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Importance of bioprotector in growth and yield of soybean plants

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Abstract

Bioprotectors are resistance inducers recently launched on the market for several plant species and may represent an important tool for increasing the productivity of the soybean crop. The present study aimed to identify the effects of H2 Protector in growth and yield of soybean plants. The experiment was carried out in an agricultural production area at Ipameri city with NS 7505 IPRO soybean cultivar in 115 days cycle. The experiment was set up following the randomized block design, three replications and $9m^2$ plot. To perform the treatments, it was used the commercial product H2 Protector with 300 ml ha^{-1} concentration and 150 L ha^{-1} flow rate with addition of mineral oil H2 Citrus. The treatments corresponded to the use of the bioprotector in different phenological stages of the soybean plants in a clinical way or overlapping applications in the previous stage: control, V4, R2, R5, V4+R2, V4+R5, R2+R5 and V4+R2+R5. The application of H2 Protector as bioprotector has biostimulant role in plants cultivated without stress conditions and must be applied with two applications to obtain high yield in soybean plants in V4+R2 or R2+R5 growth stages. The H2 Protector when applied in soybean plants in V4 growth stage increased growth and with a second application in R2 (V4+R2) resulted in higher yield of 17.3% corresponding to 636 kg ha^{-1} (kg $hectare^{-1}$). H2 Protector when used in R2+R5 growth stages increase in 18.4% soybean grain yield corresponding to 678 kg ha^{-1}. In this way, the use in two phenological stages becomes economically viable, since the costs represent 10% of the increase in productivity.

Keywords: Bioestimulant; development; *Glycine max* (L.) Merril; plant regulator; production.
Abbreviations: Fm_maximum fluorescence; F_0_fluorescence; Fv/Fm_photochemical efficiency of photosystem II; Fv'/Fm'_efficiency to absorb excitation energy by the reaction centers of PSII; NPQ_non-photochemical quenching; NS_Nideira Seeds; Pe_energy absorbed by PSII antenna complex and not used on photochemical neither heat dissipated; PSII_photosystem II; qP_photochemical quenching; R2_full bloom; R5_Start of grain filling; R9_viable harvest; V4_third leaf trifoliolate; ϕFSII_quantum yield of electron transport through PS II;.

Introduction

Soybean (*Glycine max*) is considered the most important grain crops in Brazil and one of the most socioeconomic relevant in the world (Preece et al., 2017). Brazil represents around 35% of soybean world production, and shares with United States the world largest producer position (FAO, 2020; USDA, 2020). Due to the high soybean versatility in industry, the demand increases in world market to produce animal feed meal, vegetable oils and food.

Conforming the data from CONAB (2021), there was an increase in 3.4% of cultivated area in 2020/2021 comparing to previous season. With the soybean production in 2019/2020 season, Brazil overcame United States and became the largest producer. According to FAO (Food and Agriculture Organization, 2020), Brazil will become dominant in soybean production until 2028.

The Midwest is the main region of soybean production in Brazil, with 16.4 million hectares, and due to the new technologies has been reaching higher yields in history. Goiás state produced 12.4 million tons in 2019/2020 season, superior in 9% than the last season, with average yield of 3516 kg ha^{-1} (CONAB, 2020). With a high perspective of soybean cultivation in Brazil, the higher production must be connected to a higher yield and not with the increase of cultivated area.

The areas occupied by Cerrado biome are widely used for agricultural activities and to open new areas is quite restricted by Brazilian Forest Code (Código Florestal Brasileiro). The increasing world concern with protection of natural sources inhibits the current practice of deforestation to open new areas to grow crops. The conservation of natural resources has been discussed globally and guided public policies against deforestation. As the cultivated area is limited, will be necessary develop management techniques that increase yield.

Soybean cultivation is in a high yield level, however, still below the genetic potential. Hence, a larger production associated based on increase of cultivated area is unsustainable given the obvious path of obtain larger production with the increment of yield (Oliveira, 2017; Lima et al., 2019). Thus, new strategies are necessary to cross the yield plateau, and one possible technique is the use of biostimulant to optimize the development and increase

