Scientific Note

Promissory *Passiflora* species (Passifloraceae) for its tolerance to water-salt stress

Especies promisorias de *Passiflora* (Passifloraceae) por su tolerancia al estrés hídrico-salino

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Promissory species of Passifloraceae in hydroponic conditions with water-salt stress.
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Short title: SPECIES OF PASSIFLORACEAE TOLERANT WATER-SALT STRESS

Doi: https://doi.org/10.17584/rcch.2020v14i1.10574

Received for publication: 31-01-2020  Accepted for publication: 30-03-2020

ABSTRACT

The aim of this study was to determine the tolerance of water and salt stress in four cultivated species of *Passiflora* L. Eleven accessions of four species of *Passiflora* of commercial interest from different plantations were evaluated. The experimental design was in sub-split plots, where the plot was the percentage of water saturation in relation to the requirement of the crop (33% and 100%). The subplot was established by the saturation levels of salt (sodium chloride reactive level - 99.9% purity) (EC: 1.5 and 5.5 dS m⁻¹) with the 11 accessions in a completely randomized array with five replications, where the experimental unit was one plant. Results showed that accessions m11, m13 (*P. edulis f. flavicarpa*) and m15 (*P. tarminiana*) were determined as tolerant to salinity and drought; accesses m2 (*P. edulis f. edulis*), m12 and m14 (*P. edulis f. flavicarpa*) were moderately tolerant. These observations are the basis for future studies of drought tolerance in *Passiflora*, which must be followed by field evaluations.
Additional key words: \textit{Passiflora} spp.; abiotic stress; dry weight; genetic resources; stress physiology.

RESUMEN
El objetivo de este estudio fue determinar la tolerancia al estrés hídrico y salino en cuatro especies cultivadas de \textit{Passiflora} L. Se evaluaron 11 accesiones de cuatro especies de \textit{Passiflora} de interés comercial provenientes de diferentes plantaciones. El diseño experimental fue en parcelas subdivididas, donde la parcela principal fue el porcentaje de saturación de agua en relación con los requisitos del cultivo (33\% y 100\%). La subparcela se estableció por los niveles de saturación de sal (cloruro de sodio nivel reactivo – 99,9\% de pureza) (CE: 1.50 y 5.5 dS m\(^{-1}\)) con 11 accesiones en una disposición completamente aleatoria, con cinco repeticiones, donde la unidad experimental fue una planta. Los resultados mostraron que las accesiones m11, m13 (\textit{P. edulis f. flavicarpa}) y m15 (\textit{P. tarminiana}) se consideraron tolerantes a la salinidad y la sequía; las accesiones m2 (\textit{P. edulis f. edulis}), m12 y m14 (\textit{P. edulis f. flavicarpa}) fueron moderadamente tolerantes. Estas observaciones son la base para futuros estudios de tolerancia a la sequía en \textit{Passiflora}, que deben ser seguidos por evaluaciones de campo.

Palabras clave adicionales: \textit{Passiflora} spp.; estrés abiótico; peso seco; recursos genéticos; fisiología del estrés.

INTRODUCTION
Colombia is one of the countries with the highest number of cultivated species of \textit{Passiflora} due to its variety of ecological habitats (Restrepo \textit{et al.}, 2019). A total of nine species are cultivated from sea level to 2,800 m and the most important are \textit{P. edulis f. flavicarpa} Degener (passion fruit), \textit{P. ligularis} Juss. (sweet granadilla), \textit{P. edulis f. edulis} Sims (purple passion fruit), \textit{P. maliformis} L. (stone granadilla) and \textit{P. tarminiana} Coppens (Banana passion fruit). The area under cultivation in Colombia of these species covers about 10,000 ha (Agronet, 2019). Water is essential for plants of passion fruit as it promotes good growth and increases productivity, allowing continuous and regular production with good fruit quality (Jehová \textit{et al.}, 2013).

Productivity and development of plants of the genus \textit{Passiflora} are affected by solar radiation, temperature, sunshine and soil moisture loss. Factors such as water stress and extreme conditions
of salinity (> 2.5 dS m\(^{-1}\)) limit the productive potential of passion fruit (Cavalcante, 2012). Frequent and adequate water supply allows continuous flowering and fruiting, so long as other environmental conditions are favorable. Therefore, to achieve good yield and good quality of the fruit, water should be in adequate amounts.

