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Article

Development And Grain Quality Of Soybean Cultivars Treated With Pyraclostrobin And Biostimulant

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Abstract

Products with physiological effects on plants to optimize agricultural production is increasingly used; they can change the development of plants and the chemical characteristics of seeds. The objective of this work was to evaluate the development of soybean cultivars and the protein and oil contents of soybean grains after treatment with pyraclostrobin and biostimulant. The experiment was carried out with soybean crops without irrigation. The experimental design was a randomized complete block design, with five replications, using a 3 × 3 factorial arrangement consisting of three cultivars of different maturation times (very early, M6952IPRO; early M7739IPRO; and medium, ST797IPRO), and three treatments with products that cause physiological effects (control, pyraclostrobin, and control + biostimulant). The pod, leaf, and stem dry weights, number of pods, 100-grain weight, grain yield, and oil and protein contents of the soybean plants were evaluated. The data were subjected to analysis of variance, and Tukey's test at 5% probability. The effects of the products used (pyraclostrobin and biostimulant) vary according to the cultivar and may increase the soybean leaf and pod dry weights, and grain protein contents.

Keywords: *Glycine max L.*, grain yield, oil, protein content

Introduction

Soybean (Glycine max (L.) Merrill) is one of the world's most cultivated species because of its high nutritional and economic value. In Brazil, 52.5% of soybean production is exported as grains (CONAB, 2015).

Grain quality and protein and oil contents are determinant factors of the soybean commercial value (Moraes et al., 2006; Rodrigues et al., 2010). However, few studies in Brazil are conducted aiming to increase protein and oil contents of soybean grains. These studies are important because the improvement of these characteristics adds value to the grain, increasing the competitiveness of the Brazilian soybean in the world market (Rodrigues et al., 2010).

Soybean breeding programs in Brazil have sought to increase grain yield in the last decades; however, protein and oil contents do not always show a positive correlation with increased grain yield (Bonato et al., 2000). The low protein content of soybean grains makes necessary the use of strategies for processing, such as the removing of the grain tegument for grinding, increasing their protein content and the viability of the feed manufacturing. However, this is an expensive technique, increasing the producing costs of the final product.

Products that improve agronomic performance and chemical characteristics of

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plants are commonly used in agriculture. For example, the application of pyraclostrobin (strobilurin) on soybean plants increases their photosynthetic rate, activity of the nitrate reductase enzyme, protein contents, carbon assimilation, and decreases their respiration, and ethylene synthesis, resulting in a higher grain yield (Fagan et al., 2010).

Biostimulants is also a commonly used group of synthetic products that have similar effects to plant hormones (cytokinin, gibberellin, abscisic acid, and ethylene), which usually results in increases in biomass accumulation, changes in chemical composition, and higher grain yields (Klahold et al., 2006; Moterle et al., 2008, Albrecht et al., 2012).

In this context, the objective of this work was to evaluate the development of soybean cultivars and the protein and oil contents of soybean grains after treatment with pyraclostrobin and biostimulant.

Material and methods

The experiment was conducted in Rio Verde GO, Brazil (17°47'2.41"S; 51°0'27.28"W; and altitude of 777 m). The climate of this region is Cwa, temperate humid with dry winter and hot summer, according to the classification of Köppen, and presents mean annual precipitation of 1800 mm to 2200 mm (Cardoso et al., 2015). Meteorological data of the experiment period were collected with an automatic meteorological station in the experimental area.

The soil of the area was classified as dystroferric Red Latosol (Embrapa, 2013) (Oxisol), and the soil analysis (0-20 cm layer) showed pH (CaCl2) of 5.40, 2.60 cmolc dm^{-3} of H+ + $Al^{+3,}$ 1.36 cmolc dm^{-3} Ca^{+2}, 0.73 cmolc dm^{-3} of Mg^{+2}, 0.14 cmolc dm^{-3} of K+, 6.60 mg dm^{-3} of P, 8.2 mg dm^{-3} of S, 510 g kg^{-1} of clay, 40 g kg^{-1} of silt, and 450 g kg^{-1} of sand. Soil fertilization consisted of application of 450 kg ha^{-1} of the fertilizer 00-20-20 (N-P-K) at planting. Seeding was carried out on November 25, 2013.

