ORIGINAL ARTICLE

Response of Bean Crops to Controlled Deficit Irrigation Applied at Different Stages of their Biological Cycle

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Respuesta del cultivo del frijol al riego deficitario controlado en diferentes momentos de su ciclo biológico

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ABSTRACT: The research was conducted at the National Institute of Agricultural Sciences located in Mayabeque province, Cuba, with the aim of determining the response of bean crops to controlled deficit irrigation applied at different stages of their biological cycle. The study was carried out during two planting seasons (January and October 2021) under semi-controlled conditions. Seeds of the Triunfo 70 cultivar were sown in concrete containers, and three irrigation suspension periods of 15 days were studied: during the vegetative growth stage (VG), flowering stage (FS), and grain filling stage (GFS), along with a control group irrigated at 100% of the ETc. After the irrigation suspensions, soil moisture, growth indicators, and yield components were evaluated. In VG during the second experiment, it reduced stem length, leaf number, and leaf area. Above-ground dry mass decreased in both experiments, as well as the mass of 100 grains and grams per plant. In FS, it reduced stem length, relative chlorophyll content (RCC), mass of 100 grains, and yield in grams per plant, while in GFS, only the RCC was affected. Based on the results, it can be concluded that the grain filling stage was the least sensitive to water deficiency, causing the least impact on yield.

Keywords: Soil Moisture, Relative Water Content, Relative Chlorophyll Content, Growth, Yield.

RESUMEN: Las investigaciones se realizaron en el Instituto Nacional de Ciencias Agrícolas ubicado en la provincia de Mayabeque, Cuba, con el objetivo de, determinar la respuesta del cultivo del frijol al riego deficitario controlado aplicado en diferentes momentos de su ciclo biológico. El trabajo se realizó en dos momentos de siembra (enero y octubre de 2021) en condiciones semi-controladas. Se utilizaron semillas del cultivar Triunfo 70 las que fueron sembradas en contenedores de hormigón, se estudiaron tres momentos de suspensión del riego durante 15 días, en las etapas de crecimiento vegetativo (SC), de floración (SF) y de llenado del grano (SLL) y un control regado al 100 % de la ETc. Al concluir las suspensiones del riego, se evaluaron la humedad del suelo, indicadores del crecimiento y los componentes del rendimiento. En SC en el segundo experimento redujo la longitud del tallo, el número de hojas y el área foliar. La masa seca aérea disminuyó en ambos experimentos, la masa de 100 granos y los gramos por planta. En SF, redujo la longitud del tallo, el contenido relativo de clorofilas (CRC), la masa de 100 granos y el rendimiento en gramos por planta y en SLL, solamente el CRC. De los resultados se puede concluir que la etapa menos sensible a la deficiencia hídrica fue la del llenado del que provocó la menor afectación del rendimiento.

Palabras clave: humedad del suelo, contenido relativo de agua, contenido relativo de clorofilas, crecimiento, rendimiento.

INTRODUCTION

Beans, the most significant legume in human consumption, are an essential nutritional supplement in the daily diet of over 300 million people worldwide, playing a crucial role in agricultural production systems (Calero *et al.*, 2018).

Climate change, a widely studied phenomenon, can profoundly impact agriculture, particularly due to irregular precipitation patterns (Ottaiano *et al.*, 2021).

In Central America and the Caribbean, bean cultivation often occurs in low-fertility soils, impacting crop yield (Beaver *et al.*, 2021). In Cuba, a

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significant portion of bean production is achieved under limited irrigation systems.

This region has historically been affected by extreme hydrometeorological events, with droughts being one of the most detrimental to the agricultural sector, compromising access to safe and nutritious food (Calvo *et al.*, 2018).

Drought, a natural hazard, can have severe socio-economic impacts, disrupting human activities, social development, and the environment, affecting all nations, regardless of their level of development (Ortega, 2018).

Sixty percent of the world's bean production occurs under water deficit conditions, making it a major contributor to yield reduction after diseases.

The impact of water deficit on productivity varies depending on the phenological stage at which it occurs (Reyes *et al.*, 2014).

Additionally, water deficit is a significant environmental factor affecting plant growth and development. Under water stress conditions, growth decreases proportionally to the severity and duration of the stress, but if not lethal and maintained for a certain period, the plant can recover (Rodríguez *et al.*, 2021).

In irrigated agriculture, irrigation practices are complex, requiring technical information to precisely balance applied water and crop yield (Domínguez *et al.*, 2014).

