



## Accumulation of organic solutes and enzymatic activity in cut roses (*Rosaceae*) cultivated with physiological effect products in the Sub-Middle São Francisco River Valley

Maria de Lourdes Neres da Silva<sup>1\*</sup>  Mariana Correia Santos<sup>2</sup>   
 Mayara Suzanne de Melo Barbosa<sup>2</sup>  João Henrique Ferreira Sabino<sup>2</sup>   
 Hugo Leonardo Coelho Ribeiro<sup>2</sup>  Anamaria Ribeiro Pereira Ramos<sup>2</sup>   
 Marcelle Almeida da Silva<sup>3</sup>  Márkilla Zunete Beckmann-Cavalcante<sup>3</sup> 

<sup>1</sup>Programa de Pós-graduação em Agronomia-Produção Vegetal, Universidade Federal do Vale do São Francisco (UNIVASF), 56300990, Petrolina, PE, Brasil. E-mail: malu-neres@hotmail.com. \*Corresponding author.

<sup>2</sup>Laboratório de Fisiologia Vegetal, Universidade Federal do Vale do São Francisco (UNIVASF), Petrolina, PE, Brasil.

<sup>3</sup>Universidade Federal do Vale do São Francisco (UNIVASF), Campus de Ciências Agrárias, Petrolina, PE, Brasil.

**ABSTRACT:** *The aim of the present study was to evaluate the accumulation of carbohydrates, protein, and proline as well as the activity of the antioxidant enzymes superoxide dismutase and catalase in the Ambiente cultivar of cut rose plants grown with the application of physiological effect products in the Sub-Middle São Francisco River Valley in Brazil. The experiment was performed under a mesh screen with 50% shading. The experimental design used randomized blocks with four repetitions and six treatments: T1) control (water); T2) boscalid; T3) pyraclostrobin; T4) boscalid + pyraclostrobin (T2 + T3); T5) fluxapyroxad + pyraclostrobin; T6) plant growth regulators 4-(indol-3-yl) butyric acid (IBA) + gibberellic acid (GA<sub>3</sub>) + kinetin; these treatments were applied every 15 days throughout the crop cycle. To determine the accumulation of solutes and enzymatic activity, 8 leaves was collected every 48 h. Leaves were immediately immersed in liquid nitrogen and frozen until further analysis in the laboratory. Results showed that the product combinations boscalid + pyraclostrobin and fluxapyroxad + pyraclostrobin as well as the plant growth regulators were the treatments with the most consistent responses throughout the evaluated cycle, providing a greater accumulation of solutes in rose leaves, as an osmotic adjustment strategy against stress from high temperatures, particularly when proline accumulation is observed. With regard to enzymatic activity, plant regulators showed more consistent results when compared with other treatments, increasing both superoxide dismutase and catalase activity. The marked accumulation of organic solutes and the high enzymatic activity, particularly of catalase, indicated that rose plants use such mechanisms as a defense against the region's high temperatures.*

**Key words:** *Rosa spp., strobilurins, plant growth regulators, carbohydrates, antioxidant enzymes.*

## Acúmulo de solutos orgânicos e atividade enzimática em rosa de corte (*Rosaceae*) cultivada com produtos de ação fisiológica no Vale do Submédio São Francisco

**RESUMO:** *Objetivou-se, com este estudo, avaliar o acúmulo de carboidratos, proteína e prolina, bem como a atividade de enzimas antioxidantes superóxido dismutase e catalases em plantas de rosa de corte 'Ambiente' cultivadas sob aplicação de produtos de efeitos fisiológicos no Vale do Submédio São Francisco. O experimento foi realizado em telado com 50% de sombreamento. O delineamento experimental foi em blocos casualizados com quatro repetições e seis tratamentos: T1) testemunha (água); T2) boscalida; T3) piraclostrobina; T4) boscalida + piraclostrobina (T2 + T3); T5) fluxaproxada + piraclostrobina; T6) regulador vegetal IBA + GA<sub>3</sub> + cinetina, aplicados a cada 15 dias durante todo o ciclo da cultura. Para a determinação do acúmulo de solutos e atividade enzimática foram realizadas 8 coletas de folhas a cada 48 horas. As mesmas foram imediatamente imersas em nitrogênio líquido e congeladas para análise posterior em laboratório. Os resultados demonstram que os produtos conjugados boscalida + piraclostrobina, fluxaproxada + piraclostrobina e os reguladores vegetais foram aqueles com respostas mais consistentes ao longo do ciclo avaliado, propiciando maior acúmulo de solutos nas folhas das roseiras, como estratégia de ajustamento osmótico diante do estresse por temperatura elevada, especialmente quando se observa o acúmulo de prolina. Para a atividade enzimática, os reguladores vegetais apresentam resultados mais regulares quando comparados aos demais tratamentos, aumentando a atividade da SOD e da CAT. O acúmulo acentuado de solutos orgânicos junto com a elevada atividade enzimática, especialmente da CAT, indicam que as roseiras utilizam tais mecanismos como defesa diante das temperaturas elevadas da região.*

**Palavras-chave:** *Rosa spp., estrobilurinas, reguladores vegetais, carboidratos, enzimas antioxidantes.*

## INTRODUCTION

Commercial floriculture has consistently been growing over the years, with roses, orchids, chrysanthemums, and kalanchoe being the most prominent

products currently. The high investment in technologies that influence production in all seasons has facilitated producers to migrate to high-altitude regions, such as mountain ranges, or to regions with a constant climate, such as the Brazilian Northeast (IBRAFLOR, 2017).

