

**Physiological aspects of castor bean cv. BRS Energia in response to foliar application of gibberellic and salicylic acid****Genelício Souza Carvalho Júnior<sup>†1</sup>, Rosiane de Lourdes Silva de Lima<sup>2</sup>, Hans Raj Gheyi<sup>3</sup>, Julita Maria Frota Chagas Carvalho<sup>4</sup>, Maria Roselita André Soares<sup>5</sup>, Valdinei Sofiatti<sup>4</sup>**<sup>1</sup>State University of Paraíba. Company of Brazilian Agricultural and Animal Husbandry Research (Embrapa). Rua Osvaldo Cruz, 1143, Centenário, CEP: 58.107-720, Campina Grande, Paraíba, Brazil<sup>2</sup>Federal University of Campina Grande, Academic Unit of Agricultural Engineering, Campina Grande, CEP: 58.109-970, Campina Grande, Brazil<sup>3</sup>Federal University of Recôncavo of Bahia, Nucleus of Soil and Water Engineering, Cruz das Almas, CEP: 44.380-00, Bahia, Brazil<sup>4</sup>Company of Brazilian Agricultural and Animal Husbandry Research (Embrapa). Rua Osvaldo Cruz, 1143, Centenário, CEP: 58.107-720, Campina Grande, Paraíba, Brazil<sup>5</sup>Federal University of Paraíba, Department of Biology Science. Areia, CEP: 58.397-000, Paraíba, Brazil<sup>†</sup>This paper was extracted from the thesis of the first author.**\*Corresponding author: limarosiane@yahoo.com.br****Abstract**

Gibberellic and salicylic acid may be used in crop management to influence physiology and biochemistry of plant, to improve photosynthetic capacity, and to increase crop profitability. Current analysis aims at evaluating the physiological aspects of castor bean cv. 'BRS Energia' in response to the foliar application of gibberellic and salicylic acid. Treatments consisted of the factorial distribution of five doses of gibberellic acid (0, 0.02, 0.04, 0.08, and 0.16 mg L<sup>-1</sup>) and five doses of salicylic acid (0, 0.02, 0.04, 0.08, and 0.16 mg L<sup>-1</sup>) with four replications. There were 100 experimental units and one plant per plot. High doses of gibberellic acid with or without salicylic acid reduced net photosynthesis, stomatal conductance, and transpiration, showing linear effects at 80 days after emergence. The concentrations of the photosynthetic pigments, chlorophyll a, chlorophyll b, total chlorophyll, and carotenoid content were reduced until the application of 0.09 mg L<sup>-1</sup> of gibberellic acid, associated with a dose of 0.16 mg L<sup>-1</sup> of salicylic acid. Dose of 0.12 mg L<sup>-1</sup> gibberellic acid and 0.16 mg L<sup>-1</sup> salicylic acid produced the lowest relative water content in castor bean leaves.

**Keywords:** *Ricinus communis* L., plant hormones, photosynthesizing pigments.**Abbreviations:** GA<sub>3</sub> Gibberellic acid; SA\_Salicylic acid; S\_Sum of bases; T\_Cation exchange capacity; V\_Saturation of bases; OM\_Organic matter; DAE\_Days after emergence; A\_Rate of net photosynthesis; E\_Transpiration; g<sub>s</sub> Stomatal conductance; RWC\_Relative water content; CLA\_Chlorophyll a; CLB\_Chlorophyll b; CLT\_Total chlorophyll; CAR\_Carotenoids; CNPq\_Brazilian Council for Scientific Research and Development**Introduction**

The castor bean (*Ricinus communis* L) plant is native to Ethiopia (Africa) and Afghanistan (Asia). Due to the high oil content of its seeds, it was transported to other regions and got established in numerous tropical and sub-tropical ecosystems. This plant was introduced in Brazil by the Portuguese in the 16<sup>th</sup> century, but its economic potential was discovered only in the 1960s and 1970s, because of increased demand for the product in both internal and international market. It is currently cultivated throughout Brazil (Cangemi et al., 2010). Castor bean crop is cultivated in more than a million hectares annually, with India, China, Brazil, and Russia being major world producers (Cangemi et al., 2010). Brazil is the third largest producer of castor beans in the world, the northeastern region of the country being the greatest producer. The principle regions of production are located in the states of Bahia, Ceará, Pernambuco, Piauí, and Rio Grande do Norte (CONAB, 2014). The annual production of castor beans in 2013–2014 in the northeastern region of Brazil was approximately 56,900 t of castor beans,

equivalent to 28,000 t of oil (CONAB, 2014), and was exported mainly to the European Union, the United States, Thailand, China, and Japan. Gibberellic acid (GA<sub>3</sub>) is a plant hormone that affects the physiological processes of plant growth and development, affecting characteristics such as plant height, stem diameter, number of leaves, and leaf area (Shah, 2007). Furthermore, exogenous applications of gibberellic acid influence photosynthesis, transpiration, and stomatal conductance (Misra et al., 2009; 2010), as well as photosynthetic pigments, including total chlorophyll, chlorophyll a and b (Misra et al., 2009; 2010; Ali et al., 2012), and carotenoids (Hakimeh et al., 2010; Kavina et al., 2011). It also affect the biochemical pathways producing carbohydrates and soluble proteins in plant species such as pea (El-Shraiy and Hegazi, 2009; Dawood et al., 2012).