Certain irrigation strategies, such as deficit irrigation based on plant phenological development, can reduce water frequency and quantity with minimal effects on conventional yields when validated locally (Mendoza *et al.*, 2016).

Given these considerations, this study aimed to determine the response of bean crops to controlled deficit irrigation applied at different stages of their biological cycle.

MATERIALS AND METHODS

The study was conducted over two crop cycles at the National Institute of Agricultural Sciences (INCA), located at 22°58'00"N and 82°09'00"W, at an elevation of 138 meters above sea level.

Twelve concrete containers measuring 2.60 m in length by 0.60 m in width (1.56 m2) were used, filled with Red Leached Ferralitic soil from the Mayabeque province (Hernández *et al.*, 2015), part of the Habana-Matanzas karstic plain (Castillo *et al.*, 2020).

In each container, seeds of the Triunfo 70 bean variety were sown in two rows with a spacing of 0.40 m between rows and 0.10 m between plants, resulting in 52 plants per container.

The treatments used consisted of suspending irrigation (SR) for 15 days at different stages of the plants' biological cycle. These stages were the Vegetative Growth stage from 15 to 30 days after planting (VG), the Flowering Stage from 30 to 45 days (FS), the Grain-Filling Stage from 50 to 65 days (GFS), and a control treatment in which plants were supplied with 100% of the ETc (crop evapotranspiration) throughout the period.

Irrigation was applied using an automated micro-sprinkler system, and water delivery was controlled through valves conveniently placed on the irrigation sides of each treatment. The pH and electrical conductivity values of the water applied to the crop during the experiment were 7.8 and 0.58 dS/m, respectively.

To avoid the effects of precipitation or dew during the irrigation suspension period, a transparent nylon sheet was placed over the plants without making contact with them.

1 kg of cattle manure was added to each container to improve fertility and substrate structure.

The Reference Evapotranspiration ETo (mm), the crop standard Evapotranspiration ETc (mm), and the irrigation requirements (ETc = ETo * Kc) were obtained using the CropWat 8 Program. This program was updated with a 31-year historical series of meteorological data (1990-2021) from the Tapaste weather station, which belongs to the National Meteorology Institute and is located approximately 200 meters from the experimental site. Monthly average values were used to calculate ETo and ETc.

The crop coefficients (Kc) used were: Kc beginning = 0.26, Kc middle = 1.08, and Kc end = 0.52 (Pérez *et al.*, 2021).

Growth evaluations, soil moisture, relative water content, and chlorophyll content were performed at 30, 45, and 65 days after planting (DAP), coinciding with the end of irrigation suspension periods in the vegetative growth (VG), flowering (FL), and grain filling (GF) stages.

For the determinations of relative water content, chlorophyll content (measured with a SPAD), and growth, ten replicates per treatment were taken.

The evaluations consisted of determining soil moisture, relative water content at 7 solar hours before applying replenishment irrigation, stem length and diameter, dry mass of stems, leaves, and aboveground parts, leaf area, relative, absolute, and net assimilation rates, leaf area ratio, chlorophyll content in SPAD units, number of pods per plant, number of grains per pod, 100-grain mass, grain dimensions, and plant yield.

Growth relationships were determined using the functional method (Barrientos *et al.*, 2015).

A randomized complete block design with three replications was employed, and 10 plants were sampled from each replication (30 per treatment).

Data analysis was performed using the statistical package Statgraphics Plus 5, and means were compared using the Least Significant Differences or

Tukey's multiple range tests, as appropriate. Sigma Plot 11 was used for data visualization.

RESULTS AND DISCUSSION

Figure 1 illustrates that, in all cases, soil moisture in treatments where irrigation was suspended for 15 days significantly decreased, even reaching values below 50% of that achieved in the control treatment (100% of the ETc). This behavior indicates that the plants experienced moderate to severe soil water stress during that period.

This result confirms that plants subjected to irrigation suspension at different stages of their biological cycle were exposed to periods of soil water stress, creating distinct physiological conditions for their development, consistent with previous observations by other researchers (Romero *et al.*, 2019).

The relative water content (Figure 2) exhibits a similar trend to that shown by soil moisture, confirming the presence of water stress in both the soil and the plant.

This indicator reflects the degree of water saturation in the plant under specific soil water supply conditions and is closely related to processes such as transpiration and photosynthesis. Transpiration, in particular, determines the absorption of water and nutrients into the plant, promoting the execution of other physiological processes. It is a useful indicator for measuring plant tolerance to water stress conditions (Zegaoui *et al.*, 2017).

