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# QUITOMAX® EFFECT IN BEAN PLANTS (*Phaseolus vulgaris* L.) UNDER TWO IRRIGATION REGIMES. I.GROWTH AND YIELD

## Efecto del QuitoMax® en plantas de frijol (*Phaseolus vulgaris* L.) sometidas a dos regímenes de riego. I.Crecimiento y rendimiento

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**ABSTRACT**. This work was conducted in order to evaluate the effect of QuitoMax® on the growth and yield of bean plants (*Phaseolus vulgaris* L.) under two irrigation regimes. For this seed of the variety of Tomeguín black beans planted in concrete channels of 2,60 m long and 0,60 m wide (1,56 $m^2$) in two separate rows were placed 0,40 m was used a plant spacing of 0,11 cm for a total of 44 plants per channel, each treatment had three replicates. The treatments consisted of applying 100 (R100) percent ETc. (Standard evapotranspiration) and 50 (R50) percent of ETc, counting in each irrigation treatment a variant in which 200 mg $ha^{-1}$ QuitoMax® were applied to the 20-25 days after sowing and a similar dose start flowering and another in which the bioestimulante was not applied. Evaluations were made stem length, stem diameter, number of leaflets, leaf area per plant, number of pods per plant, number of grains per pod and fresh mass of 100 grains, and the yield was estimated by unit area. The analysis of the results indicated first that the 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 improve the behavior of the different variables evaluated under insufficient water supply conditions.

*Key words*: grains, dry mass, chitosan, leaf surface, pods

**RESUMEN**. Este trabajo se realizó con el objetivo de evaluar el efecto del QuitoMax® en el crecimiento y el rendimiento de plantas de frijol (*Phaseolus vulgaris* L.) sometidas a dos regímenes de riego. Para ello se utilizaron semillas de la variedad de frijol negro Tomeguín sembradas en canaletas de hormigón de 2,60 m de largo por 0,60 m de ancho (1,56 $m^2$). 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 utilizados consistieron en aplicar el 100 (R100) por ciento de la ETc. (Evapotranspiración estándar del cultivo) y el 50 (R50) por ciento de la ETc, contándose en cada tratamiento de riego 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 longitud de los tallos, diámetro de los tallos, número de foliolos, superficie foliar por planta, número de vainas por planta, número de granos por vaina y la masa fresca de 100 granos, así como, se estimó el rendimiento por unidad de superficie. El análisis de los resultados indicó 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 mejorar el comportamiento de las diferentes variables evaluadas en condiciones insuficiente de abastecimiento hídrico.

*Palabras clave*: granos, masa seca, quitosano, superficie foliar, vainas

## INTRODUCTION

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 protein, vitamins and mineral nutrients (1).

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Proper management of plant nutrition and efficient control of the pests that affect them are two essential elements for obtaining high productivity and quality in agricultural production; As well as the indiscriminate application of chemicals can cause damage to the environment, create resistance by phytopathogenic microorganisms and cause harm to human health (2). The practice of biocontrol of diseases in plants shows a viable alternative in relation to the traditional chemical method.

Among the products studied for biocontrol, the chitosan polysaccharide found naturally in the cell wall of some fungi stands out. It has been commercially obtained from chitin, with biocompatibility, biodegradability, low toxicity, high bioactivity and microbial activity (3), as well as stimulation of growth, development and yields in crops of interest (4).

Although the mechanisms by which chitosan stimulates plant growth and development are not known, it has been suggested (5) that they are involved in physiological processes, avoiding water losses through transpiration. In this sense, the presence of stomatal closure has been demonstrated in plants sprayed with chitosan, suggesting that the stimulating effect of growth after stomatal closure could be related to an antiperspirant effect in the plant (6), indicating that the application leaf chitosan in potato reduced the effects of water stress (7).

In the bean crop, there are few studies that address the water-chitosan stress interaction. However, it has been shown that water stress damages the growth of common bean plants (*Phaseolus vulgaris* L.) and decreases nutrient content and photosynthetic pigments (8), as well as the concentration of carbohydrates in shoots; affecting the yield and its quality represented by nutrients, proteins and carbohydrates and indicates that the chitosan applied on the leaves to a concentration of 200 mg $L^{-1}$, increases the growth, the yield and its quality, in conditions of stress as well as not stressed. In the bean crop, there are few studies that address the water-chitosan stress interaction.

