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Research article

STUDY ON THE FOLIAR APPLICATION OF FITOMARE® ON DROUGHT TOLERANCE OF TOMATO PLANTS

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ABSTRACT: Tomato plants are very sensitive to drought. When grown under conditions below their water needs, there is a decrease in their development due to the negative effects produced by water deficit on the plant's physiological processes. Foliar applications of Biostimulant products rich in amino acids can activate a series of physiological and biochemical processes in the plant that can help to mitigate the negative damages from drought. In this experiment, the foliar application of Fitomare® (a product formulated from algal extracts, free amino acids and macro and micronutrients) was evaluated, as a product that is able to increase drought tolerance in tomato plants. This product was compared to a product that was formulated with free amino acids, but without plant extracts or macro and micronutrients in its composition. For this, tomato seedlings were subjected to 4 treatments: watering at 100% ETc (control), Drought (DRT), Drought + Fitomare® (foliar application; DRT+Fito), and Drought + Product rich in amino acids (foliar application; DRT+PC). At the end of the experiment, measurements were performed on the net assimilation rate of CO_2 (A_{CO2}), the stomatal conductance (g_s) and WUE (A_{CO2}/leaf transpiration), growth parameters, organic solute concentration (proline and reducing sugars), and oxidative stress (MDA). Among the most relevant results obtained, we find that the application of Fitomare® increased the tomato plant's tolerance to drought, as these plants were more developed as compared to the plants from the other drought treatments. This was due to the application of Fitomare®, which allowed the plants to maintain a more efficient use of water (A_{CO2}/E_{leaf}). The biochemical markers proline and MDA, which are used to measure the intensity of stress suffered by the plants, indicated that the plants treated with Fitomare® had the least damage from drought.

Key words: Biostimulant, Water deficit, Water relations, Gas exchange parameters.

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INTRODUCTION

Water deficit is one of the most important environmental limitations that can affect the productivity of agricultural crops. It is estimated that around 10% of the planet's surface is affected by drought and/or salinity, which causes many hectares of agricultural land to be abandoned each year [1]. In Spain, mainly in the Southeast (Alicante, Murcia and Almeria), drought has become one of the most severe environmental problems faced by the farmer. For example, the 2013/2014 growing season was considered to be the driest in the last 150 years.

Currently, scientific research on the improvement of the plant's tolerance to drought conditions have been mainly directed towards two aspects: on the one hand i) research at the molecular level in improvement programs [2], and on the other, ii) research at the agronomical level, evaluating growing techniques, genotypes, or applications of products that help plants cohabit with drought [3]. Regarding the latter, in the 2nd world congress on the use of biostimulants in Florence, 2015, the issue that the use of these products could be a good alternative to palliate the negative effects of water deficit in crops [4] was discussed.

The Biostimulants used in agriculture are products that contain substances and/or microorganisms that when applied to crops are able to stimulate physiological processes and increase the absorption and efficiency of mineral nutrients. This leads to an increase in production and quality of the fruit, and can also increase salinity and drought tolerance [5, 6, 7, 8]. Europe is the continent where these products are most applied; according to EBIC (European Biostimulants Industry Council) data, in 2012, 6.2 million hectares were treated with Biostimulants. One of the most-used biostimulants to date are those that are produced from algae. There are many publications that have shown that these types of products improve various aspects of the crops, such as seed germination, production, plant response to abiotic stresses and increased resistance to pathologies caused by insects and fungi [9, 10, 11, 12, 13, 14, 15]. The objective of this study was to evaluate the effectiveness of the application of Fitomare® (Atlántica Agrícola) on tomato plant's drought tolerance. This product was formulated with marine algae (*Ascophyllum nodosum*), enriched with amino acids and macro and micro elements that boost the effect that the algal extracts have on the plants.

MATERIALS AND METHODS

Plant material and growing conditions

For this research study, tomato plants from the variety "Optima" were used, and these were acquired from a commercial nursery (Baby Plant, Santomera, Murcia). The seedlings were transplanted to 1.5L pots with perlite used as the substrate. The experiment took place in a growth chamber under the following conditions: average daytime temperature 23-24.5 ºC, 16h photoperiod and relative humidity between 40 and 65%. During the acclimation period, the plants were watered to 100% ETc with Hoagland nutrient solution: 6 mM KNO_3, 4 mM $Ca(NO_3)_2$, 1 mM KH_2PO_4, 1 mM $MgSO_4$, 20 µM Fe^{+3} masquolate, 25 µM H_3BO_3, 2 µM $MnSO_4$, 2 µM $ZnSO_4$, 0.5 µM $CuSO_4$, 0.5 µM $(NH4)_6Mo_7O_{24}\cdot 4H_2O$.), prepared with de-ionized water.

