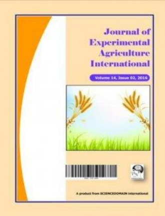

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Growth and Gas Exchanges of Arugula under Phosphate Fertilisation and Irrigation Depths

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Authors' contributions

This work was carried out in collaboration between all authors. All authors read and approved the final manuscript.

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ABSTRACT

Aims: To study the growth and gas exchanges of arugula plants under water stress and phosphate fertilisation as attenuator.

Study Design: The experimental design was randomised blocks, with treatments arranged in a 5 x 2 factorial scheme, corresponding to five doses of P_2O_5 (0, 50, 100, 150 and 200 mg dm^{-3}) and two irrigation depths, 50% of the real evapotranspiration (ETr) and 100% ETr, with four replicates, totaling 40 experimental units.

Place and Duration of Study: The experiment was carried out in a protected environment of the Center of Sciences and Agrifood Technology (CCTA) of the Federal University of Campina Grande (UFCG), Campus of Pombal-PB, Brazil, in the period from February 2 to March 31, 2015.

Methodology: Phosphorus (P) was applied 10 days before seedlings transplantation, which was

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homogenised. The source was single superphosphate, which has the following composition: 18% of P_2O_5, 16% of Calcium (Ca) and 8% of Sulfur (S). The real evapotranspiration was determined through weighing lysimetry in the treatments that received 100% ETr. For that, the weight of the pots at field capacity (WFC) (kg) was determined from the saturation through capillarity followed by drainage until constant weight, which was considered as WFC. The pots were daily weighed to obtain the actual weight (Wa) (kg) of each pot.

Results: The dose of 200 mg dm^{-3} under 100% ETr leads to the best production of fresh leaves of arugula; Phosphorus application improves the gas exchanges of arugula plants cultivated under 50% ETr, with the dose of 104 mg dm^{-3} P_2O_5 delivering the greatest gains in CO_2 assimilation rate.

Conclusion: Arugula growth and phytomass formation are reduced when plants are cultivated under 50% ETr, However, phosphorus application optimises the osmoprotection process of the arugula plants, but does not promote the increase of production under water deficit conditions.

Keywords: Eruca sativa Miller; net photosynthesis; water stress.

1. INTRODUCTION

Arugula is a relevant vegetable in the human diet and has been planted in various regions of Brazil. It is one of the most nutritious vegetables, containing minerals such as potassium, sulfur and iron, besides vitamins A and C [1].

Widely consumed in the form of salad, it is characterised by leaves with slight pungency, depending on the species and environment. In the last years, arugula has exhibited accentuated increase in both cultivation and consumption, compared with other leafy vegetables. Its main producing regions are the South and Southeast of the country, which have about 85% of the national production. Although arugula is adapted to milder climate conditions, there are small areas with this leafy vegetable in the Northeast region of Brazil, generally close to the large consuming centres [2].

The cultivation of this vegetable is mainly concentrated among medium and small producers, which gives it economic and social importance, being a factor of aggregation between the man and the land [3], which makes it a potential crop for cultivation in the semi-arid region.

However, in this region, the water balance is negative, i.e., the sum of water exits is higher than the sum of its entries. Thus, arugula cultivation can only be viable using irrigation, which can generate environmental problems and costs with the irrigation systems. Thus, it is important to use strategies to increase water use efficiency.

As a strategy to solve the problem of water stress, the plant tends to activate secondary pathways of formation of metabolites, such as glycine betaine and proline, elements considered as osmoprotectants, because they reduce leaf water potential and guarantee the absorption of water and nutrients [4,5].

However, this production depends on inorganic compounds, such as phosphorus [6], which participates in approximately 0.2% of the plant biomass, either in nucleic acids and/or in the formation of the main energy molecule, the adenosine triphosphate (ATP). Thus, it is an important element in all biochemical reactions and metabolic cycles of the plant, regardless of the type of metabolism, C3, C4 or CAM [7].

Such mitigating effect of phosphorus was analysed by Kuwahara and Souza [8] in Braquiaria brizantha, cv. ‘MG-5 Vitória', and these authors observed that water deficit caused significant reductions in stomatal conductance and net CO_2 assimilation in all treatments. However, the results of gas exchanges indicated that, effectively, the supplementation of P in the crop fertilisation promoted better recovery of the plants after a water deficit period.

