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A QUITOMAX® EFFECT IN PLANTS OF (*Phaseolus vulgaris* L.) UNDER TWO IRRIGATION REGIMES. II. PHYSIOLOGICAL VARIABLES

Efecto del QuitoMax® en plantas de (*Phaseolus vulgaris* L.) sometidas a dos regímenes de riego. II. Variables fisiológicas

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ABSTRACT. This work was carried out with the objective to evaluate the effect of QuitoMax® on different physiological variables in bean plants (*Phaseolus vulgaris* L) conducted under two irrigation regimes. The black bean, variety‶ Tomeguín″ was planted in concrete gutters of 2,60 m long by 0,60 m wide, in which two separate rows were placed at 0,40 m and a plant spacing of 0,11 cm for a total of 44 plants per channel, each treatment counted on three replicates. The treatments were: 100 (T100) percent of the ETc. (Standard crop evapotranspiration) and 50 (T50) percent of ETc, counting in each irrigation treatment with a variant in which 200 mg ha^{-1} of QuitoMax® were applied at 20-25 days after sowing and a dose similar to the beginning of flowering and another dose in which the biostimulant was not applied. The evaluations were leaf water potential, current osmotic and saturated osmotic potentials, relative water content, stomatal conductance, total chlorophylls a and b and in SPAD units, the stomata were observed, and also turgor potential is estimated from the leaf water and osmotic potentials. The results indicated first, that bean plants (*Phaseolus vulgaris* L.) are affected when subjected to a regime of insufficient irrigation and secondly, that the two applications of QuitoMax® were able to attenuate the effects of water deficiency.

Key words: chlorophyll, stomatal conductance, water potential, osmotic potential, chitosan

RESUMEN. Este trabajo se realizó con el objetivo de evaluar el efecto del QuitoMax® en diferentes variables fisiológicas en plantas de frijol (*Phaseolus vulgaris* L) sometidas a dos regímenes de riego. Se utilizó la variedad de frijol negro ‶Tomeguín″sembradas en canaletas de hormigón de 2,60 m de largo por 0,60 m de ancho, en las que se colocaron dos hileras separadas a 0,40 m y una separación entre plantas de 0,11 cm para un total de 44 plantas por canaleta, cada tratamiento contó con tres réplicas. Los tratamientos fueron: 100 (T100) por ciento de la ETc. (Evapotranspiración estándar del cultivo) y el 50 (T50) por ciento de la ETc, contándose en cada tratamiento de riego con una variante en la que se aplicaron 200 mg ha^{-1} de QuitoMax® a los 20-25 días posteriores a la siembra y una dosis similar al inicio de la floración y otra en la que no se aplicó el bioestimulante. Las evaluaciones realizadas fueron los potenciales hídrico foliar, osmótico actual, osmótico saturado, contenido relativo de agua, conductancia estomática, clorofilas a, b y totales y en unidades SPAD, se observaron los estomas, y se estimó el potencial de turgencia a partir de los potenciales hídrico foliar y el osmótico actual. Los resultados indicaron en primer lugar, que las plantas de frijol (*Phaseolus vulgaris* L.) se ven afectadas al ser sometidas a un régimen de riego insuficiente y en segundo lugar que las dos aplicaciones de QuitoMax®, fueron capaces de atenuar los efectos de la deficiencia de agua.

Palabras clave: clorofilas, conductancia estomática, potencial hídrico, potencial osmótico, quitosano

INTRODUCTION

The common bean (*Phaseolus vulgaris* L.) is a very important grain legume in the Americas and parts of Africa where it serves as a vital source of proteins, vitamins and mineral nutrients (1).

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The adequate management of the nutrition of the plants and the efficient control of the pests that affect them, constitute two essential elements to obtain a high productivity and quality of the agricultural production, as well as, that the indiscriminate application of chemical products can cause damages to the environment, create resistance on the part of phytopathogenic microorganisms and cause harm to human health (2). The practice of biocontrol of diseases in vegetables shows a viable alternative in relation to the traditional chemical method.

The sustainable management of soil fertility is one of the main concerns of several researchers (3), given the adverse impact and ecological threats posed by the use of conventional chemical fertilizers (4). In this context, biostimulants represent an interesting alternative. They consist of various substances and microorganisms that are used to improve the growth and development of plants (5).

Among the products studied for the biocontrol, the polysaccharide of chitosan found naturally in the cell wall of some fungi and which has been obtained commercially from chitin, stands out for its biocompatibility, biodegradability, low toxicity, high bioactivity and microbial activity. (6), positive results in the use of biostimulants have been verified in several investigations, among them those carried out in *Vigna unguiculata* (7).

