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Original article

Physiological efficiency of the bean plant before a water deficit

Eficiencia fisiológica de la planta de frijol ante un déficit hídrico

Michel Ruiz-Sánchez[1*], Yaumara Muñoz-Hernández[2], Roselys Rodríguez-Pérez[1], Yoerlandy Santana-Baños[2], Sara Mesa-Sotolongo[4], Max Enrique Encalada-Córdova[3], René Florido-Bacallao[5]

[1]Unidad Científico Tecnológica de Base "Los Palacios", Instituto Nacional de Ciencias Agrícolas (INCA). Carretera La Francia km 1½, Los Palacios, Pinar del Río, Cuba. CP 22 900

[2]Universidad de Pinar del Río "Hermanos Saíz Montes de Oca", Avenida José Martí No. 270, Pinar del Río, Cuba, CP 20 100

[3]Centro Meteorológico Provincial Pinar del Río (CITMA), Colón # 106, entre Maceo y Virtudes, Pinar del Río, Cuba

[4]Universidad de Loja, Ecuador, av. Pío Jaramillo Alvarado y Reinaldo Espinosa, Loja, Ecuador

[5]Instituto Nacional de Ciencias Agrícolas (INCA), carretera San José-Tapaste, km 3½, Gaveta Postal 1, San José de las Lajas, Mayabeque, Cuba. CP 32 700

ABSTRACT: The research was carried out under semi-controlled conditions from November to February of the years 2016, 2017 and 2018 in the Scientific Technological Unit of Base Los Palacios, Cuba, with the aim of evaluating the effect of the water deficit on the physiological efficiency of the bean (*Phaseolus vulgaris* L.) cv. "Delicia 364". 80 bean seeds were sown per 1.40 m^2 pot. A Completely Randomized Experimental Design was used, with three treatments and four repetitions: two with water deficit (beginning of wilting of the leaves and when the yellowing of the apex of the leaves began) and a control with irrigation at field capacity. The water deficit was applied when the plants had between three and four true leaves. After each period of stress, the foliar water potential was determined and at the time of harvest the dry mass, the agricultural yield and its components. It was found that the water deficit imposed in the vegetative phase at different intensities caused a state of water stress in the bean plant and after recovering from it, caused increases in the aerial dry mass, number of pods and agricultural yield. The water deficit in bean plants until the beginning of the yellowing of the apex of the leaves, during the vegetative phase, increases the physiological efficiency of the plant depending on irrigation, between 16-25 %, in terms of the aerial dry mass, the number of pods per plant and the agricultural yield.

Key words: stress, physiology, legume, yield.

RESUMEN: La investigación se realizó en condiciones semicontroladas de noviembre a febrero de los años 2016, 2017 y 2018 en la Unidad Científico Tecnológica de Base Los Palacios, Cuba, con el objetivo de evaluar el efecto del déficit hídrico en la eficiencia fisiológica del frijol (*Phaseolus vulgaris* L.) *cv.* "Delicia 364". Se sembraron 80 semillas de frijol por macetero de 1,40 m^2. Se utilizó un Diseño Experimental Completamente Aleatorizado, con tres tratamientos y cuatro repeticiones: dos con déficit hídrico (inicio de marchitamiento de las hojas y cuando se inició el amarillamiento del ápice de las hojas) y un testigo con riego a capacidad de campo. El déficit hídrico se aplicó cuando las plantas tenían entre tres y cuatro hojas verdaderas. Después de cada periodo de estrés se determinó el potencial hídrico foliar y en el momento de la cosecha la masa seca área, el rendimiento agrícola y sus componentes. Se encontró que el déficit hídrico impuesto en la fase vegetativa a diferentes intensidades causó un estado de estrés hídrico en la planta de frijol y después de recuperarse del mismo, provocó incrementos en la masa seca aérea, número de vainas y rendimiento agrícola. El déficit hídrico en plantas de frijol hasta el inicio del amarillamiento del ápice de las hojas, durante la fase vegetativa, incrementa la eficiencia fisiológica de la planta en función del riego, entre un 16-25 %, en cuanto a la masa seca aérea, el número de vainas por planta y el rendimiento agrícola.