Furthermore, there is information in the literature that highlights P as a potential attenuator of water stress, such as the works of Firmano et al. [9], who evaluated the soybean crop (*Glicine max*) and Kuwahara et al. [5], who evaluated plants with C4 metabolism, all with plants under water stress and P doses, based on studies of growth and gas exchanges, which can be performed to identify, in the arugula crop, the possibility of cultivation under water deficit conditions.

Thus, the objective was to study the growth and gas exchanges of arugula plants under water stress and phosphate fertilisation as attenuator.

2. MATERIALS AND METHODS

The experiment was carried out in a protected environment of the Center of Sciences and Agrifood Technology (CCTA) of the Federal University of Campina Grande (UFCG), Campus of Pombal-PB, Brazil, in the period from February 2 to March 31, 2015, at the geographic coordinates of 6º46' S and 37º47' W, mean altitude of 184 m, with mean temperature of 28°C in the municipality [10].

The experimental design was randomised blocks, with treatments arranged in a 5 x 2 factorial scheme, corresponding to five doses of P_2O_5 (0, 50, 100, 150 and 200 mg dm^{-3}) and two irrigation depths, 50% of the real evapotranspiration (ETr) and 100% ETr, with four replicates, totaling 40 experimental units. Each experimental unit consisted of a 4L pot and the soil was characterised as a Fluvic Neosol, collected in the 0-20 cm layer. The soil used in the experiment was chemically characterised according to the procedures described in Claessen [11] indicated respectively: pH = 6.02; P = 3 and 5 mg dm^{-3}; H + Al = 0.03 mmol dm^{-3}; K = 0.41 mmol dm^{-3}; Ca = 4.15 mmol dm^{-3}; Mg = 7.05 dm^{-3}; Si = 1.95 mg dm^{-3}. The results were used to plan the fertilisation.

Phosphorus (P) was applied 10 days before seedlings transplantation, which was homogenised. The source was single superphosphate, which has the following composition: 18% of P_2O_5, 16% of Calcium (Ca) and 8% of Sulfur (S).

The real evapotranspiration was determined through weighing lysimetry in the treatments that received 100% ETr. For that, the weight of the pots at field capacity (Wfc) (kg) was determined from the saturation through capillarity followed by drainage until constant weight, which was considered as WFC. The pots were daily weighed to obtain the actual weight (Wa) (kg) of each pot. With these data, equation 1 was used to determine the ETr, by dividing the subtraction of these values by the area of the pot (A) (m^2).

$$\mathrm{LI100\%ETr} = \frac{\mathrm{Wfc-Wa}}{\mathrm{A}} = \mathrm{mm} \qquad [1]$$

Fertilisations with macronutrients (except nitrogen) and micronutrients were applied according to the recommendation of Malavolta [12]. The applied quantities of nutrients were: K = 64.7 mg dm^{-3}; N = 35.6 mg dm^{-3}. Potassium nitrate and urea were applied 7 days after transplantation, and nitrogen was split into four applications to avoid leaching and ensure that no damage would be done to the crop. The P doses were applied and incorporated to the soil 10 days before transplantation, along with Ca^{2+}. Fertilisation with Mg was not necessary, because its content in the soil was adequate for the crop.

For the micronutrients, solutions were prepared for application via soil with the following compositions: B = 1.144; Cu = 2.05; Zn = 7.0; Mn = 6.052; Mo = 0.1 g/L, and Fe-EDTA 4 mL/pot, using sources that were pure (A.R.) and highly soluble in water. A single fertilisation with micronutrients was applied 15 days after transplanting the seedlings, with the application of the previously prepared solutions: Cu and Zn were used in the first solution; then B, Mn and Mo were used in the second solution, which were applied as 10 mL per pot, while the Fe-EDTA was separately applied, 4 mL per pot.

The arugula seedlings were produced on expanded polystyrene trays (200 cells), with volume of 16 mL per cell, which received two seeds. The seedlings emerged 7 days after sowing (DAS) and, at 30 DAS, two seedlings were transplanted to each pot and conducted until completing the cycle (58 DAS).

The experiment used the arugula variety 'Cultivada', which has the following characteristics: elongated and lobed leaves, with dark green color and spicy taste.