According to Rodríguez et al. (2019), the term water deficit defines an essentially ecophysiological concept and relates to the limitation of water in tissues, which can be considered almost as a synonymous of stress by drought because this refers to any limitation of ideal operation of the plant imposed by the limited availability of water.

Two types of water stress are considered: one that is due to the total lack of rain and another caused by poor distribution of the same throughout the plant growth period (Rodríguez et al., 2019); in both cases, agricultural production is seriously affected. Anthropogenic global climate changes and phenomena such as El Niño (Phenomenon of the Pacific) are typical representatives that induce abnormal distribution of rainfall in agricultural regions. When this phenomenon occurs in the Colombian regions of the Caribbean and the Andes, with the exception of Magdalena Medio, there are reductions in precipitation values in the accumulated volume during the period of occurrence of the phenomenon, near or above 20%. Regions such as Guajira, north of Cesar, some municipalities of Atlántico, Bolívar, Sucre, Córdoba, the coffee triangle, Santander and Cundinamarca, in Colombia, show a well-marked decrease in precipitation, with values equal or higher to 60% of reduction in normal rainfall (Fan et al., 2017). Some of the regions affected by the drought induced by El Niño are Passifloraceae producing areas, making production difficult, since these plants do not have sufficient tolerance to water stress conditions; therefore, it is possible to consider that fluctuations in the distribution of rainfall caused by global warming may increase the risk of plants being repeatedly exposed to drought.

Saline soils are defined as those adversely modified for the growth of most of these species due to the presence of soluble salts, exchangeable sodium or both in the root zone (Yertayeva et al., 2019). Soils affected by these salts are common in arid and semiarid regions, due to low rainfall and high evaporation (Fageria et al., 2010).

Saline stress, according to Silveira et al. (2010), is related to two types of effects: osmotic and ionic. The first effects caused by the excess of salts are biophysical in nature, with osmotic effects standing out, restricting water transport. Then, a sequence of reactions modulated by hormones is
quickly triggered, leading to restriction of stomatal opening and photosynthetic assimilation of CO₂. These effects predominate in the first phase of salt stress ("osmotic phase"), which occurs in the initial stages of plant exposure to salinity or in the presence of moderate levels of salts in contact with the root system. The same authors reported that in the “osmotic phase”, in reality, it was a physiological response, that is, acclimatization to stress than in response to the damage suffered by the salt stress itself. This is often what is commonly diagnosed as symptoms of negative effects of salt stress, which are actually normal plant physiological responses to overcome or acclimate to that adverse situation.

As the saline ions accumulate in excess in the cytosol of the cells, toxicity problems will arise (toxic or ionic phase) in plants exposed to salinity (Silveira et al., 2010). However, species differ widely in protoplasmic resistance or tissue resistance to salt stress. This ability to resist is linked mainly to the intensity of compartmentalization of the saline ions inside the vacuoles and to the maintenance of a favorable K⁺/Na⁺ balance in the cytosol. Currently, this has been one of the targets for the selection of resistant cultivars from some cultures. In this sense, this study aimed to determine the tolerance of water and salt stress in four cultivated species of *Passiflora* L.

**MATERIALS AND METHODS**

The present study was carried out between February and September, 2016, in hydroponic conditions in greenhouse. The city of Manizales is in the coffee triangle, in the center of the department of Caldas, with coordinates 5°03’23.31” N at 75°29’41.56” W, altitude of 2,130 m s.n.m., with an average external temperature of 18°C, average annual rainfall of 2,000 mm and average relative humidity of 78%.

Eleven elite accessions of four *Passiflora* species of commercial interest were evaluated, previously assessed, characterized and selected in several producing regions of Colombia, based on the improvement program of Ocampo *et al.* (2013), and different gene banks from several institutions (Tab. 1).
Table 1. Commercial *Passiflora* accessions from different gene banks in Colombia selected to evaluate abiotic stresses (salinity and drought).

