ISSN 0100-2945
DOI: http://dx.doi.org /10.1590/0100-29452022003

Revista Brasileira de
Fruticultura

*Botanic and Physiology*

# Organic acids allied with paclobutrazol modify mango tree ‘Keitt’ flowering

Luan dos Santos Silva[1], Ítalo Herbert Lucena Cavalcante[2], Jenilton Gomes da Cunha[3], Jackson Teixeira Lobo[4], Daniel Almeida Carreiro[5], Vespasiano Borges de Paiva Neto[6]

**Abstract** – The objective of this study was to evaluate the effectiveness of fulvic acids and free amino acids on paclobutrazol in the flowering of mango ‘Keitt’ cultivated in the semi-arid region. The experiment was performed from 2017 to 2018 simultaneously in two orchards with the same plants and management characteristics, located in Cabrobó, State of Pernambuco, Brazil. The experimental design was randomized blocks with four treatments and five replications of four trees. The treatments were: T1: paclobutrazol + water (control); T2: paclobutrazol + fulvic acids; T3: paclobutrazol + amino acids; T4: paclobutrazol + fulvic acids + amino acids. Applying fulvic acids plus paclobutrazol increased gas exchange efficiency, favoring flowering and fruiting. In contrast, amino acids alone or with fulvic acids decreased the flowering efficiency.

**Index terms**: *Mangifera indica* L.; PBZ; floral induction; humic substances; agricultural amino acids; vegetable gas exchange.

# Ácidos orgânicos aliados ao pacobutrazol modificam o florescimento de mangueira ‘Keitt’

**Resumo** - Objetivou-se avaliar a efetividade de ácidos fúlvicos e aminoácidos livres sobre o paclobutrazol no florescimento da mangueira ‘Keitt’ cultivada no semiárido. O experimento foi conduzido em pomar de mangueira cv. Keitt, em Cabrobó-PE, entre novembro de 2017 e setembro de 2018. O clima do local é classificado como Bswh (Köeppen), uma região semiárida. O delineamento foi em blocos, ao acaso com tratamentos distribuídos em faixas, com cinco repetições de quatro plantas cada. Os tratamentos foram: T1: paclobutrazol + água (controle); T2: paclobutrazol + ácidos fúlvicos; T3: paclobutrazol + aminoácidos livres; T4: paclobutrazol + ácidos fúlvicos + aminoácidos livres. A aplicação de ácidos fúlvicos mais paclobutrazol aumentou a eficiência das trocas gasosas, favorecendo a floração e a frutificação, enquanto aminoácido isolado ou em conjunto com os ácidos fúlvicos diminuiu a eficiência no florescimento.

**Termos para Indexação**: *Mangifera indica* L., PBZ, indução floral, substâncias húmicas, aminoácidos agrícolas, trocas gasosas vegetais.

**Corresponding author:**
luan_agronomia@hotmail.com

**Received:** January 14, 2022
**Accepted:** June 28, 2022

---

[1] Master in Phytotechnics, Federal University of Piauí, Bom Jesus - PI, Brazil. E-mail: luan_agronomia@hotmail.com (ORCID 0000-0003-4419-6669)

[2] PhD in Agronomy, Professor Researcher, Federal University of Vale do São Francisco, Petrolina – PE, Brazil. E-mail: italo.cavalcante@univasf.edu.br (ORCID 0000-0003-1610-1546)

[3] Master in Plant Production, Federal University of Vale do São Francisco, Petrolina – PE, Brazil. E-mail: jeniltongomes@hotmail.com (ORCID 0000-0003-4110-6605)

[4] Master in Plant Production, Federal University of Vale do São Francisco, Petrolina – PE, Brazil. E-mail: jackson_lob@hotmail.com (ORCID 0000-0002-6978-083X)

[5] Master in Plant Production, Federal University of Vale do São Francisco, Petrolina – PE, Brazil. E-mail: daniel.almeida.sb@gmail.com (ORCID 0000-0003-1048-7038)

[6] PhD in Agricultural Sciences, Professor Researcher, Federal University of Vale do São Francisco, Petrolina – PE, Brazil. E-mail: vespasiano.paiva@univasf.edu.br (ORCID 0000-0003-3347-7043)

## Introduction

The mango tree (*Mangifera indica* L.) cultivated in a semi-arid tropical region and not to proper management practices displays a high gibberellins synthesis (SANDIP et al., 2015). Gibberellins are hormones antagonistic to the reproductive phase, as they stimulate vegetative growth, consuming chemical energy, limiting flowering and production. Satisfactory flowering is closely related to changes in hormonal balance and carbohydrate transport for accumulation in shoots (GIBSON, 2004; UPRETI et al., 2014; PRASAD et al., 2014).

In the semi-arid tropical region, the management of the mango tree flowering comprises several aspects such as; irrigation, pruning, fertilization (OLDONI et al., 2018), use of phytoregulators (CAVALCANTE et al., 2020), branch maturation (CAVALCANTE et al., 2018) and application of nitrate salts (DAVENPORT, 2009). These practices allow scheduling the harvest for more commercially attractive periods (OLIVEIRA et al., 2015).

Paclobutrazol (PBZ) is a widely used phytoregulator to stimulate mango flowering in semi-arid conditions, as it interferes with the biosynthesis of gibberellin, inhibiting the oxidation of *ent*-caurene to *ent*-caurenoic acid (SOUMYA, et al., 2017). Furthermore, there is evidence of its influence on gas exchange, photosynthetic pigments (SUBBAIAH et al., 2018), and activation of antioxidant enzymes (SAXENA et al., 2014), stimulating the production of reserve carbohydrates for for the flowering phase (SILVA et al., 2021).

The use of organic substances, such as fulvic acids (FA) and free amino acids, is proposed to prepare mixes, mainly due to the expected effect of complexing ions and molecules, ensuring greater absorption efficiency due to increased transport in the soil to the roots and membranes permeability (BALDOTTO; BALDOTTO, 2014; SILVA, et al., 2020). In addition, these molecules have stress-reducing characteristics; amino acids promote anti-stress effects and participate in the synthesis of chlorophylls (TAIZ et al., 2017). Furthermore, FA increase the ascorbate and glutathione metabolism, promote the synthesis of flavonoids improve plants' antioxidant defense, and increase the photosynthetic capacity even when subjected to abiotic stresses (SUN et al., 2020; WANG et al., 2019).

This study aimed to evaluate the effectiveness of FA and free amino acids on the potentiation of the effect of PBZ on gas exchange rates, proline content, catalase enzyme activity (CEA), and photosynthetic pigments as mediators of flowering of ''Keitt' mango cultivated in tropical semi-arid.

## Materials and Methods

### *Characterization of the experimental area*

The study used two-and-a-half-year-old mango (*Mangifera indica* L.) trees, cultivar Keitt in the first production cycle, with uniform size and vigor. The experiment was accomplished from 2017 to 2018 in an experimental orchard located in Cabrobó (08°31'S and 39º26'W; at an altitude of 331 m above sea level), State of Pernambuco.

The climate of this region is classified as Bsh (ALVARES et al., 2013), which corresponds to a semi-arid region. During the experiment, rainfall, air temperature, and relative humidity were recorded in an automatic meteorological station installed on the orchard, and the results are shown in Figure 1.

The plants, spaced with 4.0 m between the rows and 2.0 m between the plants, were daily irrigated (drip) with four emitters per plant for nearly 2 L $h^{-1}$ each emitter. All management practices such as pruning, control of weeds, pests, and diseases, plant growth regulators for gibberellin inhibition (paclobutrazol, Cultar®), and dormancy break (calcium nitrate and potassium nitrate) were performed following the instructions of Albuquerque et al. (2002). According to plant demand, the nutrient management was performed through a fertirrigation system (SILVA et al., 2002). An additional pruning was performed 27 days after PBZ treatment to keep each plant with nearly 100 shoots, and tip pruning synchronized the vegetative flush events in the canopy.

The experiment was carried out simultaneously in two orchards with the same plant and management characteristics in an experimental unit of 7.056 m². The physical and chemical characteristics of soil in both orchards are described in Table 1. The same experiment and analyses have been used in other papers (SILVA et al., 2020, SILVA et al., 2021), but with a different approach do the results' analysis.

