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Use of Biotechnology as Generator of Resistance Mechanism to Water Stress in the Rosa Productive Chain (*Rosa indica* L. var. Classy)

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Liquid humic substances are one of the biotechnological alternatives for organic agriculture, mainly those that are obtained from recyclable organic sources such as compost and vermicompost. Therefore, the main objective of this research was to evaluate the effect of biofertilization with Liplant on the growth, development and water relations of rose plants, exposed to water stress conditions. The experiment was carried out under semicontrolled conditions in the central area of the National Institute of Agricultural Sciences (INCA), San José de las Lajas, Mayabeque province. Used were the species Rosa indica L. var. Classy; applying a completely randomized design, where the factors studied were the sprinkling with the biostimulant Liplant 1/40 v.v., and the water stress condition, with 4 treatments, 2 with control and two with stress; alternating in each case the application or not of the Liplant. The substrate used was composed of leached red ferralitic soil, combined with cachaza in proportion 3:1 v.v. It was observed that the index evaluated were significantly affected by the pre-established drought conditions, clearly showing a decrease of the biomass in the aerial parts of the plant, being more remarkable in plants without application of Liplant. Sprinkling with Liplant 1/40 v.v., allowed to increase the production of biomass, both in good wáter catering and in conditions of water deficit. This reflects that the efficient use of biotechnology helps to mitigate, in the case of rose cultivation, the negative effects of climate change.

1. Introduction

In recent years, the use of mineral fertilizers in an indiscriminate way has resulted in a loss of the optimal levels of organic matter of the soil, salinity of the same and prolonged droughts, due to the imbalance that is generated in agroecosystems. The proper use of soil organic matter is one of the basic pillars on which sustainable agriculture rests. This reason has generated a search for alternatives that allow ecologically sustainable and responsible organic nutrition, whose main condition, in addition to production to meet human needs, is to conserve and improve the environment (Arevalo et al., 2013).

In this sense, there is now a marked tendency in the world to use the combination of purely chemized agriculture and organic agriculture. Liquid humic substances are one of the alternatives for this group of products used in organic agriculture; mainly, those that are obtained from recyclable organic sources such as compost and vermicompost, given their biostimulating effects known as like-auxins analogous to the phytohormones present in plants (Penella et al., 2015 & Fontanelli et al., 2015).

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2. Materials and methods

2.1 Study Overview

The experiment, led by researchers from the Fundación Universitaria Agraria de Colombia – UNIAGRARIA in the company of the Universidad Cooperativa de Colombia - UCC, Jardín Botánico de Bogotá – JBB and the Instituto de Ciencia Animal - ICA of Cuba, was developed under semicontrolled conditions in the central area of the Instituto Nacional de Ciencias Agrícola (INCA), San José de las Lajas, province of Mayabeque; located at 23° 00' north latitude and 32°12' west longitude, and at 138 m.
The plant material used were *Rosa indica* L. var. Classy, which were planted on a substrate composed of leached red ferralitic soil, according to map 1:25 000 of the National Soils Directorate, combined with cachaza 3:1 v.v.

2.2 Culture conditions

Rose postures of 120 days of grafting were used. The transplant was carried out in pots (5 L capacity), placing one plant per pot, for a total of 120 pots, at a rate of 30 per treatments. Two applications of Liplant 1/40 v.v. (biostimulant), the first at the time of transplantation by foliar aspersion of the product at the aforementioned dilution until the drip point was reached, while the second was performed 35 days after.

2.3 Growth and development variables

The physiological variables related to growth and developments were evaluated at the beginning and end of the imposed stress period, at a rate of 5 plants per treatment chosen at random. These variables were as follows:

2.1.1. Biomass

The evaluation of growth in dry biomass of plant organs (roots, stems and leaves, expressed in g / plant), was determined in five plants per treatment. In all cases the plants were dried in an oven at 80 ºC until obtaining a constant and heavy mass in a technical balance.

