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Universidade Federal do Vale do São Francisco, Centro de Ciências Agrárias, Colegiado de Agronomia, Petrolina, Brazil

Nitrate reductase activity in the different phenophases of 'palmer' mango cultivated in the semiarid

Alana Juliete da Silva Santos, Vespasiano Borges de Paiva Neto*, Luciana Guimarães Sanches, Daniel de Almeida Carreiro, Maria Poliana Martins Pereira, Monica Cristina Rezende Zuffo Borges, Stefany Emanuella Rodrigues dos Santos, Ítalo Herbert Lucena Cavalcante

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Summary

Nitrate reductase is the enzyme that catalyzes the first reduction reaction in the nitrate assimilation process, an important nitrogen (N) source. This element is one of the macronutrients required in greater amounts by mango (*Mangifera indica* L.), exerting a great influence on the growth and development of the plants. In this context, this work aimed to evaluate nitrate reductase activity (NRa) throughout the day and characterize it in leaves of 1st and 2nd vegetative flushes and young roots of 'Palmer' mango cultivated in the Brazilian semiarid For each phenophase, leaves and roots were randomly collected from six plants in the orchard. A completely randomized design was employed, with four replications, and the NRa quantification was based on the *in vivo* evaluation method. The enzyme activity was maximum in the period of greatest solar radiation or from 10 a.m. to 11 a.m. The 2nd flush leaves and young the roots constitute the main nitrate assimilation sites in the mango crop in comparation with 1st flush leaves. Fertilization with nitrate or potassium sources in the different phenological phases benefit the NRa, which remains maximum during reproductive phase than vegetative phase.

Keywords: Phenology; Nitrogen; Enzymatic activity; *Mangifera indica* L.; Floral induction

Introduction

In semiarid conditions, mango cultivation can occur during the entire year as long as adequate plant management is performed to favor flowering (Mouco, 2015). However, the crop yield is still relatively low due to factors related to flowering and fruiting, mainly. In this case, the physiological, phytopathological, and nutritional factors related to these events must be observed so that fruit production is satisfactory (Coutinho et al., 2016).

There are tools for mango flowering management through pruning, nutritional management, and the use of growth regulators (Krishna et al., 2017). The synchronization of the canopy vegetation, better performed through pruning in all the branches of the plant, is a necessary first step in the program of flowering management, whose purpose is to leave the branches at the same physiological stage of maturity. Therefore, in commercial orchards, after a productive cycle (fruit harvest), the mango plant requires pruning, with the removal of the second branch segment (flush) formed in the previous cycle. This practice encourages the plant to form the next two flushes of the new reproductive cycle. Additionally, this practice controls the increase in the canopy diameter of each plant.

The next step is the application of paclobutrazol (PBZ) in an adequate amount and time (Wongsrisakulkaew et al., 2017). This one, by its turn, paralyzes vegetative growth and impedes the distribution of elaborate sap, leading to the accumulation of carbohydrates in the branches, resulting in maturation (Taiz et al., 2017).

For the well-succeeded stimulation of flowering, nitrate salts must be applied, via foliar spraying, after the mango sprouts reach maturity or sufficient age to overcome any inhibitory influence on the flowering response (Davenport, 2000). Potassium nitrate (KNO_3) induces the nitrate reductase activity (NRa), a key enzyme in the nitrate assimilation pathway for the synthesis of amino acids, particularly methionine (Anusuya et al., 2018), an intermediary product precursor of ethylene, which, by its turn, promotes flowering in the mango plant (Sudha, Balamohan and Soorianathasundaram, 2012; Coutinho et al., 2016).

Nitrate reductase is located in the cellular cytosol and catalyzes the reduction of nitrate to nitrite (NO_3^- to NO_2^-), a limiting step in the nitrogen (N) assimilation pathway in amino acids, proteins, and other nitrogen compounds in the cells performing a key role in the response of the plants to deficiency of this element (Kaur et al., 2015; Taiz et al., 2017).