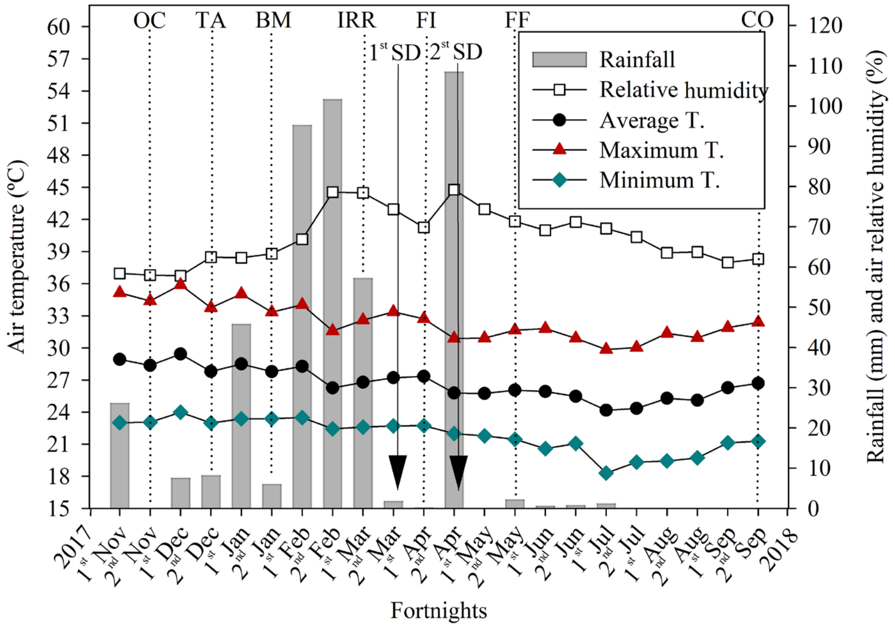

**Figure 1.** Rainfall, air temperature, and air relative humidity recorded during the experiment OC: orchard characterization; AT: Application of treatments; BM: Branches maturation; IRR: irrigation rate reduction; FI: floral induction; FF: full flowering; T.: Temperature; Sampling Date.

**Table 1**. Chemical and physical characteristics of the soil in the mango cv. Keitt orchards studied.

| Orchard | Soil layer | pH (in water) | P | $K^+$ | $Na^+$ | $Ca^{2+}$ | $Mg^{2+}$ | $Al^{3+}$ | H + Al | SB | V |
|---|---|---|---|---|---|---|---|---|---|---|---|
| - | M | - | $mg.dm^{-3}$ | $cmol_c.dm^{-3}$ | | | | | | | % |
| 1 | 0.0 - 0.2 | 4.94 | 66.62 | 0.41 | 1.22 | 2.52 | 1.43 | 0.35 | 4.29 | 5.58 | 56.55 |
| | 0.2 - 0.4 | 5.33 | 31.22 | 0.31 | 1.13 | 2.91 | 1.91 | 0.25 | 3.05 | 6.26 | 67.21 |
| 2 | 0.0 - 0.2 | 5.37 | 70.62 | 0.36 | 0.78 | 3.20 | 1.65 | 0.08 | 3.05 | 5.99 | 66.25 |
| | 0.2 - 0.4 | 6.22 | 44.28 | 0.64 | 1.70 | 3.06 | 1.92 | 0.08 | 1.98 | 7.31 | 78.70 |

| Orchard | Soil layer | CEC (pH7) | OM | FA | HA | HU | E.C. | Sand | Clay | Silt | Texture |
|---|---|---|---|---|---|---|---|---|---|---|---|
| - | m | $cmol_c.dm^{-3}$ | $g.kg^{-1}$ | | | | $dS.m^{-1}$ | $dag.kg^{-1}$ | | | |
| 1 | 0.0 - 0.2 | 9.87 | 11.13 | 0.03 | 1.44 | 4.45 | 0.06 | 51.13 | 15.90 | 32.97 | Loam |
| | 0.2 - 0.4 | 9.31 | 10.68 | 1.01 | 1.30 | 4.02 | 0.07 | 48.32 | 18.10 | 33.58 | Loam |
| 2 | 0.0 - 0.2 | 9.04 | 13.13 | 0.07 | 1.61 | 4.95 | 0.15 | 48.94 | 14.10 | 36.96 | Loam |
| | 0.2 - 0.4 | 9.29 | 9.68 | 0.84 | 1.62 | 5.53 | 0.17 | 54.81 | 16.40 | 28.79 | Sand Loam |

SB = sum of bases; OM = Organic matter; CEC = cationic exchangeable capacity [$Ca^{2+} + Mg^{2+} + Na^+ + K^+ + (H^+ + Al^{3+})$]; EC = electrical conductivity; P, K: Melich-1; H + Al: calcium acetate (extractor) 0.5M, pH 7; Al, Ca, Mg: KCl 1 M extractor; CEC: cationic exchangeable capacity.

***Experimental design and treatments application***

The experiment followed a randomized block design with four treatments, five replications per treatment, and four trees per replication. The treatments consisted of PBZ combinations with acids fulvic and free amino acids: T1: PBZ + water (control); T2: PBZ + FA; T3: PBZ + free amino acids; and T4: PBZ + FA + free amino acids.

The PBZ source used was Cultar SC® (25% i.a. PBZ), produced by Syngenta. Treatments were applied through a fertigation system once at 30 days after the production pruning when trees presented a 2.0m canopy diameter. Each tree received 2.0 g of PBZ, reaching 2.5 kg ha$^{-1}$. The sources of the acids fulvic and free amino acids used were, respectively: 16 ml per tree of Aminoagro Mol® (10% of N, 1% of $K_2O$, 8% of total organic carbon, and 33% of FA) and e 16 ml per tree of Aminoplus® (11% of N, 1% of $K_2O$, 6% of total organic carbon, and 12% of amino acids). Thus each tree received 5.28 g of FA (6.6 kg ha$^{-1}$) and 1.92 g of amino acids (2.4 kg ha$^{-1}$). The treatments were applied following the recommendations of Albuquerque et al. (2002) for mango trees and the product manufacturers.

### Analyzed variables

*Gas exchange*

A portable infrared gas analyzer IRGA (Li-COR® 6400 XT) was used to evaluate gas exchange in mature and fully expanded leaves located in the middle third of the crown. An initial characterization was carried out before applying the treatments. The valuations continued at 90, 120, and 150 days after the application of treatments, always between 10:00 and 12:00h. The experiment analyzed the following variables: liquid photosynthesis (*A*); internal concentration of $CO_2$ (*Ci*); stomatal conductance (*gs*); transpiration (*E*); carboxylation efficiency (*A/Ci*), calculated as the ratio between *A* and *Ci*; and intrinsic water use efficiency (iWUE) calculated as the ratio between *A* and *E*.

*Biochemical analysis*

The plant material collection was performed in two phases following the criteria of Malavolta et al. (1997) for nutritional analysis, during the irrigation rate reduction (IRR) (03/23/2018) and flowering (04/23/2018), between 10:00 and 11:00h. First, the leaf blade was wrapped in aluminum foil, identified and immediately deposited in liquid nitrogen, transported to the Vegetal Physiology laboratory of the Federal University of Vale do São Francisco, and kept in a freezer at an average temperature of 4 ºC until the crude extract preparation.

The crude extract was prepared as follows: 2 g of fresh leaf limb matter were weighed per sample, macerated in a pistil porcelain crucible using liquid nitrogen to a fine powder, to which 10 mL of potassium phosphate buffer solution (0.1 M and pH 7.0), containing 0.0001 M EDTA. The material obtained was filtered on a muslin tissue, placed in 2 ml Eppendorf, and centrifuged at 12,000 rpm for 15 min in a four ºC refrigerated centrifuge (Sigma 3-18K®). The precipitate was discarded, and the supernatant was used to analyze free proline (BATES, 1973) and catalase enzyme activity (BEERS; SIZER, 1952).

*Flowering*

The number of panicles per plant was counted during the practice of floral induction (FI) and at full flowering (FF), 121 and 146 days after the application of PBZ (DAP), respectively. In turn, all fruits produced by plants of each treatment were quantified, then the average individual mass of each fruit at 256 DAP was measured.

### Statistical analysis

For repeated physiological analyzes in different periods, the data were fractionated in a 4x2 complementary factorial arrangement, consisting of PBZ combinations with fulvic and free amino acids versus two evaluation periods, performed between the following phase changes: Reduction of irrigation rate (IRR ) / Floral induction (FI); and Floral Induction / Full Bloom (FF). Data were submitted to analysis of variance (ANOVA). All statistical analyses were performed using the SISVAR and SIGMAPLOT, and averages were compared by the Tukey test at $p < 0.05$.