Palabras clave: estrés, fisiología, legumbre, rendimiento.

*Author for correspondence: mich@inca.edu.cu

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INTRODUCTION

Currently, water withdrawals for agriculture represent approximately 70 % of the total water withdrawn from aquifers, rivers and lakes (1). Global environmental changes suggest that in the future there will be an increase in aridity; therefore, it is necessary to look for alternatives that allow efficient use of water and reduce its consumption, taking into account that it is a limited resource. Water scarcity (the imbalance between freshwater supply and demand) and water quality problems threaten food security and nutrition. At the same time, persistent and severe droughts, accentuated by climate change, are causing serious water deficits in agriculture, posing a greater risk to the livelihoods of rural populations by reducing agricultural yields (2). FAO's recent diagnosis predicts that demand for agricultural products will increase by 15 % over the next decade, due to population growth. How this demand is met will determine the impact that the agricultural sector will have on the natural resource base, especially land, water and biodiversity (3), which represents a threat to food security (2).

Drought caused by deficient precipitation in areas destined for agriculture has led to consider the application of water deficit to crops as an alternative to save irrigation water (4). In Cuba, beans (*Phaseolus vulgaris* L.) are an important part of consumer diet; national production covers the areas planted by the Ministry of Agriculture within its strategic projection. In 2019, an area of 197 9667 ha and a production of 240 975 t was sown, with an average yield of 1.2 t ha^{-1}. This figure includes seed production, which means a great challenge for the country's economy, to raise yields (5).

Beans are susceptible to both excess and deficit moisture during the reproductive period. Research has shown that the bean cultivar Delicia-364 is susceptible to drought (6,7), after exposing it to a terminal drought during its entire cycle, with only three irrigations in pre-flowering (R5). Even knowing this cultivar susceptibility degree, it does not mean that an irrigation management at the beginning of the vegetative phase does not contribute to the improvement of the physiological efficiency of the plant. Water deficit is considered as the reduction of the water reserve in the soil and in the root zone of plants (8). The available water in the soil is not enough to satisfy the plant's demand for a determined period and causes a similar effect at the foliar level of the plant (8) in a situation. It cannot exceed its continuous growth; that is, if the deficit does not lead to a severe hydric stress, during the recovery period physiological, biochemical and molecular mechanisms will be activated that will favor the physiological response of the plant (8,9).

Considering the above, the objective of the research was to evaluate the effect of water deficit on the physiological efficiency of the bean plant (*Phaseolus vulgaris* L.) variety Delicia 364.

MATERIALS AND METHODS

The work was carried out in areas of the Basic Scientific and Technological Unit (UCTB) "Los Palacios", belonging to the Instituto Nacional de Ciencias Agrícolas (INCA), located in the southern plains of Pinar del Río province, Cuba, at 22°44' North latitude and 83°45' West latitude, at 60 m.a.s.l., with an approximate slope of 1 %. The research was conducted in the periods from November to February of the years 2016, 2017 and 2018, under semi-controlled conditions. Twelve pots of 1.40 m2 (1.16 m wide x 1.20 m long and with a depth of 0.30 m) were used, which were exposed to the open sky and at a height of 1 m from the surface. The pots contained soil from the agricultural areas of the UCTB "Los Palacios". The soil was classified as Ferruginous Petroferric Nodular Gleysol (10), with a clay loam texture and was characterized by a slightly acid pH (6.46); low organic matter content (2.86 %); assimilable phosphorus (46.80 mg kg^{-1}) and assimilable potassium (34.63 mg kg^{-1}), interchangeable bases with typical contents for this type of soil (12). The information on climatic variables (Figure 1) was obtained from the Meteorological Station "Paso Real de San Diego", no. 317, in Los Palacios, which is located 4 km from the research area.