Similar results have been reported by other authors when subjecting plants of this crop to different soil moisture levels and treatments with various biostimulants (Dell Amico *et al.*, 2017; Estrada *et al.*, 2018).

Table I presents the dimensions of the stems, dry masses of stems, leaves, above-ground parts, and leaf surface. It is noticeable that variables related to stem growth did not differ from the control treatment in any of the variants used. This might suggest that the stress period was not sufficiently prolonged to achieve significant differentiation between treatments. However, both the dry mass of leaves and the above-ground part in treatments with irrigation suspension differed from the control treatment. This is associated with the accumulation of assimilates in these photosynthetic organs, significantly impacting the behavior of these variables.

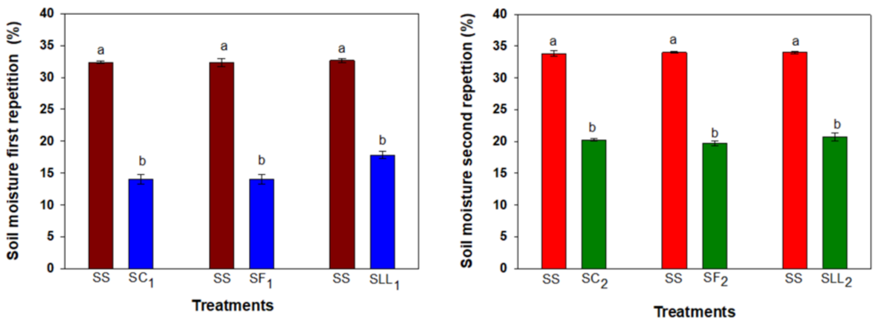

FIGURE 1. Soil Moisture at the conclusion of 15-day irrigation suspension periods (SR) during Vegetative Growth (SC), Flowering (SF), and Grain Filling (SLL) Stages. Error bars above the columns represent standard errors of the means, and different letters indicate significant differences between treatments for α ≤ 0.05 according to the LSD (Least Significant Difference) test.

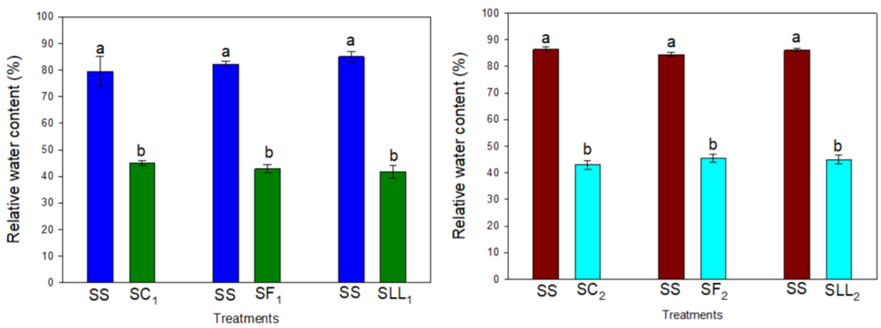

FIGURE 2. Relative water content at the conclusion of 15-day Irrigation suspension periods (SR) during Vegetative Growth (SC), Flowering (SF), and Grain Filling (SLL) Stages. Error bars above the columns represent standard errors of the means, and different letters indicate significant differences between treatments for α ≤ 0.05 according to the LSD (Least Significant Difference) test.

TABLE I. Effect of RDC treatments on three phases of bean plant (*Phaseolus vulgaris* L.) development in different morphological indicators. Different letters indicate significant differences between treatments for $\alpha \leq 0.05$ according to the LSD (Least Significant Difference) test.

