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Development of Coffee Plant under Different Doses of an Amino Acid-Based Organomineral Product

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Abstract: ***Brazil is the largest coffee producer in the world. In view of the concern for sustainable development in its economic, social and environmental dimensions, the present study was developed to examine the influence of a product based on the amino acid proline on the productive performance of coffee plants when applied foliarly. Six doses of the product applied via foliar spraying were tested. Emergence of new leaves from plagiotropic branches, number of flowers, number of young green cherries, total weight of all fruit types and polyphenoloxidase enzyme dosage were evaluated in a randomized-block experimental design with three replicates. Each plot consisted of three crop rows with 10 plants each. Significant differences were detected for all evaluated traits. The increasing doses of the proline-based product promoted an increase in the number of flowers, young green cherries and leaves emerged on the evaluated plagiotropic branches, demonstrating that the amino acid improves the performance of coffee plants. The ideal dose of the product to provide the highest coffee fruit productivity was 500 mL/ha, applied before flowering. The different doses of the amino acid concentrate were effective in increasing polyphenoloxidase activity and can be used as an instrument for improving the quality of coffee as well as an aid in conferring resistance to coffee diseases caused by microorganisms.***

Keywords: Coffee, Organomineral Fertilizer, Productivity

1. Introduction

The great importance of coffee growing in Brazil warrants constant research aimed at increasing productivity under sustainable conditions to maintain the country's competitiveness in coffee production.

In this scenario, the use of agrochemicals with low environmental impact as plant stimulants can contribute to increasing productivity [5]. Among these are products based on the amino acid proline, which is synthesized from the amino acid glutamate and whose accumulation can be an indicator of resistance to water deficiency in most plant species [15, 12, 1].

Proline, which is found in higher concentrations in the leaves, constitutes a substrate for the synthesis of hydroxyproline and betaine [13]. These molecules can influence osmotic adjustment [22], protect various enzymes against effects of inactivation by high temperatures [19], and act in the dissipation of excess reducing power, since proline biosynthesis consumes large amounts of NADPH [11].

Accumulation of proline during flowering and fruiting has been observed in many plant species [23, 24, 21, 18]. Thus, there is an indication that the application of products that promote an increase the concentration of this amino acid in the leaves can contribute to increasing coffee productivity.
The present study proposes to examine the influence of a product based on the amino acid proline on the productive performance of coffee plants when applied foliarly.

2. Material and Methods

Six doses of an organomineral fertilizer containing amino acids generated in controlled fermentation processes—that

is, a product based on amino acids (proline, glycine, betaine and glutamic acid)—were tested in a rainfed coffee plantation established with 7-year-old cultivar Catuaí Vermelho at Embrapa Meio Ambiente, in Jaguariúna/SP, Brazil.

The product was applied by foliar spraying at the following doses: control, without product application; 500 mL/ha of product in a single application before flowering; 1, 500 mL/ha of product: dose split into two applications of equal proportions (pre- and post-flowering); 3, 000 mL/ha of product: dose split into two applications of equal proportions (pre- and post-flowering); 4, 000 mL/ha of product: dose split into two applications of equal proportions (pre- and post-flowering); and 5, 000 mL/ha of product: dose split into two applications of equal proportions (pre- and post-flowering). Pre-flowering applications were carried out approximately 30 days before the beginning of the rainy season in the region, whereas the post-flowering applications were performed one week after the beginning of the first flowering cycle.

The experiment was laid out in a randomized-block design with three replicates. Each plot consisted of three rows of 10 plants each.

A backpack sprayer was used to apply the fertilizer doses.

The study considered the production resulting from the first flowering, which was very intense in the 2017/18 crop. The material was harvested when the crop showed more than 60% of ripe fruits.

The following variables were evaluated throughout the experimental period: emergence of new leaves on plagiotropic branches, number of flowers, number of young green cherries, total weight of all types of fruits (expressed in kg plant^{-1}) and polyphenoloxidase enzyme dosage.

All leaves on the plagiotropic branch that emerged during the year, identified by the bright green color, were considered new. The number of flowers was counted on the opening day of more than 80% of the flowers.