On the other hand, it has been pointed out from the results found in bean (*Phaseolus vulgaris* L.) cultivation, that one of the aspects through which chitosan was influencing the reduction of transpiration is that this product increases The levels of abscisic acid (ABA) in the treated leaves, which activates the partial closure of the stomata (9).

Likewise, it has been suggested that beans are extremely sensitive to water stress and heat (10) frequently present simultaneously in the most sensitive phenological stages of the plant for yield formation, beginning of flowering, beginning of pod growth and grain filling in rainfed areas. This type of abiotic stress decreases yields and quality of production; as well as, the water deficit significantly affects the yield given by the decrease in the number of grains and the number of pods, when it occurs during the stages of growth, flowering and grain formation.

The objective of this study was to evaluate the effect of QuitoMax® on the growth and yield of bean plants (*Phaseolus vulgaris* L.) submitted 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). Twelve concrete channels of 2,60 m long and 0,60 m wide (1,56 $m^2$) containing Ferralitic Red Leached Soil (11) were planted. In each channel, 44 black bean plants of Tomeguín variety were arranged 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 applications of QuitoMax® were made at a rate of 200 mg $ha^{-1}$. The first to 20 days after sowing and the second at the beginning of flowering. 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 replicates (three channels per treatment). The treatments tested were:

- ♦ R100, irrigated to 100 percent of the ETc. (Standard crop evapotranspiration)
- ♦ R50, irrigated at 50 percent of the ETc.
- ♦ R100 + QuitoMax®
- ♦ R50 + QuitoMax®

The irrigation was applied through an automated micro-sprinkler system and the water delivery was controlled by valves placed in each treatment.

The evapotranspiration of the reference crop (ETo) was calculated using data from a nearby meteorological station (approximately 200 m from the experiment) and the FAO Penman-Monteith method was used (12). Evapotranspiration of the crop under standard conditions (ETc) was calculated by the following equation:

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

where:
Etc. Cultivation evapotranspiration [mm $d^{-1}$],
Kc. Crop coefficient [dimensionless],
Eto. Evapotranspiration of the reference crop [mm $d^{-1}$].

The cultivation coefficients Kc employed were as follows:

Kc. Initial = 0,15, Kc. Mean = 1,10 and Kc. Final = 0,65

During the period between January 26-21 and irrigation was 3 mm daily in all treatments to ensure homogeneous initial germination and growth. From that moment the irrigation was applied according to each treatment. Effective rain was considered when it was more than 3 mm. Other cultural attentions were performed equally in both treatments.

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

Soil moisture (%) was determined weekly by a TDR (Field Time Reflectometer) Field Scout TDR 100 System, Spectrum Technologies, Inc. For each treatment 30 measurements (10 in each container) were performed at 20 cm depth.

### Evaluation of growth

The length and diameter of the stems, the number of folioles, the leaf surface and the dry masses of root, stem, leaves and total were determined at 20, 29 and 42 days after sowing (DAS).

The length of the stems was determined with a graduated ruler measured from the base of the stem to the base of the last emerged leaf, the stem diameter was determined at its base with the aid of a digitized caliper.

The foliar surface was measured using an AMP-300 leaf area integrator and the dry masses were obtained by drying in a forced draft oven at 80 °C to constant weight.