The experimental design was randomized complete block design, in a 3 × 3 factorial arrangement with five replications for each factor combination, consisting of three cultivars of different maturation times (very early, M6952IPRO; early M7739IPRO; and medium, ST797IPRO) (Table 1), and three treatments with products that cause physiological effects—products of low physiological effect (Control, T1), pyraclostrobin fungicide during the crop cycle (Pyraclostrobin, T2), and Control plus Stimulate® (GarrCo Products Inc., Converse, USA) (Control + Biostimulant, T3) (Table 2).

Table 1. Main characteristics of the soybean cultivars used in the experiment.

Characteristics	Cultivars		
	M6952IPRO	M7739IPRO	ST797IPRO
Maturation group	7.2	7.7	7.9
Cycle (days)	100	110	122
Seeding density	360,000	320,000	320,000
Gowth habit	Indeterminate	Semi-determinate	Indeterminate
Biotechnology	Intacta IPRO	Intacta IPRO	Intacta IPRO

Each plot had an area of 18 m^2 (3 m × 6 m) and evaluation area of 10 m2, with spacing of 0.5 m between plant rows. The treatments were applied using a motorized backpack sprayer, equipped with a 3 m bar with XR110015 flat jet nozzles (TeeJet, Weaton, USA) spaced 0.5 m apart, using a flow rate of 150 L ha^{-1}.

The plants were grown under no-tillage system after sorghum harvest. Weeds, pests, and fungus diseases occurring during the experiment were managed according to the technical recommendations described by Embrapa (2011).

Leaves with petioles, pods, and stems of five consecutive plants were collected in each plot at the R7 phenological stage, packaged in paper bags and dried in an oven at 65°C for 72 hours to evaluate their dry weights. The mean dry weight per plant was used for analyses.

The number of pods per plant was evaluated using five random plants of the evaluation area of the plots before harvesting. The five central meters of the two central plant rows (5 m^2) of each plot were harvested and the pods were threshed manually. Grain moisture

Table 2. Experimental treatments, active ingredients and rates, and application time.

Treatments	Active ingredients	Application rate	Application time (phenological stage)
T1. Control	Imidacloprid + Thiodicarb[8]	75 + 225 g 100 kg^{-1}	Seed treatment
	Prothioconazole + Trifloxystrobin[6]	70 + 60 g ha^{-1}	V6 (6 expanded trifoliate leaves)
	Prothioconazole + Trifloxystrobin[6]	70 + 60 g ha^{-1}	R2 (full flowering)
	Prothioconazole + Trifloxystrobin[6]	70 + 60 g ha^{-1}	R2+15 days after first application
	Cyproconazole + Trifloxystrobin[7]	96 + 225 g ha^{-1}	R2+30 days after first application
T2. Pyraclostrobin	Fipronil + Pyraclostrobin + Thiophanate-methyl[1]	25 + 2.5 + 22.5 g 100 kg^{-1}	Seed treatment
	Pyraclostrobin + Epoxiconazole[2]	66.5 + 25 g ha^{-1}	V6 (6 expanded trifoliate leaves)
	Pyraclostrobin + Fluxapyroxad[3]	166.5 + 83.5 g ha^{-1}	R2 (full flowering)
	Pyraclostrobin + Fluxapyroxad[3]	166.5 + 83.5 g ha^{-1}	R2+15 days after first application
	Pyraclostrobin + Metconazole[4]	65 + 40 g ha^{-1}	R2+30 days after first application
T3. Control + Biostimulant	(Imidacloprid + Thiodicarb)[7] + (Auxin + Gibberellin + Cytokinin)[5]	(75 + 225) + (0.025 + 0.025 + 0.045) g 100 kg^{-1}	Seed treatment
	(Prothioconazole + Trifloxystrobin)[6] + (Auxin + Gibberellin + Cytokinin)[5]	(70 + 60) + (0.0125 + 0.125 + 0.0225) g ha^{-1}	V6 (6 expanded trifoliate leaves)
	Prothioconazole + Trifloxystrobin[6]	70 + 60 g ha^{-1}	R2 (full flowering)
	Prothioconazole + Trifloxystrobin[6]	70 + 60 g ha^{-1}	R2 + 15 days after first application
	Cyproconazole + Trifloxystrobin[7]	96 + 225 g ha^{-1}	R2 + 30 days after first application