grain yield.
There are innumerous private companies that register annually biostimulants, resistance inducer and bioprotectors that are known by plant morphophysiological changes and area constituted with plant regulators, mineral nutrients, amino acids, algae and others. The activity in plants is usually beneficial for stimulate the photosynthetic metabolism and the partitioning of assimilates to grain production. The biostimulant when applied separately or combined are capable to promote positive increase in the shoot or root of soybean plants (Dos Santos et al., 2017). However, depending on the composition, the increments in development may not happen. According to Hermes et al. (2015), the biostimulant application did not modify the root length in soybean plants as hypothesized.
The biostimulant usage containing plant regulators has been the most promising by the capability that only one hormone has numerous roles, however, the usage of these substances requires special care due to the low concentration action. The use of plant regulators has been frequent to establishment and reduce flower and pod abortion in soybean plants (Borges et al., 2014; Kutschera and Wang, 2012; Nonokawa et al., 2012; Passos et al., 2011; Pelacani et al., 2016). Following Bertolin et al. (2010), the use of biostimulant composed with cytokine, indolbutyric acid and gibberellic acid increased soybean grain yield in 37%.
As reported by Matos et al. (2019), a little imperfection in the concentration of the regulator can result in reverse effect to the proposed or in farming, and result in loss, like brassinosteroids in low concentrations that stimulates the root growth and soil solution absorption and in higher concentrations has adverse effect.
The inexistence of management recommendation for cultivated plants with use of numerous biostimulant, bioprotectors and resistance inducers recently released in the market to soybean plant, stimulate researches to develop new techniques to direct crops. Thus, the use of these compounds can represent important tool to yield increase of soybean with intent to attend the increasing perspective of world grain demand.
Therefore, the work hypothesis points that the use of H2 Protector in vegetative stage contributes positive to vigorous root growth and high potential of soil solution absorption and a second utilization in reproductive stage will result in lower pod abortion and larger yield. The present work has the aim to identify the effects of the bioprotector H2 Protector in growth and yield of soybean plants.

Results and discussion

The summary of variance analysis and mean test are shown on Table 1 demonstrates the lack of significance at 5% probability to plant height and root and leaf mass ratio. The number of leaves, leaf area and stem mass ratio means differed significantly, moreover the treatments with H2 Protector in vegetative stage showed high values in these variables. The plants with higher number of leaves, leaf area and stem mass ratio were V4, V4+R2+R5 and V4+R5 with increments of 9.4%, 19.8% and 4 % when compared to control respectively.
The powerful growth of shoot without deficit in root system in plants treated with H2 Protector in V4 growth stage suggest activity of this compound in the initial growth of soybean plant. According to Basílio et al. (2021), the stages of establishment and growth for a plant are crucial to maximum potential and determinates the survival under unfavorable conditions.
The Table 2 shows the summary of variance analysis and mean test to the results of chlorophyll *a* fluorescence analysis that exhibit the lack of significance for all variables and demonstrates the integrity of photosynthesis. The results indicate the lack of stress that affects the photosynthesis and reinforce the adequate conditions for soybean growth. According to Matos et al. (2019), the abiotic stresses as drought, salinity, high temperature and high solar radiation are associated with low relative humidity and affects the photosynthetic apparatus and reduces the production of assimilates.
The summary of variance analysis and mean test are shown on Table 3 and demonstrates no significance for root growth and 100 grain mass. The plant biomass differed between the treatments and it was greater in plants that were applied H2 Protector in V4 growth stage and suggest that the product has effective role in vegetative development in soybean plants. The higher biomass found in plants in the V4+R5 treatment as 18.8% greater than control.
The number of pods were greater in plants that receive H2 Protector at reproductive stages, with attention to R5 with 35.1% more pods than the control. The yield showed large variation between the treatments, the plants at V4+R2 and R2+R5 had yield average of 17.3% and 18.4% greater than control. The results corroborates with the found by Kovalski et al. (2020), that identified variations in number of pods per plant and grain yield in soybean plants treated with biostimulant. The grain yield is the most value variable in the economic point of view and is a result of a complex sum of biochemical and morphophysiological events throughout vegetative and reproductive development and the present work identified the importance to vigorous plant growth and physiological adjustment at reproductive stage for a great yield.
The means and percentages of all significant variables are demonstrated in Figure 1. The results suggests that the H2 Protector can increases the soybean plant yield by18.4% corresponding to 678 kg ha^{-1}. The results, entirely, demonstrates that the H2 Protector exert important positive role in vegetative growth, number of pods and grain yield of soybean, however, the complexity of numerous factors that intervene and determine yield makes difficult to stablish an event that is more important that other to obtain a large yield. Despite the product be commercialized as a biological protector or resistance inducer, in the present work the role was biostimulant.
The present study corroborates, in parts, with the found by Kovalski et al. (2020) that used biostimulant and pointed to increases in plant height, number of buds, number of pods and yield in soybean plants. Furthermore, according to the authors, the larger yield is not related with the increases in the shoot but with the increases in number of pods per plant. According to the same authors, the biostimulant is more effective in soybean plants in reproductive stages. However, our work points to two possibilities of H2 Protector management: V4+R2 or R2+R5. Our results are similar regard to data variation, but different in overall, because demonstrates that yield can be larger in plants with vigorous vegetative growth treated in V4 with H2 Protector. These results confirm the biostimulant role of H2 Protector.
The canonical multivariate analysis in Figure 2 represents 79% of data variation. The closeness between V4+R2 and R2+R5 to yield is the validation that these were the