At 7, 14, 21 and 28 days after transplantation (DAT), plant growth was evaluated by measuring the number of leaves (NL), counting the photosynthetically active leaves, and plant height (PH) (cm), considering the length between the base of the plant and the tip of the longest leaf extended.

At 28 DAT, gas exchanges were determined using an infrared gas analyser, measuring the transpiration rate in the leaves (mmol of H_2O $m^{-2}s^{-1}$ (E)), intercellular CO_2 concentration (mmol of CO_2 m^2 (Ci)), stomatal conductance (mmol m2 s^{-1} (gs)) and CO_2 assimilation rate (μmol CO_2 m^{-2} s^{-1}(A)). These data were used to calculate the instantaneous water use efficiency (IWUE), based on the relationship between the values of A and E (A/E) [13]. The measurements of gas exchanges were taken from 7 to 9 a.m., in all plants, on a fully expanded leaf and without herbivory, using an artificial light source with the intensity of 1200 mmol m^{-2} s^{-1}, and CO_2 from the atmosphere at height of 3 m.

After taking the readings, the plants were harvested and separated into leaves, stem and roots to determine leaf fresh phytomass (LFP) (g), by weighing the fresh leaves on analytical scale; leaf dry phytomass (LDP) (g), by weighing the leaves after drying in forced-air oven; root dry phytomass (RDP) (g), by weighing the roots after drying in forced-air oven; and total dry phytomass (TDP) (g), obtained by the sum of LDP and RDP.

The obtained data were subjected to F test ($p<0.05$), which was conclusive for the factor irrigation depths. For P doses, polynomial regression analysis was performed using linear or quadratic models, selected based on the significance of the regression coefficients at 0.01 (**) and 0.05 (*) probability levels, obtained in the F test, and on the highest coefficient of determination (R^2). The follow-up analysis of the interaction between sources and doses was made when the effect was significant, applying regression analysis for the factor P doses in each irrigation depth, using the program Sisvar [14].

3. RESULTS AND DISCUSSION

For the number of leaves and plant height, the best results were observed in plants cultivated under adequate water conditions, 100% ETr, except in the evaluation at 7 DAT (Fig. 1A and 1B), which was due to the time of stress. For P doses, there was no significant effect, which may have occurred due to the low concentration of water present in the soil solution, which compromised the solubilisation of P and, consequently, its absorption by the root system of the plants, which limited the number of leaves and plant height.

Regarding gas exchanges, there was significant interaction for transpiration (E), net photosynthesis (A) and instantaneous water use efficiency (IWUE). The stomatal conductance (gs) suffered isolated effect of the applied P doses (Fig. 2).

For the intracellular CO_2 concentration (Ci) (Fig. 2A), there were differences only between the irrigation depths, with highest mean values in plants cultivated under ideal water conditions, on the order of 284.65 mmol mol^{-1} of CO_2, which is 5.9% higher than the values obtained for the irrigation depth of 50% ETr, equal to 267.85 mmol of CO_2 m^2. However, the observed values are equivalent to those found in C3 plants [7], which leads to the conclusion that there was no limitation in the availability of CO_2 for photosynthesis.

For leaf transpiration rate (E) (Fig. 2B), there was no significant difference between the irrigation depths, with mean values of 4.32 and 4.27 mmol of H_2O m^{-2} s^{-1}, respectively, at the lowest and highest irrigation depths (50% and 100% ETr). For the effect of P, there were different responses with the irrigation depths. When plants received irrigation depth equivalent to 50% ETr, the dose of 170 mg dm^{-3} P led to increment of about 14,52% at leaf transpiration rate. While what for the irrigation depth of 100% ETr, the highest mean values were observed at the estimated dose of 31.66 mg dm^{-3}.