Salicylic acid (SA) is currently included in the list of plant hormones, because of its activities, which are mainly involved in the thermogenesis of plants, formation of stems and roots, and regulation of several physiological processes,

such as the control of the functions of chlorophyll a and b (Noreen et al., 2011; Saeidnejad et al., 2012), total chlorophyll, carotenoids, photosynthesis, transpiration, and stomatal conductance (Liu et al., 2011). It is also involved in the regulation of relative leaf water content (Noreen et al., 2011; Saeidnejad et al., 2012).

Current research aims to evaluate the physiological characteristics of castor bean plant cv 'BRS Energia,' and their response to the application of gibberellic and salicylic acids.

## Results and Discussion

### *Effects of application of GA<sub>3</sub> and SA on gas exchanges*

Isolated or associated application of GA<sub>3</sub> and SA had a significant effect on liquid photosynthesis and stomatal conductance, whereas the application of the GA<sub>3</sub> had no significant effect on transpiration at 80 DAE (Table 2). The combined use of GA<sub>3</sub> and SA through leaf spraying had interactive effects on liquid photosynthesis (Fig 2A). The variable showed a quadratic response when 0, 0.02, and 0.04 mg L<sup>-1</sup> of SA were applied, whereas other doses triggered a linear response. Maximum net photosynthesis varied between 19 and 21 μmol CO<sub>2</sub> m<sup>-2</sup> s<sup>-1</sup> with the flexion point when doses of 0.12 and 0.10 mg L<sup>-1</sup> of GA<sub>3</sub> in combination with 0.02 and 0.04 mg L<sup>-1</sup> of SA were applied. In the absence of SA treatment, a deflexion point occurred when a dose of 0.09 mg L<sup>-1</sup> GA<sub>3</sub>, was applied, leading to a maximum of 20 μmol CO<sub>2</sub> m<sup>-2</sup> s<sup>-1</sup> of liquid photosynthesis per plant. There was a detrimental effect for the variable with the maximum doses of SA (0.16 mg L<sup>-1</sup>) and GA<sub>3</sub> (0.16 mg L<sup>-1</sup>), with maximum decrease to 14 μmol CO<sub>2</sub> m<sup>-2</sup> s<sup>-1</sup>. As the concentration of phytohormones was increased, photosynthesis decreased (Fig 2A).

The decrease in net photosynthesis may have been caused by the reduction of the leaf area due to the excess of GA<sub>3</sub> applied to the plant. Reduction in the photosynthetic area caused a natural reduction in the rate of light and CO<sub>2</sub> capture, leading to a decrease in the production of photoassimilates. The use of GA<sub>3</sub> doses above critical levels likely caused the negative response of this physiological variable, as has previously been reported that excess GA<sub>3</sub> inhibits the production of assimilates in the leaves and drastically reduces rates of net photosynthesis (Almeida; Vieira, 2010). The negative effects of high doses of GA<sub>3</sub> on net photosynthesis have also been observed by Misra et al. (2009) in vinca plants (*Catharanthus roseus* L.), and by Almeida and Vieira (2010) in tobacco plants (*Nicotiana tabacum* L.). An increase in the photosynthetic rate of the Himalayan Yew (*Taxus wallichiana* Zuccarini var. mairiei) occurred with the application of 1000 ppm of GA<sub>3</sub> (Misra et al., 2010).

Stomatal conductance (gs) is a physiological variable that measures water loss and gas exchange of a plant, as a response to factors which trigger water stress. It was strongly affected by the joint application of SA and GA<sub>3</sub> (Fig 2A). There was a quadratic response of GA<sub>3</sub> doses of 0, 0.02, and 0.08 mg L<sup>-1</sup>. The flexion point was detected when doses of 0.02 and 0.08 mg L<sup>-1</sup> SA were applied with a dose of 0.09 mg L<sup>-1</sup> of GA<sub>3</sub>, leading to a maximum stomatal conductance of 0.60 and 0.48 mol H<sub>2</sub>O m<sup>-2</sup> s<sup>-1</sup>. Furthermore, a gs of 0.45 mol H<sub>2</sub>O m<sup>-2</sup> s<sup>-1</sup> occurred at a dose of 0.09 mg L<sup>-1</sup> GA<sub>3</sub>, without SA application. When 0.16 mg L<sup>-1</sup> doses of SA were