Although the mechanisms by which chitosan stimulates the growth and development of plants are not exactly known, it has been suggested that they are involved in physiological processes, such as avoiding water losses through transpiration (8). In this sense, the presence of stomatal closure in plants sprinkled with chitosan has been demonstrated, which suggested that the stimulating effect of growth after stomatal closure could be related to an antiperspirant effect in the plant (9,10), pointing out that foliar application of chitosan in potato reduced the effects of water stress (11).

On the other hand, it has been pointed out from the results found in the cultivation of beans (*Phaseolus vulgaris* L.), that one of the aspects through which chitosan was influencing the reduction of perspiration is that this product increases the levels of abscisic acid (ABA) in the treated leaves, which activates the partial closure of the stomata (12).

Taking into account the aforementioned, the present work was carried out with the objective of evaluating the QuitoMax® effect on different physiological variables in bean plants (*Phaseolus vulgaris* L.) subjected to two irrigation regimes.

MATERIALS AND METHODS

The work was carried out during the months of January to April 2013 in the central area of the National Institute of Agricultural Sciences (INCA), for which 12 concrete gutters of 2,60 m long and 0,60 m wide were planted (1,56 m^2) that contained Ferralitic Red Leached soil (13). In each gutter, 44 black bean plants of the variety "Tomeguín" were planted in two separate rows at 0,40 m and a spacing between plants of 0,11 m.

Two irrigation treatments were used, in each of them two QuitoMax® applications were carried out at a rate of 200 mg ha^{-1}, the first at 22 days after sowing and the second at 39 days coinciding with the start of flowering, as well as, there were two treatments in which the product was not applied, giving rise to four treatments distributed according to an experimental design of random blocks with three repetitions (three gutters per treatment). The treatments tested were:

T100, irrigated at 100 percent of the ETc. (Standard crop evapotranspiration)

T50, irrigated at 50 percent of the ETc.

T100 + QuitoMax®

T50 + QuitoMax®

The irrigation was applied by means of an automated micro spray system and the water delivery was controlled by valves placed in each treatment.

Evapotranspiration of the reference culture (ETo) was calculated using data from a nearby meteorological station (approximately 200 m from the experiment) and the FAO Penman-Monteith method (14) was used. The crop evapotranspiration under standard conditions (ETc.) was calculated by the following equation:

$$Etc. = ETo * Kc. \quad [1]$$

where:

Etc. Culture evapotranspiration [mm d^{-1}],

Kc Crop coefficient [dimensionless],

ETo Evapotranspiration of the reference culture [mm d^{-1}]

The Kc cultivation coefficients used were the following:

Kc initial = 0,15; Kc medium = 1,10 and Kc. final = 0,65

The irrigation was applied by means of an automated micro spray system and the water delivery was controlled by valves placed in each treatment.

During the period between January 21 and 26, irrigation was 3 mm per day in all treatments to ensure homogeneous germination and initial growth. From that moment, irrigation was applied according to each treatment. Effective rainfall was considered when this was greater than 3 mm. Other cultural attentions were performed equally in both treatments.

The plotted data of the maximum, minimum, solar radiation and rain temperatures correspond to the decennial values obtained.

Soil moisture (%) was determined weekly, using a TDR (Time Domain Reflectrometry) Field Scout TDR 100 System, Spectrum Technologies, Inc, in each treatment 30 measurements were made (ten in each container) at 20 cm of depth.

Evaluations

The leaf water potential (Ψw) was determined with a pressure chamber (Soil Moisture Equipment Co Santa Barbara USA). The leaves used for the dew measurements were frozen in liquid nitrogen, then defrosted and the osmotic potential (Ψs) was determined, using a Wescor 5500 vapor pressure osmometer (Wescor, Logan, USA). The pressure potential (Ψp) was estimated as the difference between Ψw and Ψs.

For the measurement of the osmotic potential at maximum saturation (Ψos) the leaves were hydrated for 24 hours by placing their petioles in a container with distilled water, covered with parafilm paper and placed at low temperatures in a domestic refrigerator, subsequently frozen in liquid nitrogen and the same procedure applied to determine the Ψs was followed.

For the determination of the Relative Water Content (CRA), ten leaves were taken per treatment at 11:00 am to which the fresh mass (MF) was determined, then hydrated for 24 hours following the same procedure used to obtain it. After that time, the Saturated Mass (MFS) was determined before placing them in a stove at 80 °C until reaching a constant weight (MS). The CRA was calculated according to the following expression:

$$CRA=MF-MS/MFS-MS$$

Stomatal conductance was determined at two stages of plant development. The measurements were taken at 11:00 a.m. with an AP4 dissemination porometer in leaves well exposed to the sunlight of the upper third of the plants. A determination of the chlorophyll content were made using a chlorophyll meter SPAD-502 Plus, Minolta brand, standard model; which by reading the absorbances of the leaf in the red and near the infrared regions, calculates a numerical value in SPAD units that is proportional to the amount of chlorophyll.