Table 1. Variance analysis and mean test for plant height (PH), number of leaves (NF), leaf area (LA), leaf mass ratio (LMR), stem mass ratio (SMR) and root mass ratio (RMR) of *Glycine max* plants submitted to commercial product H2 Protector in different growth stages.

Variation Source	GL	Mean Square					
		PH (cm)	NL	LA (cm^2)	LMR	SMR	RMR
Treatment	7	7.661^{ns}	108.333^{ns}	479.45^{ns}	0.00054^{ns}	0.00160^{**}	0.00048^{ns}
Block	2	282.764^{ns}	24.582^{ns}	634.05^{ns}	0.00037^{ns}	0.00245^{ns}	0.0013^{ns}
Error	14	11.999	174.427	396.15	0.00030	0.00033	0.00022
CV (%)		4.38	17.3	13.49	5.29	3.67	8.85
Treatment		**Means**					
Control		79.44 a	69.26 b	137.82 bc	0.33 a	0.50 a	0.15 a
V4		78.36 a	75.80 a	147.79 b	0.33 a	0.50 a	0.16 a
R2		79.90 a	60.80 c	164.50 a	0.32 a	0.51 a	0.16 a
R5		77.12 a	61.93 c	149.72 ab	0.35 a	0.45 b	0.19 a
V4+R2		79.95 a	62.66 c	144.61 b	0.31 a	0.51 a	0.17 a
V4+R5		77.44 a	72.20 ab	142.70 bc	0.31 a	0.52 a	0.16 a
R2+R5		81.91 a	71.06 ab	127.71 c	0.32 a	0.52 a	0.15 a
V4+R2+R5		77.95 a	75.00 a	165.05 a	0.33 a	0.48 ab	0.18 a

*significant at 5% of probability; ** significant at 1% of probability; ns= not significant by F test. Means followed by same letter in the column do not differ from each other by Tukey test at 5% of probability.

Table 2. Analysis of variance and mean test to maximum photochemical efficiency of photosystem II (Fv/Fm), quantum yield of electron transport through PS II (ϕFSII), efficiency to absorb excitation energy by the reaction centers of PSII (Fv'/Fm'), photochemical quenching (qP), non-photochemical quenching (NPQ) and energy absorbed by PSII antenna complex and not used on photochemical neither heat dissipated (Pe) of *Glycine max* plants submitted to H2 Protector commercial product in different growth stages.

Variation Source	DF	Mean Square					
		Fv/Fm	ϕ_{PSII}	Fv'/Fm'	qP	NPQ	P_E
Treatment	7	0.00263^{ns}	0.00177^{ns}	0.00282^{ns}	0.00532^{ns}	48.463^{ns}	0.00371^{ns}
Block	2	0.00151^{ns}	0.00031^{ns}	0.00231^{ns}	0.00226^{ns}	47.505^{ns}	0.00166^{ns}
Error	14	0.00213	0.00175	0.00275	0.00727	48.020	0.00474
CV (%)		5.78	7.22	6.61	11.67	4.82	31.85
Treatment		**Means**					
Control		0.81 a	0.58 a	0.81 a	0.71 a	0.00 a	0.24 a
V4		0.80 a	0.57 a	0.79 a	0.71 a	0.01 a	0.23 a
R2		0.80 a	0.60 a	0.79 a	0.74 a	0.02 a	0.20 a
R5		0.81 a	0.61 a	0.80 a	0.75 a	0.01 a	0.19 a
V4+R2		0.82 a	0.53 a	0.81 a	0.65 a	0.02 a	0.27 a
V4+R5		0.81 a	0.58 a	0.80 a	0.72 a	0.01 a	0.22 a
R2+R5		0.81 a	0.60 a	0.80 a	0.74 a	0.01 a	0.20 a
V4+R2+R5		0.73 a	0.56 a	0.72 a	0.80 a	0.03 a	0.15 a

Statistics according to Table 1.

treatments that most increased grain production per plants. In addition, emphasizes that the variables related to vegetative growth are in opposite quadrants, and yield and number of pods represents the plant competition for assimilates between vegetative and reproductive, however, it cannot affirm that the vegetative growth opposes to reproductive, considering that the plants well stablished and of vigorous growth accumulates more supplies and can be more productive.