Treatments	Stem length (cm)	Stem diameter (mm)	Stem dry mass (g)	Leaf dry mass (g)	Dray mass aerea (g)	Leaf surface area (cm^2)
First Repetition						
100 % ETc	14.56	4.00	0.44	3.09 a	3.99 a	1312.04 a
Suspension in vegetative growth stage (SC$_1$)	13.06	3.20	0.39	1.63 b	2.03 b	995.54 b
LSD	1.57	0.27	0.11	0.09	0.182	111.2
100 % ETc	37.02	4.20	1.20	4.94 a	6.07 a	2158.83 a
Suspension in flowering stage (SF$_1$)	35.00	3.80	1.12	2.79 b	4.02 b	1759.15 b
LSD	2.85	0.30	0.08	0.17	0.20	139.93
100 % ETc	59.60	6.80	2.61	12.67a	15.27 a	3698.19 a
Suspension in grain filling stage (SLL$_1$)	55.20	6.40	1.87	6.930b	8.80 b	2244.73 b
LSD	6.68	0.55	0.31	1.20	1.25	400.18
Second repetition						
100 % ETc	53.12	0.146 a	0.95	2.97 a	3.92 a	1793.38 a
Suspension in vegetative growth stage SC$_2$)	54.50	0.106 b	0.90	2.33 b	3.23 b	1443.14 b
LSD	3.16	0.01	0.06	0.14	0.20	86.71
100 % ETc	90.86	0.46	1.33	3.28 a	4.61 a	1834.82 a
Suspension in flowering stage (SF$_2$)	74.68	0.48	1.10	2.87 b	3.97 b	1571.00 b
LSD	13.55	0.03	0.11	0.12	0.12	75.34
100 % ETc	87.60	0.48	3.87	6.72 a	10.60 a	3892.69 a
Suspension in grain filling stage (SLL$_2$)	85.20	0.48	3.81	5.17 b	8.98 b	2958.24 b
LSD	4.79	0.03	0.26	0.46	0.38	256.85

This response indicates that the dry mass of the above-ground part was determined by the dry mass of the leaves. Other authors have reported a similar behavior of stem dry mass when evaluating the effect of applying certain biostimulants along with nitrogenous fertilizers (Martínez *et al.*, 2017).

On the other hand, both the dry masses of leaves, the above-ground part, and the leaf surface showed statistically different differences in the three moments when irrigation was suspended. There is a more pronounced depression in leaf surface during the grain-filling stage, which could be a consequence of a slight leaf drop in the final stage of development in plants subjected to water deficit. This may also be associated with a decrease in leaf size, aspects that have been noted in studies correlating relative water content behavior with leaf size (Borjas *et al.*, 2015).

The response exhibited by these variables suggests that the plants have developed a potential coping mechanism to water deficit by reducing the evaporative surface. This, in turn, lowers the plant's water usage rate, thereby conserving or preventing water loss during water stress (Luna *et al.*, 2012; Valverde y Arias, 2020). Similar effects on the morphophysiological behavior of different bean genotypes under water stress conditions have been reported by other researchers (Culqui *et al.*, 2021) as well as in other crops such as *Gliricidia Sepium* Gliricidia Sepium (Valverde & Arias, 2020).

At the end of each irrigation suspension period (Table II), the analysis of the following growth relationships was performed: Relative Growth Rate (RGR), Absolute Growth Rate (AGR), Net Assimilation Rate (NAR), and Leaf Area Ratio (LAR). It was observed that the lowest rates were achieved in treatments where irrigation suspensions occurred during the vegetative (15-30 days) and the flowering (30-45 days) stages, confirming the sensitivity of the plant growth process to water deficiency. However, when the suspension occurred during the grain-filling stage (when plants have practically reached their maximum growth), the values obtained were significantly higher than those found in the earlier stages but lower than when irrigation was not interrupted.

Moreover, the Leaf Area Ratio (LAR) showed higher values in plants that were subjected to water deficiency at some point. A significant growth depression was observed when plants were subjected to irrigation suspension in the early stages of development. The Relative Growth Rate (RGR) was 42% during the vegetative growth stage (SC), 38%

TABLE II. Relative and absolute growth rates, net assimilation rate, and leaf area ratio in bean plants (*Phaseolus vulgaris* L.) at the end of the irrigation suspension period. Different letters indicate significant differences between treatments at $p \leq 0.05$ according to Tukey

Treatments	Relative growth rate (g g day^{-1})	Absolute growth rate (g day^{-1})	Net assimilation rate (g cm^{-2} day^{-1})	Leaf area ratio (cm^2 g^{-1})
First repetition				
100 % ETc	2.81 a	15.26 a	125.58 a	121.66 c
Suspension in vegetative growth stage (SC_1)	0.89 d	2.24 c	15.36 c	216.94 a
Suspension in flowering stage (SF_1)	1.29 c	3.77 c	28.14 c	204.79 a
Suspension in grain filling stage (SLL_1)	2.03 b	8.27 b	66.13 b	166.38 ab
Es $\bar{X}$	0.07	0.90	8.32	15.36
Second repetition				
100 % ETc	2.12 a	8.24 a	65.07 a	160.33 b
Suspension in vegetative growth stage (SC_2)	1.22 c	2.23 d	23.31 c	209.41 a
Suspension in flowering stage (SF_2)	1.31 c	3.80 c	27.72 c	206.41 a
Suspension in grain filling stage (SLL_2)	1.84 b	6.61 b	51.21 b	179.59 ab
Es $\bar{X}$	0.06	0.27	2.21	11.71

during the flowering stage (SF), and 13% during the grain-filling stage (SLL). The Net Assimilation Rate (NAR) varied by 64%, 57%, and 21% in the aforementioned stages, and a similar trend was observed for the Absolute Growth Rate (AGR). The Leaf Area Ratio (LAR) reflected an increase of 31%, 29%, and 12% compared to the treatment that was irrigated throughout the crop cycle. This response is consistent with the behavior of the leaf surface area.

