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Physiological response of chickpea (*Cicer arietinum* L.) in symbiosis with arbuscular mycorrhizal fungi under salinity conditions

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ABSTRACT

Objective: Evaluate the effect of salinity due to NaCl on physiological variables of chickpea plants using native strains of arbuscular mycorrhizal fungi (AMF) *Glomus cubensis* and *Rhizoglomus irregulare* during the pre-flowering stage.

Design/methodology/approach: The research was carried out under controlled weather conditions using the chickpea variety N-29 as an experimental model. The treatments consisted on the combination of four salinity levels: 0, 25, 50 and 75 mM NaCl (variation source A) and AMF (variation source B) in three levels. In total there were 12 treatments with six repetitions, which were distributed in a completely randomized experimental design. The evaluated variables were number of green and dry leaves, dry biomass per organ (leaves, root and stem), net assimilation rate (NAR), relative growth rate (RGR) and leaf area ratio (LAR).

Results: The green leaves, the NAR and the dry biomass from roots and leaves, were the variables with the greatest response in the 50 mM NaCl+*R. irregulare* treatment, with an average increase of 15% with respect to the rest of the treatments.

Limitations/implications: A decrease on the evaluated variables was observed due to the salinity effect; however, chickpea plants subjected to NaCl 50 mM inoculated with *R. irregulare* were less affected by salt stress.

Findings/conclusions. The *R. irregulare* strain was found to contribute more than the *G. cubensis* to the mitigation of the adverse effects from the salinity factor.

Keywords: *Glomeromycota*, plant development, abiotic stress, NaCl.

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INTRODUCTION

Salinity is one of the fundamental problems facing agriculture on approximately 20% of the world's irrigated lands, and salinity in these areas is increasing due to other factors such as low precipitation and bad cultural practices, with the expectation of an increase of up to 50% by the year 2050 (Shrivastava and Kumar, 2015). Saline stress seriously affects cellular

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homeostasis causing ionic toxicity which reduces the development and productivity of cultivable species (Joshi *et al.*, 2017).

To reduce the effects caused by salinization on the crop's productivity, it is necessary to use tools and mechanisms that provide tolerance to this factor (Cardona *et al.*, 2017). Arbuscular mycorrhizal fungi (AMF) can be found in most soils and can form a symbiotic association with the majority of vascular plants, which allows the improvement of water and nutrients absorption, while conferring greater tolerance to different types of stress, both biotic and abiotic (Smith and Read, 2008). During the past few years, it has been shown that the successful inoculation of plants of agricultural interest with AMF promotes growth and improves plant performance, as a result of an improved absorption of nutrients (Cauich-Cauich *et al.*, 2022).

AMF are important bioenhancers for saline soils, since arbuscular mycorrhizal colonization mitigates the damage caused by soil salinization and improves the plant's growth, nutrition, and vigor (Evelin *et al.*, 2014). The plant-AMF symbiosis varies in form and efficacy depending on the plant, species and even on the genotype of the same species (Cobb *et al.*, 2016). In plants that thrive in saline conditions, mycorrhizal colonization can also improve root hydraulic conductivity at low water potential, stimulate and change the root system morphology and increase stomatal conductance (Latef *et al.*, 2016).

Chickpea (*Cicer arietinum* L.) is a legume of commercial importance and is consumed due to its nutritional properties, representing a great option manly due to its high protein content (Aguilar-Raymundo and Velez-Ruiz, 2013). 14.2 million Mg are produced annually around the world, in 14.8 million hectares, with a productivity of 0.96 Mg ha^{-1} (FAOSTAT, 2014), India being the first country in terms of production and productivity.

Chickpea is particularly sensitive to salinity (Flower *et al.*, 2010); efforts to find the difference in salinity tolerance have been undertaken by several authors (Hirich *et al.*, 2014).

The identification of chickpea crops that form an efficient symbiosis with mycorrhizal fungi and other endophytes could improve the physiological, biochemical an agronomic response of this species, when established in saline ecosystems (Bazghaleh *et al.*, 2018). There are currently few research on the AMF and chickpea plants grown in soil salinity conditions, for this reason, the current research's objective is to evaluate the crop's physiological response when established in NaCl based salinity conditions using two strains of AMF, as possible mitigators of the adverse effect of salt stress, in the chickpea 'N-29' crop, under experimental conditions.