Nitrate reductase activity is regulated by several factors, such as the presence of nitrate, that is, its substrate (Coutinho et al., 2016; Anusuya et al., 2018), light (Oliveira et al., 2005), the water status (Oliveira et al., 2005), species, growth habit, and habitat, being lower in woody species (Rajsz et al., 2018). The regulation of NRa is necessary to avoid the accumulation of nitrite, which is toxic for the cell (Taiz et al., 2017). Furthermore, this regulation has a close relationship with photosynthesis since the step of nitrite reduction to ammonium uses reduced ferredoxin, a product of this process. In this manner, a reduction in photosynthesis could lead to nitrite accumulation in the cellular medium if the enzyme activity was not regulated (Lillo et al., 2003).

Nitrate reduction can occur in the root system or the shoot part of the plants, (Taiz et al., 2017), and it may vary among different species (Black et al., 2002). Oliveira et al. (2005) observed the enzyme activity in leaves and roots of peach palm seedlings (*Bactris gasipaes*) and verified that it was maximum in the leaves, such as observed for the grapevine crop (Mesquita et al., 2018). In maize, an alternation in NRa was observed between leaves and roots: when the enzyme activity was high in the roots it was low in the leaves, and vice-versa (Freitas et al., 2007). In the mango crop (*Mangifera indica* L.) NRa was observed in both plant tissues, although it was maximum in the roots (Souza et al., 2016), with the study being performed only at the flowering phenophase.

In the absence of NRa, NO_3^- reduction does not occur (Kaufholdt et al., 2016), limiting growth, development, and production of proteins in the plants that assimilate this element (Taiz et al., 2017).

Given the exposed, this work aimed to define the daytime of superior nitrate reductase activity and to characterize the enzyme activity in leaves of 1st and 2nd vegetative flushes and young roots of 'Palmer' mango plants in the different phenological phases of the crop, aiming to understanding how the plant distributes the nitrogen metabolism in these organs throughout its cycle.

* Corresponding author

Material and methods

The present study was performed between December 2018 and July 2019, in which period the collection of the plant material was performed in two commercial orchards at two nearby farms with 'Palmer' mango (*Mangifera indica* L.), whose general data concerning the studied areas are found in Tab. 1.

Tab. 1: General information regarding the studied areas.

Orchard	Farm	Age (years)	Spacing (m)	Size (ha)
Area 1	Sebastião da manga	8	5×8	12.54
Area 2	La bordett	7	4×6	2.2

The region climate is classified as semiarid Bsh, with a mean annual temperature of 24 °C and annual precipitation under 700 mm (Álvares et al., 2013). The climatic data referring to rainfall, solar radiation, temperature, and air relative moisture during the period of the experiment were registered and obtained from the automatic weather station installed at the Campus of Agrarian Sciences (CCA/UNIVASF).

Cultural practices such as pruning, control of weeds, pests, and diseases were performed according to the described in the technical rules for integrated mango production (Lopes et al., 2003). The application of paclobutrazol (PBZ) was performed, along with the thinning and dormancy break in the management of floral induction, according to the recommendations of Albuquerque et al. (2002). The nutritional management was performed via fertigation according to the analysis of soil and leaves to attend to the demand of the crop (Silva et al., 2002).

Aiming at defining the best time for the collection of the plant material, a daytime characterization of the NRa was performed. For that purpose, fully expanded and physiologically mature leaves of 1st and 2nd vegetative flushes and young roots were collected from plants during the floral induction phase, throughout the day every two hours, starting at 8 a.m. and finishing at 4 p.m., a period of free access to the farms. The NRa was monitored in the different mango phenological phases, and the collection of the material for this stage was performed at the time of superior activity, defined from daytime characterization. Tab. 2 describes the collection site referring to each phenophase.

Tab. 2: Collect schedule of mango leaves and roots for analyses of nitrate reductase activity, considering the pruning event as the beginning of the cycle and their respective phenophases, in two farms with similar orchards in age and management.

Phenophases (BBCH-scale*)	Collect day after pruning date	Farm	
		Sebastião da manga	La bordett
Pre-pruning [Pruning day]**	0	X	
Pruning [15 days after pruning]**	15	X	
PBZ1 [12 days after addition] (329)	97		X
PBZ 2 [32 days after addition]	117		X
Branch maturation1,**	125	X	
Pre-induction2 (510)	150	X	
Floral induction3 (510)	157	X	
Flowering (615)	196	X	
Fruiting (703)	249	X	

*(Biologische Bundesantalt, Bundessortenamt und Chemische Industrie); **Phenophase or event not mentioned in the BBCH-scale; [1]Five days after first foliar spray with 3% potassium sulfate (K_2SO_4); [2]One day before first foliar spray with 3% Potassium Nitrate (KNO_3); [3]Five days after first foliar spray with 3% Potassium Nitrate (KNO_3);

The vegetal material was obtained from six randomly sampled plants within the mango orchards. From the median portion of each plant, two leaves of the 1st flush (penultimate vegetative flush) and two leaves of the 2nd flush (last vegetative flush) exposed to solar radiation were collected, totaling 12 leaves of each type, and a portion of thin roots (absorbing) at a depth of 0-10 cm in the irrigated range of the soil.