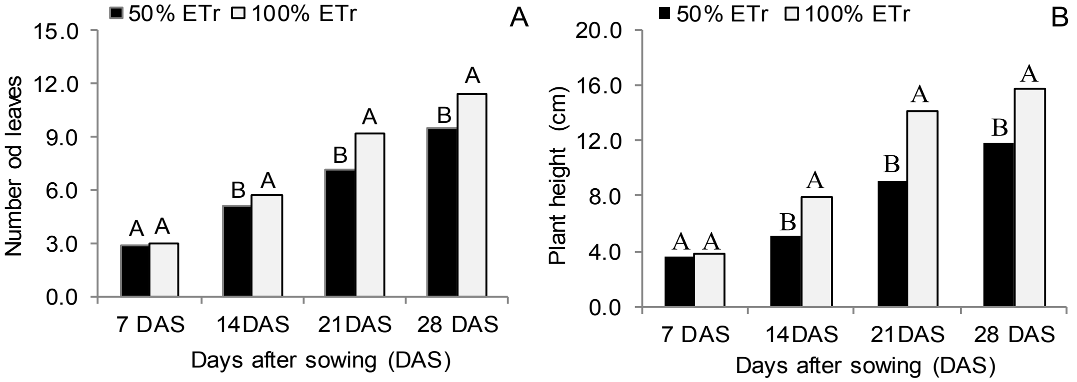

Fig. 1. Number of leaves and plants height (cm) at 7, 14, 21 and 28 days after sowing (DAS) in arugula plants cultivated under water stress conditions, 50% ETr and in adequate water conditions, 100% of ETr and doses of phosphorus

Means followed by same letter do not differ significantly by test of F at 0.05 of probability