To determine the number of flowers and the new emerged leaves, three plants were measured in each studied plot before the fruits were harvested. The number of flowers and new leaves was determined by sampling one plagiotropic branch in each of the plant strata (upper third, middle third and basal third).

Considering that the open-row crop (wide in the inter-rows and closed in the row; spacing of 3.5 × 0.6 m) had planting rows oriented in the North/South direction in a lowland area of approximately 7 ha, the number of new leaves in the different strata was counted on the crop exposure faces facing east and west (on the exposed faces of each side of the plant). Flowers in the different strata were counted on the north, south, east and west faces. Both counts generated the average number of new emerged leaves and the number of flowers, from the collections carried out in one plot.

To determine the polyphenoloxidase (PPO) activity, the enzyme extract was prepared after weighing one gram of coffee leaf from each treatment and crushing the collected leaves in a mortar with liquid nitrogen containing 0.8 g of polyvinylpyrrolidone, until a powder was obtained. Next, ten milliliters of 50 mM sodium phosphate buffer solution (pH 6.5) with 1% polyvinylpyrrolidone (w/v) and 1 mM phenylmethylsulfonyl fluoride (PMSF) were added. The solution was then centrifuged at 2000 rpm, at 4 ºC, for 20 min. The supernatants were collected and stored in a freezer (-80 ºC) until analysis.

The PPO activity was determined using 200 µL of 20 mM pyrocatechin in 100 mM sodium phosphate buffer (pH 6.8) and 10 µL of extract. Absorbance was read using Magellan software, at 420 nm, in 20 cycles of 30 s, at 30 ºC. The difference between the last and the first readings was used to calculate the activity, which was expressed in units of PPO. mg^{-1} tissue. min^{-1}, with one unit being defined as a 0.001 increase in absorbance per minute of reaction per milligram of tissue [8]. The ESTAT statistical program, developed by UNESP Jaboticabal/SP, was used to analyze the collected data. When significance was detected by ANOVA, Tukey's test was performed to compare the treatment means. RStudio software was used for the statistical evaluation of polyphenoloxidase, in triplicate. In case of significance by ANOVA, the Scott Knott test was applied.

3. Results and Discussion

Significant differences were detected for all the evaluated traits. Table 1 shows the mean numbers of new emerged leaves, young green cherries and flowers per sampling point according to the tested doses of the proline-based organomineral fertilizer.

Table 1: Mean numbers of new leaves, young green cherries and flowers

Dose of proline-based organomineral fertilizer (mL ha^{-1})	Number of new leaves	Number of young green cherries	Number of flowers
0	7.24 ab	143.92 b	65.37 c
500	8.24 ab	135.95 b	85.65 bc
1500	7.00 ab	191.40 ab	98.55 abc
3000	9.05 a	232.55 ab	123.26 ab
4000	6.61 b	246.01 ab	135.01 a
5000	8.96 a	324.94 a	121.72 ab
LSD	2.12	151.59	42.92
CV (%)	9.53	25.17	14.43

Means followed by common letters do not differ by Tukey's test at 1% probability.

The increasing doses of the proline-based organomineral fertilizer promoted an increase in number of flowers, number small green cherries and number of leaves on the evaluated plagiotropic branches, demonstrating that the product

improves the performance of the coffee plant following application of the amino acid.

Table 2 shows the mean fruit weight per evaluated plant according to the tested dose of the proline-based organomineral fertilizer.

Table 2: Mean weight of coffee fruits

Dose of proline-based organomineral fertilizer (mL ha^{-1})	Fruit weight (kg $plant^{-1}$)
0	0.77 c
500	2.23 ab
1500	2.37 a
3000	1.3 bc
4000	1.37 abc
5000	1.67 abc
LSD	0.37
CV (%)	23.23

Means followed by common letters do not differ by Tukey's test at 1% probability.

As shown in Table 2, the ideal dose of the proline-based organomineral fertilizer to promote increased production of coffee fruits is 500 mL ha^{-1}, applied before flowering.

Although plants naturally produce amino acids such as betaine and proline, among others, in situations of stress, the produced quantities may not be sufficient to meet their requirements. Thus, the additional use of a proline-based organomineral fertilizer can bring advantages to production, contributing to a greater retention of flowers and fruits and, as a result, increasing the quality and vigor of the plants.