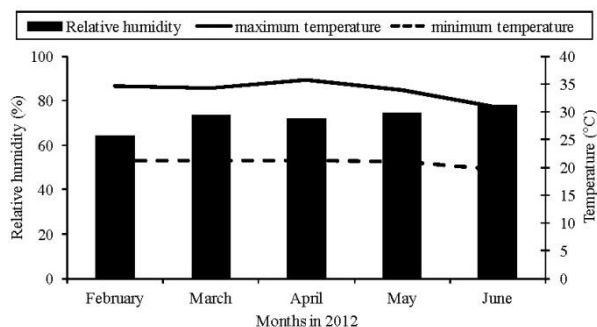
combined with the maximum GA<sub>3</sub> dose, the lowest rate of gs, 0.20 mol H<sub>2</sub>O m<sup>-2</sup> s<sup>-1</sup> was measured. In other words, high doses of the two phytohormones are harmful to plants, and drastically reduce the stomatal conductance of castor bean plants. These results suggest that further studies are required to determine optimal doses of GA and SA, application methods, and other associated factors. One of the roles of GA<sub>3</sub> in stomatal functioning is the hormone's participation in the processes that pushes calcium to the interior of the cells, and thus increases stomatal conductance (Assmann et al., 1999). Consequently, it is required for the optimal performance of the enzyme Rubisco which benefits from the increased CO<sub>2</sub> concentrations associated with improved stomatal conductance. As a rule, GA<sub>3</sub> is a prominent photosynthetic hormone, being involved in the production, translocation, and partition of photoassimilates (Shah, 2007). Studies on *Nigella sativa* L. crop show that the application of GA<sub>3</sub> greatly increases stomatal conductance and broadens the plant's photosynthetic capacity, increases production of photoassimilates, and improves overall productivity (Shah, 2007; Shah and Tak, 2011). The negative effects of GA<sub>3</sub> application on stomatal conductance were also observed in this research (Fig 2B). The isolated use of GA<sub>3</sub> and SA (Fig 2C and 2D) had negative effects on the transpiration of the castor bean cultivar 'BRS Energia' at 80 DAE. Transpiration decreased linearly when GA<sub>3</sub> was applied in the absence of SA. There was a 15% reduction in transpiration between the lowest (0 mg L<sup>-1</sup>) and the highest dose (0.16 mg L<sup>-1</sup>) of GA<sub>3</sub> (Fig 2C). Similar results were reported by Misra et al. (2010). GA<sub>3</sub> is a hormone that reacts with other hormones that regulate several metabolite processes in this cultivar (Iqbal and Basra, 2013) though there are several uncertainties and many conflicting theories regarding the role of the hormone in plant growth. Fig 2D shows the linear decrease in transpiration when SA is applied. The average decrease in transpiration was 44% between the lowest (0 mg L<sup>-1</sup>) and the highest dose (0.16 mg L<sup>-1</sup>) of SA. These results corroborate with earlier research (Liu et al., 2011).

Relative water content is a physiological variable that describes the maintenance of the plant's water potential (Fig 2E). According to the results of the experiment assay, foliar spraying with GA<sub>3</sub> is more effective with low SA doses, or isolation. According to these results, spraying with GA<sub>3</sub> is beneficial only when the growth hormone is associated with the lowest SA doses (0, 0.02 and 0.04 mg L<sup>-1</sup>) (Fig 2E). Negative effects on the relative water content occurred as from this dose when GA<sub>3</sub> doses increased. Low SA doses applied in combination with GA<sub>3</sub> increased the relative water content of the castor bean plant. This may be due to the fact that SA is an osmotic regulator and its presence increases Ca<sup>2+</sup> levels in the cellular cytosol of certain species, in addition to its role as a secondary messenger (Noreen et al., 2011). According to Ali et al. (2012), spraying with GA<sub>3</sub> increases the leaf's water potential; however, the application should be done in an appropriate dose. However, the response to GA<sub>3</sub> application mainly depends on the dose and the ability of the cells to use the hormone. The use of SA and GA<sub>3</sub> was expected to cause an increase in the plants' relative water content; however, the actual response was greatly variable. A possible explanation for this may be that generally, the magnitude of a plant's response to a particular treatment, especially to doses of GA<sub>3</sub> and SA,

**Table 1.** Chemical characteristics of the soil collected from a depth of 0–20 cm at Lagoa Seca, PB, Brazil in 2012.

pH	Ca <sup>2+</sup>	Mg <sup>2+</sup>	Na <sup>+</sup>	K <sup>+</sup>	S	H + Al	T	V	Al <sup>3+</sup>	P	OM
1:2.5	Exchangeable complex of the soil (mmol <sub>c</sub> dm <sup>-3</sup> )							%	mmol <sub>c</sub> dm <sup>-3</sup>	mg dm <sup>-3</sup>	g kg <sup>-1</sup>
6.8	3.51	1.70	0.06	0.63	5.9	0.92	6.82	86	0.0	2.37	1.26

S\_Sum of bases; T\_Cation exchange capacity; V\_Saturation of bases; OM\_Organic matter.