## Results and Discussion

The treatments had a distinct influence on proline production. The highest peak coincided with periods without rainfall and restricted water availability between the second half of March and the first half of April (Figure 1). During this period, FA treatments were more efficient in proline production than the control (T1) and amino acid (T3) treatments (Figure 2 B).

Therefore, in soil 1, only T1 showed significant superiority in the evaluated periods, decreasing the FF proline content by 62.71%. However, in soil 2, the opposite occurred. All other treatments decreased to the second date (FI - FF)(Figure 2A). This decrease is because, as the stress was more severe in the IRR, due to a 50% water availability reduction, the plants needed to increase the production of proline to withstand the stress.

As previously mentioned, plants from T2, T3, and T4 located in soil 2, and evaluated during the period of induced water stress, probably presented a more significant osmotic adjustment due to the accumulation of proline in the cytosol, reducing the osmotic potential, thus allowing the retention of water in cells even under water deficit (AL-YASI et al., 2020). Subsequently, the significant reduction in proline content may be connected to the fact that in the flowering phase, the irrigation rate was already normalized, and the plants began to degrade proline by the action of the enzymes proline dehydrogenase (PRODH) and pyrroline-5-carboxylate dehydrogenase (P5CDH) through mitochondrial route because the water status tends to normalize (TAIZ et al., 2017; REN et al., 2018).

It was observed that the proline production peak was with T2 (with FA). Hypothetically, FA can complex PBZ, due to the negative charges of humic substances that react with organic compounds containing N, in this case with triazole chemical group (PBZ) (SPOSITO, 2008). Complexation provides greater mobility to PBZ in the soil and benefits physiological plants' activity. As a result, it is believed that the proline present in greater amounts in T2 may provide a more significant energy release after the natural degradation process of this amino acid due to the resumption of the water layer, and this culminated in a more significant number of panicles per plant (Figure 3).

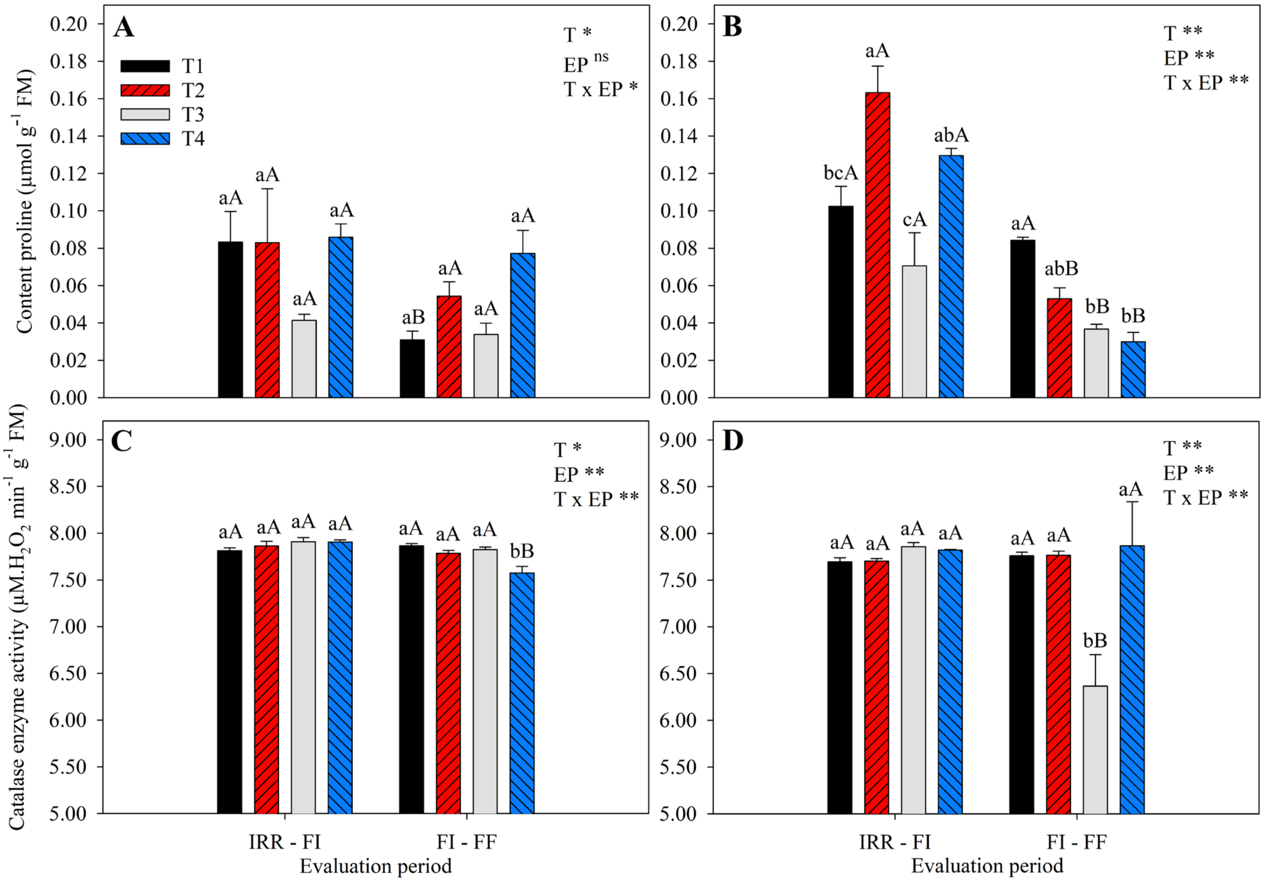

**Figure 2**. Proline content [orchard 1 (A); orchard 2 (B)], catalase enzyme activity [orchard 1 (C); orchard 2 (D)] of mango ‘Keitt’ as a function of FA and free amino acids. T1: PBZ + water (control); T2: PBZ + FA; T3: PBZ + free amino acids; and T4: PBZ + FA + free amino acids. IRR: irrigation rate reduction; FI: flowering induction; FF: full flowering; T: treatments, EP: evaluation period; T x EP: interaction of factors; *: significant at 5%, **: significant at1%; $^{ns}$: not significant; Similar capital letters ( evaluation period) and common lower case letters (treatments) do not differ at the 5% level by Tukey’s test.

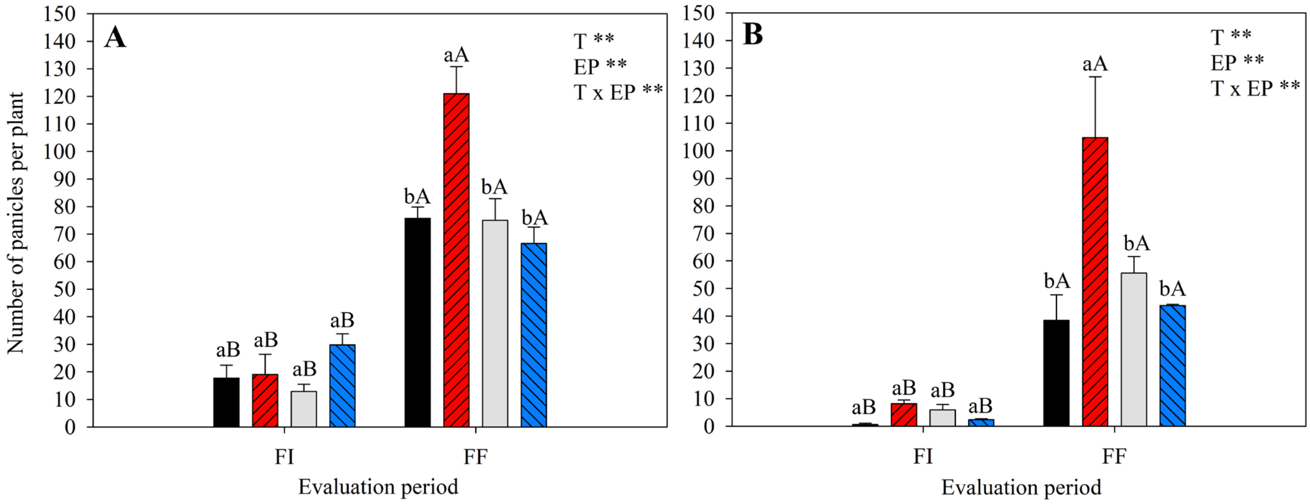

**Figure 3**. Numbers of panicles per plant [orchard 1 (A); orchard 2 (B)] of mango ‘Keitt’ as a function of FA and free amino acids. Experimental and statistical details are similar to those in Figure 2.