Another factor that can contribute to increasing productivity is the fact that plants produce polyphenolic compounds such as lignins, which, according to Rossetti [20], strengthen the cell wall; flavonoid pigments, which act as protection from ultraviolet light; as well as phenolic compounds that act against pathogens. Studies such as those carried out by Leite et al. [14] and Melo [16] reveal that polyphenoloxidases can act on phenolic compounds when the tissue is damaged with the rupture of plastids, intracellular sites where these enzymes are located. Contact with phenolic compounds can form quinones and limit the effect of diseases by promoting cell death in cells near the infection, preventing their progress, producing compounds toxic to the pathogen; and/or limit the disease by protein alkylation, forming compounds that form a barrier to pathogens.

According to Carvalho Júnior et al. [4], another aspect to be observed, in the particular case of coffee production, is that polyphenols are compounds responsible for the astringent effect on the coffee beverage, with indications of a decrease in its quality due to their presence.

Polyphenoloxidase enzymes can lead to a reduction of polyphenols, improving the quality of the beverage [9]. Studies involving sensory analysis of coffee and PPO doses revealed a relationship between the two [3, 14].

Figure 1 illustrates the PPO activity in the leaf as a function of different doses of the proline-based organomineral fertilizer.

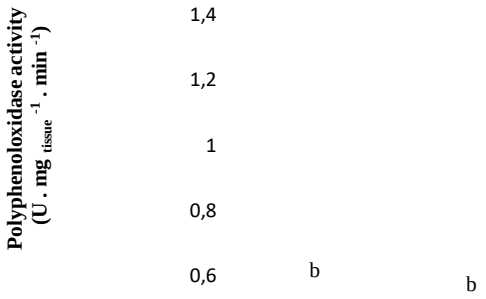

Figure 1: Polyphenoloxidase activity in coffee leaves as a function of applied doses of a proline-based organomineral fertilizer, by the Scott Knott Test at 5% probability.

As depicted in Figure 1, there was no significant difference in PPO activity between the treatments involving 0 and 500 mL ha^{-1} of the proline-based organomineral fertilizer. The results obtained with 1, 500, 3, 000, 4, 000 and 5, 000 mL ha^{-1} of the product were statistically similar and higher than the others. The tested amino acid concentrate is formed by an emulsion containing glutamic acid, proline, glycine and betaine. Proline is an example of an amino acid produced by the plant that provides response sensitivity to stress conditions (Monteiro et al., 2014) [17]. Some essential amino acids produced by the plant at different stages of growth could be supplemented to obtain the necessary conditions such as tolerance to stress and resistance to pathogens (Castro et al., 2014) [6]. In their study, Haque et al. (1971) [10] incorporated the amino acids aspartic and glutamic acid, threonine and thymine in rice and observed different rates of absorption of these amino acids at different stages of plant growth, with the greatest absorption of proline occurring in the reproductive stage.

Castro et al. (2014) [6] evaluated the use of market products with different doses of amino acids and observed positive or negative results in some plantations, which were likely due to the variety of the tested products.

Costa et al. [7] reported a 30% increase in coffee productivity following the use of fertilizers containing calcium, boron and amino acids, which confirmed the importance of these components for the development and fruiting of coffee.

Thus, the different doses of the amino acid concentrate were effective in increasing polyphenoloxidase activity at 1, 500 mL ha^{-1}, with no need for the application of a larger volume for this purpose. Its use is thus justified as an instrument for improving the quality of coffee as well as an aid in conferring resistance to coffee diseases caused by microorganisms.

4. Conclusion

Under the conditions in which this study was undertaken, the amino acid based-product influenced the productivity of the coffee plant, and the foliarly applied dose of this product that

provided the greatest productive gain was 500 mL ha^{-1}, before flowering.

The different doses of the amino acid concentrate were effective in increasing polyphenoloxidase activity. Therefore, it can be used as an instrument for improving the quality of coffee as well as an aid in conferring resistance to coffee diseases caused by microorganisms.

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