Chlorophylls *a, b* and total were determined using the acetone extraction method, performing the absorbance determinations in the spectrophotometer at wavelengths of 660 and 642,5.

To observe the stomata, a sampling was made 41 days after sowing, taking leaves from the upper part of the plant, making the observations in the central part of the leaflet and on both sides of the main rib; the process consisted in making a scraping on the leaf adaxial side, once obtained the epidermal leaf was placed on a slide, with a drop of toluidine blue, for five minutes. Subsequently, two washes of five minutes each were made, a drop of glycerin was placed and a coverslip was placed.

The samples were observed in a light optical microscope (Zeiss) and photographed with a camera (Motic) coupled to it.

The cultural and phytosanitary tasks were carried out according to what was stated in the Technical Guidelines for the cultivation of beans (15).

For the processing of the data and the comparison of means at the evaluated moments, the Statistical Program SPSS 19.0 for Windows (16) was used. The figures with the results were made using the SIGMA PLOT 11.0 program.

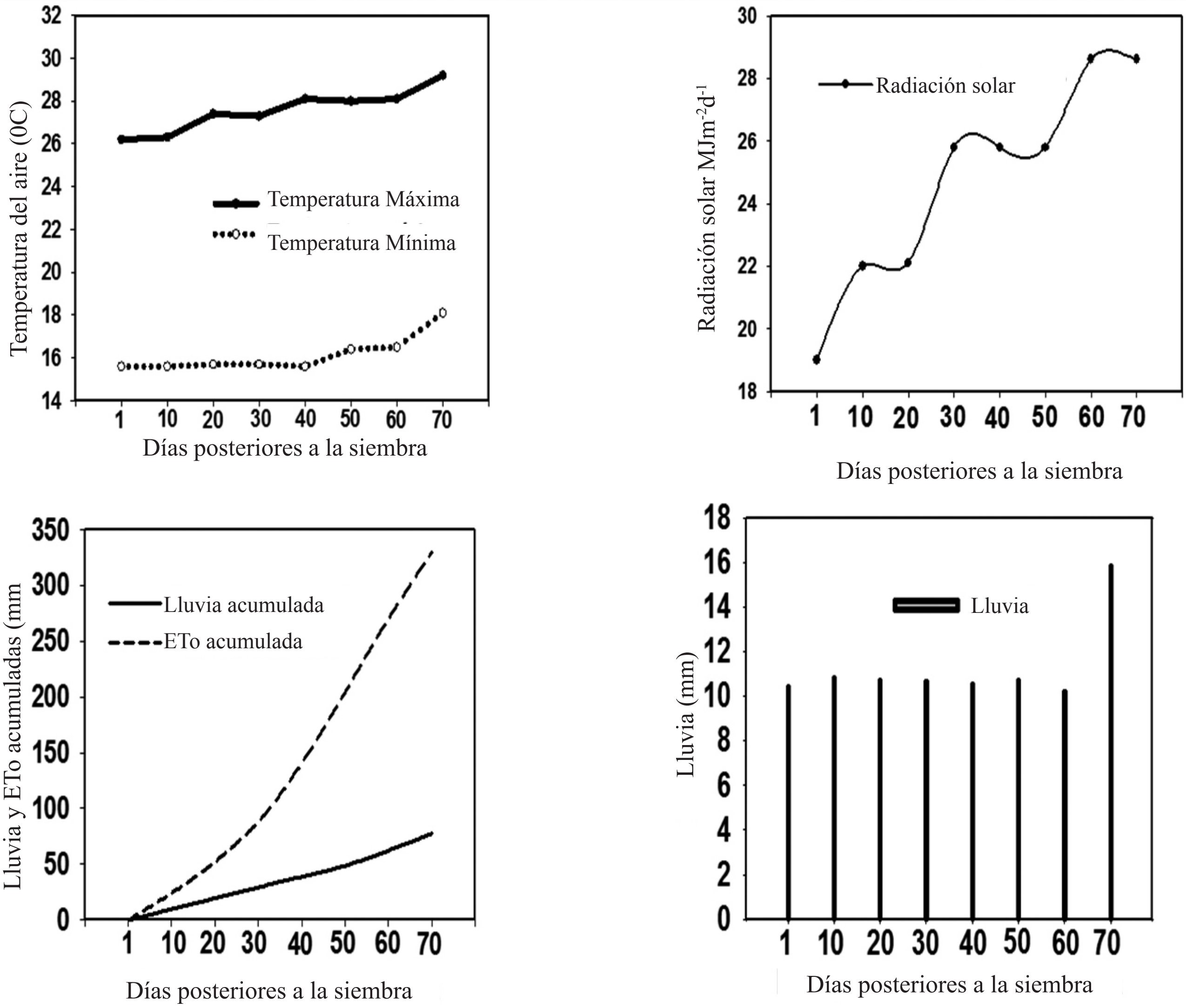
RESULTS AND DISCUSSION

The temperature and rainfall data show that the experimental period was characterized by being relatively hot and dry, as can be observed in (Figures 1A and C), mainly because the minimum and maximum temperatures had very little variation and their ranges of values were between 16 and 18 ºC minimum, between 26 and 30 ºC maximum and accumulated rainfall was 77 mm equivalent to only 6,4 mm per week.