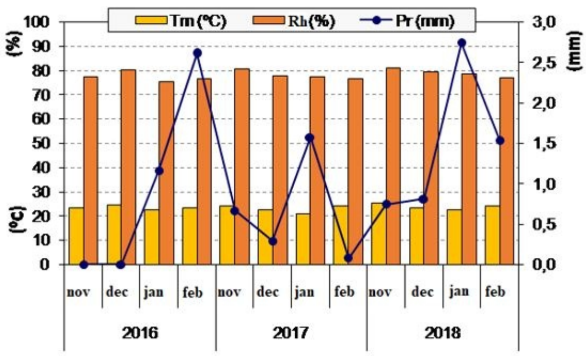

Figure 1. Behavior of meteorological variables in the periods from November to February 2016 to 2018. Monthly mean temperature (Tm), Monthly relative humidity (Hr), Monthly mean precipitation (Pr)

In each pot, four furrows were made, where twenty bean seeds (cv. Delicia 364) were deposited in each furrow at 0.1 m distance between plants, at the time of emergence, 10 plants were left in each furrow. Irrigation management allowed the establishment of the experimental treatments and for this purpose; a completely randomized experimental design was used, with three treatments and four replications: two with water deficit and a control without water deficit, which received a frequency of two weekly irrigations to maintain moisture at field capacity.

In the first irrigation (germination irrigation), 20 L per pot were applied. After emergence, the irrigation interval was four days and 20 L of water per pot was applied to the crop in each irrigation. The water deficit was applied when plants had three, four true single leaves formed (16 and 20 days after germination), and they were irrigated again when wilting or the onset of yellowing became visible at the apexes of the upper leaves (Table 1). To avoid water ingress due to rainfall, preventive conditions were created; that is, the area of the pots was covered prior to rainfall and during the night, pots were also covered.

The control treatment (Without E) showed the highest percentages of soil moisture (40.5 %) with respect to the rest of the treatments, with 28.8 % for the With E-M treatment and 21.8 % for the With E-A treatment. For this type of soil, where the research was carried out, the differences in days between the treatments exposed to stress were six days and the period without applying irrigation for the With E-M treatment was eight days and 14 days for the With E-A treatment. Fertilization was carried out as recommended in the Technical Instructions for Bean Crops (11). In addition, frequent weeding was carried out and phytosanitary control was applied whenever necessary.

Evaluations carried out

The percentage of moisture in the soil was determined before reestablishing irrigation in each treatment exposed to water deficit, for which six measurements were made in each pot diagonally, with two measurements in the center of the pot. A moisture meter (Theta Probe Soil Moisture Sensor - ML2x) was used and was expressed in percent.

As symptoms of wilting onset and yellowing of the apex of terminal leaves became visible, leaf water potential (Ψ_h) was measured with a pressure chamber (Model 615 Pressure Camber Intrument, USA). Four measurements were made on the central plants of each pot; i.e., from the central furrows. Water potential was expressed in MPa.

Plant aerial dry mass (ADM) was assessed at the end of the crop cycle by destructive sampling. Five plants were taken per pot, the samples were placed in an oven at 70 Co until constant mass. In addition, the number of pods per plant (NVP) was counted and 10 plants per pot were taken for this purpose. The agricultural yield (R) was calculated for 10 plants, based on weighing on a Ferton Electronic Balance (precision of 0.001 lb).

For each level of deficit imposed, the physiological efficiency of the bean plant in the use of irrigation water was estimated (With E-M and With E-A), with respect to the agricultural yield achieved, for which an adjustment was made to the formula (12).

$$IEF = \frac{R(without\ E) - R(with\ E)}{R(wit\ E)} \times 100$$

where:

IEF = Physiological Water Use Efficiency Index

$R_{without\ E}$ = Yield without water stress

$R_{with\ E}$ = Yield with water stress

Statistical processing and analysis

Percent moisture measurements were statistically processed and the Standard Error (SE x) of each range per treatment was determined. Data obtained from Ψ_h, ADM, NVP and agricultural yield, were subjected to a Simple Variance analysis, applying Duncan's Multiple Range docime when significant differences were found between means for the level of significance (p≤0.05). In addition, a regression analysis was performed between the mean values of leaf water potential, dry mass and agricultural yield.