A stronger relationship between NAR and RGR was found as the stress period was applied later in the plant's development, with this physiological component contributing more significantly to growth variation. This observation aligns with studies where light has been identified as the stress factor (García *et al.*, 2018).

In Figure 3, the results obtained from evaluating the relative chlorophyll content measured in SPAD units are presented. Only a slight decrease in values was found between treatments when irrigation suspension occurred during the grain-filling stage. This indicates that the plants maintained a very similar nutritional state among the treatments, primarily concerning nitrogen.

Determinations of relative chlorophyll content are currently widely used to quickly and non-destructively assess chlorophyll levels in plant leaves. These measurements are closely related to the nutritional state of the plant, primarily nitrogen.

The concentration of photosynthetic pigments is indirectly linked to leaf nitrogen concentration, allowing for the identification of nitrogen deficiency or excess. This information can serve as a technical basis for suggesting appropriate crop management to enhance photosynthetic efficiency, quality, and yield (Castañeda *et al.*, 2018).

The overall nutrient supply to the plants, especially nitrogen, was sufficient, as indicated by the chlorophyll content results. This suggests that nutrient availability, particularly nitrogen, did not pose a limitation to normal plant growth and development. This aligns with results from studies evaluating this variable in bean plants grown under different soil moisture levels and treated with foliar applications of a biostimulant (Morales *et al.*, 2017a).

This result implies that the leaf photosynthetic system maintained its integrity, ensuring favorable conditions for plant development.

The slight decrease in chlorophyll content during the grain-filling stage may be attributed to a potential inhibition of chlorophyll synthesis due to the plant's age, coupled with the activation of its degradation by the enzyme chlorophyllase (Taïbi *et al.*, 2016).

Leaf color is a significant morphological marker in breeding programs, and it is accepted that leaf color can be ancestral for progeny, making it an important approach for obtaining breeding materials (Guo *et al.*, 2018).

In Table III, the yield and its components are analyzed. Firstly, it can be observed that the treatments used in the first repetition did not affect the number of grains per pod or the weight of 100 grains. However, the number of pods was significantly affected by irrigation suspensions, with the flowering stage suspension having the greatest impact on this variable. In the second repetition, all analyzed variables showed differences between treatments.

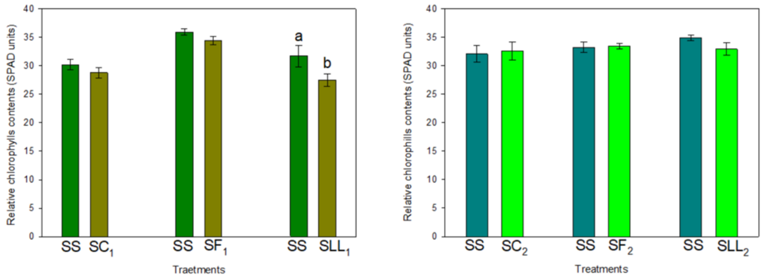

FIGURE 3. Relative chlorophyll contents at the end of irrigation suspension periods (SR) for 15 days during the vegetative growth (SC), flowering (SF), and grain-filling (SLL) stages. The bars above the columns represent standard errors of the means, and different letters indicate significant differences between treatments at $\alpha \leq 0.05$ according to the LSD (Least Significant Difference) test.