### Performance evaluation

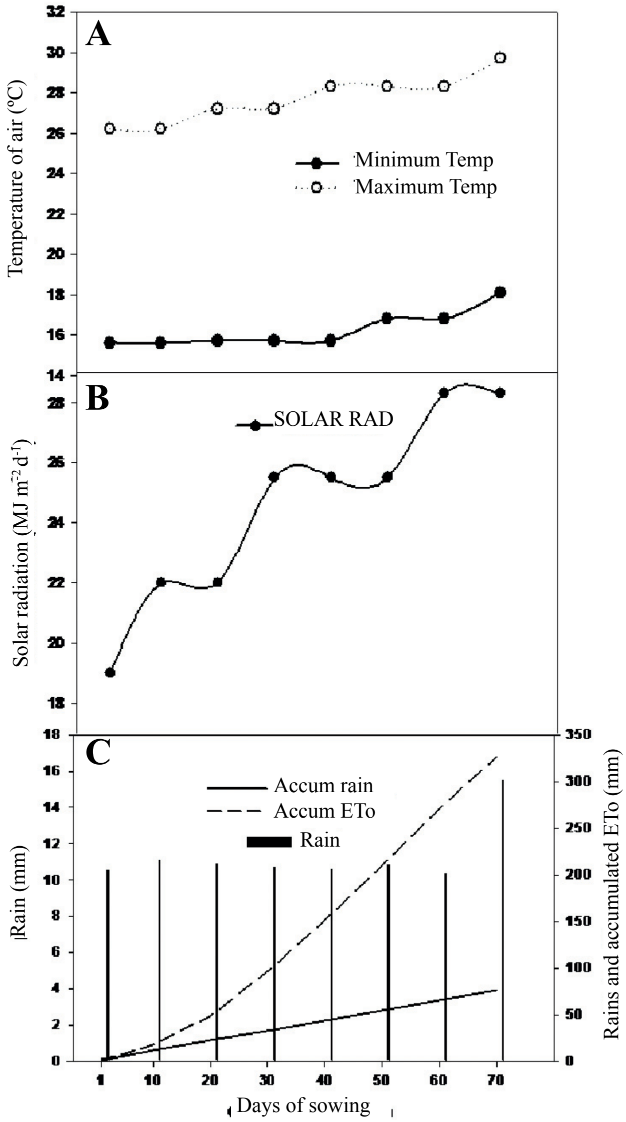
For the evaluation of the yield and its components, 10 plants were harvested at random in each channel (30 plants per treatment), which were determined the number of pods per plant, the number of grains per pod, the fresh mass of 100 grains and in addition, the total yield of each channel (g $m^{-2}$) was determined. The dry masses of the organs, the 100 grains and the yield were determined with an analytical balance with an accuracy of 0,00001g.

The cultural and phytosanitary tasks were carried out according to the Technical Guidelines for bean cultivation (13).

For the data processing and the comparison of means at each evaluated moment, it was used the SPSS 19,0 Statistical Program for Windows (14). The results were plotted using the SIGMA PLOT 11.0 program.

## RESULTADS 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 the figures (Figure 1A and C), mainly due to the fact that the minimum and maximum temperatures had very little variation and their ranges of values were between 16 and 18 ºC the minimum and between 26 and 30 ºC the maximum and the accumulated rain was 77 mm equivalent only to 6,4 mm weekly.

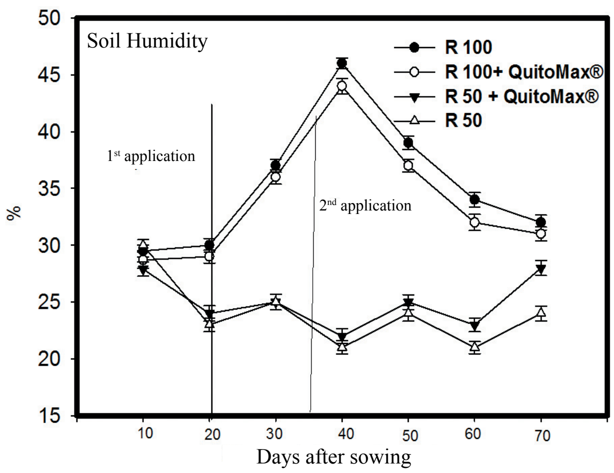



Air temperature (°C), solar radiation (MJ $m^{-2}$ $d^{-1}$), accumulated rainfall (mm) and cumulative standard evapotranspiration (ETo)

**Figure 1. Environmental conditions during the experimental period**

On the other hand, solar radiation (Figure 1B) 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. The accumulated climatic water demand (ETo) in the period was 327 mm, which represents an average daily evapotranspiration of 5 mm. In general, except for temperatures that were relatively warm, although it is argued that beans can be grown with average temperatures ranging from 15 to 27 ºC, with an optimum of 25 ºC (15), other studies have found that beans support up to 50 OC without damage to their cells (16); the values of precipitation and ETo are typical of the months in which the experiment was performed.