RESULTS AND DISCUSSION

The application of a water deficit, when plants had between three and four true single leaves formed, caused symptoms of leaf wilting and the onset of leaf yellowing, conditions that led to a state of leaf water stress in the plant, which was demonstrated by the leaf water potential (Figure 2A). The lowest values of water potentials corresponded to the treatments that showed symptoms of wilting and onset of leaf yellowing, respectively. The homogeneity of moisture in the pots was also verified through the percentage of moisture in the soil (Figure 2B).

Treatments exposed to the water deficit condition in the three years, showed the lowest values of leaf water potential, compared to the treatment without water deficit (No E). The lower values of water potential found correspond to other investigations, such is the case of the bean cultivar 'Toméguín', where 50 % of a total volume of water was applied 21 days after sowing and the water potential decreased to -0.68 MPa (13). However, these water potential values indicated that, water stress in bean plant could be considered moderate, because the lower value of Ψ_h did not exceed -0.6 MPa for the treatment with water stress and onset of leaf apex yellowing. It was reported that, if Ψ_h decreases below -0.77 MPa severe wilt symptoms were observed in bean plants (14). Therefore, it can be considered that the treatment with wilting symptoms, the stress to which these plants were exposed is light, if we take as a reference the values of the water status of plants proposed in the literature (15).

Table 1. Treatments evaluated as a function of irrigation management in bean cv. Delicia 364, from November to February 2016 to 2018

Treatments	Legend
T1- Water deficit until the onset of leaf wilting and then irrigated to field capacity.	With E-M
T2- Water deficit until the beginning of yellowing of the leaf apex and then irrigated until field capacity.	With E-A
T3- Irrigated to field capacity throughout the crop cycle, without water deficit (control).	Without E

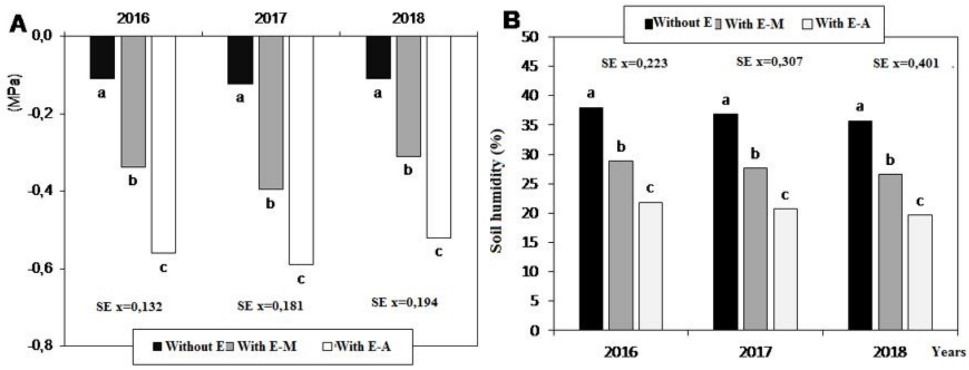

Figure 2. Percentage of soil moisture (A) and leaf water potential of bean plants cv. Delicia 364 (B), exposed to water deficit grown under semi-controlled conditions. (With E-M) with water deficit until the onset of leaf wilting; (With E-A) with water deficit until the onset of yellowing of the leaf apex; (Without E) a control without water stress. Bars with unequal letters differ significantly (p<0.05) according to Duncan's Multiple Range Test, n=5

As for the aerial dry mass after the recovery period of the plants, that is, at the time of harvest, it was observed that it was always higher in the treatments with water deficit imposed during the vegetative phase. The accumulation of ADM in the With E-M treatment ranged between 2.46 and 2.63 g, for the With E-A treatment, its behavior ranged between 2.64 and 2.82 g and in the case of the treatment (Without E) the dry mass values ranged between 1.73 and 1.81 g (Table 2).