Drought and foliar treatments applied

After 9 days of acclimation, six plants were used for the foliar application of Fitomare® at a concentration of 3% (Fito; http://www.atlanticaagricola.com/productos.php?ct=27#), with another six plants sprayed with a different product (PC), which was formulated in the lab using only free amino acids, with the same composition and concentration as Fitomare (see Table 1). However, this product did not contain algal extracts, and no macro or micronutrients were added. The drought period started the same day the products were applied. The drought treatments consisted in not watering until the humidity level of the substrate reached 40% of field capacity (FC; approximately 6 days). At this point, the products were again applied, and from this point on, the humidity of the substrate was maintained constant (40% FC) adding water everyday according to the water lost due to transpiration. The water lost was calculated by weighing the pots twice a day. The drought treatment lasted for 6 days. In parallel to these two treatments, plants watered to 100% ETc (control) and plants under drought but without any product added (drought; DRT) were grown. In total, four treatments were assayed: Control, DRT, DRT+Fito and DRT+PC.

Physiological parameters

Two days after the end of the experiment, the gas exchange parameters were measured: net CO_2 assimilation rate (A_{CO2}), stomatal conductance (g_s), leaf transpiration (E_{leaf}) and the instantaneous leaf water efficiency (WUE = A_{CO2}/E_{leaf}), using a portable gas analyzer (model CIRAS-2, PP-System, Amesbury, MA, USA). For the measurements, the equipment was set up to maintain constant light intensity (PAR: 1000 µmol m^{-2} s^{-1}) and CO_2 concentration (400 ppm) during the measurements.

Morphological parameters

At the end of the experiment, the plants were harvested and weighed, with the leaves, stem and roots weighed separately. The plant parts were briefly rinsed in deionized water and they were then dried in an oven at 60 ºC for at least 48 h. After this, they were weighed again, and milled to obtain a fine powder for further analysis in the laboratory. With these data, the total dry biomass was calculated (sum of the dry weight of leaves, stem and roots), as well as the leaf water content (fresh weight – dry weight)/dry weight, expressed as g H_2O g^{-1} dry weight (dw). The leaf area was determined with a leaf area meter (model LI-3100C, LI-COR).

Biochemical parameters

At the end of the experiment, the concentrations of proline, reducing sugars and malondialdehyde (MDA) were measured in leaves. The proline was extracted from the dry leaf tissue with sulfosalicylic acid (3%) and was quantified according to the protocol described by Bates et al. [16]. On their part, the reducing sugars were extracted from dry leaf tissue with ethanol (80%) and was quantified according to protocols described by Nelson [17] and Somogy [18]. In order to determine if oxidative damage was present in the plants, lipid peroxidation was analyzed through the measurement of malondialdehyde (MDA) using the method by Hodges et al. [19]. Samples of fresh leaf tissue were taken, and homogenized with 10% trichloroacetic acid (TCA) and quantified with thiobarbituric acid (TBA).

Statistical analysis

The statistical analysis included an analysis of variance (ANOVA) calculated with the SPSS statistical program v19 (Chicago, IL, USA). Six plants were used per treatment, randomly distributed between two trays, which were placed on tables in the growth chamber. When the results were significant ($p<0.05$), the means where further analyzed using Duncan's multiple range test.

Table 1. Aminogram of the two products applied to tomato plants undergoing drought treatment.

	Aminogram* (%)			
Free amino acids	***Asp***	0,168	***Lys***	0,358
	Glu	0,650	***Pro***	0,101
	Ala	0,127	***Ser***	0,08
	Arg	0,082	***Thr***	0,088
	Phe	0,058	***Val***	0,09
	Gly	0,175		

*Aspartic acid (Asp), Glutamic acid (Glu), Alanine (Ala), Arginine (Arg), Phenylalanine (Phe), Glycine (Gly), Lysine (Lis), Proline (Pro), Serine (Ser), Threonine (Thr), Valine (Val).

RESULTS

Morphological parameters and water relations

The control tomato plants had a leaf biomass and area of around 9 g and 744 cm^2, respectively. Drought led to a decrease in growth (Figure 1) but the plants that were treated with Fitomare® experienced a reduction in growth that was lesser as compared to the other treatments. Then, the plants treated with Fitomare® had a decrease in growth rate that was 26% as compared to 35% from the other two treatments (DRT and DRT+PC). As for the water content (grams of H_2O per each gram of the plant's dry weight), no significant differences were found among treatment.