Climate components play an important role in the life of living organisms and of nature as a whole. It has therefore been argued that the direct impacts of climate change on natural, economic and social systems (17, 18) Due to high temperatures and changes in rainfall patterns, are becoming more evident, with the primary production sector being one of the most negatively affected. Figure 2 shows the variations of the soil moisture content, where it was observed that in the irrigation treatments R100 and R100 + QuitoMax® the soil moisture always remained above 27 % and at 40 DAS reached maximum values of about 46 % with very few differences between them.




The bars on 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 deep**

As for R100 and R100 + 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 irrigation treatments applied to the crop.

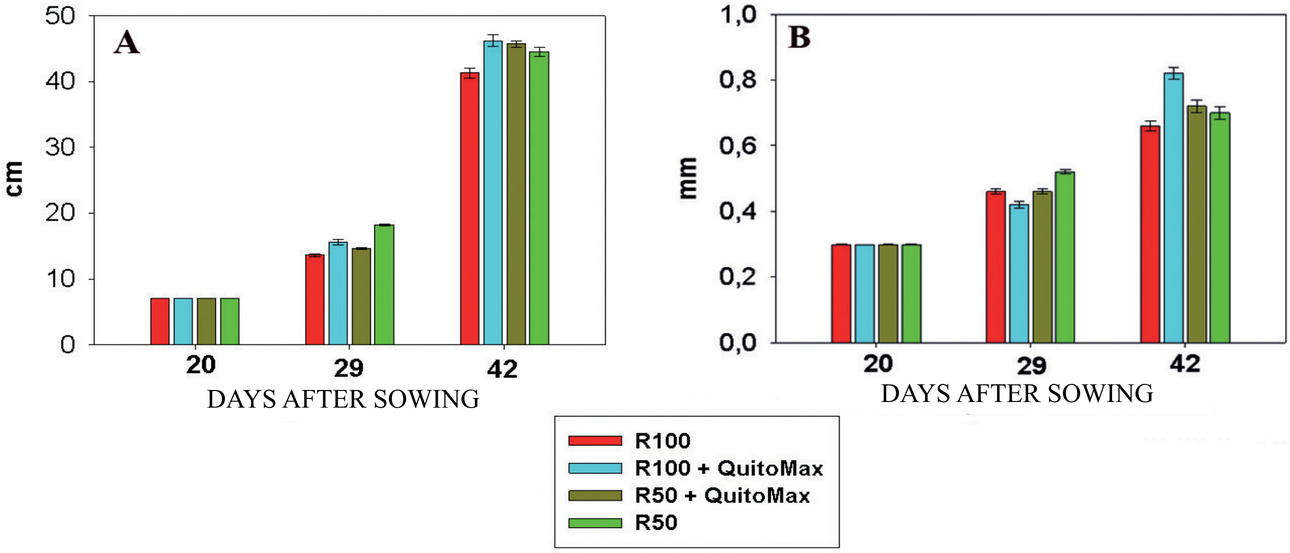
As shown in Figure 3, both the length of the stems and their diameters showed a very similar behavior, showing at 29 days after sowing the highest values in the treatment with less water supply (R50) and without the application of QuitoMax®; as well as, at 42 days, the treatment with the highest availability of water (R100) and without the application of product, showed the lowest values with significant differences with respect to the other treatments.

In both variables, the treatment in which in addition to having the greater availability of water, the biostimulant product (R100 + QuitoMax®) was added, presenting the highest absolute values, even with significant differences in the diameter of stems when analyzing the response shown by these variables, it was observed that this behavior coincides with that reported by other authors who found favorable results in the growth expressed by the length of stems and roots, their fresh and dry masses, leaf surface and the content of chlorophyll in bean (*Phaseolus vulgaris superstryke*) cultivation (4).