Considering the above, the foliar water deficit found did not exceed the physiological limits that would condition a negative effect on the plant. At the same time, it possibly led to the activation of the response mechanisms to this condition, which is called "evasion" (16). This mechanism allows the plant to make osmotic adjustment at the cellular level (16,17), with the purpose of maintaining cell turgor and the processes that depend on it, such as cell expansion and growth, the opening of stomata and photosynthesis, as well as the maintenance of a water potential gradient favorable to the entry of water into the plant (16). These mechanisms, after the restoration of irrigation, favored the recovery of the plant and contributed to greater growth and development.

The water deficit imposed in the vegetative phase led to wilting and the beginning of yellowing of the leaf apex and caused a differential effect during the three years in which the research was carried out on the bean plants (cv. Delicia 364). A positive behavior was observed at the end of the cycle, in favor of this irrigation variant, with respect to the control treatment without water deficit (No E), in the growth variables (ADM) and yield variables (number of pods per plant and grains per plant) (Figures 3 and 4). These results disagree with those of several authors (6,18), who have worked with water deficit in this crop, and who assure that it always decreases the dry mass and, finally, affects the grain yield.

In the case of pod number per plant for the treatment With E-M, it was maintained throughout the study between 10.9 and 11.7 pods; while for the treatment With E-A it ranged between 12.0 and 14.3 pods, these two treatments statistically surpassing the control (Without E), which showed lower values between 8.8 and 9.6 pods per plant. The highest number of pods per plant was found in the three consecutive years when the water deficit reached the onset of yellowing of the leaf apex, followed by the leaf wilting treatment. The increase in the number of pods per plant was due to a possible increase in the root system, as a mechanism to absorb water, and this, after irrigation was restored, conditioned a greater surface area of soil explored to absorb water and nutrients. Nevertheless, the number of pods per plant in all treatments was below the potential values of the variety (11,19,20). In addition, the recovery period allowed a greater accumulation of aerial dry mass and reserves for the formation of yield components.

Table 2. Production of aerial dry mass and number of pods per plant of bean cv. Delicia 364, exposed to deficit grown under semi-controlled conditions

Treatment	Aerial dry mass (g plant^{-1})			Pod number per plant		
	2016	2017	2018	2016	2017	2018
Without E	1.81 c	1.76 c	17.3 c	8.80 c	9.80 c	9.60 c
With E-M	2.60 b	2.46 b	2.63 b	11.73 b	10.90 b	11.12 b
With E-A	2.82 a	2.64 a	2.71 a	14.30 a	12.00 a	12.82 a
SE x	0.018	0.015	0.019	0.055	0.046	0.049

With E-M) with water deficit until the onset of leaf wilting; (With E-A) with water deficit until the onset of yellowing of the leaf apex; (Without E) control without water stress; (SEx) Standard Error

Means with equal letters do not differ significantly, according to Duncan's test for p<0.05, n=5.

On the other hand, the grain yield of plants when exposed to different intensities of water stress (wilting of leaves and yellowing beginning of the leaf apex) without affecting the leaf area (without loss of plant tissue), allowed an increase in yield when compared to the control treatment, with best treatments. Those are exposed to water deficit during the three years of study (Figure 3A). The increase in grain yield indicated a physiological efficiency of the plant (Figure 3B), since the water deficit led to the activation of response mechanisms to this condition, with the purpose of tolerating or surviving the imposed water deficit.

The physiological efficiency in the use of water was always higher in the treatments that showed the yellowing beginning of the apex of leaves (With E-A), with respect to wilting (With E-M). In this regard, they reported that, although the production of total dry mass (biological productivity) of the plant is significant, it is also necessary to guarantee that part of this total production is destined to the economically useful part of the plant (21). It is important to highlight that the evident resumption of growth or recovery of the plant, once irrigation was restored, was evidenced by the greater accumulation of dry mass and number of pods per plant, indicators that are determinant (22) in the yield achieved. Grain yield during the three years of the variants withered leaves of the plants behaved between 160.6 g and 179.8 g. Meanwhile, the variant, yellowing of the apex of plant leaves, ranged between 181.6 and 206.9 g, and the control was lower than the treatments, ranging between 105.7 and 120.0 g, which shows the positive effect of causing a water deficit in this crop in the early stages of its cycle (vegetative phase).