After the collection of the material, laboratory analysis were performed in the laboratories of Analytical Chemistry and Plant Physiology of the Universidade Federal do Vale do São Francisco (UNIVASF), Campus of Agricultural Sciences (CCA), located in the city of Petrolina- PE. Leaves and roots were stored in plastic bags and aluminum foil, respectively, both stored in a cool box containing ice. The *in vivo* nitrate reductase activity (NRa) was determined according to the method described by Majerowicz et al. (2003), with a few modifications. The leaves underwent a washing sequence with running water, 1% detergent, running water, and distilled water, in this order. The roots were washed in a sieve only with running and distilled water to avoid loss of the vegetal material.

After drying, 7 mm discs were removed from the leaf blade, forming a compound sample for both leaf types. Each simple sample analyzed contained 60 discs and was weighed to obtain the mass. The roots were chopped into small fragments, forming a compound sample. Each simple sample contained 0.5 g of roots. The portions of leaves and roots were placed in falcon tubes wrapped in aluminum foil, into which were added 4 mL of a phosphate buffer solution 0.05 M, pH 7.5, potassium nitrate (KNO_3) 0.05 M, and1% n-propanol. Afterward, the plant tissue submerged in the solution was subjected to three vacuum sessions, each during 1-minute (min), with 30-second (s) intervals. The tubes were then properly sealed and incubated for 60 minutes at 30 ºC.

After the incubation period, the tubes were subjected to immersion in ice for two minutes. The amount of nitrite (NO_2^-) released into the incubation medium was determined in 2 mL aliquots with the addition of 1 mL of N-naphthyl-ethylene-diamine at 0.2% (m/v) and 1 mL of Sulfanilamide at 1% (m/v), reacting for 30 minutes. The samples were analyzed in an EVEN® UV-VIS spectrophotometer at 540 nm. The enzyme activity was expressed in µmol of nitrite released per 1 g of fresh leaf per hour of incubation ($\mu mol \cdot NO_2^- . g^{-1} \cdot h^{-1}$), calculated based on the linear equation obtained through the nitrite standard curve, previously prepared.

A completely randomized design was used in the experiment, with four replications. The obtained data were subjected to analysis of variance by the F test, and the means were compared by Tukey's test with $p<0.05$ using the R software (R Development Core Team, 2018). The graphics were generated in the Sigmaplot software (10.0, Systat Software, San Jose, CA).

Results

Nitrate reductase activity (NRa) in the 'Palmer' mango plant was observed throughout the day in mature leaves of 1st and 2nd vegetative flushes and young roots of plants in the floral induction phase, indicating two sites of N reduction (shoot and root) (Fig. 1).

For the 2nd flush leaves and roots, the NRa can be observed from the early morning hours, with a peak at 10 a.m., decreasing from noon. The 1st flush leaves presented a small variation in the enzyme activity in the morning, with no evident activity peak in the monitored hours, although with an equal trend for NRa reduction from noon (Fig. 1). It is possible to observe that the activity peak coincided with the time of greatest solar radiation (Fig. 1).