With the expansion of the ornamental flower sector, the Sub-Middle São Francisco River Valley, a well-established producer of fruits, could be a new agricultural frontier for the production of flowers, such as cut roses (*Rosaceae*). However, considering the natural requirements of the rose bush, which, for its ideal development, requires temperatures between 17 °C and 18 °C during the night and between 23 °C and 25 °C during the day, it is necessary to use technologies that enable the introduction of this culture to environments with an adverse climate (BARBOSA et al., 2005).

In conditions of high luminous intensity and high temperatures, such as those present in the semiarid northeastern Brazil, plants tend to present responses that assist in maintaining their physiological processes because in situations of stress, changes in their metabolism may occur. These responses will require osmotic adjustment using the strategy of accumulating organic substances, such as carbohydrates, protein, proline, and other free amino acids (MOURA et al., 2016; TAIZ; ZEIGER, 2017). Another mechanism used by plants that are subjected to high temperatures is the activation of the enzymatic antioxidant system, particularly superoxide dismutase (SOD) and catalase (CAT), which are enzymes specialized in the deactivation of reactive oxygen species (BARBOSA et al., 2014; AMARO et al., 2018). Therefore, rose plants can both synthesize organic solutes and increase antioxidant activity in response to the stress caused by the region's climate.

With the aim to introduce cut rose cultivation to the Sub-Middle São Francisco River Valley, the foliar application of products from the strobilurin, carboxamide, and anilide chemical groups as well as of plant growth regulators may be a viable technology, because studies have shown that these molecules act by modifying the growth physiology and metabolism of the plants upon application, thereby enabling better crop development and yield. Additionally, they promote an increase in the activity of the antioxidant system in plants, minimizing the damage caused by stress (RAMOS et al., 2013; RAMOS et al., 2015; MACEDO et al., 2017, AMARO et al., 2018).

However, there is no data on the activity of these products with regard to the possible physiological effects caused to plants used in floriculture. Therefore, the present study aimed to evaluate the influence of pyraclostrobin, boscalid, and fluxapyroxad as well as the plant growth regulators kinetin, gibberellic acid ( $GA_3$ ), and 4-(indol-3-yl) butyric acid (IBA) on the synthesis of carbohydrates, protein, and proline and

on the activity of SOD and CAT in cut rose plants of the Ambiance cultivar grown in the Sub-Middle São Francisco River Valley.

## MATERIALS AND METHODS

The research was conducted from April 2016 to April 2017. The cultivation was conducted at the geographical coordinates 09°21' South latitude and 40°34' West longitude, in a semiarid tropical climate (BSWh according to the KÖPPEN classification), with an annual rainfall of approximately 400 mm, majorly distributed between November and April (ALVARES et al., 2014). The area's soil was classified as an orthic quartz-sand neosol. Temperature and relative humidity data were obtained from HOBO U12-012 TEM/RH/Light/External Data Logger devices installed inside the mesh screen; whereas, precipitation data were obtained from a meteorological station installed approximately 500 m from the site of the experiment. The mean temperature recorded for the period of collections inside the screen was 28.9 °C, with a minimum of 21.3 °C and a maximum of 41 °C. The mean relative humidity of the air was 57%, with a minimum of 19% and a maximum of 91%, whereas precipitation was 41.4 mm.

The experiment was installed under a mesh screen (50% shading; 14.0 m wide × 25.0 m long × 3.0 m high). Rose seedlings of the Ambiance cultivar were transplanted by stake propagation in April 2016. The cultivation was conducted in a single row with a 1.0-m spacing between lines and 0.25-m spacing between plants, irrigated with a drip system (flow rate of 3.3 L.h<sup>-1</sup>, one emitter per plant). Calcium nitrate (2.3 kg/15 L of water) was used via weekly fertigation and mixed foliar fertilizer (40 mL/20 L of water) was used every 15 days as fertilizers, according to the recommendations for this crop (VILLAS BÔAS et al., 2008) as well as to results obtained from a chemical analysis of the soil performed 60 days before implantation.

The experiment was divided into randomized blocks, with four repetitions and 10 plants per repetition, 2 of which were used as borders at the extremities and 8 central ones, to collect material for further laboratory analysis. Treatments applied were as follows: T1) control (water application); T2) boscalid (0.15 g.L<sup>-1</sup>); T3) pyraclostrobin (0.8 mL.L<sup>-1</sup>); T4) boscalid + pyraclostrobin (T2 + T3); T5) fluxapyroxad + pyraclostrobin (2.5 mL.L<sup>-1</sup>); and T6) the growth regulators IBA (0.005%) +  $GA_3$  (0.005%) + kinetin (0.009%) at 1 mL.L<sup>-1</sup>. The first application of

the treatments was performed 32 days after planting the seedlings in the field and subsequent applications were performed every 15 days for the entire duration of the experiment (345 days). Vegetable oil at 0.5% was added to the products to protect them against losses due to evaporation, drift, or washing. A plastic curtain was used to prevent drift between treatments.