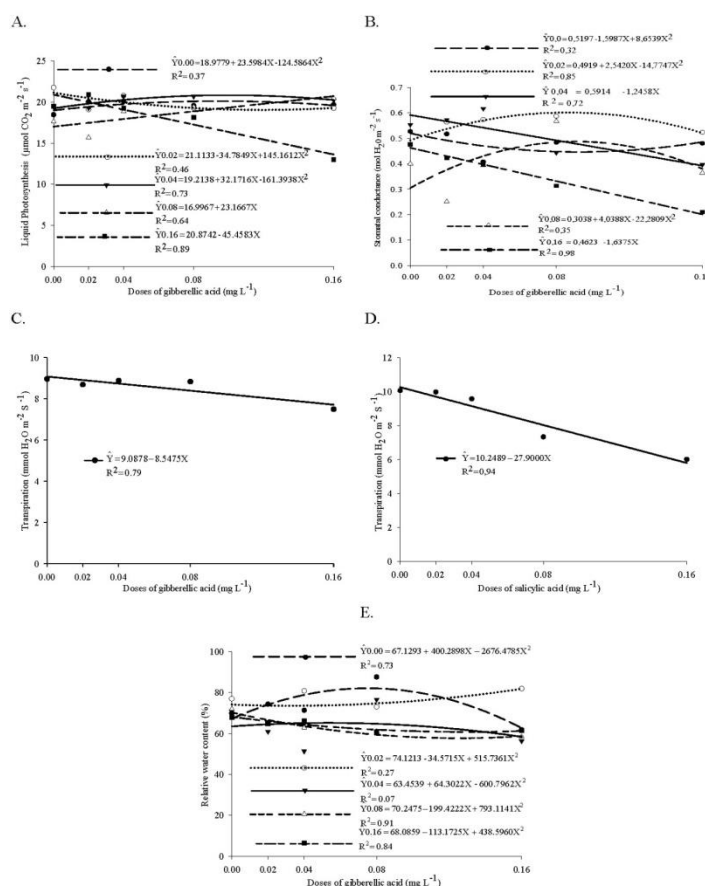


**Fig 1.** Maximum and minimum temperatures (°C) and relative air humidity (%) within the greenhouse of the Embrapa Algodão, Campina Grande PB Brazil, during the experimental period in 2012.

**Table 2.** Summary of analysis of variance (mean squares) for the variables: net photosynthesis (*A*), stomatal conductance (*gs*), transpiration (*E*), and relative water content (*RWC*) in castor bean plants ‘BRS Energia’ in response to the application of different doses of gibberellic (GA<sub>3</sub>) and salicylic (SA) acids at 80 DAE. Campina Grande Brazil, 2012.

FV	GL	A	gs	E	RWA
GA <sub>3</sub>	4	4.10 <sup>ns</sup>	0.02 <sup>ns</sup>	5.575*	184**
SA	4	12.25*	0.09**	49.82**	513.2**
GA <sub>3</sub> x SA	16	10.16**	0.02*	2.96 <sup>ns</sup>	163.3**
Residues	50	3.65	0.01	1.97	15.9
Standard error	-	1.10	0.06	0.81	2.30
C.V (%)	-	9.93	22.08	16.36	5.87

\*Significance at 0.05 probability of F test \*\* significant at 0.01 probability; ns - not significant.