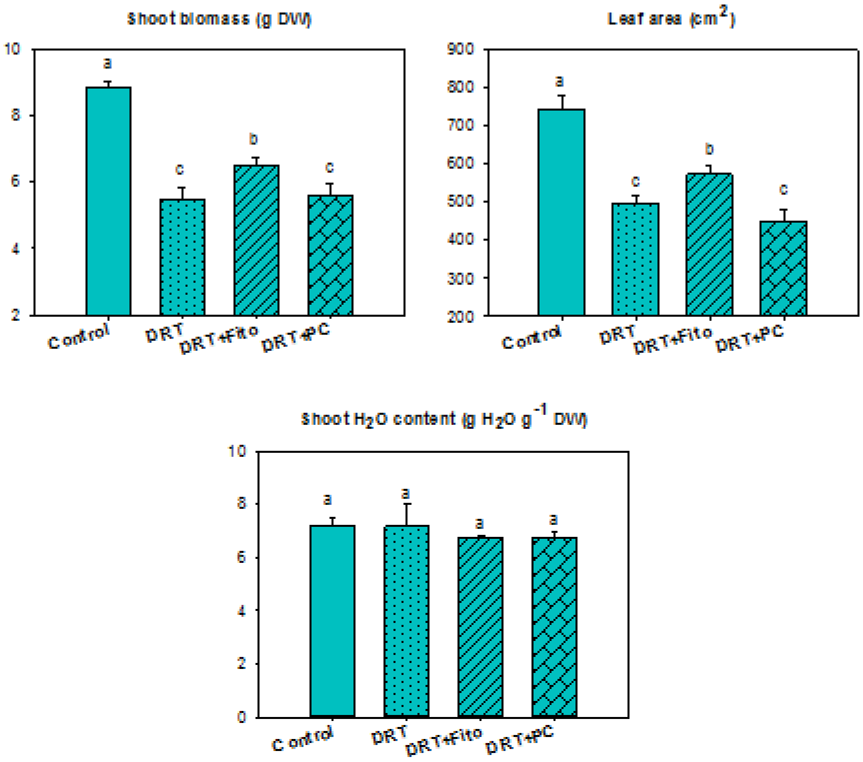

Figure 1. Morphological parameters: leaf biomass (g dw; dry weight), leaf area (cm^2) and shoot water content (g H_2O g^{-1} DW) after 12 days in drought set at 40% of its ETc. The different *lower case letters* indicate significant differences among treatments at $p< 0.05$ as established by Duncan's multiple range test. The vertical bar indicates the standard error of the mean (*n*=*6*).

Physiological parameters

The control plants showed normal A_{CO2} and g_s values of 8.3 µmol m^{-2} s^{-1} and 517 mmol m^{-2} s^{-1}, respectively. On the other hand, the plants subjected to drought treatments had reductions in both parameters, but these reductions were less in the plants sprayed with Fitomare® (Figure 2).

When looking at the A_{CO2} data obtained, we observed that the plants from the DRT+Fito treatment only experienced a reduction of 34% with respect to the control, while the plants from the DRT and DRT+PC treatments experienced a reduction of 97% and 81%, respectively. As for the water use efficiency (WUE), we observed that from the three groups treated with drought, the ones treated with Fitomare® were the ones that had the highest values (3.27 µmol CO_2 $mmol^{-1}$ H_2O).