Nitrate reductase activity was monitored in the different phenophases of the mango crop, verifying, after harvesting and before pruning (pre-pruning), maximum nitrate reductase activity in the root, statistically differing from the leaves of 1st and 2nd flushes, which

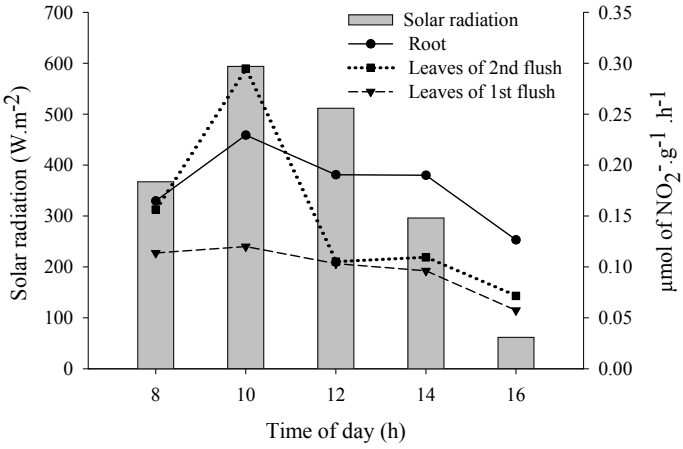

Fig. 1: Daytime variation of nitrate reductase activity in leaves of 1st and 2nd vegetative flushes and young roots of 'Palmer' mango and mean values of solar radiation obtained from the weather station at the Campus of Agricultural Sciences (CCA), of the Universidade Federal do Vale do São Francisco (UNIVASF)

did not differ from each other (Fig. 2). It can be observed that after pruning the NRa was not quantified in the 2nd flush leaves because this management removes this material. It is importante to affirm that these two analyzed flushes were generated in the previous vegetative cycle, while from branch maturation onwards, the analyzed flushes (1st and 2nd flushes) were from the new vegetative cycle.

In the phase of plant 'locking', an increase in the NRa of the leaves was verified to the detriment of the roots. 12 days after the application (DAA) of PBZ, the superior NRa was found in the 1st flush leaves, which was statistically different only from the roots. 2nd flush leaves and roots did not differ from each other (Fig. 2). At 32 DAA, the enzyme activity was maximum in 1st and 2nd flushes leaves, not differing statistically from each other, although differing from the roots (Fig. 2).

In the branch maturation phase, the NRa was statistically equal in the 2nd flush leaves and roots, being much maximum than in the 1st flush leaves, which differed statistically from the remaining plant tissues (Fig. 2).

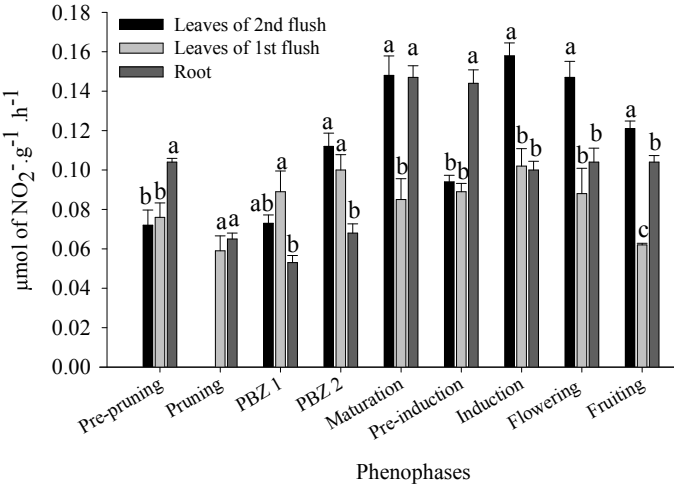

Fig. 2: Nitrate reductase activity in leaves of the 1st and 2nd vegetative flushes and young roots of 'Palmer" mango along different phenophases. Means ± SD followed by the same letters on the bars, into each phenophases singly, do not differ significantly by the Tukey test at 5% probability.

It can be observed that the NRa was influenced by the foliar spraying with KNO_3 in 2nd flush leaves and roots of mango. The 1st flush leaves exhibited a reduced alteration in enzyme activity in the periods of pre and post floral induction (Fig. 2).

In pre-induction, the superior NRa occurred at the roots, statistically differing from the leaves of 1st and 2nd flush, which were different from each other. After the induction there was a decrease in root NRa, equaling the 1st flush leaves, and an increase in enzyme activity in the 2nd flush leaves, making this the main NO_3^- reduction site in the mango plant after the exogenous increment of KNO_3, via foliar spraying.

In the flowering phase, the NRa found in the 2nd flush leaves was significantly maximum in relation to the 1st flush leaves and roots (Fig. 2). Although this difference between plant materials does exist, it is observed that the NRa begins to decrease in the 2nd flush leaves, in comparison to the previous phase.