After 1 year of cultivation, during the 23<sup>rd</sup> cycle of application of the treatments, leaves were collected every 48 h, always in the morning, to evaluate the accumulation of organic solutes and the enzymatic activity. Therefore, these data were evaluated on day 0 before the application of treatments in the cycle and at 1, 3, 5, 7, 9, 11, and 13 days after application (DAA), totaling eight collections of 24 samples each (overall 192 samples), comprising a 15-day cycle corresponding to the interval between treatment applications, in a 6 × 8 factorial scheme (6 treatments evaluated at 8 moments). The collected leaves were immediately wrapped in aluminum foil and immersed in liquid nitrogen, following which these were stored in a freezer at a temperature of -20 °C. In the laboratory, soluble carbohydrates (DUBOIS et al., 1956), protein (BRADFORD, 1976), and free proline (BATES, 1973) levels as well as SOD enzyme activity (GIANNOPOLIS; RIES, 1977) and CAT enzyme activity (BEERS JUNIOR; SIZER, 1952) were quantified.

The concentration of soluble carbohydrates was determined in test tubes containing 500 µL of crude leaf extract + 500 µL of phenol + 2.5 mL of sulphuric acid. Thereafter, the tubes were left undisturbed for 10 min. Subsequently, they were stirred in a vortex and left in a tray with water at a controlled temperature of 25 °C for 10 min. The absorbance was measured in a spectrophotometer at 490 nm. A mixture of 500 µL of deionized water + 500 µL of phenol + 2.5 mL of sulphuric acid was used as the blank.

The BRADFORD method was used for protein determination. A mixture of 100 µL of raw extract + 1 mL of the BRADFORD reagent was placed in test tubes. This was followed by vortex agitation, 15-min incubation, and absorbance reading in a spectrophotometer at 595 nm. A mixture of 100 µL of deionized water + 1 mL of the BRADFORD reagent was used as the blank. For the determination of free proline, 1 mL of raw extract + 1 mL of acid ninhydrine + 1 mL of glacial acetic acid were added to threaded test tubes. Tubes were closed and shaken in a vortex, following which they were placed in a boiling water bath for 1 h at 100 °C. Thereafter, they were removed from the water bath and immediately

cooled in an ice bath. Subsequently, 2 mL of toluene was added to each tube, followed by another vortex stirring. The resulting supernatant was aspirated using a pipette and the absorbance was measured in a spectrophotometer at 520 nm. The blank consisted of 2 mL of toluene.

The SOD activity was measured in the laboratory under reduced luminosity by adding the following to test tubes appropriately identified according to each sample: 25 µL of crude leaf extract + 75 µL of extraction buffer; 1660 µL of reaction medium; 200 µL of NBT; and 40 µL of riboflavin. Tubes with the prepared samples were exposed to lighting with a 30 W fluorescent lamp for 5 min. After this period of exposure, ambient lighting was reduced and the tubes were wrapped in aluminum foil, agitated in a vortex, and the absorbance was measured in a spectrophotometer at 560 nm. Only the extraction buffer, along with the other reagents but without the crude extract, was used as both the clear and the dark blank. The SOD activity was determined by the indirect method, using calculations proposed by GIANNOPOLIS and RIES (1977).

For CAT, the extraction buffer and 500 mM hydrogen peroxide were used as reagents. During the sample readings, the extraction buffer was immersed in a water bath at 30 °C. In the cuvette, for each sample to be read on the spectrophotometer, 1390 µL of extraction buffer was added, followed by 50 µL of crude extract. Inside the spectrophotometer, 60 µL of hydrogen peroxide was added. The solution was stirred thrice using a pipette and the absorbance was quickly measured at 240 nm. The extraction buffer, including the hydrogen peroxide but not the crude extract, was used as the blank, in the same proportions of the samples to be evaluated.

Data obtained were submitted to an analysis of variance, with an F-test at 5% error probability and grouping of means using the Scott-Knott test, in a factorial scheme conducted using SISVAR version 5.6 statistical software (FERREIRA, 2014).

## RESULTS

For carbohydrates (Table 1), it was observed that on the 1<sup>st</sup> DAA, T4 and T5 showed increased carbohydrate content, and along with T3 and T6, these treatments showed the greatest accumulation of this solute, differing from T2 and the control group. On the 5<sup>th</sup> day of evaluation, results for T5 were higher than those for the other treatments, whereas on the 9<sup>th</sup> DAA, T6 was the treatment with the best mean value. At the end of the evaluation cycle,

Table 1 - Accumulation of organic solutes - carbohydrate, protein, and proline - in rose leaves of the Ambiance cultivar daily and on the days before (day 0) and after application of the physiological effect products (days 1 to 13). DAA: Days after Application. Petrolina – PE, 2017.