On the other hand, solar radiation showed a range of values between 19 and 28 Mj m^{-2} d^{-1} and the highest values were presented at the end of the experiment. Cumulative climatic water demand (ETo) in the period was 327 mm, which represents an average daily evapotranspiration of 5 mm.

In general, except the temperatures that were relatively warm, although it is suggested that the beans can be grown with average temperatures ranging from 15 to 27 ºC, with an optimum of 25 ºC (17), although in other works it has been found that the beans supports up to 50 °C without damage to its cells (18). The precipitation and ETo values are typical of the months in which the experiment was carried out.

Climate components play an important role in the life of living organisms and of nature as a whole (19,20), which is why it has been suggested that the direct impacts of climate change on natural, economic and social systems, due to high temperatures and changes in rainfall patterns, they are increasingly evident, with the primary production sector being one of the most negatively affected.



Air temperature (°C), solar radiation (MJ m^{-2} d^{-1}), rain, accumulated rain (mm) and accumulated standard Evapotranspiration (ETo) (mm)

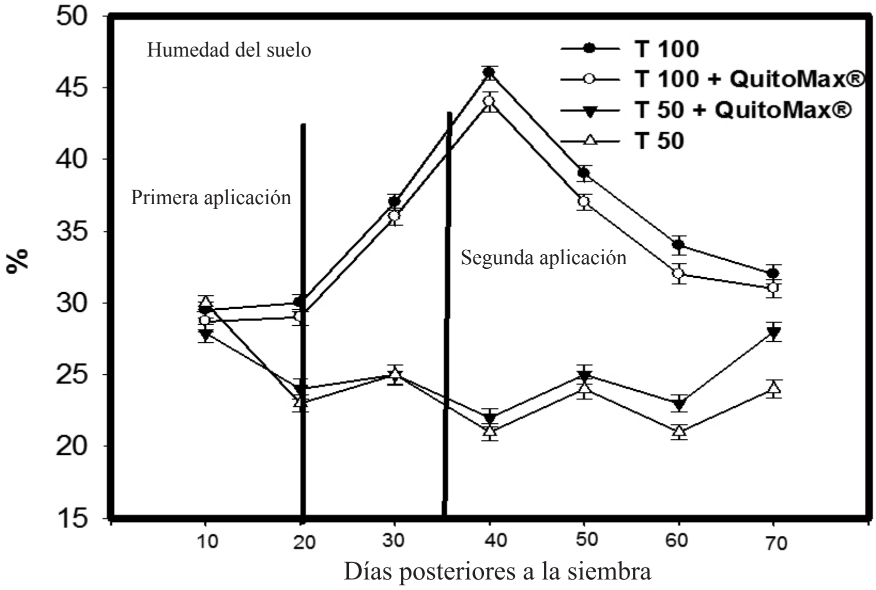
Figure 1. Environmental conditions during the experimental period

Figure 2 shows the variations in the moisture content of the soil, where it was observed that in the irrigation treatments T100 and T100+QuitoMax® the soil moisture always remained above 27 % and at 40 DAS it reached maximum values of around 46 % with very little difference between the two.

As for T100 and T100+QuitoMax®, the values of this variable were between 20 and 30 % and only differences between them were found from the 40 DAS. These results show the effect of the irrigation treatments applied to the crop.

The leaf water potential showed a different response only after eight days of the highest values applied in the plants that received a better water supply (Figure 3A).It is appreciated that the differences between the treatments were maintained until 20 days after the product application showing the greatest potential in the best-supplied plants without the addition of QuitoMax® with difference that under this same level of soil moisture was received the product dose, a similar response showed the treatments with less water supply, but with a differentiated potential higher in the plants treated with the biostimulant. These results confirm those raised by other authors who have pointed out the potential of chitosan in adverse conditions of different origin (21,22).

The current osmotic potential differentiated the response of the plants in two well-defined groups according to the water availability with which the plants counted but with no difference between the two treatments in each of the cases (Figure 3B), an issue that must be given by a higher concentration of solutes in these plants due to less hydration of their tissues, as could be seen in the behavior of the leaf water potential.