In the fruiting phase, the NRa was maximum in the 2nd flush leaves, followed by roots and 1st flush leaves (Fig. 2). Although it has been maximum in 2nd flush leaves, the enzyme activity continued to reduce in comparison with the previous phases. In this stage, the remobilization of foliar N for fruit development continues.

It can be observed that the cumulative nitrate reductase activity in the analyzed leaves and roots of mango is lower during the vegetative phase when compared with reproductive phase, and in general, the NRA variation is small in the leaves of the 2nd flush (Fig. 3).

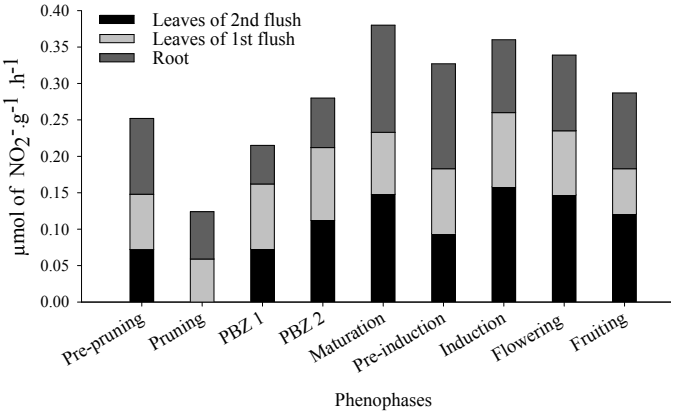

Fig. 3: Distribution of the nitrate reductase activity between leaves of the 1st and 2nd flushes and root of the 'Palmer' mango in the different phenophases along the vegetative and reproductive cycle in commercial orchard.
PBZ 1: 12 days after PBZ application; PBZ 2: 32 days after PBZ application; NRa: nitrate reductase activity in µmol of NO_2^- · fresh matter^{-1} · h^{-1}.

Discussion

In the present study, a maximum NRa activity was verified at 10 a.m., a similar behavior to that observed by Oliveira et al. (2005) in peach palm leaves (*Bactris gasipaes*), in which the maximum NRa was also verified at the referred time, three hours after the beginning of the luminous period. Furthermore, the authors also observed a decline in the enzyme activity after this time. Additionally, for sweet orange (*Citrus sinensis*) cv. Valência, the maximum NRa was also observed at this same time, corresponding to maximum photosynthesis (Dovis et al., 2014). Such behavior occurs due to the effect of light on the enzyme activity, which may be either direct, in its activation, or indirect, via photosynthetic process, providing the necessary energy for nitrate assimilation (Martuscello et al., 2016).

Among the climatic factors monitored on the day of the experiment,

solar radiation seems to be the one that most influenced the activity of the nitrate reductase enzyme. SANTOS et al. (2014) inferred that the enzyme is more active in the maximum intensity hours of global solar radiation. It is possible that in mango, NRa presents the same behavior pattern of other species such as tomato (TUCKER et al., 2004) and annatto (PATRA et al., 2019), for which it has been shown that NRa exhibit circadian rhythm. According to TUCKER et al. (2004), regulatory mechanisms acting at different levels ranging from transcription to protein degradation. Therefore additional investigations to confirm this phenomenon in mango plants are required.

In that perspective, KHOURI (2007) reported that the maximum NRa values and probably also the maximum absorption of NO_3^- occur during the periods of high temperature and evaporative demand. These conditions can increase the influx of water and NO_3^- to the leaves, favoring the activity of the enzyme in these tissues, since NO_3^- is translocated to the shoot part via xylem through transpiration flow (TAIZ et al., 2017), thus increasing the availability of the substrate for the enzyme.

Given the management that is performed in the mango crop, in the pruning act there is a reduction in the number of flushes, especially the second, making impossible the quantification of NRa in its leaves (Fig. 2); however, in general, the nitrate absorbed by the roots can be assimilated in the root tissues or in leaf tissues, depending on their availability (OLIVEIRA et al., 2011). On the other hand, it is highlighted that the evaluated plants did not receive nitrogen fertilization via fertigation or foliar spraying after harvest (pre-pruning phase), which may be a factor for the low enzyme activity in the leaves of 1st and 2nd flushes. Furthermore, the low NRa in these tissues might have occurred as a function of the low NO_3^- concentrations due to the exportation of N by the fruits harvested in the previous season, approximately two months before the evaluation, since this element is the second most extract macronutrient through harvest in the mango crop (NASCIMENTO et al., 2005).