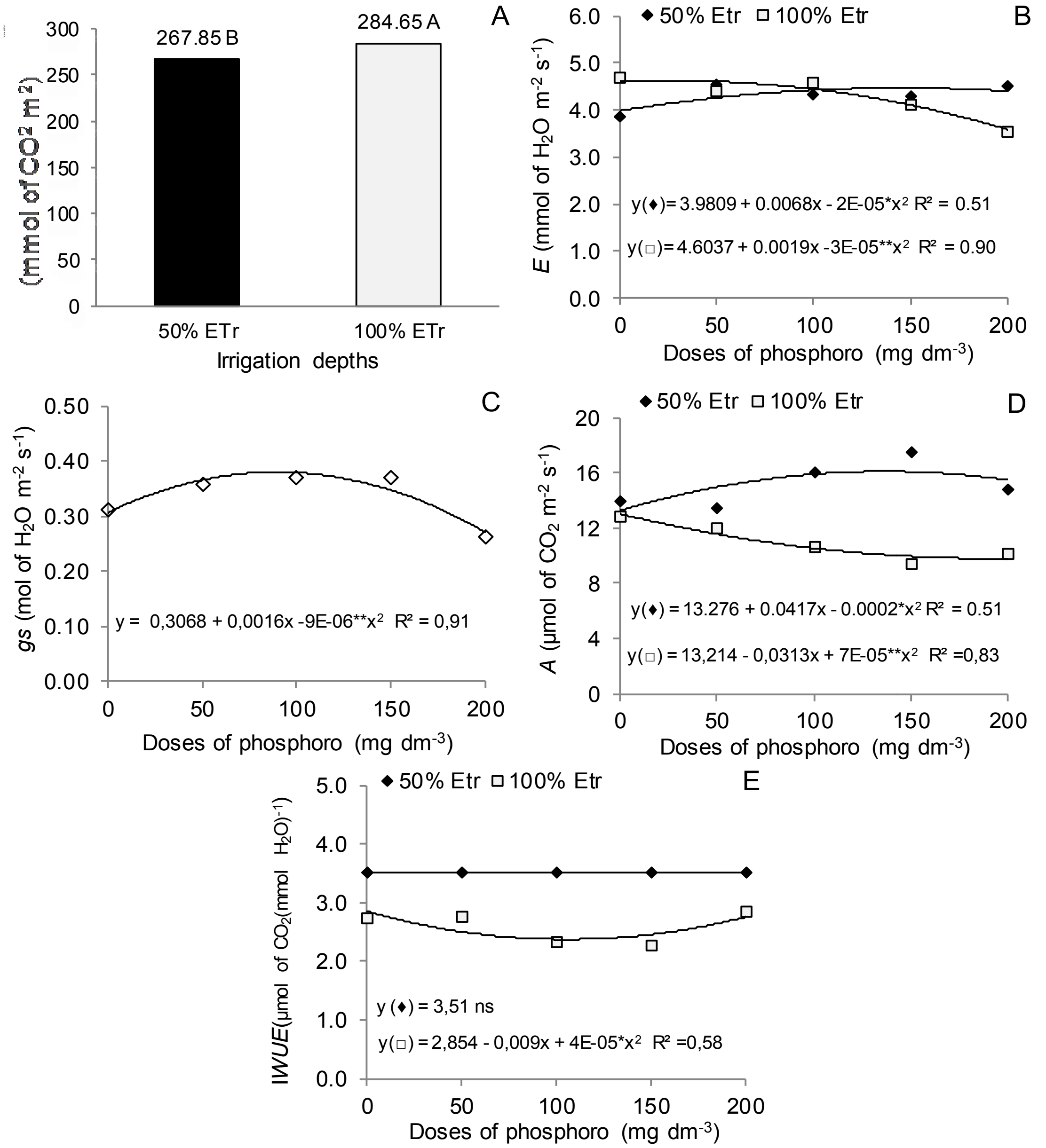

Fig. 2. Plants of arugula cultivated under water stress conditions, 50% of ETr and in water conditions suitable for the cultivation of arugula, 100% ETr and doses of phosphorus at 28 days after transplant (DAT) for: intercellular CO2 concentration (A), transpiration rate in the leaves (B), stomatal conductance (C), liquid photosynthesis (D) and water use efficiency (E)
**P<0.05; **P<0.01*

For stomatal conductance (gs), there was significant effect only of the applied P_2O_5 doses, and the estimated dose of 88.89 mg dm^{-3} led to the highest value (Fig. 2C), equal to 0.38 mmol m^{-2} s^{-1}.

The effects of P on stomatal conductance and especially on plant transpiration can be related to mechanisms of tolerance when plants were under the lowest irrigation depth. Hence, the increase in the P dose may have caused energetic conditions to guarantee stomatal opening and transpiration, in order to increase the inflow of CO_2 used in photosynthesis, as shown in Fig. 2D. Under water deficit conditions, the net photosynthesis increased until the estimated P dose of 104 mg dm^{-3}, which is interesting, because this dose led to a photosynthesis value of 15.45 μmol of CO_2 m^{-2} s^{-1}, which is higher than that observed in plants under 100% ETr (10.98 μmol of CO_2 m^{-2} s^{-1}), evidencing the influence of P on water stress. Such effect, however, may be related to the need to produce osmoprotective compounds [4,5], which may be confirmed in the study on phytomass formation.

It is known that gas exchanges are regulated by the stomatal movement, in which the absorption of CO_2 is dependent on a higher transpiration, notably in C3 plants, thus evidencing that the reduction in transpiration limits the CO_2 entry in the substomatal chamber [15]. Hence, plants need to have higher water use efficiency, for maximum CO_2 with a minimum water loss [7], which did not occur in arugula plants under water stress at the irrigation depth of 50% ETr, because although the regression equation fitted, the values of instantaneous water use efficiency (IWUE) ranged from 2.4 to 3.0 μmol of CO_2 (mmol H_2O) (Fig. 2E).

For leaf fresh phytomass (LFP), leaf dry phytomass (LDP), root dry phytomass (RDP) and total dry phytomass (TDP), there were interactions between the irrigation depths and P doses, and the best results were observed in arugula plants cultivated under irrigation depth of 100% ETr (Fig. 3 A, B, C and D). The greater availability guarantees conditions of turgor and flow of nutrients, which allow plant growth and development [7].

According to the effect of P at each irrigation depth, for plants irrigated with 100% ETr, the increase in the P doses led to linear increments in leaf fresh phytomass, leaf dry phytomass and total dry phytomass, on the order of 21.9%, 14.5% and 12.2%, respectively, for every increment of 50 mg dm^{-3} in the applied P dose.