| Species                  | Origin    | Identification     |
|--------------------------|-----------|--------------------|
| *P. edulis* f. *edulis* Sims | Colombia-UNAL | m2–Cumbia2014 |
| *P. maliformis* L.        | Colombia-UNAL | m3–Cholupa2014 |
| *P. edulis* f. *flavicarpa* D. | Colombia-UNAL | m4–Calfla04/2014 |
| *P. edulis* f. *flavicarpa* D. | Colombia-UNAL | m6–Caufla01/2014 |
| *P. edulis* f. *flavicarpa* D. | Colombia-UNAL | m7–Tolfla02/2012 |
| *P. edulis* f. *flavicarpa* D. | Colombia-UNAL | m9–Atlafla01/2011 |
| *P. edulis* f. *flavicarpa* D. | Colombia-UNAL | m11–Valfla10/2010 |
| *P. edulis* f. *flavicarpa* D. | Colombia-UNAL | m12–Valluna/2014 |
| *P. edulis* f. *flavicarpa* D. | Colombia-UNAL | m13–Huifla07/2014 |
| *P. edulis* f. *flavicarpa* D. | Colombia-UCAL | m14–Calfia01/2015 |
| *P. tarminiana* C & B      | Colombia-UCAL | m15–Calmol01/2015 |

Source: Hurtado-Salazar et al. (2017).

The experimental design was completely randomized, in an array of sub-split plots, where the plot was the percentage of water saturation in relation to the requirement of the crop (100% and 33%). The subplot consisted of saturation levels of salt (sodium chloride reactive level - 99.9% purity) (EC: 1.5 dS m\(^{-1}\) and 5.5 dS m\(^{-1}\)) and each sub-subplot contained the 11 accessions of *Passiflora* in a completely randomized array with five replicates, where the experimental unit consisted of one plant.

Were planted 240 seeds of each accession, germinated in hydroponic bed, using rice husk as substrate, which was kept constantly wet until the time of transplantation. After 15 days of germination, 54 seedlings of each accession were selected to ensure greater uniformity of the root system.

Subsequently, the plants were transferred to hydroponic beds of 6 m length and 1.2 m wide to ensure irrigation sheet according to the treatments (percentage of water saturation 100 and 33% and saturation levels of salt EC: 1.5 and 5.5 dS m\(^{-1}\)), according to the experimental design. A conduit system was used in vertical trellis, with 12 cal.-galvanized wire, 2 m from the hydroponic greenhouse bed soil. The plants were conducted on a single stem, with weekly pruning of side branches. When the main stem surpassed the wire at 20 cm, they were bent down, forming a loop. Secondary and tertiary branches were maintained.

The modified Hoagland & Arnon’s universal nutrient solution (1950) by Niu et al. (2015), was used. To ensure the salinity of the treatment, each solution was brought to a value of EC: 1.5 dS m\(^{-1}\)
and 5.5 dS m\(^{-1}\), with the addition of reagent level sodium chloride (99.9% purity). To evaluate the interaction between the percentage of water saturation and salinity, electrical conductivity was added to the different treatments (1.5 dS m\(^{-1}\) and 5.5 dS m\(^{-1}\)) to the percentage of water of 100% and 33% for each of the experimental units of the accessions evaluated.

The production efficiency index (PEI) of dry matter to assess the effect of water and saline stress was estimated. This index allowed to classify the tolerant genotypes. This ratio can be calculated as described below (Fageria et al., 2010):

\[
PEI = \left( \frac{PANS}{PMANS} \right) \times \left( \frac{PBNS}{PMBNS} \right)
\]

where PEI – efficiency index of production of dry matter, PANS – production of dry matter with high salinity; PMANS – average production of the experiment with high salinity; PBNS – production with low salinity; PMBNS – average production of the experiment with low salinity.

Analysis of variance was performed and the averages were compared using the Tukey test, with a confidence level of 95 using SAS statistical package (Statistical Analysis System, 2013).

RESULTS AND DISCUSSION

According to Fageria et al. (2010) and their proposal of the production efficiency index of the dry weight of the aerial part. When the EIP is greater than 1.0, cultivars are classified as tolerant; in the range of 0.5 to 1.0 moderate salinity tolerance; if less than 0.5, the genotypes are classified as sensitive to salinity; therefore, it is worth noting how three accessions are tolerant and also how the same number of accessions are moderately tolerant (Tab. 2).