It is believed that the maximum enzyme activity in the roots may have occurred as a function of the applications of calcium nitrate, via fertigation, performed during fruit development in the previous cycle, which was not required by the plant, being stored in the root vacuoles for later metabolization. According to PRADO (2013), fruit crops assimilate NO_3^- mainly in the roots, with an increment in the leaves when there is an increase in the availability of this element. The mango plant seems to present great storing capacity of reserve substances, besides presenting a root system that, although being little dense, occupies a large soil volume (CASTRO NETO, 1995). Likewise, in the grapevine crop, the younger leaves presented high nitrate reductase activity after harvest, which was similar to the accumulation of sucrose and starch (HUNTER and RUFFNER, 1997). According to these authors, the increase in the enzyme activity could be related to the annual accumulation of nitrogen in the crop. This difference between the mango and grapevine crops could occur as a function of the N management in the annual cycle; besides, the location of the nitrate assimilation tissue in the plants varies according to the species and may indicate different ecological adaptations (BLACK et al., 2002).

At 15 days after pruning, in spite of the plant material analyzed, the nitrate reductase presented a statistically equal activity (Fig. 2). In the production pruning, performed just after harvest, the main objectives are to clean out dry and sick branches, besides promoting the central opening of the plant to allow a maximum internal lighting, favoring vegetative growth for the next harvest; in this manner, all resources are directed towards vegetative growth (MOUCO, 2015). After this cultural practice, nitrogen fertilization was performed, although not observing an increase in the NRa of the plant tissues. This may have occurred because the N source provided was ammonium sulfate, which does not contain nitrate (NO_3^-) in its composition, the main substrate for the activation of the enzyme (MARTUSCELLO et al., 2016). Generally, ammonium sulfate and urea are the main N sources provided in this phase for the mango crop. Nitrate salts are applied at the dormancy break phase of the buds, via foliar spraying, and during fruit development, via fertigation.

With regard to the NRa quantification in the post-PBZ evaluations, a growth regulator applied to inhibit branch growth and promote bud maturation, increasing the potential of the plant in producing reproductive sprouts (DAVENPORT, 2007), in the present study the use of this regulator demonstrated to favor a maximum activity in the leaves (in spite of the flush) than in the roots (Fig. 2). PBZ has been widely employed in mango farming to restrict vegetative growth, favor carbohydrate accumulation, and promote flowering. It works by blocking the synthesis of gibberellin, which inhibits flowering and stimulates vegetative growth (TAIZ et al., 2017). The lower activity of the enzyme in the roots, in both evaluated days, may be related to the lower metabolic activity in the root system, since PBZ is applied via root system, affecting root growth and leading to a reduction in drainage force, affecting energy consumption and demand, since the energy expenditure for NO_3^- reduction in the root tissues is high and roots are considered less efficient assimilation sites than the shoot part (CARELLI and FAHL, 1991), since the leaves are the synthesis center of ATP and reducing agents (TAIZ et al., 2017).

The maximum NRa in 1st and 2nd flushes leaves may be related to the increase in the carbohydrate content in these sites, a primary energy source, even if most sugars are directed to branch maturation, since the availability of carbohydrates increases the nitrate reduction rate (KLEPPER et al., 1971). Although PBZ application decreases the root water potential and consequently reduces the transpiration rates, the adequate dose of the product should not decrease the production of photoassimilates (SOUZA et al., 2016), maintaining the plant metabolism in these conditions, what seems to have occurred in the studied plants when observing that the nitrate reductase enzyme maintained its activity, especially considering that the demand for nitrogen compounds should decrease in this locking phase of the plant when there is no generation of new tissues.

SOUZA et al. (2016) characterized nitrate reductase activity in leaves and roots of 'Palmer' mango after PBZ application via fertigation and found a maximum activity in the root system, differently from what was observed in the present work. Furthermore, they observed that there was no significant effect of the application method and doses of PBZ via fertigation on NRa.