-----Soluble Carbohydrates (Conc. mmol/gMF)-----								
Treatment	Day 0	Day 1	Day 3	Day 5	Day 7	Day 9	Day 11	Day 13
T1	1.8 bB	1.7 bB	1.6 aB	1.6 bB	3.4 aA	1.5 bB	2.2 aB	1.6 bB
T2	1.5 bB	1.4 bB	1.6 aB	2.4 bA	3.9 aA	2.0 bB	2.0 aB	2.5 aA
T3	3.3 aA	2.4 aA	1.7 aA	2.2 bA	3.0 aA	2.0 bA	2.6 aA	2.3 aA
T4	1.7 bA	2.4 aA	1.8 aA	2.1 bA	3.0 aA	1.7 bA	1.6 aA	2.2 aA
T5	2.4 bA	2.6 aA	2.0 aA	3.5 aA	3.1 aA	2.0 bA	2.4 aA	1.4 bA
T6	2.6 aA	2.2 aA	2.3 aA	2.0 bA	3.3 aA	4.0 aA	2.1 aA	1.7 bA
CV%	15.2	19.0	19.1	14.6	26.1	19.7	26.6	26.5
-----INTERACTION: Treatment * DAA: 4.3*-----								
-----Protein (Conc. mmol/gMF)-----								
Treatment	Day 0	Day 1	Day 3	Day 5	Day 7	Day 9	Day 11	Day 13
T1	3.4 BA	3.9 aA	4.2 aA	4.3 aA	2.3 bB	4.1 aA	2.3 cB	3.6 aA
T2	2.7 BC	3.1 BC	4.0 aB	2.3 bC	3.9 aB	2.3 bC	5.2 aA	2.4 bC
T3	8.9 aA	3.4 aB	4.4 aB	2.2 bC	1.9 BC	3.2 bB	3.3 bB	2.6 bC
T4	4.4 bA	1.9 cB	3.1 bB	2.3 bB	2.8 aB	2.6 bB	3.2 bB	3.8 aA
T5	1.6 bB	1.8 cB	4.2 aA	2.4 bB	1.5 bB	2.9 bB	2.5 cB	2.4 bB
T6	2.7 bA	2.6 bA	3.6 bA	2.3 bB	1.5 bB	1.8 bB	1.7 cB	3.5 aA
CV%	24.0	16.1	13.7	24.3	24.2	24.41	22.4	25.0
-----INTERACTION: Treatment * DAA: 7.9*-----								
-----Free Proline (Conc. mmol/gMF)-----								
Treatment	Day 0	Day 1	Day 3	Day 5	Day 7	Day 9	Day 11	Day 13
T1	7.8 bB	5.6 bB	5.9 bB	3.6 bC	4.0 BC	5.7 bB	5.0 bC	11.7 CA
T2	7.8 bB	1.7 cc	3.1 cc	4.9 bB	5.7 bB	13.1 aA	3.3 bC	6.4 dB
T3	3.2 CA	5.0 bA	5.1 bA	4.3 bA	5.3 bA	7.1 bA	5.8 bA	4.8 dA
T4	8.3 bB	6.7 aB	7.4 aB	6.9 aB	8.0 aB	5.1 bB	8.9 aB	17.1 aA
T5	3.1 cc	4.6 BC	3.5 cc	6.2 aB	7.6 aB	4.6 BC	3.7 bC	15.5 aA
T6	10.9 aA	3.9 bB	4.2 cB	4.8 bB	6.3 bB	6.5 bB	4.4 bB	10.3 bA
CV%	24.7	23.0	18.3	20.8	22.1	25.7	21.9	27.7
-----INTERACTION: Treatment * DAA: 9.2*-----								

\*Significant to 5% probability by an F-test. Means followed by different lowercase letters within a column and different uppercase letters within a row differ from each other according to the Scott-Knott test at 5% probability. Lowercase letters within a column compare treatments to each other; uppercase letters within a line compare treatment over the days. Treatments: T1: control group (water application); T2: boscalid; T3: pyraclostrobin; T4: mixture of boscalid + pyraclostrobin (T2 + T3); T5: flupyroxad + pyraclostrobin; T6: kinetin + GA<sub>3</sub> + IBA.

T2, T3, and T4 were the treatments that significantly differed from the remaining ones.

On analyzing the behavior of isolated treatments over the days, only the control (T1) and boscalid (T2) groups showed variation over the period evaluated; the control group significantly differed from the other treatments on the 7<sup>th</sup> DAA, whereas the boscalid group (T2) was statistically superior to the other products on the 5<sup>th</sup>, 7<sup>th</sup>, and 11<sup>th</sup> DAA. The other treatments maintained a constant performance, without considerable variance throughout the period evaluated (Table 1).

Regarding protein accumulation (Table 1), it was observed that on most of the evaluated days there was no statistical difference between the control group and the tested products. Over the days, the control group maintained consistent results practically during the entire observation period, in contrast with the other treatments that exhibited an oscillating behavior.

For the accumulation of proline (Table 1), it was observed that the treatment with boscalid + pyraclostrobin (T4) significantly differed from the others during most of the study period and

that this treatment provided the most consistent result, followed by treatment with fluxapyroxad + pyraclostrobin (T5).

When observing the performance of the treatments in isolation over the study period, it was observed that T3 showed no significant differences on any day, thereby maintaining a consistent performance both before and after application of the products, whereas T4 presented the highest mean on the 13<sup>th</sup> DAA, but remained constant on the other days of evaluation.

Regarding SOD activity, it was observed that in the 1<sup>st</sup> DAA, there was an increase in SOD activity, recording the highest means for the cycle, where T6 was the treatment that promoted the highest SOD activity (Figure 1, means followed by lowercase letters).