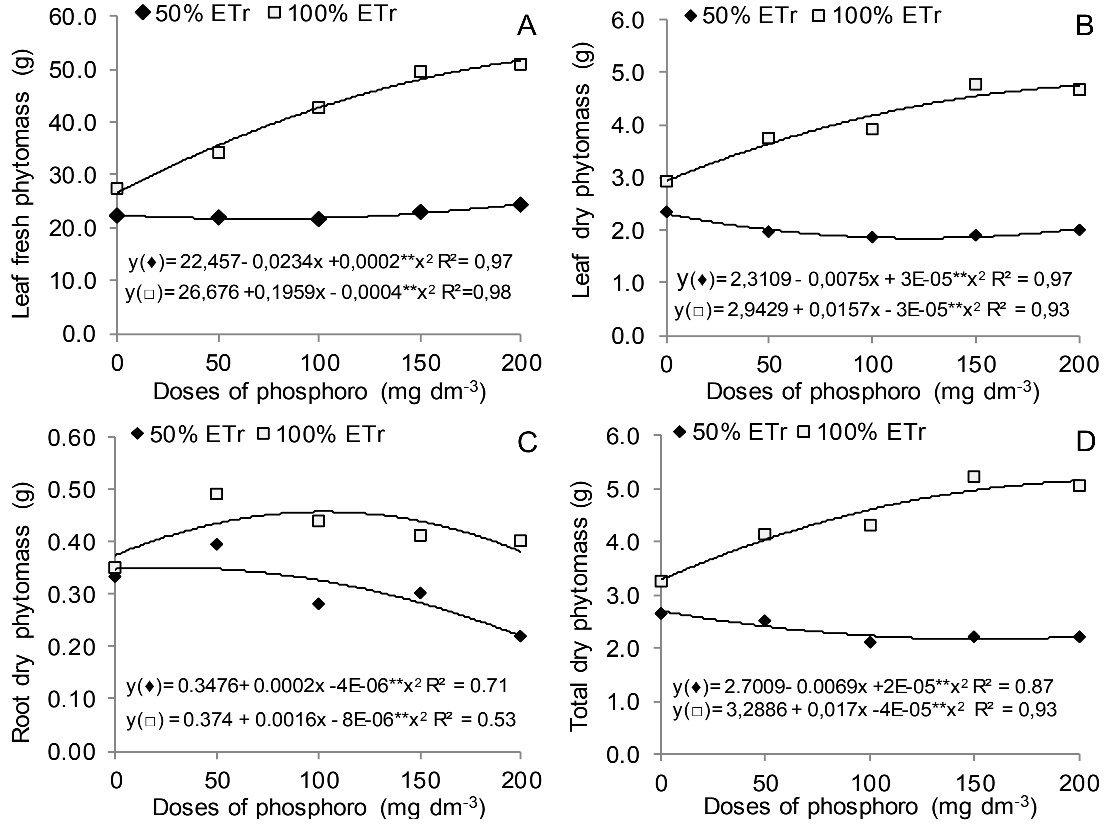

Fig. 3. Plants of arugula cultivated under water stress conditions, 50% of ETr and in water conditions suitable for the cultivation of arugula, 100% ETr and doses of phosphorus at 28 days after transplant (DAT) for: fresh leaf phytomass (A), dry leaf phytomass (B), dry root phytomass (C) and total dry phytomass (D)

***P<0.01*

In this case, the irrigation depth of 100% ETr, combined with the fertilisation of 200 mg dm^{-3} of P_2O_5, promoted the best production of the arugula crop. At the irrigation depth of 100% ETr, it is also observed that the ideal P dose was 100 mg dm^{-3} of P_2O_5 for the root dry phytomass, which may be related to the limitation caused by the container used in the cultivation, because the arugula plants were grown in pots. This situation was also observed in the irrigation depth of 50% of the ETr, however, from the dose of 25 mg dm^{-3} of P_2O_5.

At irrigation depth of 50% ETr, the formation of leaf fresh and dry phytomasses was little influenced by the increment in the P dose, although there was significant effect on the physiological variables when plants were cultivated under water stress. Thus, the theory that the increase in net photosynthesis and transpiration was due to the need to form secondary compounds for protection against water deficit has been confirmed. That being confirmed, there were reductions in total dry phytomass on the order of 4.6% for every increase of 50 mg dm^{-3} in the P dose. In this case, it is possible to claim that P helps the mechanisms of tolerance; however, it was not sufficient to maintain the total dry phytomass of the plants, which was limited by the water deficit.

4. CONCLUSION

The dose of 200 mg dm^{-3} under 100% ETr leads to the best production of fresh leaves of arugula;

Arugula growth and phytomass formation are reduced when plants are cultivated under 50% ETr, However, phosphorus application optimises the osmoprotection process of the arugula plants, but does not promote increase of production under water deficit conditions.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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