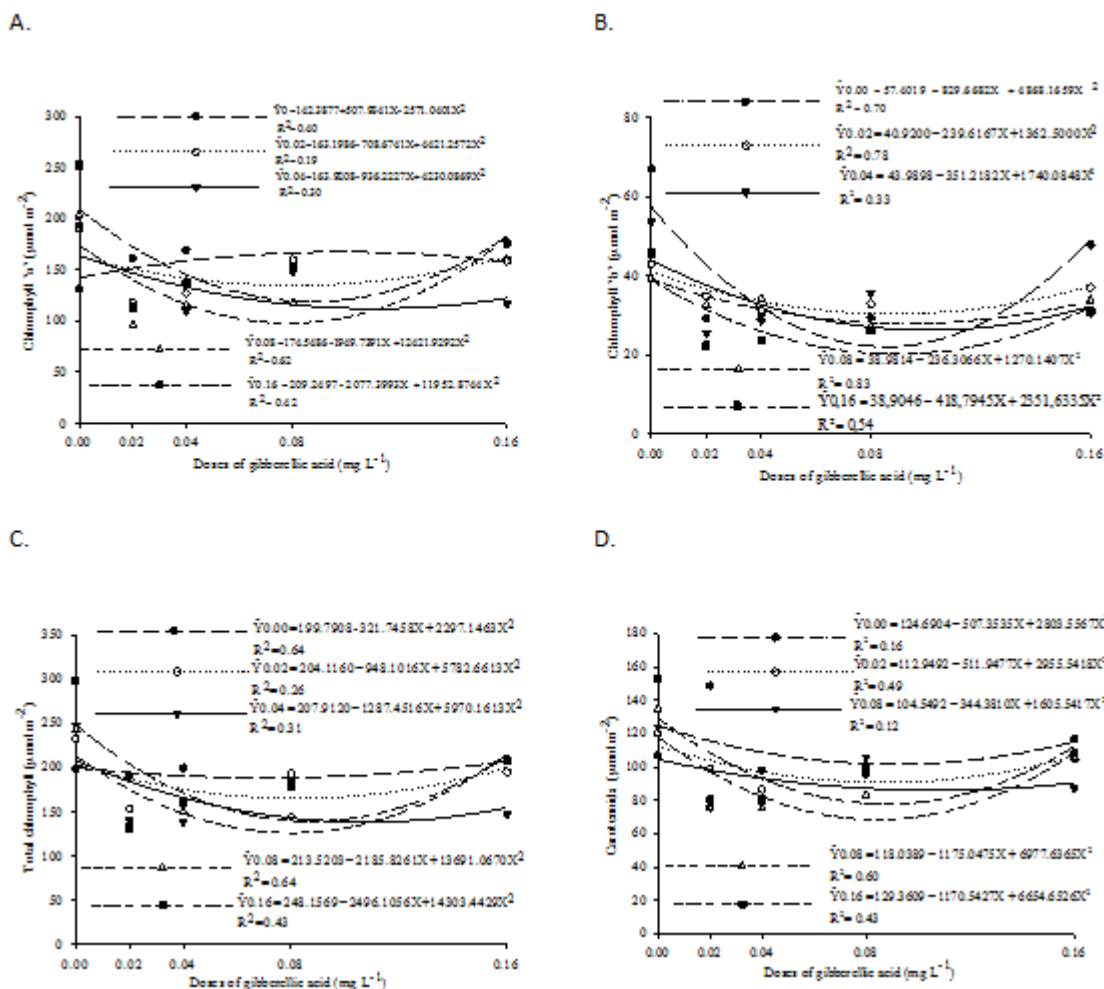


**Fig 2.** Net photosynthesis (A), Stomatal conductance (B), Transpiration (C and D), and Relative water content (E) in the castor bean cultivar ‘BRS Energia,’ in response to the application of doses of gibberellic acid and salicylic acid at 80 DAE. Campina Grande Brazil, 2012.

**Table 3.** Summary of analysis of variance (mean square) for the variables Chlorophyll *a* (CLA), Chlorophyll *b* (CLB), Carotenoids (CAR) and Total Chlorophyll (CLT) in castor bean plants cultivar BRS Energia in response to the application of different levels of gibberellic ( $GA_3$ ) and salicylic (SA) acid at 80 DAE. Campina Grande, Brazil, 2012.

FV	GL	CLA	CLB	CAR	CLT
$GA_3$	4	12131.2**	1147**	3904.7**	20429.4**
SA	4	1976.92**	237.2**	880.4 <sup>ns</sup>	1945.5**
$GA_3 \times SA$	16	2400.21**	111*	928**	2113.6**
Residue	50	327.30	48.8	353	505.6
C.V (%)	-	12.05	20.02	18.43	12.15

\*significant at 0.05 probability by F test; \*\* significant at 0.01 probability; ns- not significant.



**Fig 3.** Concentrations of chlorophyll a (A), chlorophyll b (B), total chlorophyll (C) and carotenoids (D) in castor bean plant cultivar ‘BRS Energia’ as a response to the application of doses of  $GA_3$  with SA at 80 DAE. Campina Grande, Brazil, 2012.

also depends on the plant’s growth phase, assay conditions, and environment.

#### Effects of application of $GA_3$ and SA on photosynthesizing pigments

There were significant interactions between treatments for the following variables: photosynthesizing pigments Chlorophyll *a* (CLA), Chlorophyll *b* (CLB), total chlorophyll (CLT), and Carotenoid (CAR), with significant isolated effects associated with  $GA_3$  and SA application, excepting carotenoids for SA (Table 3). Quadratic effects occurred for all interactions where SA doses were isolated and  $GA_3$  doses were diversified (Fig 3A). A flexion point at 0.10 mg L<sup>-1</sup> of  $GA_3$  was detected when the phytohormone dose was applied without salicylic acid. In fact, this treatment was associated

with a maximum 167 µmol m<sup>-2</sup> of chlorophyll *a*. However, chlorophyll *a* content was lowest (98 µmol m<sup>-2</sup>) when 0.08 mg L<sup>-1</sup> of SA was applied with 0.08 mg L<sup>-1</sup> of  $GA_3$ .

The negative effect of the inadequate application of  $GA_3$  is discernible in the plants’ metabolic processes. As a rule, the efficiency of the photosynthetic process is due to the quantity and maintenance of the various types of chlorophyll. Inadequate  $GA_3$  doses generally leads to a decrease in photosynthetic rates, changes in the reaction centers of Photosystem II, and the inhibition of the enzymatic processes of the Calvin system (Ouzounidou et al., 2010). With regard to the negative effects of  $GA_3$  on chlorophyll *a* concentrations, several authors report that excessive doses of the hormone affect the chlorophyll *a* production pathway, and thus seriously compromise the plant’s metabolism. Similar results have been reported in several studies. Misra et al.