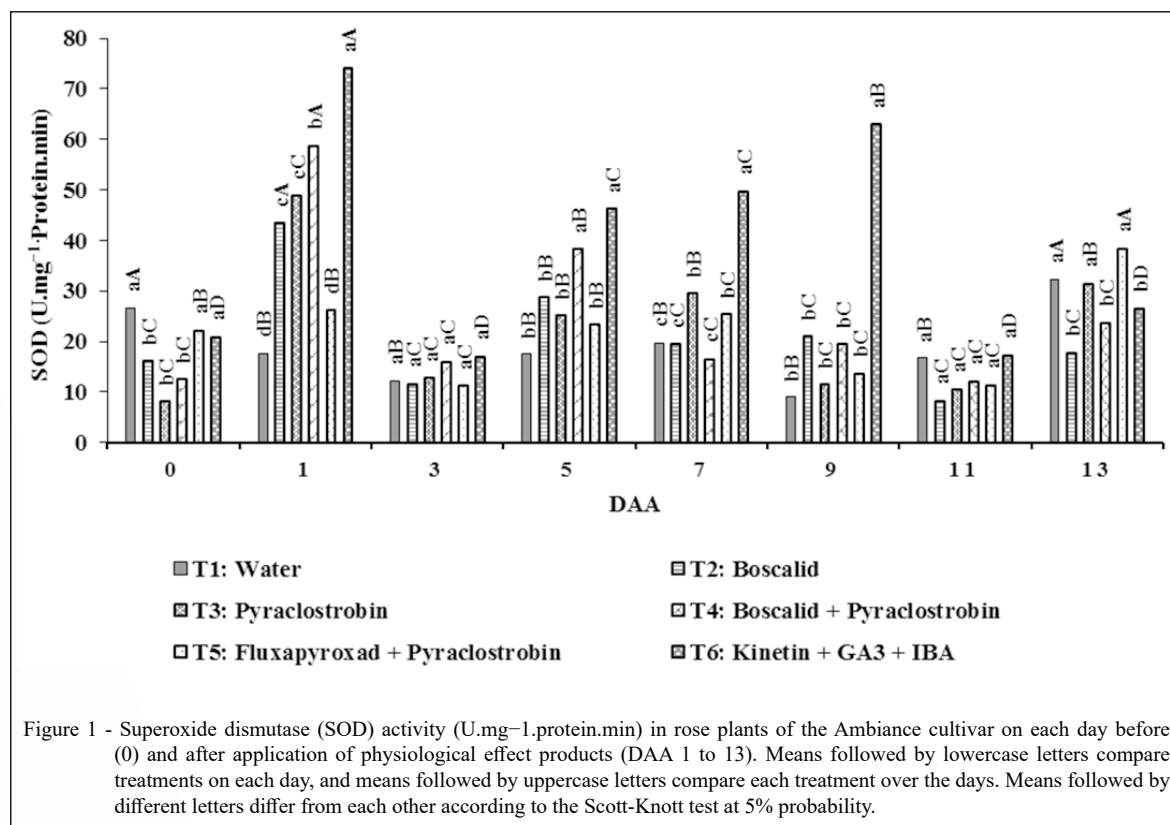
On the 3<sup>rd</sup> DAA of the products, there was no significant difference between the treatments, whereas on 5<sup>th</sup>, 7<sup>th</sup>, and 9<sup>th</sup> DAAs of the evaluation, T6 significantly differed from the other treatments, being the product with the most consistent results for the SOD activity in the evaluated cycle.

Over the study period (Figure 1, means followed by capital letters), the influence of each

treatment for promoting SOD activity was recorded and it was observed that the control group presented better performance on day 0 (before application) and 13<sup>th</sup> DAA, whereas T2, T3, T4, and T6 presented results similar to each other, with the highest mean for SOD activity being recorded on the 1<sup>st</sup> DAA, with lower mean values in the following days, suggesting a more immediate action of such products in the promotion of SOD activity. Treatment with fluxapyroxad + pyraclostrobin (T5) promoted higher SOD activity on the 13<sup>th</sup> DAA, suggesting that the product exhibits a later action in terms of SOD activity.

With regard to CAT activity in the rose plants treated with physiological effect products, it was observed that on day 0 (before treatment application), the treatments presented similar mean values, not differing from each other (Figure 2, means followed by lowercase letters). For the remaining days of evaluation, it was noticed that there was a variation in the performance of treatments on CAT activity, with T5 and T6 being the most consistent treatments throughout the cycle.

Observing the behavior of each treatment alone over the days (Figure 2, means followed by



capital letters), it is noted that the control group and that for the treatment with boscalid only (T2) did not vary over the period, with T1 maintaining basically the same means for CAT activity. Although, a variation in T2 means is noticeable over the evaluation days, it was not statistically significant. For the treatment with pyraclostrobin alone (T3), the highest mean recorded occurred on the 11th DAA, with consistent mean values on the other days. T4 maintained consistent performance virtually throughout the entire cycle, with a higher average only on the 13th DAA. For treatment with fluxapyroxad + pyraclostrobin (T5), it was observed that the highest means occurred on the 3<sup>rd</sup>, 5<sup>th</sup>, and 9<sup>th</sup> DAA, unlike the other groups.

Finally, treatment with plant regulators (T6) promoted consistent mean values for CAT activity from the 3<sup>rd</sup> to the 11<sup>th</sup> DAA, with the highest means observed on the 1<sup>st</sup> and 13<sup>th</sup> DAA. Considering these results; although, plant regulators exhibited a greater consistency in promoting greater SOD and CAT activity, no well-defined pattern was observed for the action of other treatments, and a sharp fluctuation of these treatments was noted in

the promotion of enzyme activity in the tested plants. Moreover; although, the highest SOD activity was observed on the 1<sup>st</sup> DAA, the highest CAT activity was observed on the 13<sup>th</sup> DAA. The SOD plays a role in the first line of defense of the plant by reducing superoxide and producing hydrogen peroxide, which will be in turn used by CAT in the cell peroxisomes; the results obtained suggested a joint action of both enzymes, acting in the defense of the plant against oxidative stress and ensuring the protection to the photosynthetic apparatus.