When evaluating the results obtained regarding the number of leaflets per plant (Figure 4A), it can be seen that at 29 days after sowing, the plants treated with QuitoMax® and with less availability of water without product significantly exceeded the treatment with greater quantity of available water and that did not receive the applications of QuitoMax® emphasizing the treatments with the smaller availability of water. However, at 42 days the number of leaflets was higher in the treatments that received the applications of the product and with more water available.

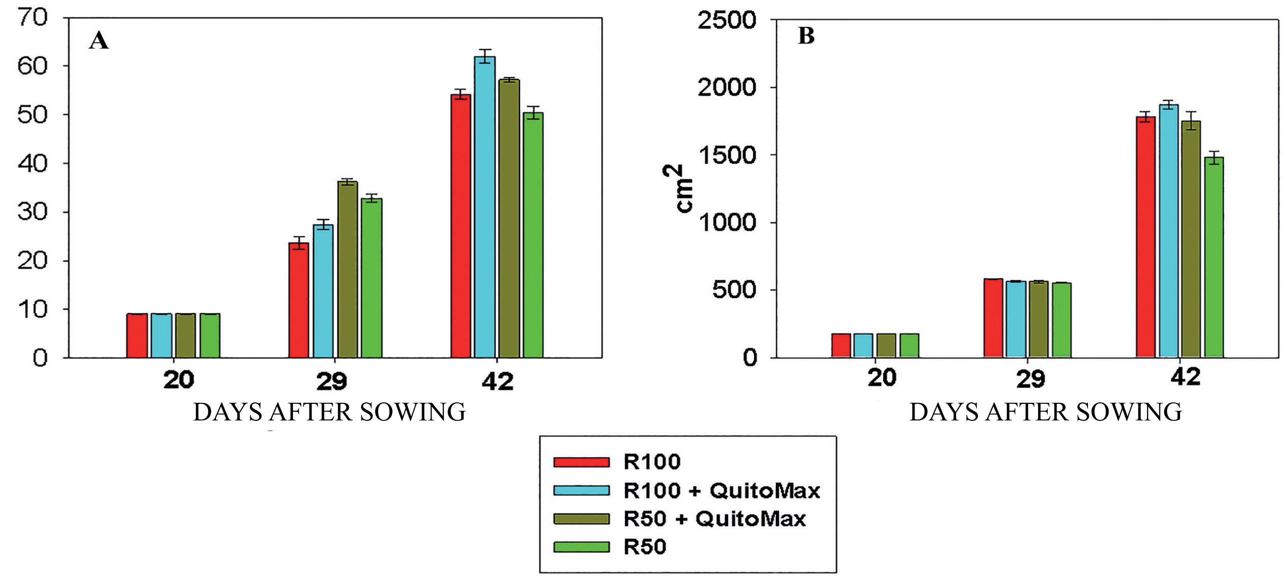
The foliar surface (Figure 4B) showed a similar behavior between the treatments at 29 days, but at 42 the response was in agreement with the behavior of the number of leaflets, which is logical if one takes into account that the second variable depends to a great extent of the first, as long as the size of the leaflets is not altered by treatments.

The water deficit is one of the factors that more quickly and with greater intensity alters the growth of the plants, thus it was demonstrated that the use of irrigation regimes with different levels of water supply caused a decrease in both the plant height, and leaf number, leaf surface and total chlorophyll content as the plants received a smaller amount of water (19).




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

**Figure 3. Length (A) and diameter of stems (B) of bean plants treated with QuitoMax® and subjected to two levels of water supply**