As the proline has exceptional conformational rigidity, its most significant accumulation occurs in plants under stress conditions. The proline synthesis mechanism occurs through the glutamate and ornithine pathways. However, the glutamate pathway is the most common, in which it is reduced to glutamate-seminaldehyde (GSA) by the enzyme pyrroline-5-carboxylate synthetase (P5CS) and spontaneously converted to pyrroline-5-carboxylate (P5C), where later, P5C reductase (P5CR) further reduces the P5C intermediate to proline (SZABADOS; SAVOURÉ, 2010).

In contrast, especially in recovery periods, the degradation of proline, which occurs in the mitochondria, provides energy to the respiratory process. Such an effect results from the sequential action of proline dehydrogenase (PDH) or proline oxidase (POX), producing P5C from proline, and P5C dehydrogenase (P5CDH), which converts P5C into glutamate. Therefore, PDH and P5CDH use FAD and NAD+ as electron acceptors and generate FADH2 and NADH, respectively, delivering electrons for mitochondrial respiration (SAIBI et al., 2015).

Literature articles claim PBZ has a pleiotropic effect on plants, acting on an antioxidative enzyme activation, which is proportional to the PBZ dose. Therefore, they suggest that when plants are under adverse conditions, this phytoregulator could act in their defense against the formation of free radicals, preventing the leakage of ions/damage to the membrane (SAXENA et al., 2014; SRIVASTAV et al., 2010). However, the treatments showed little statistical difference in the enzyme catalase activity responsible for withstanding the plant stress in this experiment. Furthermore, during IRR there was no significant difference between treatments (Figure 3 C-D).

In the following period, the T4 catalase activity in soil 1, and T3 in soil 2 was lower than in the other treatments, both with the presence of amino acids, possibly indicating that PBZ molecule was already at low levels due to the lower absorption efficiency by the plant throughout time, provided by these treatments. The response of PBZ to catalase activity was probably more prominent soon after its application as demonstrated by Srivastav et al. (2010) and Saxena et al. (2014). Thus, it is inferred that proline acted as the first line of defense against the effects of water stress, especially in T2 and T4 during IRR. The data suggest that proline acted as a first-defense line against the water stress effects, mainly on T2 and T4 during IRR, observing a higher variability in proline levels and CAT activity preservation.

Srivastav et al. (2010) evaluated PBZ doses and salinity levels. The authors also observed that the mango tree increased catalase activity proportionally to PBZ dose under conditions with and without salinity. Furthermore, catalase minimized membrane damage proportionally to the applied dose due to the control of free radicals ($H_2O_2$) and lipid peroxidation arising from the stressful conditions necessary for flowering. Saxena et al. (2014), evaluating the effect of PBZ on mango leaves during flowering, observed a positive correlation between PBZ doses and the content of antioxidative enzymes containing iron (CEA and POX).

On the other hand, according to the results shown in Figure 3 C-D, the hypothesis arises that the increase in catalase activity may be a response to the stress of the phytoregulator itself. While it interferes with the biosynthetic pathway of gibberellic acid, catalase probably is activated to minimize negative impacts on plant physiology. So when the level of PBZ in the plant reduces after a long time of application, the level of catalase activity also decreases, this being related only to the effects of PBZ, as other stressors, such as high temperatures, affect the plant during the production cycle.

Figure 4 presents the effect of treatments on gas exchange. In IRR, T1 and T4 plants responded more severely to stress, reducing the net photosynthetic activity to less than 5 μmol $CO_2$ $m^{-2}$ $s^{-1}$ in both soils, and those from T3 were 147.13% higher than the control in soil 1 and 130.86% in soil 2. . Besides that, T2 and T3 were superior to control and T4 treatments in photosynthetic rates during IRR.

It should be highlighted that mango trees had their water supply reduced by 50%. Water supply reduction is a recommended cultural practice for mango in semi-arid regions, allowing a faster and more uniform branch maturation (BM). However, the reduction cannot be total, as plants need to photosynthesize to accumulate carbohydrates without vegetating (ALBUQUERQUE et al., 2002).

The PBZ efficiency can explain the different treatments' responses to prepare the plants for stress, as the plant should already stop its vegetative development, avoiding energy expenditure and thus, increasing carbohydrate accumulation necessary for flowering. (SILVA et al., 2020; SILVA et al., 2021) observed that mango trees treated just with PBZ and PBZ + AF and amino acids had a late gibberellin reduction due to the slow PBZ absorption. For this reason, the carbohydrates accumulation during the branches' maturation was jeopardized due to the energy consumption associated with the vegetative growth.

With the proximity of whole FF, treatments T1 and T4 increased the photosynthetic rate in both soils, T3 only in soil 1, and T2 remained stable. For effective FI, plants have to increase the production of carbohydrates for BM through photosynthesis due to the demand for panicle emission. However, the sharp increase of 193 and 143% (soil 1) and 224 and 140% (soil 2) in T1 and T4, respectively, between FI and FF, demonstrates that plants had low carbohydrate reserves, so they needed to increase photosynthetic activity too much, but too late (Figure 4 A-B). The AF and ammino acids treatments promoted a better branch maturation due to the satisfactory carbohydrate production and accumulation, as observed in previous studies published by our research group (Silva et al., 2021).

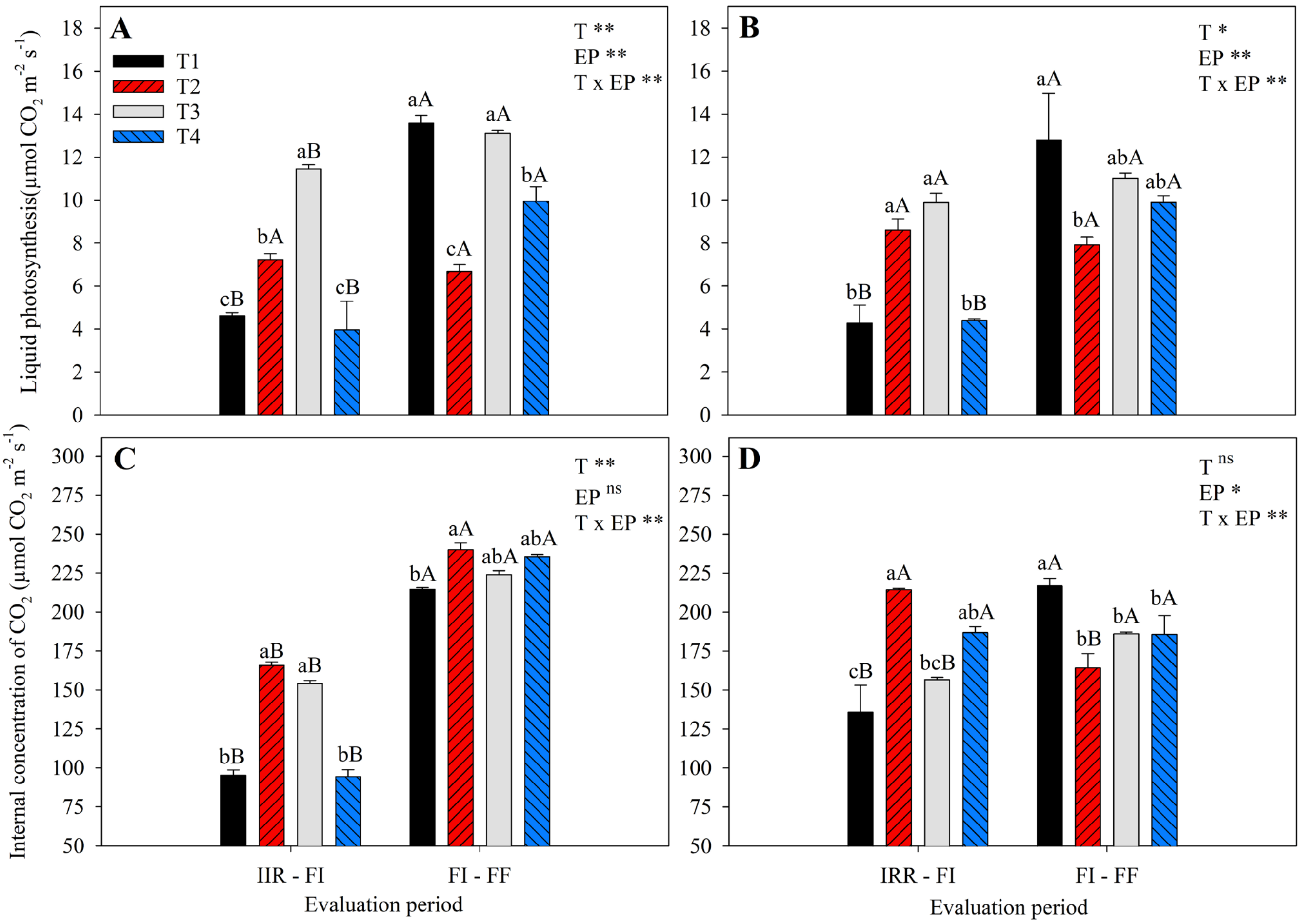

**Figure 4.** Liquid photosynthesis [orchard 1 (A); orchard 2 (B)] and internal concentration of $CO_2$ [orchard 1 (C); orchard 2 (D)] of mango 'Keitt' as a function of FA and free amino acids.
Experimental and statistical details are similar to those in Figure 2.