Typically, it can be observed that the products acted in different ways during the experimental cycle. The combination of boscalid + pyraclostrobin presented more satisfactory results in the accumulation of organic solutes, particularly for proline, whereas for enzymatic activity, the plant regulators were notable compared with other treatments.

## DISCUSSION

There were different responses from the rose plants in relation to the solutes evaluated and the products applied. Stress caused by high

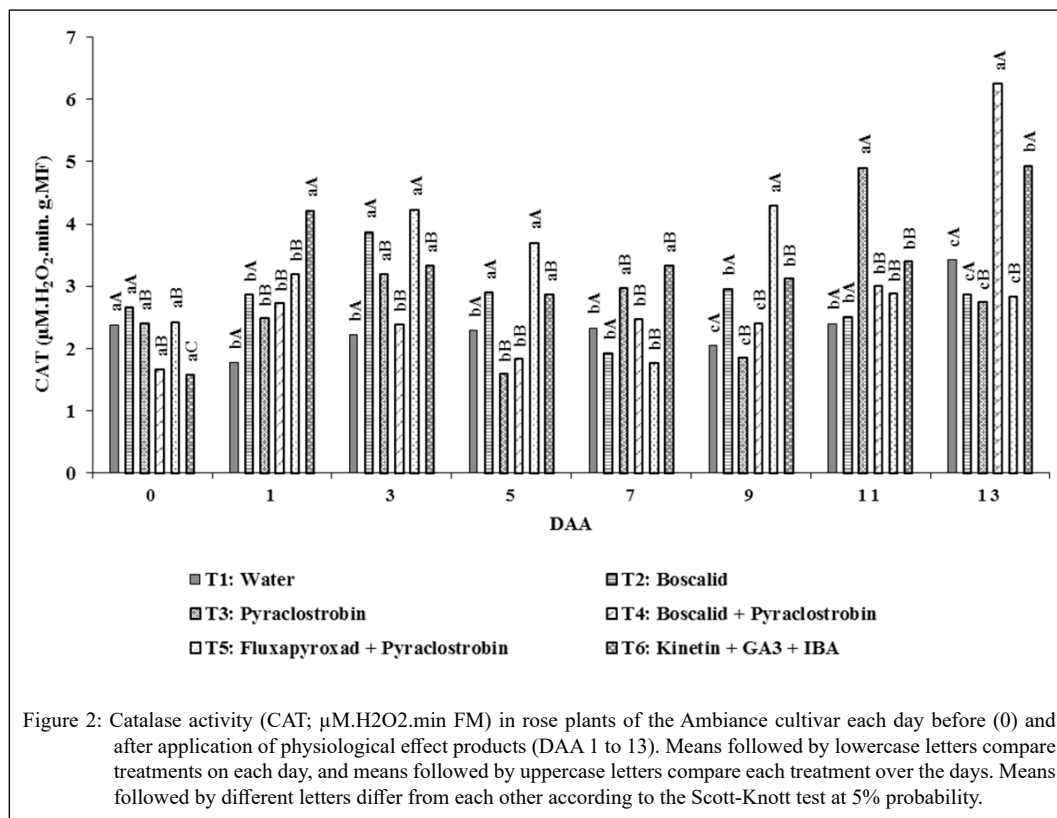


Figure 2: Catalase activity (CAT;  $\mu\text{M.H}_2\text{O}_2.\text{min FM}$ ) in rose plants of the Ambiance cultivar each day before (0) and after application of physiological effect products (DAA 1 to 13). Means followed by lowercase letters compare treatments on each day, and means followed by uppercase letters compare each treatment over the days. Means followed by different letters differ from each other according to the Scott-Knott test at 5% probability.

temperatures, salinity, or water deficiency or excess can induce plants to increase the synthesis of organic solutes, such as carbohydrates, protein, and proline, in response to stress. This is considered a beneficial effect, because an increase in the production of these compounds will facilitate osmotic regulation; and consequently, the acclimatization of plants to these conditions (PIMENTEL, 2004).

In the present study, it can be inferred that there was an increase in the production of organic solutes in the rose bushes studied, particularly for those plants treated with product combinations, such as boscalid + pyraclostrobin, fluxapyroxad + pyraclostrobin, and plant regulators. RAMOS et al. (2015) observed a high accumulation of carbohydrates in tomato plants treated with boscalid alone and with a mixture of boscalid and pyraclostrobin. The plant regulators, according to that study, provided a substantial accumulation of carbohydrates in the plants tested.

Carbohydrates are considered key elements for plants because they can be stored as polysaccharides, thus exhibiting low osmotic activity, or in the form of soluble sugars and hygroscopes. This enables the plants subjected to stress to use carbohydrates for the production of osmotically active compounds via their degradation (TAIZ; ZEIGER, 2017).