The results obtained in this research do not coincide with those reported by other authors (4), who state that when this crop was subjected to a water deficit in this vegetative phase, grain yield and its components did not decrease. A similar response was found in another study (7), where the yield was statistically higher when the crop did not suffer from water deficit during the vegetative cycle of the crop. The higher yield achieved in the treatment with E-A can be attributed to the component number of pods per plant and to the higher accumulation of dry mass per plant due to the effect of the imposed hydric deficit. Among other factors, similar results were reported by other authors (21,23) and at the same time, they contradict with other reported studies (6). Yield levels can be higher or lower, depending on the intensity of the hydric deficit (24) and the time of exposure of plants to this condition (25), besides their own genetic capacity to tolerate this condition.

In other research related to the water deficit by default in the cultivation of beans, several authors (26) reported increases in grain yield and its components. In this respect, other authors in this same crop (27) and in others different to this one, as in tomato (28); in rice (26), do not coincide their criteria with the results obtained in this investigation, where the deficit of water imposed in the soil, instead of diminishing the yield in grain, motivated its increase. This result was achieved with cv. Delicia 364, under the edaphoclimatic conditions of Los Palacios, in the province of Pinar del Río. This result could be indicating that the beginning of leaf wilting is enough to promote the activation of antioxidant mechanisms or the production of signals at a biochemical or molecular level, which allow the plant to recover (8,24). Given that the water deficit was caused in the vegetative stage, it should not be ruled out that root deepening and, in general, the interaction of the shoot/root ratio could have influenced the exploration of a larger soil volume and greater access to water and mineral nutrition.

The increase in physiological efficiency of water use by bean plants, even when the loss of turgor in leaves manifested flaccidity (wilting of leaves). It could be indicating a behavior sufficiently visible to promote the activation of antioxidant mechanisms or the production of signals at the biochemical or molecular level, which allow the plant to recover and thus promote the efficiency of water use by the plant. It was reported that the condition of water deficit activates the production of ascorbate and glutathione, while decreasing the production of peroxide and oxidative damage and, therefore, the plant can recover from the imposed water deficit, as long as there is no unfavorable imbalance in the production of reactive oxygen species and the production of antioxidant molecules (9,24).

The regression analysis between Ψ_h and aerial dry mass (ADM) in the three years of research (2016, 2017 and

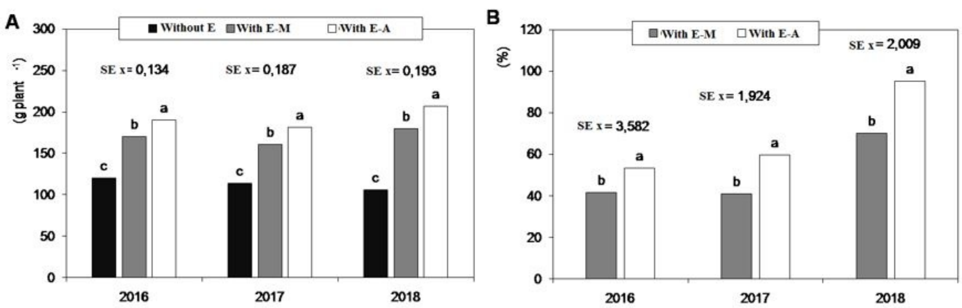

Figure 3. Agricultural yield per plant of bean *cv*. Delicia 364 (A) and physiological efficiency of plants (B), exposed to water deficit grown under semi-controlled conditions. (With E-M) with water deficit until the beginning of leaf wilting; (With E-A) with water deficit until the beginning of yellowing of leaf apex; (Without E) control without water deficit; (SEx) Standard Error Bars with unequal letters differ significantly (p<0.05) according to Duncan's Multiple Range Test, n= 10

2018), showed a coefficient of determination higher than R^2=0.810 and correlation coefficients (r) exceeded 0.905, indicating a direct and positive linear relationship between the variables, statistically significant for a confidence level of 95 % (Figure 4).