MATERIALS AND METHODS

Location and experimental conditions

The experiment was established in a growth chamber with artificial lightning, with a photoperiod of 12 h, a daytime temperature of 26 °C and a nighttime temperature of 18 °C, until the flowering phenophase. The seeds used were treated previously with a 1% sodium hypochlorite solution, exposed for 10 min and then washed five times with sterile distilled water. Next, they were pre-germinated in sterile petri dishes at room temperature for 4 d, until the radicle and plumule emission. Subsequently, they were transplanted into

plastic containers with 700 g of Fluvisol soil (Hernández *et al*., 2015). The AMF inoculate was applied during the transplant.

A completely randomized design with factorial arrangement was used where two factors were evaluated: the salinity factor (A), with four levels corresponding to different concentrations of NaCl: 0, 25, 50 and 75 mM and a second factor (B) which was the soil inoculation with AMF strains in the next three levels: without AMF, *Glomus cubense* (Rodríguez *et al*., 2011) and *Rhizoglomus irregulare* (Sieverding *et al*., 2014), at a rate of 2 g per plant (45-50 spores g^{-1}). 12 treatments with 10 biological replicates were evaluated. The different saline solutions were applied twice per week, at a rate of 50 mL per plant. In the treatments that did not contain salinity, the same amount of water was applied and with the same frequency.

Evaluated variables

The number of green and dry leaves were evaluated, counting the existing quantity; and the leaf area using the Photoshop CS6 software. Likewise, the growth rate: net assimilation rate (NAR) through the relationship between values of the leaf area and the dry weight of the plant, expressed in g dm^{-2} d^{-1}; the relative growth rate (RGR) by using the formula:

$$RGR = \left[\left(final\ height - initial\ height \right) / number\ of\ elapsed\ days \right]$$

and the leaf area ratio (LAR) according to the methodology described by Beadle (1993). The dry biomass per organ (root, stem, and leaves) was determined according to the methodology described by Cardona *et al*. (2016).

Statistical analysis

For data processing, accomplishment of the theorical assumptions of homogeneity of variance was verified. Subsequently, a double classification analysis of variance was carried out, based on a linear model of fixed effects (Fisher, 1937) and in cases where there were significant differences between the averages, these were compared by using the Tukey test ($p \leq 0.05$) (Tukey, 1960). The determined indicators were standard error in the mean of treatments, coefficient of variation and unadjusted coefficient of determination for each variation source and for the interaction. The professional statistical package STATISTICA version 8.0 for Windows was used for all these analyses (StatSoft, 2008).

RESULTS AND DISCUSSION

In the green leaf's variable, there were significant differences between the established treatments ($p=0.0177$) (Figure 1). The greatest contribution to the total variability found in this variable was attributed to the salinity factor, contributing 52% ($R^2=0.52$), however, AMF contributed 36% ($R^2=0.36$), with a significant interaction between both, salinity factor and AMF, which contributed 10% ($R^2=0.1$).

In the dry leaves variable (Figure 1), salinity contributed 56% ($R^2=0.56$) of the total variability of the treatments; AMF contributed 27% ($R^2=0.27$) and there was also a highly significant interaction, contributing 16% ($R^2=0.16$). The obtained result demonstrates the