TABLE III. Performance and its components of bean plants (*Phaseolus vulgaris* L.) subjected to irrigation suspensions at different stages of their biological cycle. Different letters indicate significant differences between treatments at $p \leq 0.05$ according to Tukey

Treatments	Pods per plant	Beans per pod	100-grain weight (g)	Grain length (mm)	Grain width (mm)	Grain thickness (mm)	Production per plant (g)
First repetition							
100 % ETc	9.23 a	6.30	20.18	9.64 a	6.26 a	4.35 a	11.73 a
Suspension in vegetative growth stage (SC_1)	7.76 b	6.48	19.56	9.68 a	6.36 a	4.51 a	9.84 bc
Suspension in flowering stage (SF_1)	7.08 c	6.60	19.62	9.46 a	6.33 a	4.60 a	9.17 c
Suspension in grain filling stage (SLL_1)	8.09 b	6.50	19.57	9.18 b	5.82 b	3.93 b	10.29 b
Es $\bar{X}$	0.09	0.11	0.22	0.09	0.05	0.06	0.26
Second repetition							
100 % ETc	10.40 a	5.49 a	18.98 a	9.86 a	6.45 a	4.99 a	10.83 a
Suspension in vegetative growth stage (SC_2)	9.13 b	5.40 ab	18.31 b	9.63 ab	6.15 b	4.78 b	9.03 c
Suspension in flowering stage (SF_2)	9.21 b	5.22 c	18.14 b	9.52 b	6.15 b	4.78 b	8.72 c
Suspension in grain filling stage (SLL_2)	10.19 a	5.37 bc	18.59 ab	9.41 b	6.01 b	4.41 c	10.17 b
ES$\bar{X}$	0.30	0.06	0.19	0.10	0.06	0.05	0.36

When evaluating grain size in terms of length, width, and thickness, it was noted that the treatment in which irrigation suspension occurred during the grain-filling stage was the only one that caused a significant decrease in grain dimensions. This led to a lower yield compared to the treatment without irrigation suspension.

Lastly, plant production was significantly affected when irrigation was suspended during the flowering stage, followed by the suspension during the vegetative stage. Although not significantly different from the latter treatment, irrigation suspension during the grain-filling stage resulted in the least impact on this variable.

The number of pods was the variable that determined plant production, with its effect being more noticeable in the treatment with irrigation suspension during the grain-filling stage, showing the highest values after the control treatment.

Notably, this treatment presented the lowest values for grain size dimensions, variables that likely influenced grain weight and thus contributed to the differences with the treatment that was irrigated throughout the crop cycle with the addition of 100% of the accumulated standard crop evapotranspiration from preceding irrigations. However, the values for this variable did not differ statistically between both treatments, as did the number of pods per plant, albeit with lower absolute values in the water-stressed treatment.

This aspect may be related to the plant's reduced capacity to achieve full cell growth that makes up the grain.

The grain dimensions found in this study align with those reported by other authors when evaluating this variable in commercial beans under Mexican conditions (Morales *et al.*, 2017b).

Other authors have reported that soil water deficiency during the flowering and early grain formation period of beans reduced plant yield per plant (Romero *et al.*, 2019).

It is well known that soil water deficiency affects various processes that ultimately determine plant productivity, such as gaseous exchange characterized by stomatal conductance and carbon assimilation, as well as transpiration, which plays an important role in nutrient absorption and movement through the plant. These aspects may explain the behavior shown by plants grown under the aforementioned conditions (Aguilar *et al.*, 2017).

The slight difference in yield between plants with irrigation suspension during the grain-filling stage and the well-irrigated treatment is interesting in two directions. Firstly, it defines this stage as the least sensitive to water deficiency. Secondly, according to other authors, under such conditions, the synthesis of metabolites with the ability to inhibit the synthesis of some enzymes involved in carbohydrate metabolism increases, resulting in the obtaining of beans with a greater hypoglycemic effect. (Herrera *et al.*, 2019).

It was also found that when stress occurred during the vegetative growth stage, the number of grains per pod was not affected, contrary to what happened when irrigation was suspended during flowering and also during grain filling when new pods still emerge. This may be because soil water deficiency affects nutrient absorption, transpiration, gaseous exchange between the environment and the plant, biomass production, and, consequently, photosynthetic efficiency, grain formation, and weight, leading to a decrease in yield. (Castañeda *et al.*, 2006).

The reduction in yield and its components under soil water deficiency conditions has been attributed to the abscission of reproductive structures and the limitation of assimilates for grain formation and filling. (Ishiyaku y Aliyu, 2013).

CONCLUSIONS

It can be noted that the suspension of irrigation for 15 days during the vegetative growth, flowering, and grain filling stages of Triunfo 70 bean plants caused water deficiency in the soil, negatively impacting relative water content, dry matter accumulation in the aboveground part, growth relationships, and per-plant production. It can also be observed that irrigation suspension during the grain filling stage was the least sensitive variant to water deficiency, causing the least impact on production compared to well-irrigated treatment throughout the biological cycle of the crop.

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