During flowering, T1 was superior to the treatment containing FA (T2). This result may be linked to slower PBZ absorption, which delayed BM and made the plant have to produce chemical energy in a very late phenological stage, which is not beneficial for flowering. On the other hand, according to Prasad et al. (2014), the high photosynthesis in this period may represent the higher mango tree's carbohydrates requirements for bud differentiation and panicle formation.

Silva et al. (2021) demonstrated that when FA complexes PBZ, PBZ absorption presents a higher efficiency. In addition, the plant reduces the biosynthesis of gibberellin, increases the accumulation of carbohydrates in the maturation phase of branches, and supplies their demand for conversion of shoots into panicles, increasing productivity.

Orabi et al. (2010) found that PBZ can indirectly reduce photorespiration through lower RuBisCO oxygenase activity, enhancing net photosynthesis. One of the steps in photorespiration is the breakdown of hydrogen peroxide by catalase. However, at 90 days, the catalase enzyme activity level was the same for all treatments (Figure 2 C-D). Therefore, the difference in net photosynthesis between treatments can only be attributed to gross photosynthesis or mitochondrial respiration since that net photosynthesis results from the relationship between raw photosynthesis, respiration, and photorespiration (PEIXOTO et al., 2011).

Subbaiah et al. (2018), researching the effects of PBZ on photosynthetic pigments in the Banganpalli mango tree, observed an increase in chlorophyll content conditioned by PBZ. These pigments are constituents of the antenna complex, responsible for absorbing light and exciting electrons in the photochemical phase of photosynthesis (TAIZ et al., 2017), so the content increase can add to the photosynthetic performance of the plant, especially in the maturation phase of mango branches, when high performance is needed for the production of carbohydrates that must be accumulated in this phase to provide a good flowering (PRASAD et al., 2014).

One of the main primary substrates of photosynthesis is carbon from $CO_2$, so the more $CO_2$ in the mesophyll, the greater the activity of RuBisCO in converting carbon into organic acids, mainly from C3 respiratory metabolism (such as mango), as they do not store $CO_2$ in the cells of the bundle sheath, therefore, is dependent on the influx of this gas by the stomata (KERBAUY, 2004; SANTOS et al., 2015).

Thus, the plants submitted to T2 excelled in *Ci* content, as they presented 73.93 and 57.65% higher content than the control in soil 1 and 2, respectively, during IRR (Figure 4 C-D), which is even higher than other treatments. Thus, it is evident that using isolated FA was more beneficial maintaining carbon in the mesophyll of mango leaves under water restriction, possibly the level of photorespiration; hence the carbon consumption was lower in this treatment.

In addition, an adequate *Ci* in C3 plants prevents photorespiration from occurring through the consumption of $O_2$ and the loss of previously fixed $CO_2$ (TAIZ et al., 2017), preventing a reasonable expenditure of energy and nutrients at a crucial moment of the accumulation of reserves, preceding flowering.

At 120 DAP, close to FF, there was the only difference between T1 and T2 in soil 1, with T2 being 11.78% higher (Figure 4 C). However, in soil 2, the opposite occurred. T1 was significantly higher than other treatments (Figure 4 D). The higher T1 result is related to the high photosynthesis activity in the same period, given the need for stomatal opening and $CO_2$ absorption to replace the carbohydrate consumed during the branch BM characterized mainly by induced water stress.

As for carboxylation efficiency during IRR, T3, with the presence of amino acid, was superior to the other treatments (Figure 5 A-B), which is congruent with the high photosynthetic rates (Figure 4 A-B).

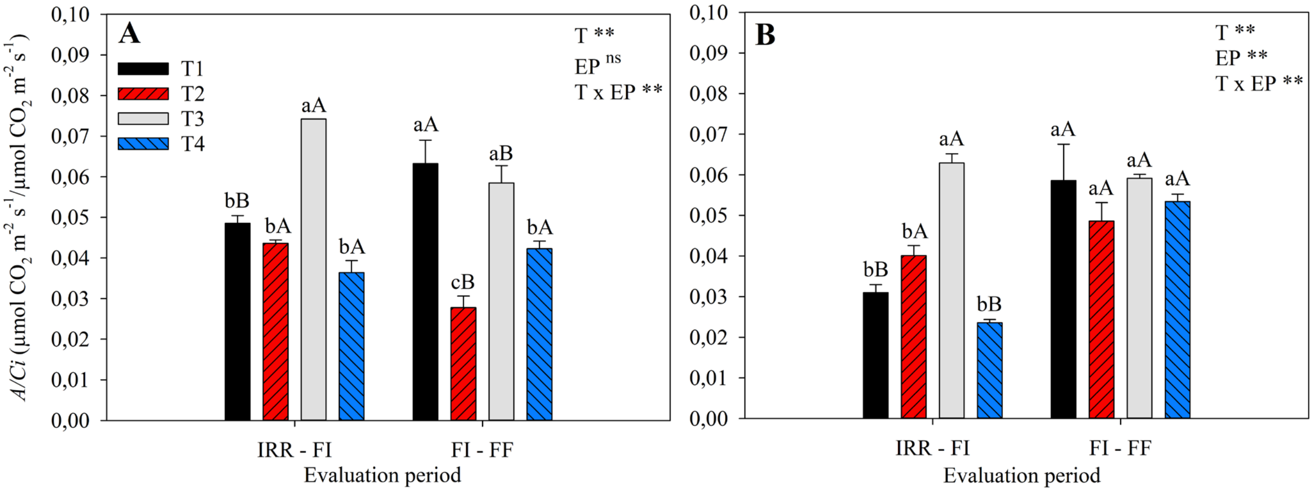

**Figure 5**. Efficiency of carboxylation [orchard 1 (A); orchard 2 (B)] of mango 'Keitt' as a function of FA and free amino acids. Experimental and statistical details are similar to those in Figure 2.

However, the amino acid provided may have acted directly to improve the photosynthetic system and combat stress. These are already known functions (HEINEMANN et al., 2020) and are not conditioned to the increased PBZ absorption, as previously described.

During FI, plants submitted to the control treatment that had added *Ci* and net photosynthesis according to IRR maintained the same behavior in carboxylation efficiency, indicating suppression of the deleterious effects of water stress plant can activate the photosynthetic system more efficiently to reverse the damage.

It is generally known that gs accompany the increase in *Ci* contents; therefore, the stomatal restriction is the main limiting factor in the photosynthetic performance of plants, as there is a proportional relationship between stomatal opening and $CO_2$ diffusion to the substomatic chamber (Santos et al., 2013). Thus, the stomata remained more open, allowing diffusion to the leaf, as shown in Figure 6 A-B, in which there was a significant increase in the *gs* of the IRR until the flowering period for T1 and T4 plants.

One factor that explains the difference between these phases is the water regime combining high temperatures and low relative humidity: water availability differed even if the temperature variation was minimal. In the IRR phase, there was a 50% irrigation reduction. During flowering, fell about 110mm of rain (Figure 1). Besides the irrigation, rainfall can rehydrate the plant crown, which favors the maintenance of open stomata, resulting in increased *gs*.

Furthermore, the directly proportional behavior of the gas exchange variables mentioned above was already expected, given the strong relationship (MUDO et al., 2020). This premise is confirmed as *E* correlates with *gs*: the higher the index or time of stomatal opening, the greater the plants' transpiration, so the tendency was also repeated, as highlighted in Figure 6 C-D.