The bars above the mean values represent the confidence interval of the means, α = 0,5

Figure 2. Seasonal variation of soil water content in treatments at 20 cm depth

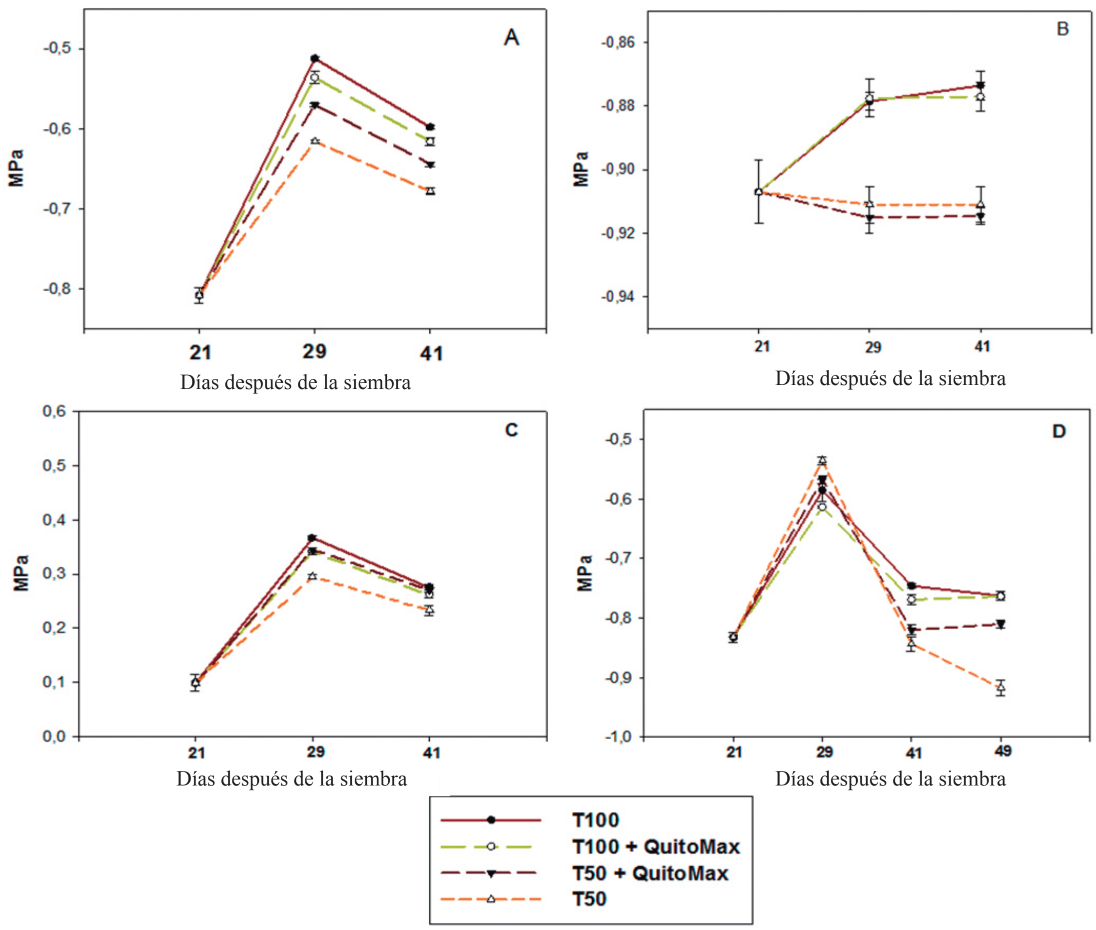


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Figure 3. Leaf water potential (A), osmotic potential (B), turgor potential (C) and saturated osmotic potential in bean plants treated with QuitoMax® and subjected to two levels of water supply

On the other hand, the turgor potential showed a similar behavior between the best supplied water treatments and that with the 50 % reduction of the available water received the QuitoMax® applications without differences between them (Figure 3C), which they were differentiated from the less supplied with water and without the biostimulant, ratifying the criterion of the positive effects of the chitosan derivatives before adverse conditions for the plants.