(2010) reported that the Himalayan Yew (*Taxus wallichiana* Zuccarini var. mairei), when treated with GA<sub>3</sub>, exhibited a drastic reduction in chlorophyll a concentration. Similar effects were confirmed in case of lily (*Lilium longiflorum* Thunb.) with the application of the hormone (Ali et al., 2012). Chlorophyll b rates were negatively affected by the application of combined doses of SA and GA<sub>3</sub> (Fig 3B). There was a quadratic response to doses of SA applied in isolation and doses of GA<sub>3</sub> varied, with the average deflexion point occurring when a dose 0.09 mg L<sup>-1</sup> of GA<sub>3</sub> was applied. The minimum concentration of chlorophyll b per plant ranged between 20 and 30 μmol m<sup>-2</sup>. These rates occurred when doses 0.16 and 0.02 mg L<sup>-1</sup> SA were combined with a 0.09 mg L<sup>-1</sup> dose of GA<sub>3</sub>. Similar results were reported by Misra et al. (2010) and Ali et al. (2012), who demonstrated that the application of 1000 ppm of GA<sub>3</sub> considerably decreased the chlorophyll b content of the leaves of the Himalayan Yew (*Taxus wallichiana* Zuccarini var. mairei) and the roselle (*Hibiscus sabdariffa* L.).

Similarly, total chlorophyll (Fig 3C) was significantly reduced by the application of GA<sub>3</sub> and SA at 80 DAE. Minimum levels of total chlorophyll were reached when SA of any dosage was applied in combination with GA<sub>3</sub>. Furthermore, CLT decreased quadratically in response to the combined application of GA<sub>3</sub> and SA. However, total chlorophyll decreased with a GA<sub>3</sub> dose of 0.09 mg L<sup>-1</sup> (Fig 3C). In this study, the mean concentration of CLT was 152 μmol m<sup>-2</sup>. This decrease in total chlorophyll in response to GA<sub>3</sub> application has also been reported to occur in plants as the pea (El-Shraiy; Hegazi, 2009) and the roselle (Ali et al., 2012). However, different results were reported by Kavina et al. (2011) for peppermint (*Mentha piperita* Linn.). Carotenoid content was greatly affected by the joint application of SA and GA<sub>3</sub> (Fig 3D). Two treatments induced a quadratic effect, while each treatment possessed a deflexion point. A carotenoid content of 22 μmol m<sup>-2</sup> plant<sup>-1</sup> was associated with the application of 0 mg L<sup>-1</sup> of SA and 0.09 mg L<sup>-1</sup> of GA<sub>3</sub>. When 0.02 and 0.08 mg L<sup>-1</sup> of SA was applied with 0.09 mg L<sup>-1</sup> of GA<sub>3</sub>, carotenoid content ranged between 30 and 28 μmol m<sup>-2</sup> plant<sup>-1</sup>. However, the minimum carotenoid content was 20 μmol m<sup>-2</sup> plant<sup>-1</sup>, associated with the application of 0.16 mg L<sup>-1</sup> of SA and 0.09 mg L<sup>-1</sup> of GA<sub>3</sub>. These results indicate that the two phytohormones affected carotenoid content in the castor bean plant cultivar BRS Energia (Fig 3D), which deviates from the results of studies conducted on lily and peppermint (Kavina et al., 2011).

Chlorophyll a, b, and total content, along with carotenoid content depend primarily on the dosages of the hormones applied (Hakimeh et al., 2011), as the hormones influence the biosynthesis of terpenoids by affecting the metabolic route regulated by porphyrins and isoprenoids. Carotenoids are pigments that participate in the plants' stress-tolerance system. According to Mansouri et al. (2011), the decrease in this pigment is probably due to the same mechanism which affects chlorophyll, since the two pigments are controlled and accumulated by the same mechanism. Changes in chlorophyll and carotenoid rates seem to be associated with the activity of ribulose-1,5-biphosphate carboxylase/oxygenase (Rubisco), the synthesis of which is restricted in response to excessive GA<sub>3</sub> activity.

## Materials and Methods

### Location and crop plant

The experiment was performed in a greenhouse between February and June of 2012, at the Centro Nacional de Pesquisa de Algodão da Empresa Brasileira de Pesquisa

Agropecuária (Embrapa Algodão), in Campina Grande PB Brazil (7°13'1"S and 35°52'31"W, altitude: 551 m). According to Köppen's classification, the climate is As, or rather, hot and humid with rainfall in autumn and winter. The rainy season lasts from April to June, with an average rainfall of 800 mm (1974-2004). Maximum and minimum yearly mean temperatures are about 28.7°C and 19.8°C, respectively, with slight variations occurring throughout the year. During the experiment, maximum and minimum temperatures, as well as the relative humidity inside the greenhouse were all measured daily using a thermohygrograph (Fig 1). Soil samples for plant cultivation, were collected from the experimental field of the Empresa Estadual de Pesquisa da Paraíba (EMEPA) in Lagoa Seca, PB - Brazil, from 0–20 cm layer and analysed in the laboratory of Soil and Water Analysis at the Universidade Federal de Campina Grande (UFCG). The soil was classified as Sandy Regolithic Neosol (EMBRAPA, 2006), slightly acidic, with low concentrations of P, Ca, K, and organic matter (Table 1). The recommendations of the Laboratory of Soil Analysis for crop fertilization were followed: 80 kg ha<sup>-1</sup> N, 90 kg ha<sup>-1</sup> P<sub>2</sub>O<sub>5</sub>, and 60 kg ha<sup>-1</sup> K<sub>2</sub>O, in the form of urea, simple superphosphate, and potassium chloride, respectively. Nitrogen fertilization was conducted in two phases: the first dose was applied as basal dose to the soil, and the second as topdressing on the 30<sup>th</sup> day after the emergence (DAE) of the plantlet.