H2 Protector beyond fertilization role shows traces of salycilic acid and ethylene that can act like elicitors induction of metabolic events that favors the vegetative development. Despite of salycilic acid and ethylene exert inhibition of growth, leaf senescence induction and activation of defense mechanism and helps plants pass through low water potential in soil (Trevenet et al., 2017; Taiz et al., 2017), in the present work these events were not identified.

Although the H2 Protector is commercialized as a resistance inducer, the product can act like a biostimulant in plants cultivated in non-stress condition. The results of chlorophyll *a* fluorescence demonstrates the lack of stress and in these situations relief any bioprotector role, therefore, is unlikely that the compound had exert protector activity in soybean plants through activation of defense mechanism.

The data points that H2 Protector acted like biostimulant to soybean plants, and ethylene and salycilic acid hormones could made tissues more sensitive to other hormones like brassinosteroid, gibberellin, cytokine and auxin that are crucial to establishment, vegetative growth and reduction of pod abortion (Matos et al., 2019). According to Taiz et al.

Table 3. Analysis of variance and mean test for root length (CR), biomass (BM), number of pods (NV), 1000 grain mass (M1000) and yield (Y) of *Glycine max* plants submitted to H2 Protector commercial product in different growth stages.

Variation Source	DF	Mean Square				
		CR (cm)	BM (g)	NV	M1000 (g)	PD (Kg ha^{-1})
Treatment	7	2.076^{ns}	23.525^{ns}	90.103^{ns}	1.118^{ns}	613348^{**}
Block	2	2.248^{ns}	2.727^{ns}	204.012^{ns}	1.258^{ns}	71377^{ns}
Error	14	7.792	13.990	35.433	0.6212	60666
CV (%)		12.96	15.93	10.25	4.51	6.52
Treatment		Médias				
Control		21.49 a	21.94 c	49.06 d	174.3 a	3693.54 c
V4		22.38 a	27.77 a	53.93 cd	166.7 a	3043.40 e
R2		21.78 a	22.97 c	63.20 ab	180.0 a	3963.70 b
R5		20.72 a	18.30 d	66.26 a	184.5 a	3649.76 c
V4+R2		20.47 a	23.24 bc	59.06 bc	179.3 a	4331.28 a
V4+R5		21.18 a	26.07 ab	57.86 bc	170.0 a	3362.62 d
R2+R5		21.23 a	23.97 bc	54.73 c	171.1 a	4371.95 a
V4+R2+R5		22.97 a	23.58 bc	60.60 b	170.6 a	3823.23 bc

Statistics according to table 1.