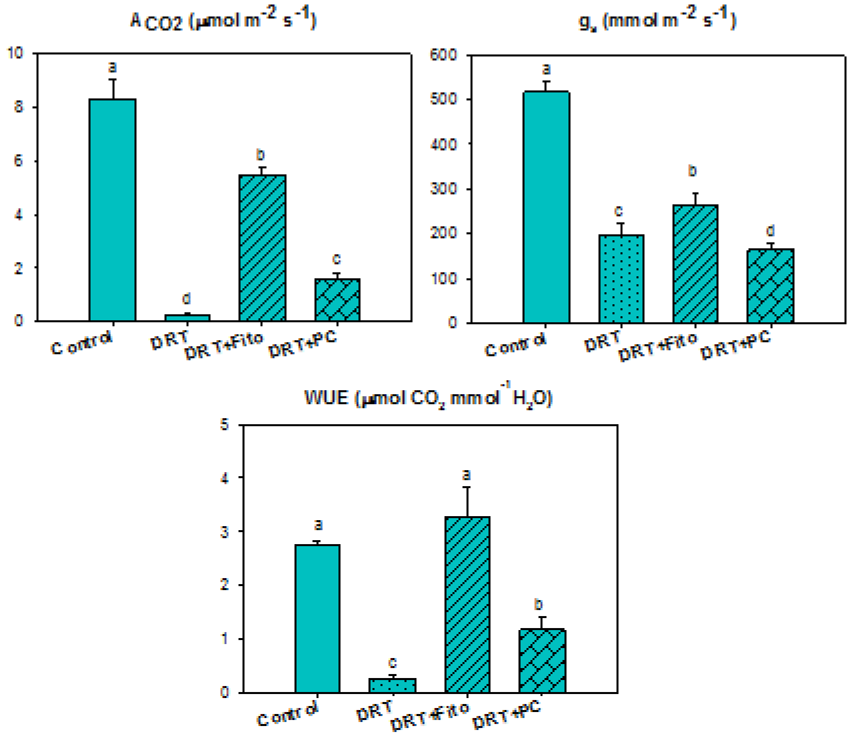

Figure 2. Physiological parameters: net assimilation rate of CO_2 (A_{CO2}; µmol m^{-2} s^{-1}), stomatal conductance (g_s; mmol m^{-2} s^{-1}) and water use efficiency (WUE, CO_2 $mmol^{-1}$ H_2O) after 12 days of drought at 40% ETc. The different *lower case letters* indicate significant differences among treatments at $p < 0.05$ as established by Duncan's multiple range test. The vertical bar indicates the standard error of the mean (*n=6*).

Biochemical parameters: proline and reducing sugar concentration in leafs

The concentration of certain foliar osmolytes such as proline and reducing sugars from control plants were 1.69 and 30.4 mg g^{-1} dw, respectively. Drought conditions led to an increase in the concentration of reducing sugars in the leaf, with this increase being less in plants that were treated foliarly with Fitomare® (Figure 3). As for foliar proline, only a significant increase was observed in the concentration of this osmolyte in the DRT+PC treatment.

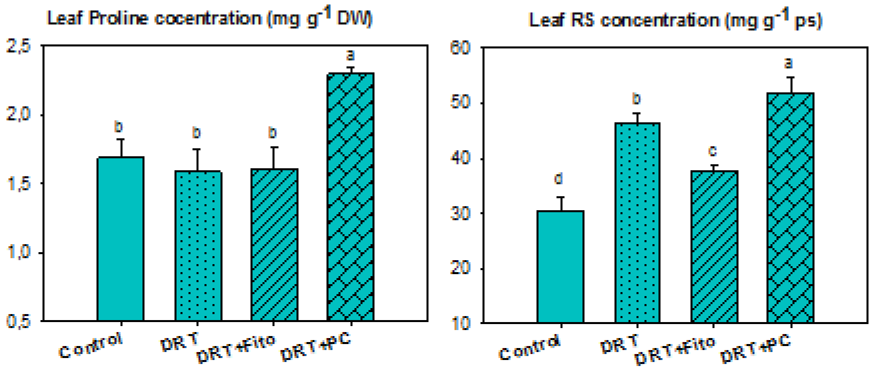

Figure 3. Biochemical parameters: foliar proline concentration (mg g^{-1} dry weight) and foliar concentration of reducing sugars (RS; mg g^{-1} dry weight) after 12 days of drought at 40% of its ETc. The different *lower case letters* indicate significant differences among treatments at $p < 0.05$ as established by Duncan's multiple range test. The vertical bar indicates the standard error of the mean (*n=6*).

Oxidative stress: determination of MDA in leafs

The oxidative stress felt by the tomato plants as a consequence of the drought treatment shows that there was an increase in the concentration of MDA with respect to the control plants (Figure 4). However, the plants treated with Fitomare® had a less pronounced increase (5.85 nmol g^{-1} fw). This is in agreement with what is observed in photographs found in figure 4, where we can see that the DRT+Fito plants have less damage from drought that the other two treatments, where yellowing, loss of turgor and rolled leaves can be observed.