### Treatments, experiment conduction and plant materials

The experiment was in completely randomized design, and treatments consisted of a 5 x 5 factorial scheme, the factors were composed of five doses of GA<sub>3</sub> (0, 0.02, 0.04, 0.08, and 0.16 mg L<sup>-1</sup>) and five doses of SA (0, 0.02, 0.04, 0.08, and 0.16 mg L<sup>-1</sup>), with four replications. A 30 L recipient was considered to be one experimental unit. Cultivar 'BRS Energia' was used in the current study, the plant has a short growth cycle, low stature, and a mean seed productivity of 1800 kg ha<sup>-1</sup> when cultivated using a rainfed farming regime. Treatments were divided into two applications, the first was carried out at 30 days after emergence (DAE), and the second at 50 DAE. The foliar application of GA<sub>3</sub> and SA was done by spraying, directing sprayer to the abaxial and adaxial faces of the plants' leaves. A surfactant was added to the spraying solution to improve the efficiency of GA<sub>3</sub> and SA absorption by the leaf surface (Sávio et al., 2011). A manual compression sprayer with a high molar mass polyethylene tank with a capacity of 3 L was used, along with a pistol pump with a nozzle diameter of 34 mm.

The plants were irrigated daily, according to water requirement, which was estimated by the difference between the applied and drained volumes. Other crop management practices consisted of manual elimination of weeds and the control of fungal diseases through application of fungicides. Irrigation was done using medium quality C<sub>2</sub>S<sub>1</sub> pap water stored in a cistern at the Centro Nacional de Pesquisa de Algodão. Analysis by the Laboratory of Soil and Water Analysis of Embrapa - Algodão, showed that the water was alkaline (average pH 7.7), with moderate chloride levels (266.25 mg L<sup>-1</sup>). It was also rich in CaCO<sub>3</sub> (92.50 mg L<sup>-1</sup>), Ca<sup>2+</sup> soluble at 29 mg L<sup>-1</sup>, Mg<sup>2+</sup> equivalent to 30.60 mg L<sup>-1</sup>, and contained low levels of Na<sup>+</sup> (98.90 mg L<sup>-1</sup>). The water was somewhat saline (CE = 730 μS cm<sup>-1</sup>), with a low concentration of sodium (RAS = 3 mmol L<sup>-1</sup>)<sup>0.5</sup>.

## Measurements

Gas exchange was evaluated at 80 days after emergence (DAE), using measurements of the following characteristics: rate of net photosynthesis (A) ( $\mu\text{mol m}^{-2} \text{s}^{-1}$ ), transpiration (E) ( $\text{mmol of H}_2\text{O m}^{-2} \text{s}^{-1}$ ), and stomatal conductance (gs) ( $\text{mol m}^{-2} \text{s}^{-1}$ ) (Prado; Moraes, 1997), relative water content (RWC) (Weatherly, 1950), and concentration of photosynthetic pigments (chlorophyll a (CLA), b (CLB), total chlorophyll (CLT), and carotenoids (CAR)) in the castor bean leaves (Arnon, 1949).

## Data analysis

Data were analyzed by analysis of variance (F test) and regression tests, significance at 0.05 probability level, using the statistics software SISVAR 5.3 (Ferreira, 1996).

## Conclusions

High doses of gibberellic acid, used in conjunction with salicylic acid or isolated, reduced net photosynthesis, stomatal conductance, and transpiration. The effects were linear at 80 days after emergence of castor bean.

The photosynthetic pigments chlorophyll a, b and total and carotenoids are reduced by the application of up to 0.09 mg L<sup>-1</sup> of GA<sub>3</sub> in combination with 0.16 mg L<sup>-1</sup> of SA, whereas the lowest relative water content in the castor bean leaves were achieved by using a dose of 0.12 mg L<sup>-1</sup> of GA<sub>3</sub> and 0.16 mg L<sup>-1</sup> of SA.

## Acknowledgments

The authors would like to thank The Brazilian Council for Scientific Research and Development (CNPq) for granting the PNPd scholarship to the second author. - EDITAL MEC/CAPES and MCT/CNPq/FINEP N° 28/2010 – PNPd/CNPq/UFMG.