It was observed that, as water potential decreased with water intensification, the accumulation of aerial mass increased. This response can be considered positive, as long as the symptoms of water stress intensity do not exceed the onset of yellowing at the apex of the leaves.

The relationship between Ψ_h and agricultural yield in the three years, showed a coefficient of determination higher than R^2=0.93 and the correlation coefficients exceeded 0.965, indicating a direct and positive linear relationship between the variables, statistically significant for a confidence level of 95 %. It was observed that, as water potential increased, grain yield per plant increased.

The intercept of the points describing the related variables (Figure 4), conformed the three treatments evaluated and indicated that, the lowest values of Ψh, ADM and agricultural yield correspond to the control (No E), during the three years of research. The relationship found between Ψh, ADM and agricultural yield corroborates what was reported by other authors, regarding the increase of ADM and yield as a function of a better hydric condition of the plant, after the recovery period; that is, at the time of harvest (25).

The water deficit condition (until the beginning of leaf wilting and until the yellowing beginning) applied to crop contributes on average about 10 and 12 days without irrigation, respectively. It is evidenced through the leaf water potential in bean plants (*cv*. Delicia 364) a water deficit state, with a higher physiological efficiency of the plant depending on the irrigation between 16-25 % compared to the treatment without water deficit.

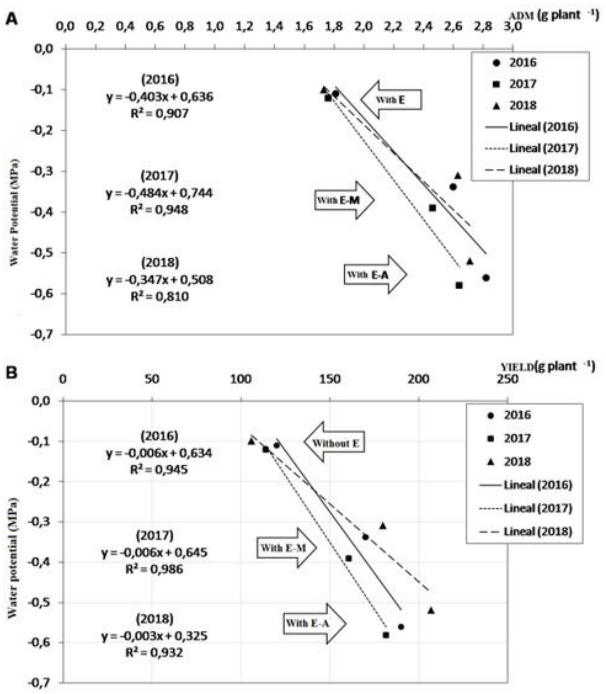

Figure 4. Relationship between leaf water potential and aerial dry mass (A) and agricultural yield (B) in bean plants *cv*. Delicia 364, exposed to water deficit grown under semi-controlled conditions. R^2=regression coefficient With E-M) with water deficit until the onset of leaf wilting; (With E-A) with water deficit until the onset of yellowing of the leaf apex; (Without E) control without water deficit Arrows indicate groupings by treatments

CONCLUSIONS

- The water potential of bean plants (*cv*. Delicia 364) shows a state of leaf water deficit, which is characterized as light, when wilting symptoms are observed, and moderate to severe when yellowing of the leaf apex is observed.
- Water deficit in bean plants (*cv*. Delicia 364) until the onset of yellowing of the leaf apex during the vegetative phase increases aerial dry mass, number of pods per plant and agricultural yield at the end of its cycle, with a physiological efficiency depending on irrigation between 16-25 %.
- There is a direct relationship between water potential, dry mass accumulation and agricultural yield of bean plants (*cv*. Delicia 364), which favors their productivity at the end of the vegetative cycle.

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