In the branch maturation phase, the NRa in the 2nd flush leaves resembled that of the roots (Fig. 2) and, in this period, it is common to employ 3% potassium sulfate (K_2SO_4) in two or more foliar sprayings, after leaf maturation, around 60 days after PBZ application, with a 7-day interval between them. Four K_2SO_4 applications were performed in the studied plants. To the detriment of this result, it is worth noting that potassium (K) is the product component used and, such as N, it is the nutrient that determines gain in the activation of nitrate reductase and, consequently, NO_3^- reduction, an already seen fact in cucumber plants (RUIZ and ROMERO, 2000). Furthermore, this ion interferes with the potassium/nitrogen relation, inhibiting vegetative growth and collaborating with branch maturation in mango, thus improving bud fertility (SILVA and VILELA, 2004).

Potassium an osmotically active ion involved in the osmoregulation of guard cells, and its concentration in these cells is responsible for the stomatal opening and closing mechanisms (TAIZ et al., 2017). The increase in the concentration of K in the guard cells reduces their osmotic potential, allowing the inlet of water and, as a function of the high cell turgor, the stomata open (TAIZ et al., 2017). In this manner, there is an increase in stomatal conductance, and it may favor NO_3^- transportation to the shoot part, inducing an elevated nitrate reductase activity in the 2nd flush leaves. According to RUIZ and ROMERO (2002), K facilitates the absorption and transportation

of NO_3^- to the shoot part of the plant, explaining the increase of NRa with the increment of this element in the 2nd flush leaves.

Furthermore, 1st flush leaves presented lower NRa, 2nd flush leaves are stronger drains if compared to 1st flush leaves, and, consequently, receiving more substrate, activating nitrate reductase, and favoring its activity. Alternatively, the decrease in the enzyme activity with leaf age can be attributed to changes in the general metabolic activity and foliar ontogenesis (Black et al., 2002). This fact has been already demonstrated in leaves of the mango variety Dasehri, originated from India (Ananthanarayanan and Chacko, 1983), in which the NRa was high in the initial phase of development, with later reduction and stabilization.

Another management performed in the floral induction process is the reduction of the irrigation water depth along with branch maturation. Water deficit decreases nitrate reductase activity due to the decrease in the water flow through the transpiration current and, consequently, the nitrate flow toward the leaves (Oliveira et al., 2011), since the enzyme is highly dependent on its substrate. However, this management did not seem to interfere with the NRa of the 2nd flush leaves and roots, perhaps because when the analysis was performed the irrigation water depth was reduced in 25% of the total applied, not yet a limiting factor to the activity of the enzyme. Besides the foliar spraying with a potassium nitrate, efficient in favoring the activity of the enzyme.

Dormancy break is performed through the foliar spraying of nitrate salts to stimulate reproductive sprouting (Jameel et al., 2018). In the studied plants, 3% potassium nitrate (KNO_3) was used for this purpose. This element triggers the formation of nitrate reductase, which reduces nitrate and leads to the synthesis of amino acids, especially methionine, which is the precursor of ethylene. This, by its turn, induces flowering (Coutinho et al., 2016). In this manner, it is inferred that this processes, mediated by the NRa as a function of foliar spraying with KNO_3, occurs more substantially in the roots and 2nd flush leaves, in the periods of pre and post floral induction, respectively (Fig. 2), showing a modulation capacity in the NRa by the plant for the benefit of the application of nitrate salts (Taiz et al., 2017), since the enzyme is induced by its substrate (Martuscello et al., 2016), possibly explaining the activity increase in the 2nd flush leaves after the spraying, in comparison with the roots. According to Konishi and Yanagisawa (2011), the expression of NR genes is rapidly stimulated in several plants in the presence of NO_3^-.

There is a report that in grapevine leaves and roots there was an increase of the NRa in the presence of calcium nitrate ($Ca(NO_3)_2$), being evident that the shoot part is the preferential N assimilation site in this crop (Mesquita et al., 2018), as well as in peach palm seedlings (*Bactris gasipaes*) (Oliveira et al., 2005). As for young coffee plants (*Coffea arabica* L.) irrigated with solutions containing different nitrate concentrations, the NRa was maximum in the roots than in the leaves (Carelli and Fahl, 1991).