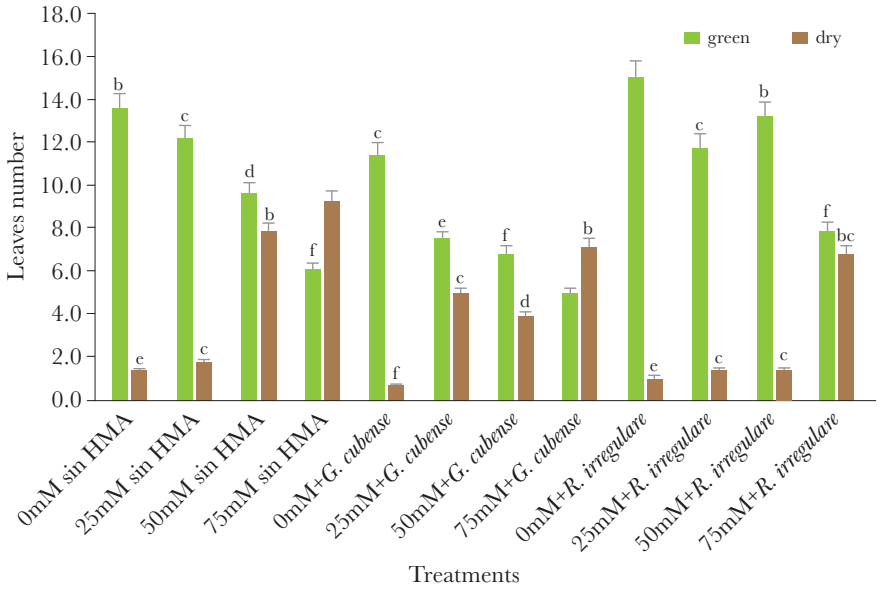

Figure 1. Number of green and dry leaves from the chickpea crop exposed to different NaCl levels inoculated with AMF. The vertical bars indicate the standard error of the means.

existence of a considerable sensitivity of the variety used in salinity, which can be degraded with the use of R. irregulare in agricultural practices in soils affected by this stressful condition. Cardona *et al.* (2016) indicated the use of these variables as precise indicators of salinity tolerance in the chickpea crops.

The number of green leaves decreased in the rest of the treatments, and the lowest values were registered on those plants that were under the NaCl effect without inoculation. Salinity is one of the main factors of environmental stress that directly affects the crop's development and productivity (Cardona *et al.*, 2017). Multiple studies have been aimed to finding alternatives that can mitigate the stress effects on the plants, including the use of AMF. Harris-Valle *et al.* (2011) suggest that native AMF corporations allow a better growth and water balance for the plants, by increasing leaf water by more than 82% under salinity conditions of 40 mM NaCl. Nakmee *et al.* (2016), found an increase of 23% in the biomass and 29% in the dry weight of sorghum grain (*Sorghum bicolor* L.) compared to the regulation without AMF inoculation.

Meriño *et al.* (2018) evaluated these indicators in different chickpea crops under salt stress effect and found a significant reduction in the number of green leaves at 50 mM NaCl. Elhindi *et al.* (2017), when evaluating the mycorrhizal effect under different levels of induced salt, like the established in the current essay, found significant reductions in the number of leaves, number of branches and dry matter from the organs in both mycorrhized plants and non-mycorrhized plants, which demonstrates the susceptibility of this species to the aforementioned abiotic factor.

The amount of dry biomass in the three measured organs (root, stem and leaves) also showed a significant decrease as NaCl concentration increased; however, when *R. irregulare* was applied, the effects decreased in more than 10% on the treatments inoculated with

this strain, obtaining a greater biomass from leaves and roots. The stem biomass was not significantly affected in the salinity treatments, but it did increase with the AMF inoculation (Table 1). In each evaluated organ, the total variability found (CV) was explained, in at least 97% (R^2), by the treatment's effects, which shows that with the linear model of fixed effects, used for statistical processing, was feasible to establish the differences between treatments.

These significant differences in the biomass production in the different salinity levels in both mycorrhized plants and non-mycorrhized plants could be related to the fact that the plants that were at the 75 mM saline level, did not overcome the possible nutritional interference generated in the absorbent complex of the soil by the Na^+ and Cl^- cations with respect to the essential nutrients required by the plants, or due to ionic toxicity caused by the low selectivity of the membranes or the non-activation of physiological, biochemical and/or molecular mechanisms of tolerances to the salinity conditions (Argentel *et al.*, 2016).

However, the plants that were in symbiosis with *R. irregulare* at a 50 mM NaCl concentration, presented a greater biomass. The current result shows the importance of the use of *R. irregulare* as a mitigator of the effects of salt stress in the variety used as an experimental model. In this regard, Cardona *et al.* (2017) reported similar results to the ones found in the current research, in Castilla Blackberry (*Rubus glaucus* Benth) in symbiosis with AMF exposed to different salt concentrations. Datta and Kulkarni (2014) found similar results to the ones reported on the current research, but on *Acaica arábica* crops under salt stress in mycorrhizal symbiosis and under similar conditions to the ones presented here.

Table 1. Dry biomass by organs (root, stems and leaves) from the chickpea crops inoculated with two AMF strains subjected to NaCl stress.