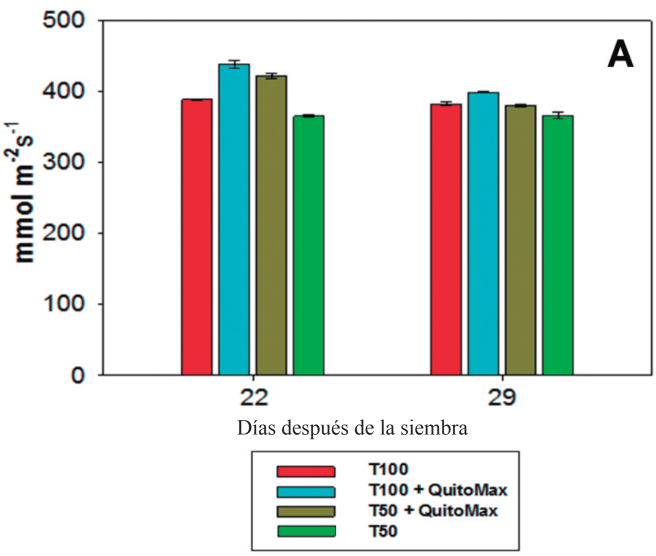
The saturated osmotic potential, although after eight days of taxes, the treatments did not show a very different behavior among them (Figure 3D), from that moment on they began to differentiate and in the last evaluation a clear difference between the treatments better supplied with water and those less favored was evidenced, with differences in this last condition between the treaty with the biostimulant and the one that was not treated, differences that seem to indicate the presence of an osmotic adjustment or perhaps a strong stomatal regulation as a way to avoid water losses via transpiration.

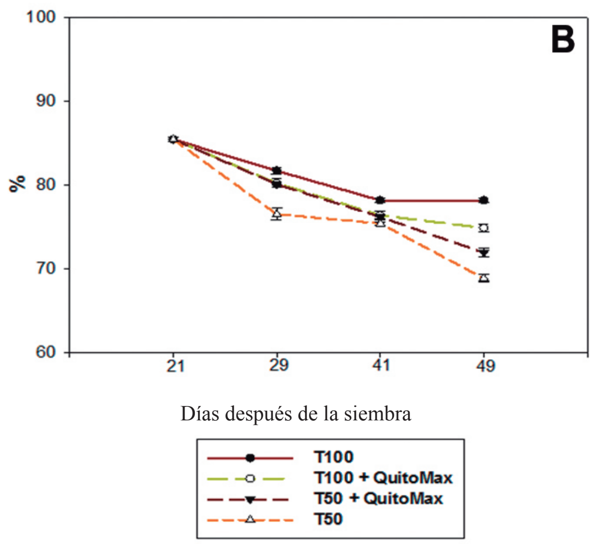
This plant response could be related to the synthesis of abscisic acid (ABA) that occurs when they are in the presence of this type stress (23), a question that has been raised by other authors who have reported that it has been shown that drought causes an increase in the biosynthesis and accumulation of ABA by activating the genes that code for the enzymes involved in the biosynthesis of this hormone. This increase in ABA is the signal that allows amplifying other signaling cascades that apparently regulate the water balance in the plant and tolerance to stress. In this way, the synthesis and regulation of this bioregulator constitutes a response mechanism to the water deficit of the soil.

Stomatal conductance measured at two stages of crop development showed the lowest values in the treatment that received 50 % of the water needed to cover its evaporative demand (Figure 4A), while this variable was favored with the application of QuitoMax® to reflect these treatments values higher or similar to that of the plants that received 100 % of water.

On the other hand, the Relative Water Content measured in four stages of the crop development until 49 days after sowing (Figure 4B), showed the tendency to decrease as the plants were aging, as well as the values extremes were found in the treatments that did not receive biostimulant applications, while the behavior of the plants treated with the product was similar until 41 days; moment from which all the treatments were differentiated from each other with the highest values in the treatments with greater water availability in the soil, occupying an intermediate position those who received the QuitoMax® applications.

The behavior of these variables in different water conditions in the soil is well known for different types of plants as well as for beans in which a reduction in stomatal conductance has been found as a result of the stomatal regulations that occur when the water availability in the soil constitutes a limitation for the proper development of plants (24,25). This explains, as has been suggested, from studies carried out with different varieties of *Phaseolus vulgaris* and *Vigna unguiculata*, respectively, which under these conditions produces a reduction in stomatal conductance, transpiration and photosynthesis.



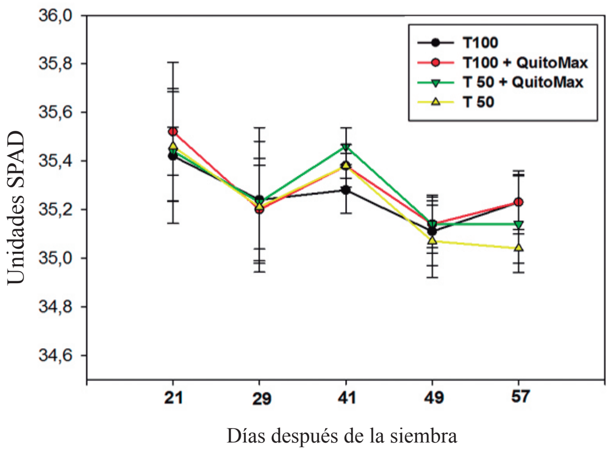


The bars above the mean values represent the confidence interval of the means, α = 0.5

Figure 4. Stomatal conductance (A) and relative water content (B) in bean plants treated with QuitoMax® and subjected to two levels of water supply

This paper highlights the treatments with the application of biostimulant in which this variable was favored and probably other processes related plants with respect to reduced availability of water in the soil without the biostimulant application.