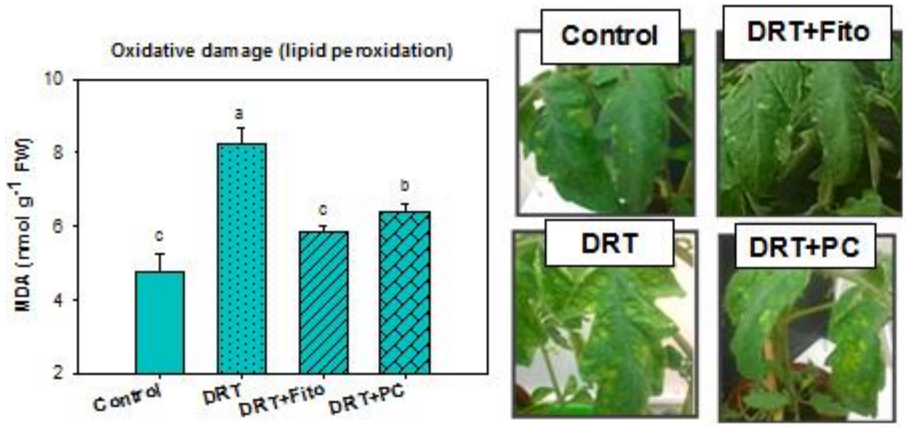

Figure 4. Oxidative damage (foliar MDA concentration nmol g^{-1} dry weight; dw) after 12 days of drought at 40% of its ETc. The different *lower case letters* indicate significant differences among treatments at $p < 0.05$ as established by Duncan's multiple range test. The vertical bar indicates the standard error of the mean (*n=6*).

DISCUSSION

The drought treatments imposed in our experiment resulted in a decrease in plant growth as was expected, as when plants suffer water deficit, they experience physiological and biochemical alterations that negatively affect their growth [20]. However, the foliar application of Fitomare® improved plant growth with respect to the DRT and DRT+PC treatments. Also, the foliar application of PC, a product that had the same aminogram as Fitomare®, did not improve plant growth as compared to the drought treatment without the foliar application. This shows that the application of products that are formulated from vegetable extracts with amino acids, and complemented with macro and micronutrients are more efficient for reducing the adverse effects caused by moderate drought, as compared to products that are formulated only with free amino acids.

The benefits of Fitomare® as a treatment for drought could be explained by the effects that the application of this product had on water use by these plants (WUE= A_{CO2}/E_{leaf}). Some authors have suggested that the maintenance of WUE in plants under drought is crucial for the maintenance of good plant development [21]. At the physiological level, one of the first responses of plants to water deficit is the closing of stomata, and this could be due to the loss of cellular turgor (passive closure) or through active closing, which is hormonally regulated once the plant has detected the stress [22]. In this experiment, the data shows that the stomatal closure was not due to leaf dehydration, as their water content was not affected by drought (Figure 2). This was due to the fact that the intensity of drought was moderate, and the plants put into action a series of mechanisms that allowed them to maintain a good water status. However, these mechanisms seemed to be more efficient in plants treated with Fitomare®, as these did not have a reduced WUE. The stomatal closure brings with it a decrease in the net assimilation rate of CO_2, but in this case (application of Fitomare®), the reduction of E_{leaf} was similar to the reduction of A_{CO2}. In the other drought treatments, however, a decrease in the water use efficiency was observed, as a consequence of the A_{CO2} decreasing more than the E_{leaf}, indicating that the decrease of A_{CO2} was not regulated by stomas, but it was also affected by non-stomatal causes, which could have produced damages to the photosynthetic machinery.

The changes induced by water stress to osmolyte content depend on the duration, intensity and progression of the stress, meaning that variations in the content of proline could be due to adaptive responses in the plants in order to establish osmotic homeostasis in the medium term (osmotic adjustment). However, this could also be due to the plant's defense strategy to mitigate the damaging effects due to long-term stress, after a prolonged exposure to drought [23]. In tomato plants, specifically, proline is considered to be a biomarker, which indicates that the plant has suffered water stress.

Then, the greater the concentration of proline in the leaves, the greater the stress felt by the plants. The experimental data showed there was high accumulation of proline in the DRT+PC treatment, and a high accumulation of reducing sugars in the DRT and DRT+PC treatments, which support the idea that these plants suffered greater damage due to water stress as compared to the plants treated with Fitomare®. Also, this is confirmed by the data from figure 4, which shows that the plants from treatments DRT and DRT+PC had a greater concentration of MDA (oxidative stress biomarker) as compared to the plants treated with Fitomare®, again confirming what was mentioned above, which is that the plants from treatments DRT and DRT+PC suffered greater damage due to drought as compared to plants treated with Fitomare®.

CONCLUSIONS

With the data gathered form this experiment, we can conclude that the foliar application of Fitomare® could partially mitigate the damage caused by moderate drought on tomato seedlings, with the application resulting in greater plant development as compared to the plants from the other drought treatments. This was due to the fact that the application of Fitomare® helped maintain the good functioning of physiological processes (regulation of WUE), circumventing damage on the leaves. The results then suggest that future research on to the application of algal extracts should aim to obtain formulated products where these extracts are combined with amino acids, and macro and micronutrients in order the boost the effects of these biostimulant products.

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