Treatments NaCl (mM), AMF	Dry biomass (g) (x±ES)			
	Leaves	Stem	Root	Total
0mM, without AMF	0.68±0.02 a	0.17±0.01 a	0.24±0.02 a	1.08±0.02 a
25mM, without AMF	0.61±0.05 ab	0.12±0.01 bc	0.19±0.01 bc	0.92±0.06 abc
50mM, without AMF	0.52±0.03 bcd	0.11±0.00 bcd	0.15±0.01 d	0.78±0.03 cd
75mM without AMF	0.43±0.09 cd	0.08±0.01 e	0.15±0.01 d	0.65±0.10 de
0mM+*G. cubense*	0.65±0.01 ab	0.11±0.01 bcd	0.18±0.02 bcd	0.94±0.02 ab
25mM+*G. cubense*	0.61±0.02 ab	0.09±0.00 cd	0.17±0.01 cd	0.87±0.03 bc
50mM+*G. cubense*	0.56±0.04 abc	0.12±0.01 bc	0.15±0.01 d	0.82±0.04 bc
75mM+*G. cubense*	0.51±0.02 bcd	0.12±0.01 bc	0.19±0.02 bcd	0.81±0.04 bc
0mM+*R. irregulare*	0.69±0.07 a	0.14±0.01 ab	0.22±0.01 ab	1.04±0.09 a
25mM+*R. irregulare*	0.59±0.02 ab	0.14±0.01 ab	0.22±0.02 ab	0.95±0.04 ab
50mM+*R. irregulare*	0.67±0.03 a	0.14±0.01 ab	0.24±0.01 a	1.05±0.03 a
75mM+*R. irregulare*	0.39±0.06 d	0.09±0.01 cd	0.15±0.01 d	0.62±0.06 e
CV	3.5	2.9	4.1	5.1
R^2	0.98	0.99	0.99	0.97

Different letters in the same column indicate significant differences, by Tukey ($p \leq 0.05$). ESx: Standard error of the mean.

The initial leaf area did not show significant differences, perhaps due to the little or no absorption of toxic cations and/or anions. However, the negative effect of salt stress at the end of the experiment was greater, with a variation percentage greater than 18%.

At the end of the experiment, a significant increase in the leaf area was found, against salinity, with a greater response when the plants where in symbiosis with *R. irregulare* at 50 mM NaCl. The previous results show that the inoculation with *R. irregulare* allowed the plants to acquire an adaptation to saline stress as the experiment developed.

These results agree with the those obtained by Abdul (2011) in the bean crops, which showed a 25% decrease in the leaf area after 10 d of applying 60 mM NaCl. Likewise, Abdolahpour and Lotfi (2014), observed a significant loss, greater than 40% of the leaf area with 75 mM NaCl in chickpea.

The net assimilation rate (NAR) decreased significantly in treatments where the highest concentration of NaCl (75 mM) was applied, even when AMF was inoculated. The percentage of variability found in this indicator was 14% (Table 2).

The fact that the NAR remained similar in the 0 mM NaCl and 50 mM+*R. irregulare* treatments demonstrates the contribution of this strain to the mitigation of the adverse effects of NaCl on the chickpea crop development.

The RGR and LAR decreased as NaCl concentration and stress exposure time increased. In both variables, the total variability found (CV) was explained in more than 97% (R^2) by the effect of the established treatments. The reduction on the treatments with 75 mM NaCl with and without AMF, were approximately 50% when compared to the treatments with 0 mM NaCl (Table 2). In return, plants subjected to concentrations of 50 mM with *R. irregulare* showed similar response to those of the controls.