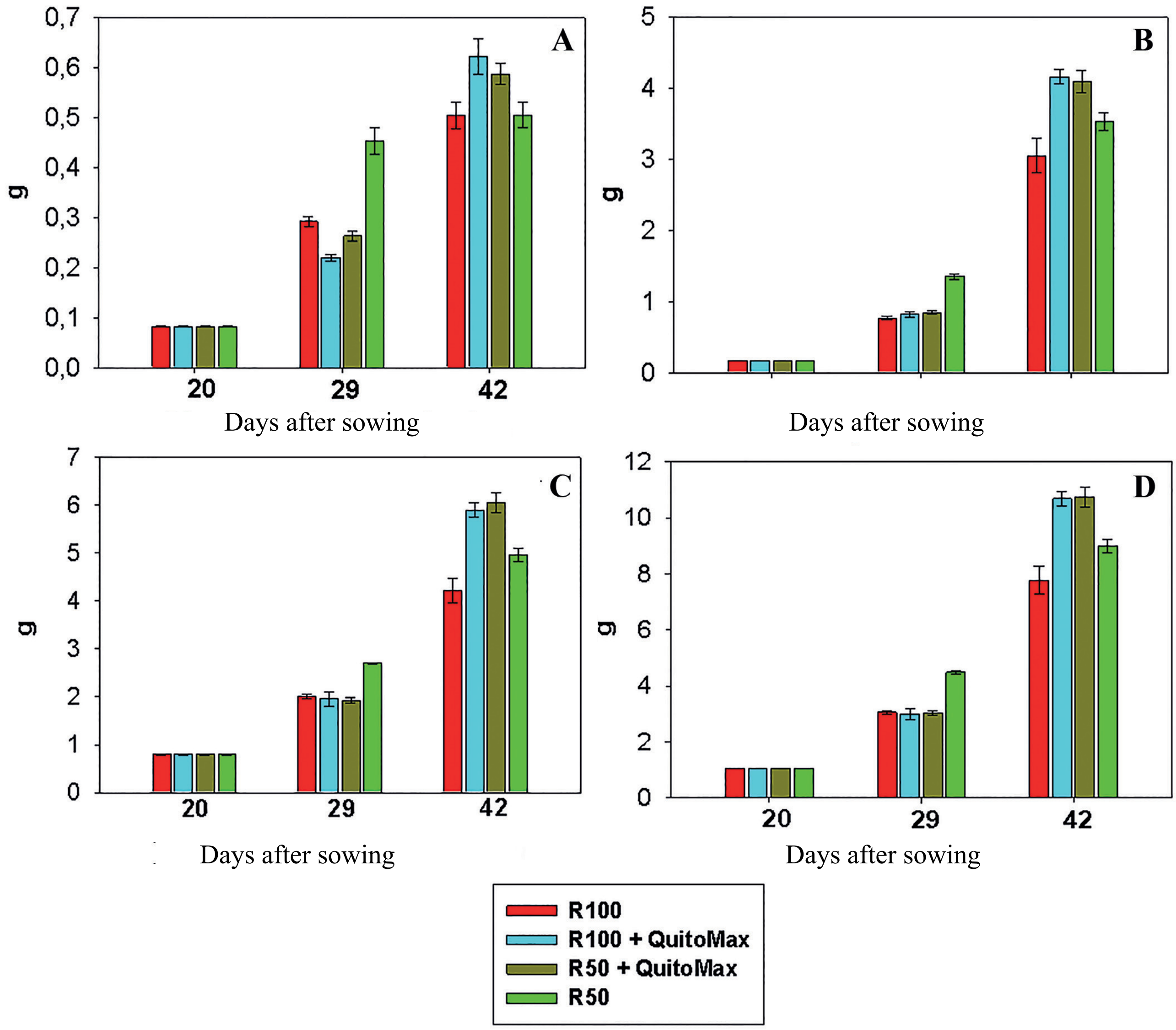

The bars on the mean values represent the confidence interval of the means, α = 0,5

**Figure 4. Number of leaflets (A) and leaf surface (B) of bean plants treated with QuitoMax® and subjected to two levels of water supply**

When the dry mass of the different organs was evaluated (Figures 5A, B, C), it was observed that at 29 days after sowing, the treatment with the lowest water supply and without applications of the biostimulant (R50) was the one that showed the highest values of dry mass with significant differences with respect to the other treatments; However, at 42 days were the two treatments that had the applications of QuitoMax® which reflected the highest values with significant differences with respect to the others.

The treatment (R100) showed the lowest values in different organs, which is in agreement with the behavior reflected by the length and the diameter of the stems.

The response found in relation to the total dry mass (Figure 5D) is closely related to that shown by the indicators that gave rise to it.