Commercial products and concentrations of active ingredients (g L-1): 1Standak Top® - Fipronil 250 + Pyraclostrobin 25 + Thiophanate methyl 225; 2Opera® - Epoxiconazole 50 + Pyraclostrobin 133; 3Orkestra® - Fluxapyroxad 167 + Pyraclostrobin 333; 4Opera Ultra® - Metconazole 80 + Pyraclostrobin 130; 5Stimulate® - (Auxin: 4-Indol-3-ylbutyric acid 0.05 + Gibberellin: Gibberellic acid 0.05 + Cytokinin: Kinetin 0.09); 6Fox® - Prothioconazole 175 + Trifloxystrobin 150; 7Sphere Max® - Cyproconazole 160 + Trifloxystrobin 375; 8Cropstar® - Imidacloprid 150 + Thiodicarb 450.

was evaluated using an electric moisture meter, and the 100-grain weight (g), and grain yield (kg ha^{-1}) with 13% moisture content were evaluated.

Oil and protein contents were analyzed according to the recommendation of the Brazilian Compendium of Animal Feed (Brasil, 2013). Protein contents (percentage of crude protein) were evaluated using the Kjeldahl method. The grain oil was extracted by the continuous extraction method in a Soxhlet-type device using petroleum ether as solvent, and the oil contents were expressed as percentages in relation to the grain weight.

The results were subjected to analysis of variance and the means of the treatments were compared by the Tukey's test at 5% probability.

Results and discussion

The interactions between the factors (cultivars × treatments) presented significant effect for leaf dry weight, pod dry weight, and grain protein content, according to the analysis of variance (Table 3).

Stem dry weight, and grain yield presented significant differences for the cultivar factor (Table 3).

Total precipitation during the experiment was 1011 mm (Figure 1); this volume is above the necessary to obtain high grain yields of soybean crops (450 to 800 mm) when it is well distributed throughout the crop development (Embrapa, 2011). However, the precipitation distribution was not uniform, with a water stress period from December 21, 2013 to February 13, 2014, with precipitation of 121 mm in this period (Figure 1), which is below the normal volume, since the average precipitation in January is 250 mm (INMET, 2015).

The cultivars M6952IPRO and M7739IPRO reached the phenological stage R5.1 at approximately 50-60 days after sowing (DAS), and the ST797IPRO at 70 DAS. The water stress occurred from 20 to 80 DAS.

The cultivar M7739IPRO presented the highest stem dry weight per plant (41.80 g), followed by ST797IPRO (33.10 g), and M6952IPRO

Table 3. Analysis of variance of stem dry weight (SDW), leaf dry weight (LDW), pod dry weight (PDW), number of pods per plant (NPP) 100-grain weight (100GW), grain yield (GY), grain protein content (GPC), and grain oil content (GOC) of soybean plants depending on the cultivar (C) and treatment with products that cause physiological effects (T).

Source of variation	F calculated							
	SDW	LDW	PDW	NPP	100GW	GY	GPC	GOC
Cultivar	25.1**	26.4**	5.7	0.8	0.3	10.9 *	4.1	1.0
Treatments	0.6	3.7	1.9	1.9	0.3	1.9	1.0	2.9
Cultivar × Treatments	1.9	4.9*	5.1**	0.5	0.2	0.7	4.0*	2.0
Coefficient of variation (%)	16.3	21.7	14.3	25.1	14.9	12.5	3.1	6.0
Overall mean	34.1	18.8	86.5	46.9	10.9	1946	33.7	21.2