The total chlorophylls measured at different development times of the plants reflected a similar behavior among all the treatments, since there were no differences between them and at the moments when the values were more distant, the differences did not exceed 0.2 SPAD Units (Figure 5).



The bars above the mean values represent the confidence interval of the means, α = 0.5

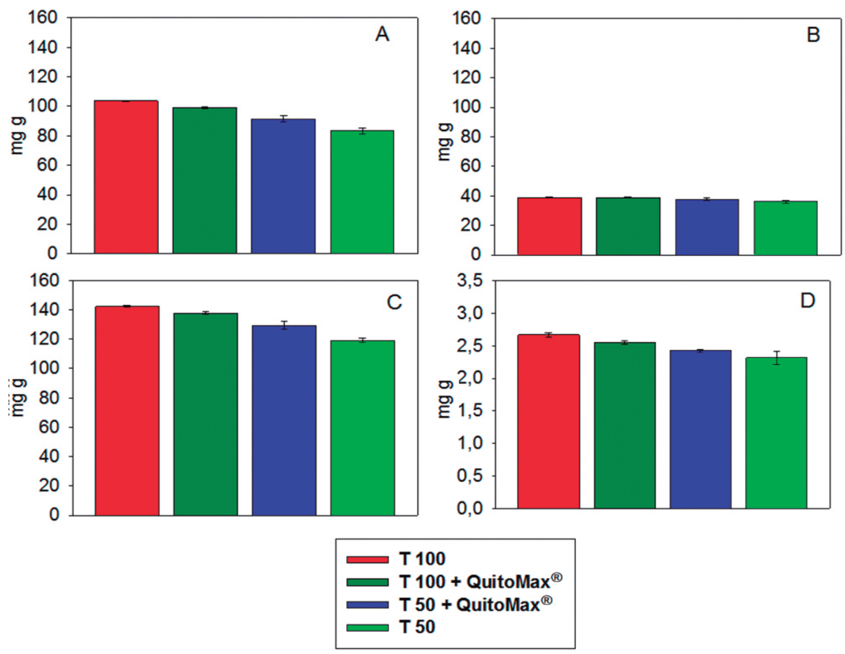
Figure 5. Total chlorophylls in bean plants treated with QuitoMax® and subjected to two levels of water supply

This result indicates that the plants in general were sufficiently supplied with nutrients, mainly nitrogen, so the availability of nutrients was not a limitation for their normal development.

On the other hand, it can be inferred that the photosynthetic system of the leaves maintained its integrity, thus guaranteeing favorable conditions for the development of the plants, aspects that have been pointed out by other authors from works carried out in *Coffea canephora* Pierre (26).

As shown in Figure 6A, chlorophyll *a* showed differences between the treatments with a descending behavior from the plants well supplied with water to those moderately supplied, with differences in turn among those that even when receiving the QuitoMax® applications had different water content in the soil, while chlorophyll *b* showed a similar behavior among the treatments, although with higher values in the treatments better supplied with water and with the lowest value in which the biostimulant was not received and had a lower amount of water. (Figure 6B)

When evaluating the behavior of the total chlorophylls it was possible to appreciate that all the treatments were different from each other (Figure 6C), showing a concordant response with that shown by chlorophyll *a*, which indicates that it was this type of chlorophyll that made the greatest contribution to total chlorophylls.



The bars above the mean values represent the confidence interval of the means, α = 0.5

Figure 6. Chlorophyll *a* (A), chlorophyll *b* (B), total chlorophylls (C) and chlorophyll a/b ratio (D) in bean plants treated with QuitoMax® and subjected to two levels of water supply

On the other hand, the chlorophyll *a*/chlorophyll *b* ratio followed the same behavior as chlorophyll *a*, showing the highest value in the plants that did not lack water during the crop cycle (Figure 6D), followed by the treatments sprinkled with QuitoMax® and the lower ratio in the treatment without product and with less water availability. In general it can be said that the ratio between both types of chlorophyll was not high.

It has been shown that the water deficit causes a decrease both in the growth and development of the plants, as well as in the chlorophyll content (27,28) as well as in the chlorophyll *a*/chlorophyll *b* ratio, indicating that this decrease in the proportion between both chlorophyll types may be related to stomatal limitations (24).