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

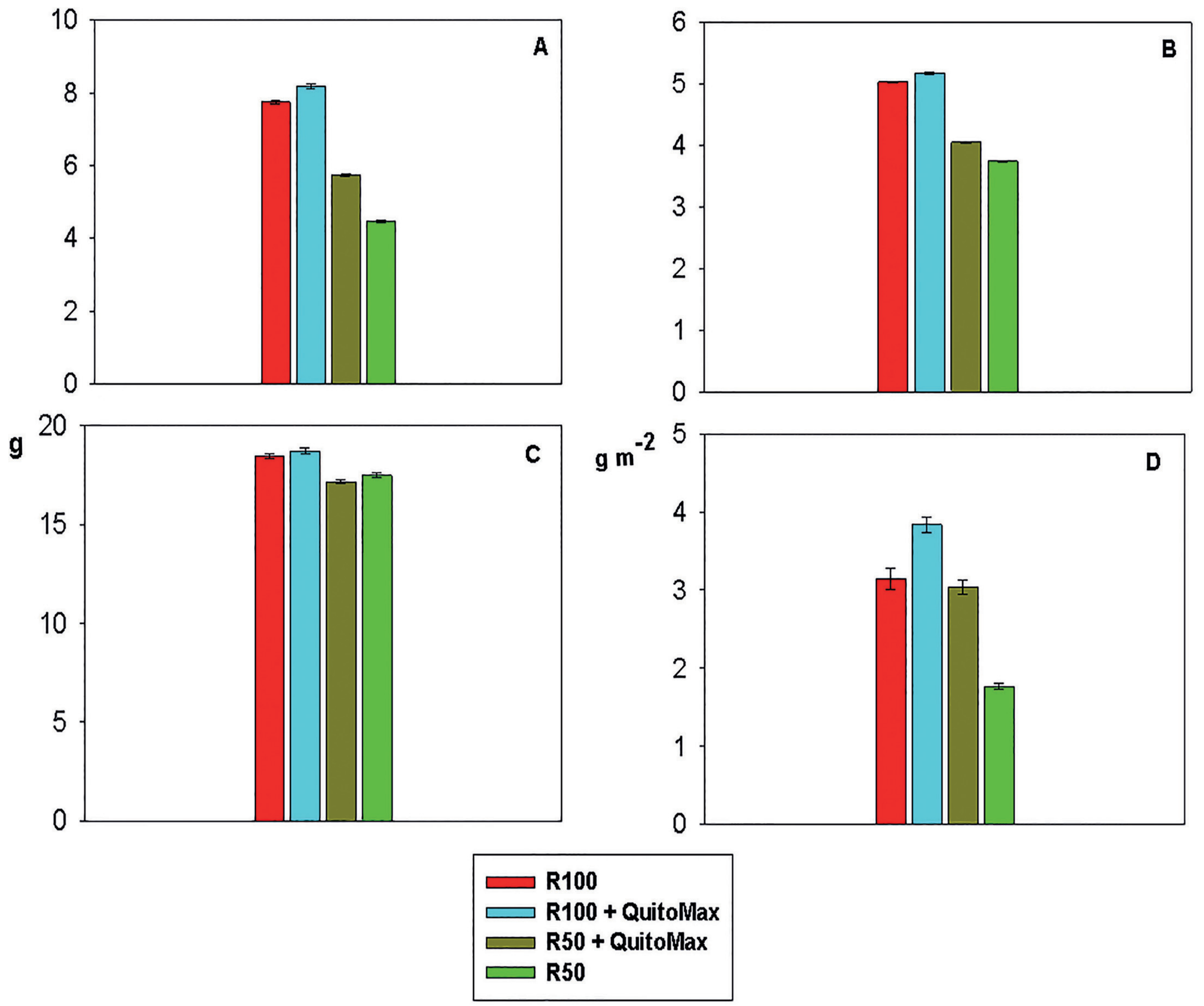
**Figure 5. Dry mass of the root (A), stem (B), leaves (C) and total (D) of bean plants treated with QuitoMax® and subjected to two levels of water supply**

It can be seen in the figures that were first the leaves followed by the stems which contributed most to the accumulation of dry matter by the plants.