** and * = significant at 1% and 5% by the F test at 5% probability, respectively.

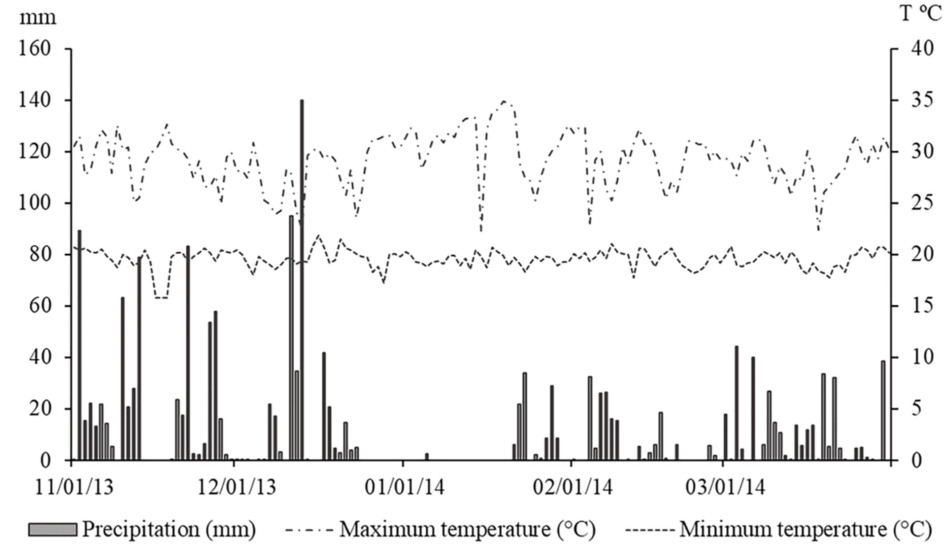

Figure 1. Precipitation and temperature during the period of the experiment. Rio Verde GO, Brazil (2013/2014).

(27.52 g) (Table 4).

The stem dry weight of the cultivar M7739IPRO increased 20% to 34%, compared to the other cultivars, denoting that this variable depends on specific characteristic of each cultivar. Variations in plant height, and stem dry weight can occur, for example, as a function of the cultivar maturation time. Late-maturing soybean cultivars have longer vegetative development time and, consequently, longer

Table 4. Stem dry weight, leaf dry weight, and pod dry weight of soybean cultivars depending on treatments with products that cause physiological effects.

Cultivars	Treatments			Mean
	Control	Pyraclostrobin	Biostimulant	
		Stem dry weight (g per plant)		
ST797IPRO	35.42	33.50	30.38	33.10 B
M6952IPRO	23.45	30.35	28.76	27.50 C
M7739IPRO	44.85	40.99	39.52	41.80 A
Mean	34.57	34.95	32.88	
		Leaf dry weight (g per plant)		
ST797IPRO	16.46 aAB	13.60 aB	11.76 aB	13.94
M6952IPRO	11.66 bB	19.23 aAB	22.79 aA	17.90
M7739IPRO	21.83 aA	24.70 aA	27.50 aA	24.68
Mean	16.65	19.17	20.68	
		Pod dry weight (g per plant)		
ST797IPRO	84.28 aAB	82.48 aA	59.20 bB	75.32
M6952IPRO	70.02 bB	101.40 aA	100.38 aA	90.60
M7739IPRO	96.26 aA	93.80 aA	90.45 aA	93.51
Mean	83.53	92.56	83.34	

Means followed by the same uppercase letter in the columns, and lowercase letters in the rows do not differ by the Tukey's test at 5% probability.

period of biomass accumulation (Marchiori et al., 1999).

The interactions between the factors (cultivars × treatments) presented significant effect for leaf dry weight at the R7 phenological stage (Table 4). The applications of pyraclostrobin and biostimulant increased the leaf dry weight of the cultivar M6952 IPRO in 65% and 95%, respectively, when compared with the control, presenting the highest means. The use of pyraclostrobin in soybean and wheat plants improves the permanence time of leaves in the plants (Lenz et al., 2011; Takano et al., 2015). This is due to the physiological effects of pyraclostrobin, which increase net photosynthesis, biomass production, and activity of the nitrate reductase enzyme, and decrease ethylene production (Fagan et al., 2010).

Swoboda & Pedersen (2009) evaluated foliar applications of pyraclostrobin in soybean crops and observed a 10% increase in plant dry weight in relation to the standard treatment. The effect of the biostimulant Stimulate® was evaluated by Oliveira et al. (2015) on common bean crops, and Rós et al. (2015) on the sweet potato crops, also showing increases in leaf dry weight, and shoot dry weight.

The number of pods per plant, and 100-grain weight presented no significant differences, and no effect by the interaction between the factors. The grain yields found were difference between cultivars (Table 5).

Table 5. Number of pods per plant, 100-grain weight, and grain yield of soybean cultivars depending on treatments with products that cause physiological effects.

Cultivars	Treatments			Mean
	Control	Pyraclostrobin	Biostimulant	
Number of pods per plant				
ST797IPRO	53.28	40.80	49.10	48.76
M6952IPRO	43.00	39.24	52.20	43.78
M7739IPRO	45.50	47.48	51.84	48.29
Mean	47.28	42.5	51.04	
100-grain weight (g)				
ST797IPRO	11.29	10.97	11.25	11.17
M6952IPRO	10.20	10.88	11.16	10.74
M7739IPRO	10.40	10.93	10.99	10.78
Mean	10.64	10.93	11.13	
Grain yield (kg ha^{-1})				
ST797IPRO	2047.51	2304.75	2172.36	2174.80 A
M6952IPRO	1738.69	2037.39	1915.36	1897.20 B
M7739IPRO	1767.41	1729.78	1808.36	1768.50 B
Mean	1851.2	2023.9	1965.4	

Means followed by the same uppercase letter in the columns, and lowercase letters in the rows do not differ by the Tukey's test at 5% probability.