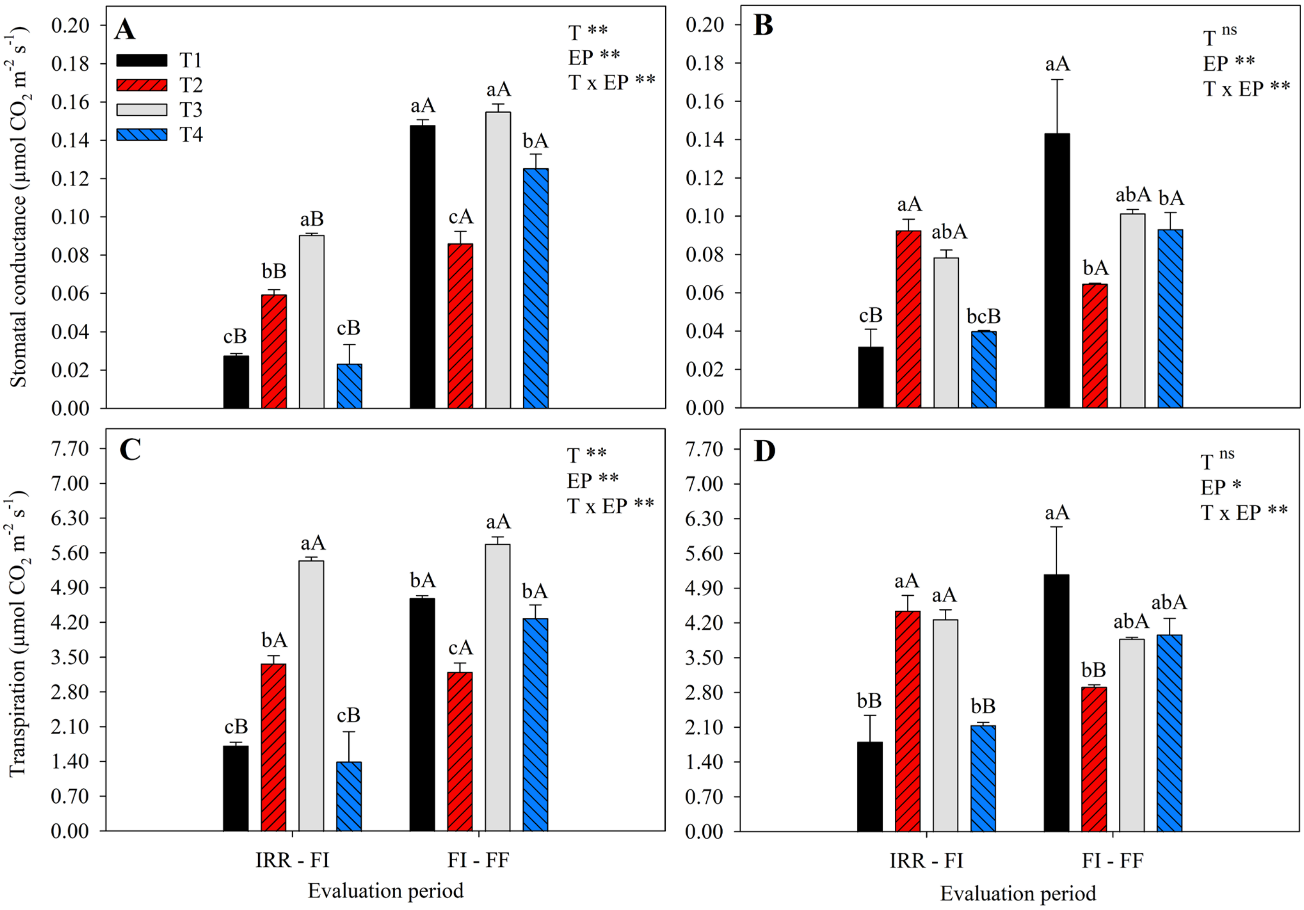

**Figure 6**. Stomatal conductance [orchard 1 (A); orchard 2 (B)] and transpiration [orchard 1 (C) e orchard 2 (D)] of mango ‘Keitt’ as a function of FA and free amino acids. Experimental and statistical details are similar to those in Figure 2.

During IRR the control treatment (T1) and the one with FA combined with amino acids (T4) had *E* of less than 2.5 µmol $CO_2$ $m^{-2}$ $s^{-1}$. This behavior can be related to slower absorption of PBZ and consequent damage from water stress. Both results can be explained by the questions listed below.

T1, since the PBZ supplied has not been complexed by FA or amino acids, reduces the mobility of PBZ in the soil and consequently restricts its absorption by the plant (SILVA et al., 2020). While T4, which combines the two organic molecules, promotes competition between them since AF can bind to amino acids (which have two protonable $NH_2$ groups) by cation exchange and complexation (BALDOTTO; BALDOTTO, 2014). This reduced the complexation possibility with PBZ, delaying. This delays the phytoregulator transport of the in the soil, and its absorption by the plant.

Afterward, the treatments influenced the plants’ intrinsic water use efficiency. For example, plants from T1 and T4 had higher iWUE values than T2 and T3 in soil 1 (Figure 7 A) and T2 and T4 in soil 2 during the water stress (Figure 7 B).

Plants submitted to the control treatment, even with higher water use efficiency in both soils (Figure 7), had less photosynthesis than plants submitted to T2 and T3 (Figure 4 AB). Although the more intense water use generated more chemical energy for the plant during the water restriction phase, which is desirable for the crop, the use of FA alone promoted a higher water deficiency resistance. Thus, in this case, water use efficiency is only interesting from a biological point of view, as the plant restricts its metabolism to overcome adversity. However, from an agronomic point of view, the lower water use efficiency can provide a more significant accumulation of dry mass, as occurs in this experiment.

In the subsequent phase (FI – FF), there was only a significant difference between treatments in soil 1, and the most remarkable difference was between T1 and T2, with the control being 38.69% higher than T2 (Figure 7 A).

So far, it is clear that the management of FI using phytoregulators combined with organic acids has a distinct influence on plant physiology. As expected, the number of panicles per plant also pointed out different responses to using organic acids and amino acids (Figure 8).

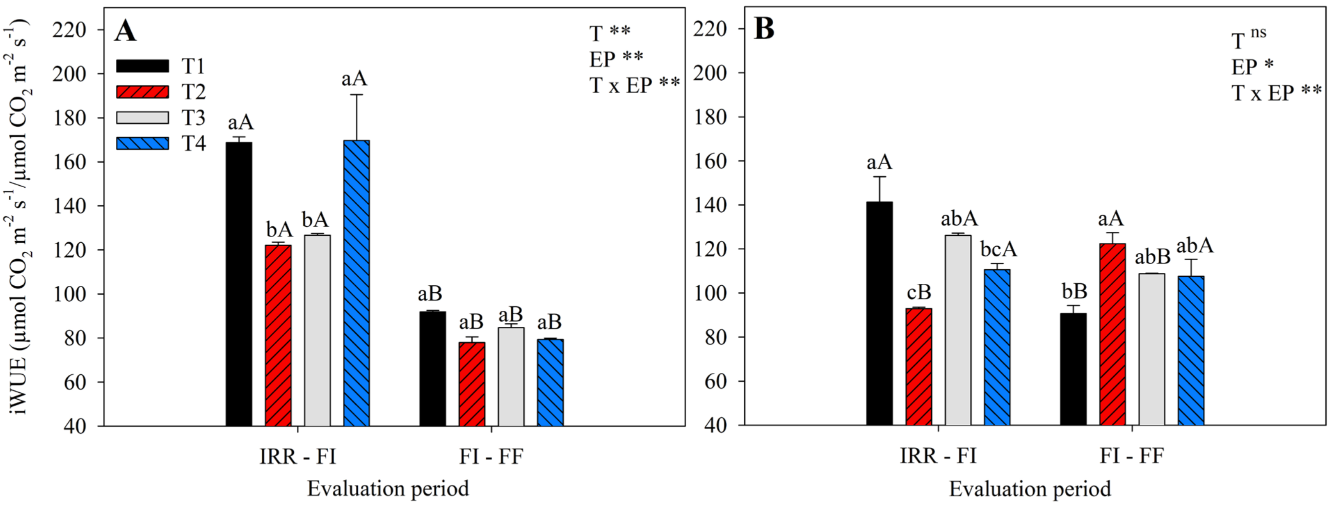

**Figure 7**. Intrinsic water use efficiency [orchard 1 (A); orchard 2 (B)] of mango ‘Keitt’ as a function of FA and free amino acids. Experimental and statistical details are similar to those in Figure 2.