With regard to proteins, it is reported that temperature extremes as well as other abiotic stresses can cause disorders in protein structure. To avoid or at least minimize the issues caused by stress, plants have developed mechanisms, such as osmotic adjustment, hydration maintenance, and thermal tolerance improvement, which can result in even higher tolerance when plants are re-exposed to high temperatures, a situation that could be lethal (TAIZ; ZEIGER, 2017).

The observed decrease in protein synthesis may be related to the activity of proteolytic enzymes that act via catabolizing the proteins of plant reserves, resulting in a decrease in their synthesis when plants are subjected to stress. This contributes to an increase in the synthesis of amino acids, such as proline (TAIZ; ZEIGER, 2017). In the present study, a marked proline accumulation was observed, particularly for plants treated with a mixture of boscalid + pyraclostrobin. Synthesis of this solute is important for the plant, considering its function as an osmoregulator in responses modulated by stress. However, it appears to act as a metabolic signal as well, regulating metabolites pools and redox balance as well as controlling gene expressions and

influencing plant growth and development. Proline appears to play a role in protecting the integrity of proteins and increasing the activity of several enzymes (SZABADOS; SAVOURÉ, 2010).

Therefore, it can be observed that the synthesis of organic solutes functions as a measure used by the plant to signal the stress condition as well as primarily a form of protection to ensure that the plant can continue to perform its basic functions of growth, development, and production. Therefore, the substances tested in this experiment that showed the most promotion of the carbohydrate, protein, and proline synthesis can be considered effective in helping rose plants to resist the stresses suffered in the semiarid region where the experiment was conducted.

On evaluating the responses of rose plants to oxidative stress through SOD and CAT activity, it was observed that the product based on the plant regulators kinetin + GA<sub>3</sub> + IBA provided the highest activity for both enzymes. This finding is different from that reported by AMARO et al. (2018), in which SOD and CAT activity was higher in Japanese cucumber plants treated with strobilurins (azoxystrobin), with boscalid, and with a mixture of boscalid + pyraclostrobin. However, the climatic conditions were different, as that study was conducted under milder temperatures (humid subtropical climate) when compared with the present study with rose plants, conducted under high temperatures (semiarid tropical climate); this difference may have influenced the distinctions observed between the results. MACEDO (2015) reported that SOD and CAT activity was higher in plants that received applications of fluxapyroxad + pyraclostrobin, followed by plants treated with azoxystrobin.

Conversely, CARRIJO (2014) tested physiological effect products in soybean plants and observed that the highest enzymatic activity, SOD and CAT included, was obtained in plants treated with pyraclostrobin alone, whereas those treated with applications of fluxapyroxad, both alone and in combination with pyraclostrobin, showed the lowest activity for antioxidant enzymes. Therefore, it appears that each plant species responds differently to physiological effect products, in addition to the influence from the temperature difference between the study sites.

## CONCLUSION

The products tested show different results in relation to the variables evaluated for the rose

plants. Overall, product combinations exhibited the best performance in the accumulation of organic solutes, particularly in the results observed for proline, whereas plant regulators promoted higher enzymatic activity in response to oxidative stress in rose plants of the Ambiance cultivar grown in the Sub-Middle São Francisco River Valley. Therefore, these treatments are recommended to assist in a possible introduction of a cut rose crop into that semiarid region.

## ACKNOWLEDGMENTS

The authors thank the Fundação de Amparo à Ciência e Tecnologia do Estado de Pernambuco (FACEPE) process No. APQ-1062-5.01/14 and the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), Brasil for the scholarship grant of the first author.

## DECLARATION OF CONFLICT OF INTERESTS

The authors declare no conflicts of interest. The founding sponsors had no role in defining the study; in the collection, analysis or interpretation of data; writing the manuscript; or deciding to publish the results.