The cultivar ST797IPRO showed the highest grain yield when compared to the other cultivars evaluated.

The biostimulant had no effect on the number of pods per plant, 100-grain weight, and grain yield under conditions of water stress. Moterle et al. (2008) found different results using the same biostimulant in adverse climatic conditions—the use of the biostimulant increased the grain yield and 1000-grain weight, compared to the control. However, the effectiveness of the biostimulant decreases when climatic conditions are favorable (without water stress), and it may not provide benefits to the crop. Contrastingly, Klahold et al. (2006), and Bertolin et al. (2010) evaluated this biostimulant in soybean crops under favorable climatic conditions and found an increase in grain yield when compared to the control treatment.

The pyraclostrobin application had no effect on the grain yield, and 100-grain weight. According to Swoboda & Pedersen (2009), applications of pyraclostrobin in soybean crops affect positively the 100-grain weight of plants but have no effects on grain yield, or oil and protein contents.

The grain protein contents of the soybean cultivars were affected by the interaction between cultivars and treatments. However, the grain oil contents were not affected by this interaction (Table 6).

The cultivars evaluated presented low protein content, not meeting the requirements of the external market. The soybean grains must contain 41.5% and 43% of protein to be classified as Normal and HyPro, respectively (Moraes et al., 2006).

The protein content of the cultivar M7739IPRO, which presents a 110-day cycle, was affected positively using pyraclostrobin and biostimulant. The use of these products increased its grain crude protein contents. The application of the biostimulant resulted in a higher protein content in relation to the control treatment, with an increase of 2.64%. However, the treatment with pyraclostrobin resulted in an intermediate protein content that was statistically equal to the control treatment (Table 6).

Table 6. Protein, and oil contents of grains of soybean cultivars depending on treatments with products that cause physiological effects.

Cultivars	Treatments			Mean
	Control	Pyraclostrobin	Biostimulant	
		Grain protein content (%)		
ST797IPRO	32.85 aA	33.33 aA	32.95 aB	33.04
M6952IPRO	34.35 aA	33.96 aA	32.85 aB	33.72
M7739IPRO	32.83 bA	34.51 abA	35.47 aA	34.27
Mean	33.34	33.93	33.75	
		Grain oil content (%)		
ST797IPRO	21.44	22.17	20.48	21.36
M6952IPRO	20.19	22.76	21.28	21.40
M7739IPRO	20.17	20.62	21.47	20.75
Mean	20.6	21.85	21.07	

Means followed by the same uppercase letter in the columns, and lowercase letters in the rows do not differ by the Tukey's test at 5% probability.

The use of biostimulant and pyraclostrobin had no effect on the protein content of the cultivars M6952IPRO and ST797IPRO, these effects were significant only for the medium-maturing (110 days) cultivar, M7739IPRO.

According to Bertolin et al. (2009), biostimulants have no effect on crude protein content, and yield of soybean grains. However, recent studies indicate that the use of biostimulants (auxin + gibberellin + cytokinin) may affect grain protein accumulation; Albrecht et al. (2012) tested different biostimulant rates and found effects on oil and protein contents by the action of the biostimulant, mainly increasing the protein content and decreasing the oil content.

According to Albrecht et al. (2008), climatic conditions presenting low water availability and high temperatures during the reproductive stage of the soybean plants are favorable to increase grain protein accumulation. Similar climatic conditions to those observed in the present study were reported by Albrecht et al. (2008). The soybean genotype also determines grain oil and protein contents, which show differences between cultivars, with some presenting higher protein contents and others higher oil contents (Rodrigues et al., 2014).

Conclusions

The use of pyraclostrobin and biostimulant do not affect the pods per plant, 100-grain weight, grain yield, and grain oil content of the M6952IPRO, M7739IPRO, ST797IPRO soybean cultivars.

The effects of the products used (pyraclostrobin and biostimulant) vary according to the cultivar and may increase the soybean leaf and pod dry weights, and grain protein contents.

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