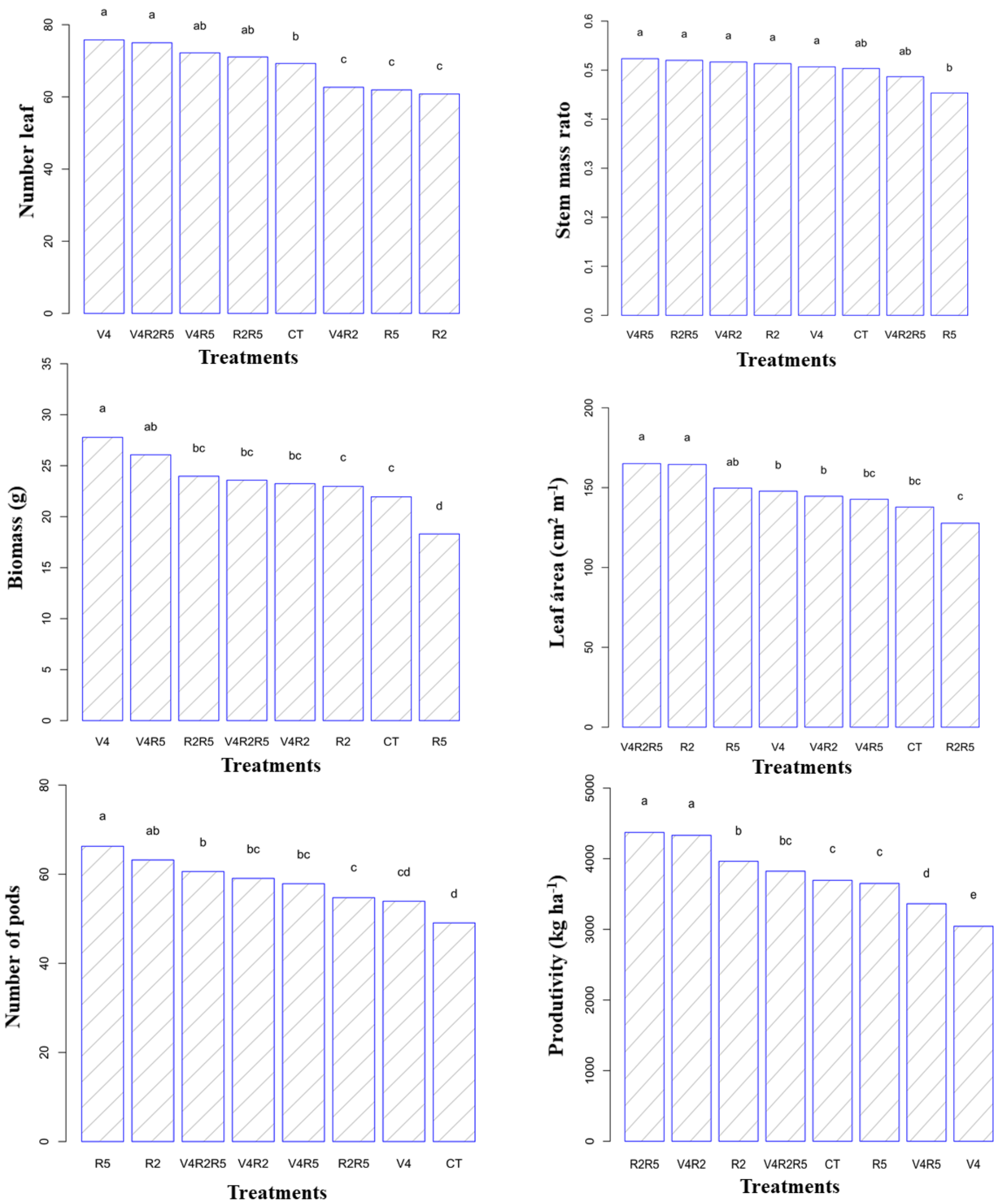

Figure 1. Mean test and percentage values of number of leaves, stem mass ratio, biomass, foliar leaf, number of pods and yield of *Glycine max* plants submitted to H2 Protector commercial product in different growth stages. The means followed with the same letter not differs between themselves by Tukey test at 5% of probability.

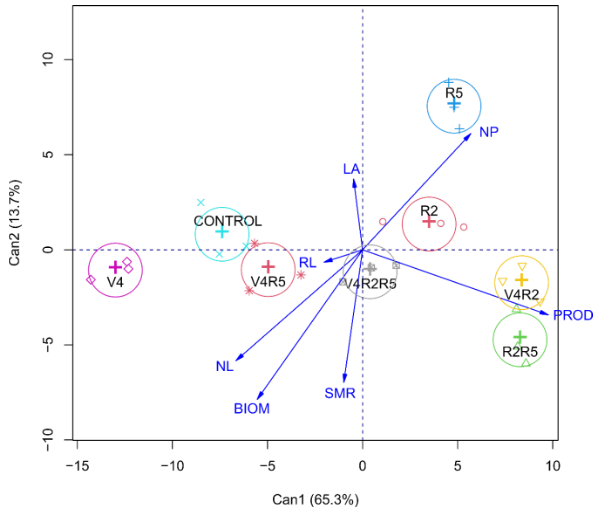

Figure 2. Canonical analysis for biomass (BIOM), number of leaves (NL), root length (RL), leaf area (LA), number of pods (NP), grain yield (PROD) and stem mass ratio (SMR) of *Glycine max* plants submitted to H2 Protector commercial production in different growth stages: Control, V4, R2, R5 and V4+R2, V4+R5, R2+R5 and V4+R2+R5.

(2017), ethylene can be more sensitive to others hormones action so that the events controlled by other hormones are maximized.