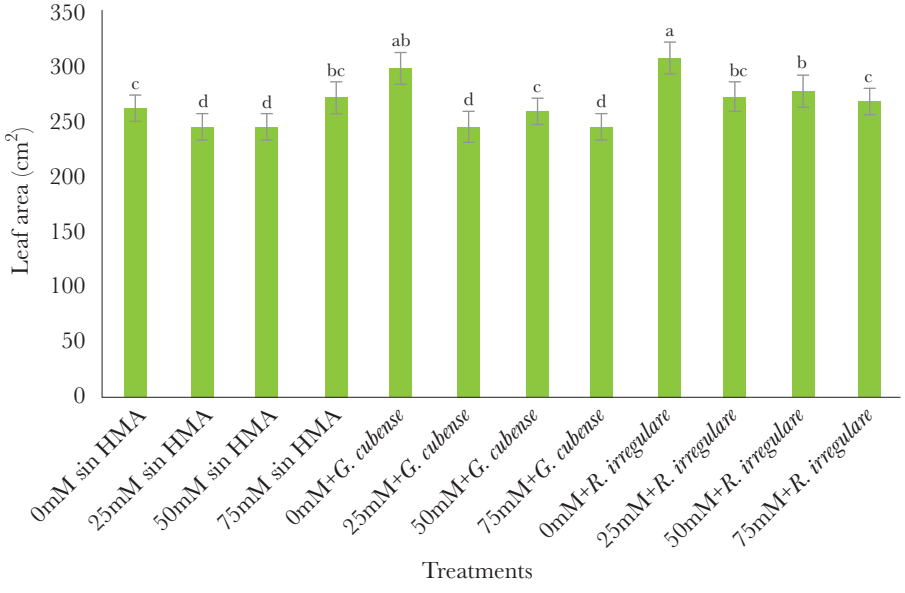

Figure 2. Chickpea plants leaf area in symbiosis with AMF exposed to different salt concentrations. (Different letters in the columns indicate significant differences, by Tukey ($P \leq 0.05$). Coefficient of variation: CV = 19.3%).

Table 2. Growth and development indicators.

Treatments NaCl (mM), AMF	Development indicators (X±ESx)		
	RGR (cm day^{-1})	LAR (cm^2)	NAR (mg cm^{-2} dm^{-1})
0mM without AMF	4.99±0.18 a	134.00±2.21 bc	0.33±0.0014 a
25mM without AMF	4.26±0.48 abc	127.47±2.58 bc	0.26±0.0011 c
50mM without AMF	3.87±0.23 abc	125.46±2.40 c	0.26±0.0011 c
75mM without AMF	2.75±0.70 bc	125.36±4.39 c	0.18±0.001 d
0mM+*G. cubense*	4.91±0.40 a	142.03±3.15 abc	0.30±0.002 b
25mM+*G. cubense*	4.44±0.30 abc	131.22±1.52 bc	0.25±0.001 c
50mM+*G. cubense*	3.86±0.38 abc	129.75±2.43 bc	0.22±0.001 c
75mM+*G. cubense*	3.68±0.22 abc	127.26±4.78 bc	0.21±0.001 cd
0mM+*R. irregulare*	5,06±0.40 a	158.64±4.75 a	0.32±0.0013 a
25mM+*R. irregulare*	4.59±0.43 ab	148.20±4.04 ab	0.27±0.0011 bc
50mM+*R. irregulare*	4.87±0.04 a	145.93±2.82 abc	0.32±0.0013 a
75mM+*R. irregulare*	2.65±0.49 c	126.94±2.21 bc	0.15±0.0006 e
CV	7.21	5.3	14.5
R^2	0.99	0.98	0.72

Different letters in the same column indicate significant differences, by Tukey ($p \leq 0.05$). ESx: standard error of the mean. RGR: relative growth rate; LAR: leaf area relation; NAR: net assimilation rate.

A similar trend can be observed in the LAR, where there was a significant reduction in the treatments with NaCl without inoculation and those inoculated with *G. cubense*, without significant differences between them. When the plants grew in symbiosis with *R. irregulare*, the leaf surface increased with respect to the breathing/respiratory mass (total biomass), not being so in those treatments subjected to 75 mM NaCl in which the greatest reductions are shown in this indicator.

Balliu *et al.* (2015), found that the relative growth rate decreased less when the plants where in symbiosis with the mycorrhizae under saline conditions, being the non-mycorrhizal plants the most affected. In experiments carried out by Miranda *et al.* (2011) it was shown that the leaf area ratio and the photosynthetic rate in the mycorrhized plants increased with respect to non-mycorrhized plants under saline conditions.

The research carried out revealed the variation in the response of the chickpea 'N-29' variety to salinity and the feasibility of using AMF to mitigate issues related to soil salinity. This result will contribute to increase the use of saline soils and food self-sufficiency in regions affected by this type of stress, mainly in areas where other species fail to reach their productive genetic potential and food production needs are more than necessary, essential.

CONCLUSIONS

In the chickpea 'N-29' variety, there was a reduction in the development variables as the saline concentration increased; however, these effects decreased with the use of *R. irregulare*.

Given the salinity conditions dictated, the variables with a greater response to stress were number of green leaves, the dry biomass of roots and leaves, and the net assimilation rate (NAR). These variables can be used as reference indicators for salinity tolerance in chickpea when arbuscular mycorrhizal fungi are used.

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