## References

- Ali HM, Siddiqui MH, Basalah MO, Al-Wahaibi MH, Sakran AM, Al-Amri A (2012) Effects of gibberellic acid on growth and photosynthetic pigments of *Hibiscus sabdariffa* L. under salt stress. Afr J Biotechnol. 11:800-804.
- Almeida AQ, Vieira EL (2010) Gibberellin action on growth, development and production of tobacco. Sci Agrar Parana. 9:45-57.
- Arnon, DI (1949) Copper enzymes in isolated chloroplasts: polyphenoloxidase in *Beta vulgaris*. Plant Physiol. 24:1-15.
- Assmann SM, Armstrong F (1999) Hormonal regulation of ion transporters: the guard cell system. In: Hooykaas, PJJ, Hall MA, Libbenga KR (eds.), Biochemistry and molecular biology of plant hormones. Elsevier, Amsterdam, 337-361.
- Cangemi JM, Santos AM, Claro Neto AS (2010) Revolução verde da mamona. Quím Nova Esc. 32:3-8.
- CONAB (2014) Companhia Nacional de Abastecimento. Acompanhamento da safra brasileira: V.1 - Safra 2013/14 N.11 - Décimo primeiro levantamento Agosto/2014. Available at <http://www.bcsf.com.br/Editais2014/Safra%202014%2011Lev%20antamento.pdf>. on 12/08/2014.
- Dawood MG, Sadak MS, Hozayen M (2012). Physiological role of salicylic acid in improving performance, yield and some biochemical aspects of sunflower plant grown under newly reclaimed sandy soil. Aust J Basic Appl Sci. 6:82-89.
- El-Shraiy AM, Hegazi, AM (2009) Effect of acetylsalicylic acid, indole-3- bytric acid and gibberellic acid on plant growth and yield of pea (*Pisum sativum* L.). Aust J Basic Appl Sci. 3:3514-3523.
- Empresa Brasileira de Pesquisa Agropecuária – EMBRAPA. Centro Nacional de Pesquisa de Solos. Sistema brasileiro de classificação de solos. 2. ed. Rio de Janeiro: Embrapa Solos, 2006. 306 p.
- Ferreira, P. V (1996) 2<sup>nd</sup>. Estatística experimental aplicada à agronomia. Maceió: Edufal, 606p.
- Hakimeh M, Zahra A, Ryszard A (2011) The response of terpenoids to exogenous gibberellic acid in *Cannabis sativa* L. at vegetative stage. Acta Physiol Plant. 33:1085-1091.
- Iqbal M, Basra SMA (2013) Gibberellic acid mediated induction of salt tolerance in wheat plants: growth, ionic partitioning, photosynthesis, yield and hormonal homeostasis. Environ Exper Bot. 86:76-85.
- Kavina J, Gopi R, Panneerselvam R (2011) Traditional and nontraditional plant growth regulators alter the growth and photosynthetic pigments in *Mentha piperita* Linn. Int J of Environ Sci. 1:124-134.
- Liu C, Guo J, Cui Y, Lü T, Zhang X, Shi G (2011) Effects of cadmium and salicylic acid on growth, spectral reflectance and photosynthesis of castor bean seedlings. Plant Soil. 344:131-141.
- Misra A, Srivastava NK, Srivastava AK, Khan A (2009) Influence of ethereal and gibberellic acid on carbon metabolism, growth, and alkaloids accumulation in *Catharanthus roseus* L. Afr J Pharm Pharmacol. 3:515-520.
- Misra A, Srivastava NK, Srivastava AK, Chattopadhyay SK (2010) Influence of gibberellic acid and *Arbuscular mycorrhizae* inoculation on carbon metabolism, growth, and diterpene accumulation in *Taxus wallichiana* Zuccarini var. Mairei. J Biophys. 2:22-27.
- Noreen S, Ashraf M, Akram NA (2011) Does exogenous application of salicylic acid improve growth and some key physiological attributes in sunflower plants subjected to salt stress? J Appl Bot and Food Qual. 84:169-177.
- Prado CHBA, Moraes JAPV (1997) Photosynthetic capacity and specific leaf mass in twenty woody species of Cerrado vegetation under field conditions. Photosynthetica. 33:103-112.
- Ouzounidou G, Ilias I, Giannakoula A, Papadopoulou P (2010) Comparative study on the effects of various plant growth regulators on growth, quality and physiology of *Capsicum annuum*. Pakistan J Bot. 42:805-814.
- Sadeghipour O, Aghaei P (2012) Impact of exogenous salicylic acid application on some traits of common bean (*Phaseolus vulgaris* L.) under water stress conditions. Intl J Agri Crop Sci. 4:685-690.
- Saeidnejad AH, Mardani H, Naghibolghora M (2012) Protective effects of salicylic acid on physiological parameters and antioxidants response in maize seedlings under salinity stress. JAEBS. 2:364-373.
- Sávio FL, Silva JC, Teixeira IR, Borém A (2011) Produção de biomassa e conteúdo de silício em gramíneas forrageiras sob diferentes fontes de silicato. Semina. 32:103-110.
- Shah SH (2007) Photosynthetic and yield responses of *Nigella sativa* L. to pre-sowing seed treatment with ga<sub>3</sub>. Turkish J Biol. 31:103-107.
- Shah SH, Tak HI (2011) Evaluation of soaking and spray treatments with GA<sub>3</sub> to black cumin (*Nigella sativa* L.) in relation to growth, seed and oil yields. Genet Plant Physiol. 1:119-129.
- Weatherley PE (1950) Studies in the water relations of cotton plant. I The field measurement of water deficits in leaves. New Phytol. 49:81-97.