stress on the antioxidant system, osmolytes and secondary metabolites of *Saposhnikovia divaricata* seedlings. *Acta Physiologiae Plantarum*, 40:191. https://doi.org/10.1007/s11738-018-2762-0

Morosan, M., Al Hassan, M., Naranjo, M.A., López-Gresa, M.P., Boscaiu, M., & Vicente, O. (2017). Comparative analysis of drought responses in *Phaseolus vulgaris* (common bean) and *P. coccineus* (runner bean) cultivars. *The EuroBiotech Journal*, 1:247-252. https://doi.org/10.24190/ISSN2564-615X/2017/03.09

Morr, C., German, B., Kinsella, J., Regenstein, J., Buren, J.V., Kilara, A., Lewis, B., & Mangino, M. (1985). A collaborative study to develop a standardized food protein solubility procedure. *Journal of Food Science*, 50:1715-1718. https://doi.org/10.1111/j.1365-2621.1985.tb10572.x

Muhie, S.H. (2022). Optimization of photosynthesis for sustainable crop production. *CABI Agriculture and Bioscience*, 3, 50. https://doi.org/10.1186/s43170-022-00117-3

Ozturk, M., Unal, B.T., García-Caparrós, P., Khursheed, A., Gul, A., & Hasanuzzaman, M. (2020). Osmoregulation and its actions during the drought stress in plants. *Physiologia Plantarum*, 1-15. https://doi.org/10.1111/ppl.13297

R Core Team (2022). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. https://www.R-project.org/

Rao, S.R., Qayyum, A., Razzaq, A., Ahmad, M., Mahmood, I., & Sher, A. (2012). Role of foliar application of salicylic acid and L-tryptophan in drought tolerance of maize. *The Journal of Animal & Plant Sciences*, 22:768-772. http://thejaps.org.pk/docs/v-22-3/42.pdf

Rehman, A., Safeer, M., Qamar, R., Mohsin-Altaf, M., Sarwar, N., Farooq, O., Mazher-Iqbal, M., and Ahmad, S. (2019). Exogenous application of salicylic acid ameliorates growth and yield of sunflower (*Helianthus annuus* L.) in saline soil. *Agrociencia*, 53:207-217. http://www.colpos.mx/agrocien/Bimestral/2019/feb-mar/art-6.pdf

Reyes-Matamoros, J., Martínez-Moreno, D., Rueda-Luna, R., & Rodríguez-Ramírez, T. (2014). Efecto del estrés hídrico en plantas de frijol (*Phaseolus vulgaris* L.) en condiciones de invernadero. *Revista Iberoamericana de Ciencias*, 1:191-203. http://www.reibci.org/publicados/2014/julio/2200132.pdf

Surabhi, G.K., and Rout, A. (2020). Glycine betaine and crop abiotic stress tolerance: an update. *In*: Roychoudhury, A., Tripathi, D.K. (Eds). Protective chemical agents in the amelioration of plant abiotic stress: biochemical and molecular perspectives. John Wiley & Sons, NJ, USA. pp: 24-52. https://doi.org/10.1002/9781119552154.ch2

Teniente-Martínez, G., González-Cruz, L., Cariño-Cortes, R., & Bernardino-Nicanor, A. (2016). Caracterización de las proteínas del frijol ayocote (*Phaseolus coccineus* L.). *Investigación y Desarrollo en Ciencia y Tecnología de Alimentos*, 1:1-6. http://www.fcb.uanl.mx/IDCyTA/files/volume1/1/1/1.pdf

Yan, X., Liu, J., Wu, K.X., Yang, N., Pan, L.B., Ying, C., Liu, Y. & Zhong-Hua, T. (2022). Comparative analysis of endogenous hormones and metabolite profiles in early-spring flowering plants and unflowered plants revealing the strategy of blossom. *Journal of Plant Growth Regulation*, 41:2421-2434. https://doi.org/10.1007/s00344-021-10452-w