2.1.2. Leaf surface

It was determined under the disc method (Watson and Watson, quoted by Ortiz et al., 2015). For this purpose, six plants were treated per treatment and ten leaves per plant were selected as follows: three of the basal part, three of the apical part and four of the middle part. From these sheets 40 discs were obtained homogeneously, with a perforator of known area, for a total of 240 discs per treatment. Subsequently, they were dried in an oven at 80 ºC until reaching a constant mass; with the values of the dry mass and leaf surface of the disks and the total dry mass of the leaves the leaf surface was determined by means of the following equation:

$$LA = (AD \times DLM) / (DMD) \quad (1)$$

Where: Leaf Area (LA expressed in cm^2), Area of 40 discs (AD expressed in cm^2), Dry Leaf Mass (DLM of the plant expressed in g) and Dry Mass of the Discs (DMD expressed in g).

2.1.3. Growth rates

From the Leaf Surface (leaf area) and biomass according to Beadle (1993), were calculated the indicators of Relative Growth Rate (RGR) and the Net Assimilation Rate (NAR) using the following expressions:

$$RGR = (\ln M_2 – M_1) / (t_2\text{-}t_1) \quad (2)$$

Expressed in: g/(g days)

$$NAR = [(M_2 – M_1) / (SF_2 – SF_1)]\ [(\ln SF_2 – SF_1) / (t_2\text{-}t_1)] \quad (3)$$

Expressed in: g m^2 $days^{-1}$

Where: M_1 (total dry mass at the beginning of the stress period), M_2 (total dry mass at the end of the stress period), LA_1 (leaf surface at the end of the stress period), LA_2 (leaf surface at the beginning of the stress period), t_1 (Time at the beginning of the stress period / 40 days) and t_2 (Time at the end of the stress period / 50 days).

2.1.4. Assessment of the water status of plants

On days 4, 7 and 10 after the establishment of the water stress period, physiological determinations were made concerning the hydric relations to 5 plants by treatments chosen at random. These variables were determined in well-developed leaves of the upper third of the plants; in the specific case of potentials at dawn (before sunrise).
Leaf hydric potential (Ψ_f): Leaf hydric potential in MPa was determined using a Sholander pressure chamber according to the technique described by Taíz and Zeiger (2003) and nitrogen gas was used.
Pressure Potential (Ψ_p): The pressure potential was calculated from the difference between leaf hydric potential and leaf osmotic potential, using the following equation:

$$\Psi_p = \Psi_f - \Psi_0 \text{ (MPa)} \quad (4)$$

Osmotic potential (Ψ_0) and saturated osmotic potential (Ψ_{0S}): The osmotic potential was determined in the same samples of the hydric potential; a steam pressure osmometer of type Wescor 5520, calibrated with solutions of known osmolality was used. After measured their hydric potential, the leaves were wrapped in aluminum foil and were and introduced into liquid nitrogen to freeze below 50°C, then stored in the cold at 4°C until analysis.
Considering that the physiological variable, osmotic potential at full turgidity (or saturated osmotic potential as it is more commonly called) expresses the ability of a plant to perform osmotic adjustment under certain stress conditions. Its determination was made very similar to that of Ψ_0, only with saturated samples.

2.4 Context of experimental design and statistical analysis

The treatments were distributed in a completely randomized design, where the factors studied were aspersion with the biostimulant Liplant 1/40 v.v. and the water stress condition, as described below:

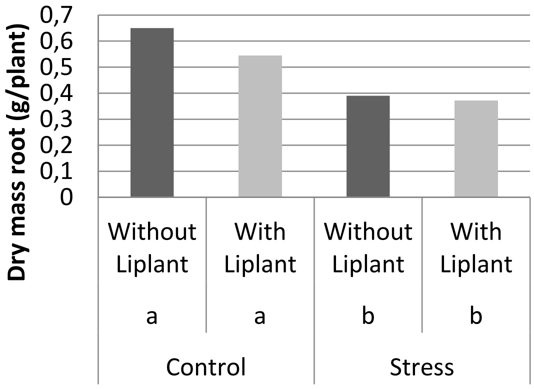
- Control without Liplant 1/40 v.v. (with irrigation on alternate days)
- Control with Liplant 1/40 v.v (with irrigation every other day)
- Water stress without Liplant 1/40 v.v. (no irrigation since 40 days)
- Water stress with Liplant 1/40 v.v. (no irrigation since 40 days)

To perform the Statistical Analysis, the primary and graphic data of each of the indicators evaluated were processed using the Microsoft Excel tool. In the processing of the results of the different experiments, we took as a reference the one proposed by Suárez (2011), who describes a completely randomized analysis of variance (ANOVA) with a significance level of 95 % ($\alpha = 0.05$), using the Multiple Rank test as discriminant using the Statgraphics Centurion statistical package.