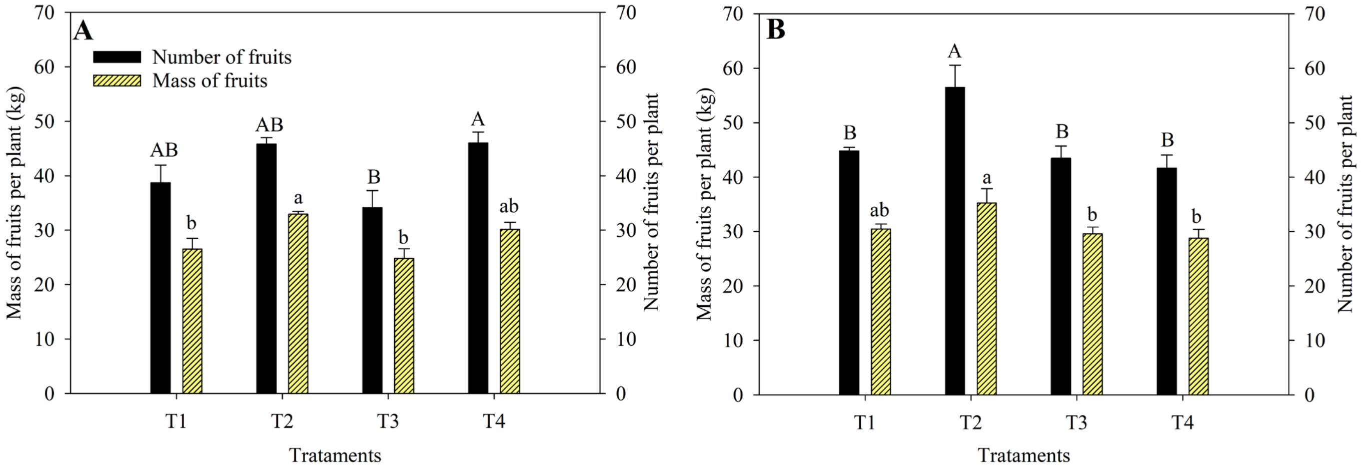

**Figure 8**. Mass and number of fruits per plant [orchard 1 (A); orchard 2 (B)] of mango ‘Keitt’ as a function of FA and free amino acids. Similar capital letters (number of fruits) and common lower case letters (Mass of fruits) do not differ at the 5% level by Tukey’s test. Experimental details are similar to those in Figure 2.

At first, there was no difference between the treatments for panicle emission during the FI itself, corresponding to the application phase of calcium and potassium nitrates to stimulate ethylene production and, consequently, differentiation of the shoots. However, in FF, it is possible to highlight the use of FA due to the significant difference in the total number of panicles emitted by plants.

FA possibly increases soil mobility, absorption speed, and total absorption and generates less PBZ residue in the soil after harvest compared to the control (without fulvic acid), using only amino acids and amino acids together with FA. This result is due to the PBZ complexation with FA, avoiding unnecessary energy expenditure to activate plant defense mechanisms (SILVA et al., 2020).

The low photosynthesis levels close to FF (Figure 3 A-B) highlights that T2 provided good BM due to the accumulation of sufficient reserves in the previous phases (SILVA et al., 2021), and therefore culminated in a high floral load. Such superiority in the flowering of plants subjected to treatment with PBZ + FA is observable by the difference of 59.56 and 138.71% more panicles than plants from T1 (soil 1) and T4 (soil 2), respectively.

The hydrophobic and hydrophilic interactions between PBZ and organic matter (MILFONT et al., 2008) may be the reason for the highlight of the isolated use of FA (T2) in increasing the absorption of PBZ by the mango tree (Silva et al., 2020 and 2021).

In addition, the use of FA stood out in the mass of fruits per plant compared to the treatment with amino acids only (T3), with the control (T1) in soil 1 and FA + amino acids (T4) in soil 2, as highlighted in Figure 8. The number of fruits per plant also confirms the FA superiority (T2), mainly in soil 2, which produced approximately 11, 13, and 14 more fruits than T1, T3, and T4, respectively. However, the unit mass was similar among all treatments.

Finally, although the floral load of the plants in soil 1, regardless of treatment, was numerically more significant than in soil 2 (Figure 2), they managed to equal and even overcome the fruit yields (Figure 8) of soil 1. Therefore, the floral load also has to be consistent with the soil fertilization, as soil 2 contained 10.86% more base saturation (V%) than soil 1, which is a characteristic widely used to measure the fertility of the soil, and in addition, the V% of soils recommended for the development of hoses in the region is around 80% (CORREIA et al., 2018).

These results reinforce the need to search for technologies that manage plants' stress allowing their full development.

## Conclusion

The application of fulvic acids combined with paclobutrazol increased the gas exchange efficiency of mango cv. Keitt, favoring the flowering process. At the same time, using amino acids alone or together with fulvic acids decreases flowering efficiency.

## Acknowledgments

The authors thank CNPq (National Council for Scientific and Technological Development) for granting the research scholarship and Clorofila Agropecuária (Cabrobó, Pernambuco, Brazil) for the structural support necessary to accomplish the experiments.

Funding details: This work was supported by the [National Council for Scientific and Technological Development] under Grant [number 304388/20165].

## References

ALBUQUERQUE, J.A.S.; MEDINA, V.D.; MOUCO, M.A.C. Indução floral. *In*: GENÚ, P.J.C.; PINTO, A.C.Q. (ed.). **A cultura da mangueira**. Brasília: Embrapa Informação Tecnológica, 2002. p.259-76.

ALVARES, C.A.; STAPE, J.L.; SENTELHAS, P.C.; GONÇALVES, J.L.M.; SPAROVEK. G. Köppen's climate classification map for Brazil. **Meteorologische Zeitschrift**, Berlin, v.22, n.6, p.711-28, 2013.

AL-YASI, H.; ATTIA H.; ALAMER, K.; HASSAN, F.; ALI, E.; ELSHAZLY, S.; SIDDIQUE, K.H.S.; HESSINI. K. Impact of drought on growth, photosynthesis, osmotic adjustment, and cell wall elasticity in Damask rose. **Plant Physiology Biochemistry**, Lancaster, v.150, n.1, p.133-9, 2020.

BALDOTTO, M.A.; BALDOTTO, L.E.B. Ácidos húmicos. **Revista Ceres**, Viçosa, MG, v.61, n.1, p.856-81, 2014.

BEERS, R.F.; SIZER, I.W. A spectrophotometric method for measuring the breakdown of hydrogen peroxide by catalase. **Journal of Biological Chemistry**, Rockville, v.195, n.1, p.133-40, 1952.

CAVALCANTE, Í.H.L.; SANTOS, G.N.F.; SILVA, M.A.; MARTINS, R.D.S.; LIMA, A.M.N.; MODESTO, P.I.R.; ALCOBIA, A.M.; SILVA, T.R.S.; AMARIZ, R.A.; BECKMANN-CAVALCANTE, M.Z. A new approach to induce mango shoot maturation in Brazilian semi-arid environment. **Journal of Applied Botany and Food Quality**, Kiel, v.91, n.1, p.281-6, 2018.

CAVALCANTE, Í.H.L., SILVA, G.J.N.; CAVACINI, J.A.; AMARIZ, R.A.; FREITAS, S.T.; SOUSA, K.Â.O.; SILVA, M.A.; CUNHA, J.G. Metconazole on inhibition of gibberellin biosynthesis and flowering management in mango. **Erwerbs-Obstbau**, Osnabrück, v.62, n.1, p.89-95, 2020.

CORREIA, M.A.R.; FLORES, R.A.; PRADO, R.M.; NATALE, W; ROZANE, D.E.; VALE., D.W.D. USINg Limestone to improve soil fertility and growth of mango (*Mangifera Indica* L.). **Communications in Soil Science and Plant Analysis**, Athens, v.49, n.8, p.903-912, 2018.

DAVENPORT, T.L. Reproductive physiology. *In*: LITZ, R. (ed.). **The mango**: botany, production and uses. London: CABI, 2009. p.97-169.