The total dry weight per accession decreased by approximately 21.2% in water (33% humidity) and saline (5.5 dS m\(^{-1}\)) stress conditions, except in tolerant accessions m15, m13 and m11, which showed dry matter accumulation higher than other accessions evaluated under similar conditions of maximum stress (33% moisture and 5.5 dS m\(^{-1}\)) and optimum humidity and electrical conductivity (100% and 5.5 dS m\(^{-1}\)). The accessions m2 (\textit{P. edulis} f. \textit{edulis}), m12 (\textit{P. edulis} f. \textit{flavicarpa}), and m14 (\textit{P. edulis} f. \textit{flavicarpa}) obtained PEI values between 0.5 and 1.0 and were classified as moderately tolerant.
Table 2. Influence of salinity in the dry matter of the aerial part (g) of 11 accessions of *Passiflora* and its classification in tolerance to salinity, according to the Production Efficiency Index (PEI).

| Accession | Level of salinity (dS m\(^{-1}\)) | PEI  | Classification\(^1\) |
|-----------|---------------------------------|------|---------------------|
|           | Control (1.5) (100% humidity) (g) |      |                     |
| m2        | 5.41 c                           | 0.51 e | MT                  |
| m3        | 4.00 d                           | 0.32 f | S                   |
| m4        | 1.61 ef                          | 0.09 g | S                   |
| m6        | 1.51 f                           | 0.29 f | S                   |
| m7        | 2.42 e                           | 0.25 fg| S                   |
| m9        | 3.42 d                           | 0.37 ef| S                   |
| m11       | 6.37 b                           | 3.76 a | T                   |
| m12       | 5.42 c                           | 0.50 e | MT                  |
| m13       | 6.42 b                           | 1.86 c | T                   |
| m14       | 7.42 a                           | 0.85 d | MT                  |
| m15       | 5.50 c                           | 2.61 b | T                   |
| Mean      | 4.45                             | 1.68g |                     |

\(^1\) T= tolerant, MT= moderately tolerant, MS= moderately sensitive S= sensitive. Methodology according to Fageria *et al.* (2010). *The values followed by different letters differ significantly (P<0.05) according to Tukey test.

Cellular osmoregulation gives plants the ability to tolerate conditions of lack of water and high salinity, with the expression of adaptive mechanisms that prevent the reduction of photosynthesis, changes in translocation and distribution of photoassimilates, and decreased performance. These events are of great importance in the normal growth and development of plants and, consequently, in crop productivity.

On the other hand, sensitive accessions showed a 45% decrease in the accumulation of dry weight. A study of *P. edulis* by Gomes *et al.* (2012), shows a reduction in dry matter content in the leaves, stem and roots when plants were exposed to water stress. Rodríguez *et al.* (2019), concluded that exposure of *P. ligularis* to drought resulted in a significant decrease in leaf area, number of leaves per plant and the length of the branches. However, root-air part ratio, based on dry weight and specific weight of the leaves, augmented with the increase of water stress in plants. The distribution pattern of dry leaves, branches, stems and roots was altered with drought.

The negative effect of water deficit is reflected in growth reduction of the aerial part, since much of assimilates are invested in the growth of the root system. Modifications in the root system occur due to the fact that root volume increases in water stress conditions, which favors water
uptake and decrease of the aerial part in order to reduce the rate of leaf transpiration (Rodríguez et al., 2019). Nevertheless, Hurtado-Salazar et al. (2018) found that the accession m15 (P. tarminiana) may be able to survive with salinity up to 5.5 dS m\(^{-1}\) (NaCl), which can be considered as high, with the removal of toxic ions. Possibly, the excretion of excess salt was done through epidermal glands. Sodium, in addition to other inorganic ions, is used for osmotic adjustment.

**CONCLUSION**

According to the PEI (Efficiency Index of Production of dry matter) methodology, accessions m11 (P. edulis f. flavicarpa), m13 (P. edulis f. flavicarpa) and m15 (P. tarminiana) were classified as salt tolerant, and accessions m2 (P. edulis f. edulis), m12 (P. edulis f. flavicarpa) and m14 (P. edulis f. flavicarpa) were classified as moderately tolerant. It is recommended to make studies of the tolerance mechanisms of the plants that presented potential such as stomatal closure, expression and signaling of abscisic acid. At the cellular level the osmotic adjustment, and the modification of gene expression under these levels of abiotic stress.

**Conflict of interests:** The manuscript was prepared and reviewed with the participation of the authors, who declare that there exists no conflict of interest that puts at risk the validity of the presented results.

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