3. Results and discussion

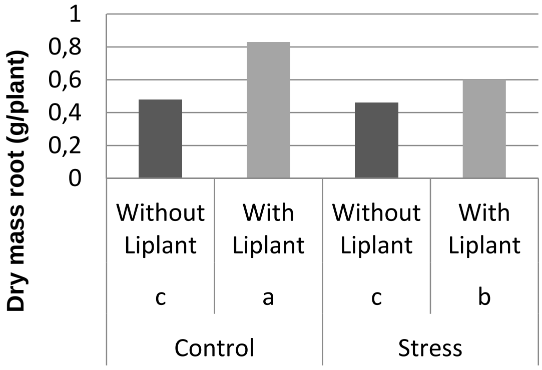
3.1 Biomass

When analyzing the influence of water stress on growth, a decrease in the total biomass of stressed plants was found in relation to the control. The dry mass of the root and the leaves were the most affected by stress and possibly the biomass decreases of these organs determined the reduction of the total biomass, since the dry mass of the stem although affected, was in much smaller amount; and only occurred when the plants were moistened with solutions of Liplant 1/40 v.v, and exposed to water deficit, but still exceeded the values presented in the plants without application of the biostimulant (Figures one to four).
It is noteworthy that, under the conditions studied, spraying with Liplant 1/40 v.v., showed significant contributions in the dry mass of the stem and leaves, which positively influenced a significant increase in the total biomass of the crop, differences which were indistinct to the presence or not of water stress, except for the biomass of the roots, in which there were no differences because of the aspersion.
To the above it is possible to be added that, although the application of the Liplant was carried out in a foliar form (as indicated above), until reaching the point of drip of said dilution, indicating that the excess Liplant fell to the ground, incorporating itself to the solution of the same and enriching the microbial flora. This biostimulant already integrated to the soil could be absorbed by the roots stimulating the development of the radical system and incorporating as a nutrient, which subsequently implied a general increase in the biomass of the plants. In this regard Duarte et al. (2010) argues that the variation in nutrient content in plants is closely related to the variation of dry mass (biomass).



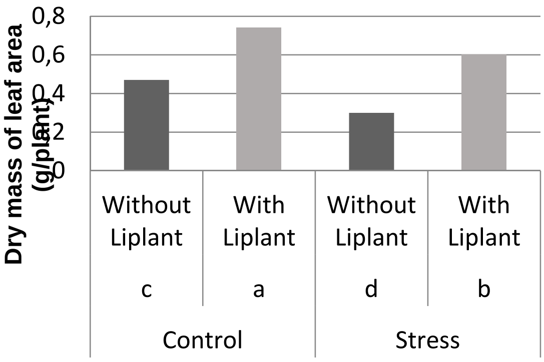
Sx= 0.02 and Cv= 0.746

Figure 1. Dry root mass.



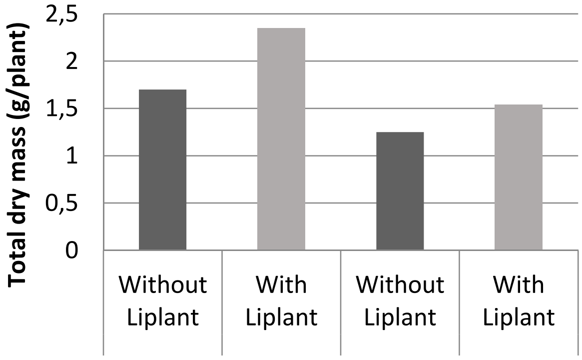
Sx= 0.03 and C.v= 0.10

Figure 2. Dry mass on the stem



Sx= 0.02 and Cv. = 0.706

Figure 3. Dry mass of leaf area.