GIBSON, S.I. Sugar and phytohormone response pathways: navigating a signalling network. **Journal of Experimental Botany**, Oxford, v.55, n.395, p.253-64, 2004.

HEINEMANN, B.; KÜNZLER, P.; EUBEL, H.; BRAUN, H.P.; HILDEBRANDT, T.M. Estimating the number of protein molecules in a plant cell: protein and amino acid homeostasis during drought. **Plant Physiology**, Oxford, v.185, n.2, p.385-404, 2021.

MALAVOLTA, E.; VITTI, G.C.; OLIVEIRA, S.A. **Avaliação do estado nutricional das plantas**: princípios e aplicações. Piracicaba: Potafos, 1997. p.319.

MILFONT, M.L.; ANTONINO, A.C.D.; MARTINS, J.M.F.; NETTO, A.M.; GOUVEIA, E.R.; CORREA, M.M. Transporte do paclobutrazol em colunas de solos. **Revista Brasileira de Ciências do Solo**, Viçosa, MG, v.32, n.5, p.2165-75, 2008.

MUDO, L.E.D.; LOBO, J.T.; CARREIRO, D.D.A.; CAVACINI, J.A.; SILVA, L.S.; CAVALCANTE, Í.H.L. Leaf gas exchange and flowering of mango sprayed with biostimulant in semi-arid region. **Revista Caatinga**, Mossoró, v.33, n.2, p.332-40, 2020.

OLDONI, F.C.A.; LIMA, A.M.N.; CAVALCANTE, Í.H.L.; SOUSA, K.S.M.; CARNEIRO, M.A.; CARVALHO, I.R.B. Boron fertilizing management on fruit production and quality of mango cv.Palmer in semiarid. **Revista Brasileira de Fruticultura**, Jaboticabal, v.40, n.3. p.1-8, 2018.

OLIVEIRA, M.B.; PEREIRA, M.C.T.; MIZOBUTSI, G.P.; MAIA, V.M.; SILVA, J.F.; OLIVEIRA, J.A.A. COSTA, I.J.S.; NIETSCHE, S.; SANTOS, E.F.; MOUCO. M.A.C. Paclobutrazol and tip pruning in the management of "Palmer" mango trees in the semiarid region of Brazil. **Acta Horticulturae**, Leuven, v.1075, n.1, p.149-56, 2015.

ORABI, S.A.; SALMAN, S.R.; SHALABY, M.A. Increasing resistance to oxidative damage in cucumber (*Cucumis sativus* L.) plants by exogenous application of salicylic acid and paclobutrazol. **World Journal of Agricultural Sciences**, Albany, v.6, n.3, p.252-9, 2010.

PEIXOTO, C.P.; CRUZ, T.V.; PEIXOTO, M.F.S. Análise quantitativa do crescimento de plantas: Conceitos e prática. **Enciclopédia Biosfera**, Goiânia, v.7, n.13, p.51-76, 2011.

PRASAD, S.S.; REDDY, Y.T.N.; UPRETI, K.K.; RAJESHWARA, A.N.Studies on changes in carbohydrate metabolism in regular bearing and "Off" season bearing cultivars of mango (*Mangifera indica* L.) during flowering. **International Journal Fruit Science**, Chicago, v.14, n.4, p.437-59, 2014.

REN, Y.; MIAO, M.; MENG, Y.; CAO, J.; FAN, T.; YUE, J.; XIAO, F.; LIU, Y.; CAO, S. DFR1-Mediated inhibition of proline degradation pathway regulates drought and freezing tolerance in Arabidopsis. **Cell Reports**, Cambridge, v.23, n.13, p.3960-74, 2018.

SAIBI, W., FEKI, K., YACOUBI, I., BRINI, F. Bridging between proline structure, functions, metabolism, and involvement in organism physiology. **Applied Biochemistry and Biotechnology**, Denver, v.176, n.8, p.2107-19, 2015.

SANTOS, M.R; MARTINEZ, M.A.; DONATO. S.L.R. Gas exchanges of 'Tommy Atkins' mango trees under different irrigation treatments. **Bioscience Journal**, Uberlândia, v.29, n.5, p.1141-53, 2013.

SANTOS, M.R.; NEVES, B.R.; SILVA, B.L.; DONATO, S.L.R. Yield, water use efficiency and physiological characteristic of "Tommy Atkins" mango under partial rootzone drying irrigation system. **Journal of Water Resource and Protection**, Irvine, v.7, n.13, p.1029-37, 2015.

SAXENA, P.; SINGH, V.K; PATHAK, N.Antioxidative enzymes and biochemical changes in paclobutrazol induced flowering in mango (*M. indica*) cultivars. **Journal of Environmental Biology**, Lucknow, v.35, n.6, p.1061-6, 2014.

SILVA, D.J.; QUAGGIO, J.A.; PINTO, P.D.C.; PINTO, A.D.Q.; MAGALHÃES, A.D.J. Nutrição e adubação. *In*: GENÚ, P.J.C.; PINTO, C.A.Q. (eds.). **A cultura da mangueira**. Brasília: Embrapa Informação Tecnológica, 2002. p.191-222.

SILVA, L.S.; SILVA, P.T.S.; CAVALCANTE, Í.H.L. Impact of fulvic acid and free amino acids on paclobutrazol absorption by 'Keitt' mango. **Revista Ambiente & Água**, Taubaté, v.15, n.4, p.1-8, 2020.

SILVA, L.S.; SOUSA, K.Â.O.; PEREIRA, E.C.V.; ROLIM, L.A.; CUNHA, J.G.; SANTOS, M.C.; SILVA, M.A.; CAVALCANTE, Í.H.L. Advances in mango 'Keitt' production system: PBZ interaction with fulvic acids and free amino acids. **Scientia Horticulturae**, Amsterdam, v.277, n.1, p.109787, 2021.

SOUMYA, P.R.; KUMAR, P.; PAL. M.; Paclobutrazol: a novel plant growth regulator and multi-stress ameliorant. **Indian Journal of Plant Physiology**, New Delhi, v.22, n.3, p.267-278. 2017. DOI: 10.1007/s40502-017-0316-x.

SPOSITO, G. **The chemistry of soils**. Oxford: Oxford University Press, 2008. 328 p.

SRIVASTAV, M.; KISHOR, A.; DAHUJA, A.; SHARMA, R.R. Effect of paclobutrazol and salinity on ion leakage, proline content and activities of antioxidant enzymes in mango (*Mangifera indica* L.). **Scientia Horticulturae**, Amsterdam, v.125, n.4, p.785-8, 2010.

SUBBAIAH, K.V., REDDY, N.N.; PADMAVATHAMMA, A.S.; REDDY, M.L.N.; RAO, A.D.; MANJULA R.; REDDY, A.G.K. Effect of paclobutrazol on hermaphrodite flowers, leaf chlorophyll. **International Journal of Current Microbiology and Applied Sciences**, Tamilnadu, v.7, n.4, p.1-7, 2018.

SUN, J.; QIU, C.; DING, Y.; WANG, Y.; SUN, L.; FAN, K.; GAI, Z.; DONG, G.G.; WANG, J.; LI, X.; SONG, L; DING, Z. Fulvic acid ameliorates drought stress-induced damage in tea plants by regulating the ascorbate metabolism and flavonoids biosynthesis. **BMC Genomics**, London, v.21, n.1, p.1-13, 2020.

SZABADOS, L.; SAVOURÉ, A. Proline: a multifunctional amino acid. **Trends in Plant Science**, v.15, n.2, p.89-97, 2010.

TAIZ, L.; ZEIGER, E.; MØLLER, I.M.; MURPHY, A. **Fisiologia e desenvolvimento vegetal**. Porto Alegre: Artmed, 2017. 858 p.

UPRETI, K.K.; PRASAD, S.S.; BINDU, G. Regulatory roles of phytohormones and carbohydrates of flowering in mango. *In*: RAVISHANKAR, H.; SINGH, V.K.; MISRA, A.K.; MISHRA, M. (ed.). **Physiological of flowering in perennial fruit crops**. Lucknow: Souvenir, 2014. p.164-72.

WANG, Y.; YANG, R.; ZHENG, J.; SHEN, Z.; XU, X. Exogenous foliar application of fulvic acid alleviate cadmium toxicity in lettuce (*Lactuca sativa* L.). **Ecotoxicology and Environmental Safety**, Amsterdam, v.167, n.1, p.10-19, 2019.