Materials and methods

Experiment design

The experiment was carried out in an agricultural production area at Ipameri city, Goiás in 20/21 season. The region has a tropical Aw climate according to Köppen classification, characterized as a tropical savanna, with rainy summer and dry winter (Köppen and Geiger, 1928). The soil of experimental field is classified as yellow-red Oxisoil (Embrapa, 2018). Soil chemical analysis revealed the following result: pH in water – 5.37; pH in $CaCl_2$ – 4.84; P (Mehlich-1) – 4.1 mg dm^{-3}; K (Mehlich-1) – 187.52 mg dm^{-3}; Ca2+ - 2.22 cmolc dm^{-3}; Mg^{2+} - 1.17 cmolc dm-3; Al^{3+} - <0.1; H+Al – 1.57 cmolc dm-3; Effective CTC – 3.97 cmolc dm^{-3}; Total CTC at pH 7.00 – 5.44 cmolc dm^{-3}; Organic Matter - 33g dm^{-3}; and V – 71%. After chemical analysis of the soil, fertility was corrected according to the recommendation for the crop (PROCHNOW et al., 2010). The cultivation of soybean NS 7505 IPRO was done within a cycle of 115 days.

The sowing occurred in November when the accumulated precipitation overcame 80 mm. The research took place in a commercial area of soybean cultivation without the use of irrigation. The soybean seed were treated with fungicides and insecticides and sown in 0.5 m spacing and 15 seeds per linear meter. The experiment was set up following the randomized block design with eight treatments, three replications and $9m^2$ per plot (3 m x 3 m). For the applications it was used the commercial product H2 Protector according to information provided by the company and registered with government control bodies (H2 Agroscience, 2023) with composition: 2% of sulfur, 4% of copper, 0.2% of zinc, 1% of manganese and 5-sulfosalicylic acid 0.002%, ethylenediaminetetraacetic acid 0.02% and salicyaldehyde salt 0.002% in concentrations of 300 ml ha^{-1} and 150 L ha^{-1} flow rate with addition of 100 ml of mineral oil H2 Citrus in soybean plants in different growth stages: control, V4, R2, R5, V4+R2, V4+R5, R2+R5 and V4+R2+R5.

Tried to achieve maximum uniformity during the applications through one pulverization in each growth stage. The growth analysis were performed in the same day for every treatment after 17 days after the last application in R5 stage. At the R9 stage, were performed the pod number and yield.

Growth variables: Plant height was measured from the root-stem transition zone at soil level (crown) to the tip of the stem using a graded ruler. The root length was measured through the root tip to the stem close to soil surface. The number of leaves was obtained by counting. The area of the trifoliate leaf full extended as determined with the equipment LI-3100 Area Meter, LI-COR, USA expressed in cm^2. The destructive analysis were performed separating roots, stem and leaves and set up in an oven at 72º C to dry until achieve constant mass and then weighted. With the dry mass was calculated the ratio of root, stem and leaf mass by the division of each part for the biomass.

Fluorescence variables: The maximum photochemical efficiency of photosystem II (PSII) (Fv/Fm), quantum yield of electron transport through PS II (ϕFSII), efficiency to absorb excitation energy by the reaction centers of PSII (Fv'/Fm'), photochemical quenching (qP), non-photochemical quenching (NPQ) and energy absorbed by PSII antenna complex and not used on photochemical neither heat dissipated (Pe) were measured using fluorometer with pulse modulated (Junior-PAM, Germany) before 5am. The leaves were exposed to weak pulse of far-red light (1-2 µmol m^{-2} s^{-1}), to determinate the minimum fluorescence (F_0). Then, a saturate pulse of light, with 6000 µmol (photons) m^{-2} s^{-1} irradiance for 1 second was applied to estimate the maximum fluorescence (Fm).

Yield variables: number of pods, 100 grain mass and yield were measured at R9 growth stage and adjusted to 13% grain moisture. The harvest of each plot was done manually.

Statistical proceedings: the data was submitted to variance analysis with Tukey test to compere the means. The canonical multivariate analysis it was made using Candisc package in Software R 4.0.1 (R Core Team, 2020) and RBIO (Bhering, 2017).

Conclusions

The use of H2 Protector commercialized as a bioprotector has biostimulant role in plants cultivated in the lack of stress and, must be used in two applications to obtain high yield in soybean plants at V4+R2 or R2+R5. H2 Protector when used in soybean plants at V4 stage increased the vegetative growth and with an additional application at R2 (V4+R2) resulted in higher yield of 17.3% corresponding to 636 kg ha^{-1}. H2 Protector when used in reproductive stages R2+R5 increased the yield of soybean plants in 18.4% corresponding to an increase of 678 kg ha^{-1}.

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