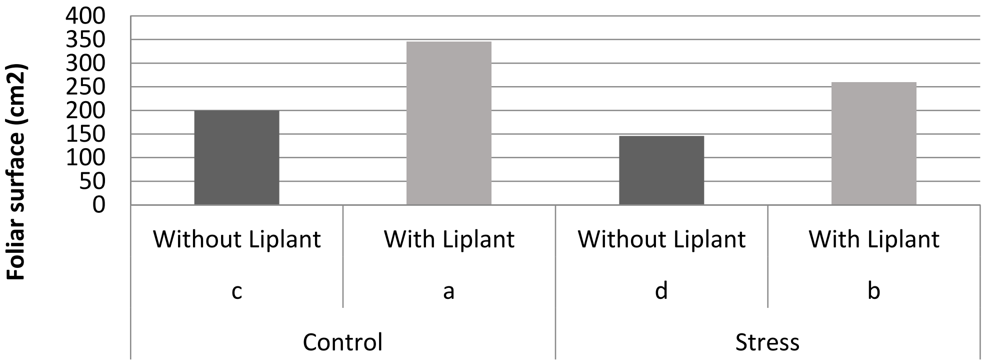


Sx= 0.06 and Cv. = 0.65

Figure 4. Total dry mass.

3.2 Leaf surface

For this variable, in the plants grown under conditions of water stress the leaf area was reduced, while the plants that were submitted to the dilution Liplant 1/40 v.v., both grown as control and under stress conditions, showed a better behavior, see Figure 5.



Sx= 7.91 and Cv. = 0.58

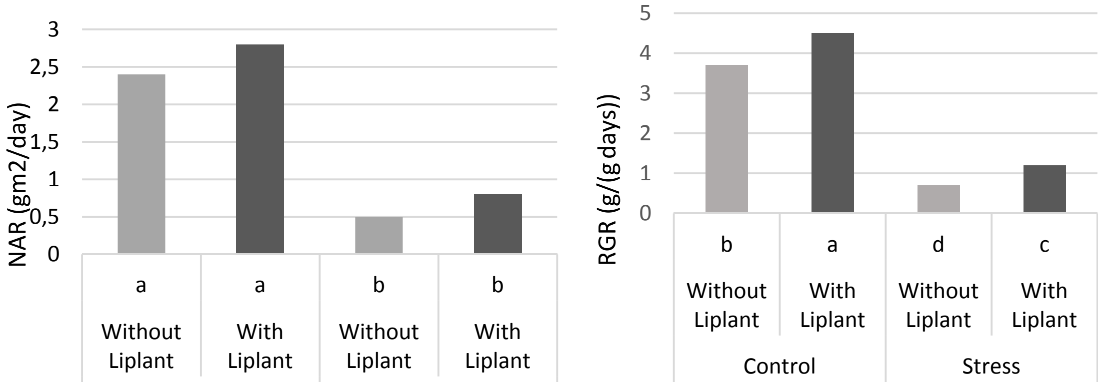
Figure 5. Foliar surface in the development of the crop in plants that were subjected to stress by suspension of the water supply.

In the same way, the decrease in the leaf surface in the stressed plants could be mainly due to the presence of a smaller number of leaves, because visually it was noticed a fall in the older leaves when the water stress became more intense. In this regard, Suárez (2016) argues that leaf area is one of the first indicators to be affected by stress, because cell expansion is very dependent on the water status of plants.

Also, the decrease in the number of leaves and leaf area in plants subjected to the stress regime was interpreted as an indirect measure of the dry mass of the aerial part. Indicating that plants treated with Liplant 1/40 v.v., made a more efficient use of solar radiation and therefore increased those processes dependent on light such as: photosynthesis, reduction of NO_3^- to NH_4^+ and assimilation of NH_4^+, processes necessary for the synthesis of amino acids of the plants. In addition, respiratory activity could be increased by an increase in respiratory substrates emerging from photosynthesis. The fact that the processes mentioned above were favored brought as resulted in an increase in the production of biomass.