The fact that the plants showed the best response to the accumulation of dry matter in their organs during the 42-day treatment period when 50 % of the water (R50) was supplied, compared to the one that received all the necessary water (R100), it is in agreement with other authors who have indicated that the growth of the plants is not susceptible to water deficit in all conditions and stages of its growth (20); the role of growth regulators in the behavior of these variables in relation to water stress conditions has also been highlighted (21), which explains the response shown by treatments with different water supply with the application of QuitoMax®.

Affectations on the increase of dry mass as a consequence of a limited water supply were reported for the cultivation of Sesbania [*Sesbania Sesban* (L.) Merril.] (22).

Figure 6 shows the response found in assessing performance and its components. It can be seen in Figures 6A and B that the treatments best supplied with water were those that reached a greater number of pods per plant and number of grains per pod, being statistically different from the less well-fed ones, being in turn among the latter the one that did not receive the applications of QuitoMax® the lowest values of these variables reached.




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

**Figure 6. Number of pods per plant (A), number of grains per pod (B), fresh mass of 100 grains (C) and yield (D) of bean plants treated with QuitoMax® and subjected to two levels of water supply**

It was also observed that the best available water treatment and the applications of the biostimulant (R100 + QuitoMax®) was the one that reflected the higher values of these variables.

The fresh mass of 100 grains was also favored in the treatments better hydrated, slightly excelling the treatment R100 + QuitoMax®.

On the other hand, the performance achieved with the different treatments is the reflection of the response of the evaluated components, highlighting the treatments that received the applications of QuitoMax®. In the first place, in addition to the product, there was a greater availability of water by showing significantly higher yields than the other treatments and the one with less water supply and the application of the product was able to match its performance with the best supplied without product. The least stocked and without product was, the one that showed the lowest performance.

Similar responses, regarding the number of pods and number of grains per pod during the application on growth regulators on plants were reported when evaluating the effect of the application of Biobras-16 on bean (*Phaseolus vulgaris* L.) (2,3).

It has also been pointed out that drought conditions in the soil produce a significant decrease in the yield components associated with the production of grains and legumes; (24, 25), as well as when a restricted irrigation regime was applied (26).

Increases in crop yield stimulated by the application of chitosan have also been reported in evaluating the effect of foliar applications of cowpea (*Vigna unguiculata* L) (27).

These responses of the plants could be related as has been suggested by other authors (28), with stomatic limitations that affect the photosynthetic capacity of the plants and found that at 17 days of drought, photosynthesis began to register values near zero when the soil moisture content was reduced to about 40 % of the field capacity and that under such conditions the proportion of chlorophyll *a*/chlorophyll *b* was significantly lower while the content of malondialdehyde was significantly higher in plants under water stress.

Another aspect that could affect the yield and its components in the conditions of fewer water supplies is the one related to the abscission of reproductive structures, that take place in periods of lack of water which leads to a limitation of photo assimilates for the formation and filling of the grains (29).

Also, these results may be explained by a possible accumulation of "ASR" proteins, which have been reported from observations made in a plant cell from plants subjected to water stress (30), the presence of a surprising concentration of the same in the cytoplasm. It has been known of the action of these proteins to grant resistance to water stress, so it is pointed out that several laboratories are trying to generate transgenic planes, by inserting the gene that codes for this protein.

The greatest abundance of this protein would be found at the root, which is where the primary signal of water scarcity is most quickly detected. It is likely that a molecular signal is transported by the xylem to the aerial part.

The gene encoding this protein would not be found in plant species originating in tropical and rainy areas. What surprised these researchers was to find it in abundant concentrations in the cytoplasm of the cell, because it is not common in this type of protein, which behaves as a factor of gene transcription.

From this observation, it can be deduced that it would have some function in the cytoplasm.

In order to express a hypothesis, it is likely to work in the cytoplasm as 'chaperone', that is, it would act in a way that would avoid the processes of denaturation of proteins already formed. It is assumed that ASR aids in the folding of other proteins, so that they acquire the correct conformation in space. This would happen in view of the risk that the plant poses to limit stress situations, in this case water.

## CONCLUSIONS

- The QuitoMax® applied at two moments of the development of the crop at doses of 200 mg ha$^{-1}$ increases the growth and the yield of the plants.
- 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 on plants.

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