When observing the status of the stomata in the different treatments used, it could be seen that they present a very similar state, where there seems to be an incipient degree of openness, which could be related to the different degree of polymerization and acetylation of the chitosan used or with the doses of QuitoMax® used, which in general were low in relation to those used by other authors who have found that the chitosan application stimulates the stomatal closure and therefore exerting an antiperspirant effect (9,10, 29), although it is noteworthy that other authors in works carried out in *Glycine max* and in *Zea mays* reported that chitosan stimulated stomatal opening and transpiration (30).

CONCLUSIONS

- In conclusion, it can be pointed out that QuitoMax® applied at two stages of the crop development at a dose of 200 mg ha^{-1} favors the water status of the plants grown under conditions of water deficiency in the soil.
- On the other hand, it is suggested to continue the studies related to this subject taking into account that QuitoMax® could be a promising material used to reduce the harmful effect of water stress in plants.

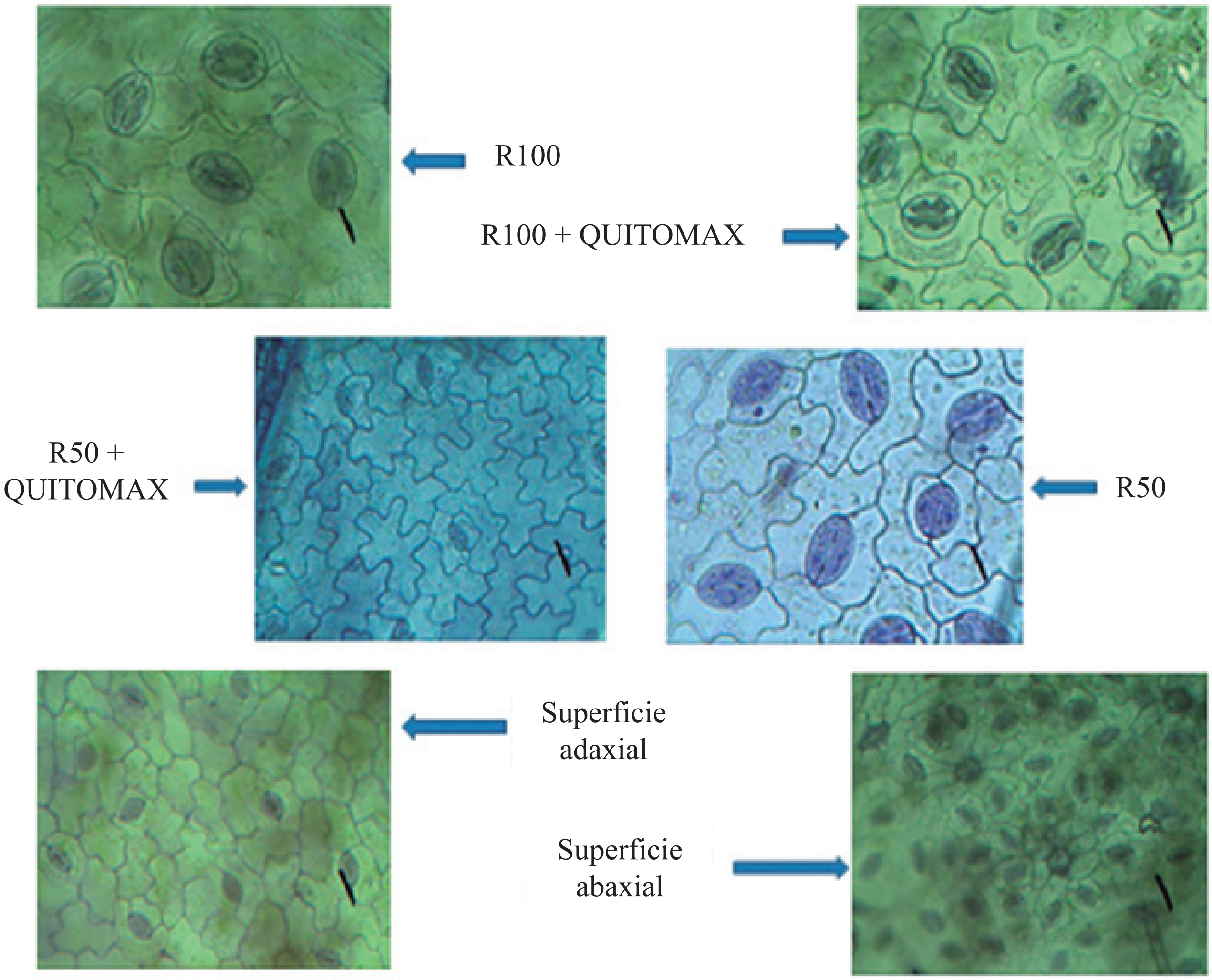


Figure 7. Status of stomata in bean plants treated with QuitoMax® and subjected to two levels of water supply

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