3.3 Growth rates

The characteristic shown in Figure 6 reveals the behavior of two variables that indicate the aspect related to the growth of the crop; first, the Relative Growth Rate (RGR) and secondly the Net Assimilation Rate (NAR) during the 10 days in which the plants were subjected to stress condition.



Sx= 0.18 and Cv. = 12.17%

Sx = 0.14 and Cv. = 14.93%

Figure 6. Indices of growth of the crop.

In the case of the plants sprayed with Liplant 1/40 v.v., a difference was observed with respect to those not sprayed and grown under the same conditions of water supply (for the RGR), whereas these results were not similar for the analysis of the NAR, so it can be said that, for this specific case, it was not possible to increase the efficiency of the photosynthetic apparatus in the rose cultivation at least in the studied conditions; coinciding with the results obtained by Suárez et al. (2017 and 2016) for the crops of *Heliantus annus* L. and *Zea mays* L., Suárez et al. (2016) for the cultivations of *Avena sativa* L. and *Lolium multiflorum* Lam. in hydroponic culture; as well as those obtained by Ortiz et al. (2015) in *Zea mays* L.

3.4 Assessment of the water status of plants

3.4.1 Water potential and pressure potential

When this condition was checked during the 10 days of the stress treatments, a slight decrease of the water potential (Ψ) and of the pressure potential (Ψp) at 4 days, measured at dawn, was observed. The differences caused by the sprinkling of dilutions of Liplant 1/40 v.v., at this time of evaluation (4 days) were hardly perceptible in the crops.

It is noteworthy that, at 7 days of stress treatments, very pronounced differences were found because of the water deficit, being this the moment of the evaluations where it was observed that the spraying of the dilution 1/40 v.v. of Liplant began to play a prominent role under these conditions, because in the plants well supplied of water were not differences by the effect of the sprays.

Thus, the decrease in foliar turgor clearly expressed by decreases in the pressure potential was a characteristic symptom presented by the plant as it increased the degree of stress, in this case the reduction of this indicator was of one 70% in untreated stressed plants, while in stressed plants treated with Liplant, these decreases were avoided up to approximately 40% for the benefits caused by Humic Substances - SH.

At ten days of stress, and coinciding with the final moment of the evaluations, it was possible to verify according to the results presenting the potential of leaf turgor (pressure potential) that the plants were in the presence of a severe water deficit stress, and that values were reached in the non-sprinkler stressed plants very close to the point of loss of turgor that coincides with the zero value. Although, it is not less true that the pressure potential in the plants sprayed with this solution had a marked decrease in the absence of irrigation for 10 days, if it is of special note that their values were in an acceptable level of turgor considering the stress treatment imposed.

These benefits caused by the spraying of Liplant 1/40 v.v., in the foliar turgor of stressed plants together with the nutritional and other benefits of the biostimulant; allowed without doubt to be responsible for the highest values in the production of total biomass of plants sprinkled on plants not sprinkled when there is a presence of soil moisture deficit.

3.4.2 Osmotic potential

The potential physiological variable osmotic to full turgidity or saturated osmotic potential, as it is more commonly called, expresses the ability of a plant to perform osmotic adjustment in certain stress conditions and is one of the most important mechanisms of development by plants for maintain the potential gradient between soil and leaf tissue, which allows or not the flow of water and the maintenance of turgor in tissues in the event that this resource is limited or not in the soil in a way available to the normal assimilation of the crop according to (Cigasova et al., 2016).

At the same time, the results of the saturated osmotic potential indicated that there was no significant difference between the treatments at any of the evaluated moments, so that the rose cultivation under conditions of water deficit as applied in this study did not develop osmotic adjustment, and hence the explanation that the plants that were not benefited with the spraying with Liplant in stress conditions, presented the values of turgor close to zero.

4. Conclusion

As a result, it was observed that the pre-established drought conditions significantly affected the evaluated indices, clearly showing a decrease of the biomass in the aerial parts of the plant, being more remarkable in plants without Liplant application. This allowed to infer that the sprinkled plants have a greater disposition to face the water stress and to synthesize structural biomass.

Finally, it was evident show that rose plants under conditions of water stress do not develop osmotic adjustment, which leads to a temporary or permanent dehydration of the crop, thus affecting its metabolism.

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