## REFERENCES

- ALVARES, C.A. et al. Köppen's climate classification map for Brazil. *Meteorologische Zeitschrift*, v.22, p.711-728, 2014. Available from: <[http://www.lerf.eco.br/img/publicacoes/Alvares\\_etal\\_2014.pdf](http://www.lerf.eco.br/img/publicacoes/Alvares_etal_2014.pdf)>. Accessed: Jan. 22, 2018. doi: 10.1127/0941-2948/2013/0507.
- AMARO, A.C.E. et al. Effects of the fungicides azoxystrobin, pyraclostrobin and boscalid on the physiology of Japanese cucumber. *Scientia Horticulturae*, v.228 p.66-75, 2018. Available from: <<https://www.sciencedirect.com/science/article/pii/S0304423817306209>>. Accessed: Jan. 5, 2018. doi: 10.1016/j.scienta.2017.10.016.
- BARBOSA, J.G. et al. Cultivo de rosas. *Informe Agropecuário*, Belo Horizonte, v.26, n.227, p.20-29, 2005. Available from: <[http://www.epamig.br/index.php?option=com\\_content&task=view&id=1268](http://www.epamig.br/index.php?option=com_content&task=view&id=1268)>. Accessed: Jul. 21, 2016.
- BARBOSA, M.R. et al. Enzymatic generation and detoxification of reactive oxygen species in plants. *Ciência Rural*, v.44, n.3, p.453-460, 2014. Available from: <[http://www.scielo.br/scielo.php?pid=S010384782014000300011&script=sci\\_abstract&tlng=pt](http://www.scielo.br/scielo.php?pid=S010384782014000300011&script=sci_abstract&tlng=pt)>. Accessed: Jan. 10, 2019. doi: 10.1590/S0103-84782014000300011.
- BATES, L.S. Rapid determination of free proline for water-stress studies. *Plant and Soil*, v.39, p.205-207, 1973. Available from: <<https://link.springer.com/article/10.1007/BF00018060>>. Accessed: Oct. 8, 2017. doi: 10.1007/BF00018060.
- BEERS JUNIOR, R. F.; SIZER, I. W. A. A spectrophotometric method for measuring the breakdown of hydrogen peroxidase by catalase. *Journal of Biological Chemistry*, v.195, n.2, p.133-140, 1952. Available from: <<https://www.ncbi.nlm.nih.gov/pubmed/14938361>>. Accessed: Nov. 22, 2017.
- BRADFORD, M. M. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry*, v.72, p.248-254, 1976. Available from: <<https://www.sciencedirect.com/science/article/pii/0003269776905273>>. Accessed: Oct. 8, 2017. doi: 10.1016/0003-2697(76)90527-3.
- CARRIJO, D. R. Physiological effects caused by the fungicide Fluxapyroxade, isolated and in combination with Pyraclostrobin, on soybean crop. 2014, 72f. Dissertation (Master's Degree in Phytotechnics) — Graduate Course in Agronomy, School of Agriculture “Luiz de Queiroz”, University of São Paulo, Piracicaba.
- DUBOIS, M. et al. Colorimetric method for determination of sugars and related substances. *Analytical Chemistry*, v.28, p.350-356, 1956. Available from: <<https://pubs.acs.org/doi/abs/10.1021/ac60111a017>>. Accessed: Oct. 12, 2017. doi: 10.1021/ac60111a017.
- FERREIRA, D.F. Sisvar: a Guide for its Bootstrap procedures in multiple comparisons. *Science and Agrotechnology*, v.38, n.2, p.109-112, 2014. Available from: <<http://dx.doi.org/10.1590/S1413-70542014000200001>>. Accessed: Sep. 12, 2017. doi: 10.1590/S1413-70542014000200001.
- GIANNOPOLITIS, O.; RIES, S.K. Superoxide dismutase: I. Occurrence in higher plants. *Plant Physiology*, v.59, n.2, p.309-314, 1977. Available from: <<https://www.ncbi.nlm.nih.gov/pubmed/16659839>>. Accessed: Nov. 22, 2017.
- IBRAFLOR. Instituto Brasileiro de Floricultura. Boletim Ibraflor 2017. Available from: <<http://www.ibraflor.com/publicacoes/vw.php?cod=280>>. Accessed: Oct. 30, 2018.
- MACEDO, A. C. et al. Strobilurin and boscalid in the quality of net melon fruits. *Semina: Ciências Agrárias*, v.38, n.2, p.543-550. 2017. Available from: <<http://dx.doi.org/10.5433/1679-0359.2017v38n2p543>>. Accessed: Jan. 3, 2018. doi: 10.5433/1679-0359.2017v38n2p543.
- MACEDO, A. C. Fungicides of physiological effects on metabolism and development of lacy melon plants under protected cultivation. 2015, 102f. Thesis (PhD in Horticulture) — Graduate course in Agronomy, Faculty of Agronomic Sciences, Universidade Estadual Paulista, Botucatu.
- MOURA, A.R. et al. Water relations and organic solutes in young plants of *Jatropha curcas* L. Under different water regimes. *Ciência Florestal*, v.26, n.2, p.345-354, 2016. Available from: <<http://dx.doi.org/10.5902/1980509822735>>. Accessed: Dec. 20, 2017. doi: 10.5902/1980509822735.
- PIMENTEL, C. *The relationship of the plant to water*. Seropaedic: EDUR, 2004. 191p.
- RAMOS, A.R.P. et al. Quality of tomato fruits' Giuliana treated with products of physiological effects. *Semina: Ciências Agrárias*, v.34, n.6, p.3543-3552, 2013. Available from: <<https://www.researchgate.net/publication/262013520>>. Accessed: Set. 15, 2017. doi: 10.5433/1679-0359.2013v34n6Sup1p3543.
- RAMOS, A.R.P. et al. Accumulation of carbohydrates in the development of tomato plants treated with different chemical products. *Semina: Ciências Agrárias*, v.36, n.2, p.705-718,



2015. Available from: <<http://www.uel.br/revistas/uel/index.php/semagrarias/article/view/16300>>. Accessed: Nov. 28, 2016. doi: 10.5433/1679-0359.2015v36n2p705.

SZABADOS, L.; SAVOURÉ, A. Proline: a multifunctional amino acid. **Trends in Plant Science**, v.15, n.2, p.89-97, 2010. Available from: <<https://www.ncbi.nlm.nih.gov/pubmed/20036181>>. Accessed: Jan. 12, 2018. doi: 10.1016/j.tplants.2009.11.009.

TAIZ, L.; ZEIGER, E. **Physiology and plant development**. 6.ed. Porto Alegre: Artmed, 2017. 888p.

VILLAS BÔAS, R. L. et al. Export of nutrients and quality of rose cultivars in the field and in a protected environment. **Horticultura Brasileira**, v.26, n.4, 2008. Available from: <<http://www.scielo.br/pdf/hb/v26n4/v26n4a18.pdf>>. Accessed: Mar. 21, 2016. doi: 10.1590/S0102-05362008000400018