The preference regarding the nitrate reduction site may vary according to the species, but it is not constant, since it is influenced by environmental and physiological factors (Andrews, 1986), such as the irradiance regime, so that in the period of greater photosynthetically active radiation (PAR) nitrate reduction is maximum in the leaves compared to the roots, and when of the reduction in the PAR, the NRa concentrates in the root tissue (Carelli and Fahl, 2006). Photosynthetic tissues are the most adequate place for nitrate reduction due to their energy consumption, a previously discussed fact. However, there are several reports of NRa in root tissues (Martuscello et al., 2016), such as in the present study.

The decrease in root NRa at floral induction and in the subsequent phases (Fig. 2) may also be related to the amount of energy made available in these tissues as a function of the energy invested in the formation of panicles and fruits, which are more efficient drains than the roots, in these phenophases (Taiz et al., 2017).

Regarding mango flowering, this is the phase in which there is a demand for an adequate nitrogen supply, which is also related to the age and physiological stage of the plant during the agricultural year. Before flowering, there are high values of nitrogen content, while in full bloom and fruit formation there are minor contents (Nascimento et al., 2005), which may explain the NRa behavior in the referred phenophases.

Changes in the foliar N content and the photosynthetic capacity during plant development can result in N depletion by drains, such as flowers and fruits (Urban et al., 2004), which may be the cause of the beginning of a reduced enzyme activity in the 2nd flush leaves, closer to the inflorescences, acting as a carbohydrate source (energy) for the development of flowers and fruits, consequently reducing NRa in this site, since the photosynthetic rates of the plants are fundamental for the increase in the activity of the enzyme (Hunter and Ruffner, 1997). Souza et al. (2016) observed in the 'Palmer' mango that at the flowering phase there was a decrease in the content of total soluble sugars, reducing sugars, total proteins, and in the content of sucrose, and inferred that this may have occurred due to the energy demand for the formation of inflorescences, source/drain ratio.

As previously demonstrated, throughout the evaluated phenophases, in general, there was a reduction in NRa, and such a fact can be better observed in the fruiting phase, in which it is inferred that as a function of the growing N demand, the remobilization of foliar N to fruit development continues in this stage and, as a consequence, there is a decrease of this element in the plant organs evaluated in this study. On the other hand, the degradation of foliar proteins generates a greater number of amino acids that act as regulators, decreasing NRa probably through the action inhibition of NO_3^- transporters in the cell membrane, since circulating amino acids in the phloem can control the NO_3^- absorption rate by the roots (Imsande and Touraine, 1994). This fact can justify the lower NRA activity in 1st flush leaves comparing with 2nd flush leaves, whereas those will always be older than these, and, probably with superior degradation of proteins.

In the roots, there is little variation in the activity of the enzyme between the phases of floral induction, flowering, and fruiting (Fig. 2). It is suggested that this may have occurred due to the soil application of nitrate calcium, performed when the plants presented a defined flowering and early green fruits, acting as an available substrate for the maintenance of NRa in the roots and increase in the shoot part during these phenophases, with the 2nd flush leaves being the preferential site for the reduction and assimilation of NO_3^- in the mango crop, in these phenological stages.

Another factor that may have contributed to maintaining nitrate reductase activity in the shoot part at the fruiting phase was the soil fertilization with potassium sulfate and potassium chloride, performed in the orchard during the fruit development stage. According to Ruiz and Romero (2002), the application of K-based fertilizers facilitates the capture and transportation of NO_3^- to the shoot part, since this element is an accompanying cation of NO_3^- in the xylem (Blevins, 1985).

By performing a general NRa analysis, an opposed behavior could be verified between the vegetative and reproductive phases when the contribution of each monitored organs was grouped (Fig. 3). In the vegetative phase, it is possible to evaluate the consequence of pruning, in which there is no compensation of enzyme activity by the 1st flush leaves and roots as a function of the elimination of the 2nd flush leaves. Regarding the increase in NRa from 32 days after PBZ application, it seems to have occurred as a function of the supply with a potassium source, which favors the absorption and transportation of NO_3^- in the plant and, consequently, the activity of the enzyme. The reproductive phase coincided with the foliar and fertigation application of a nitric fertilizer, in which the enzyme activity begins to decrease from flowering, verifying, at the end of fruiting, a similar

level to that observed at the beginning of the cycle, that is, at pre-pruning (Fig. 3).

Nutrition can affect the yield of the mango crop. Nutrient absorption by the crop varies as a function of the age and phenological stage of the plant. For this reason, it is important to know the dynamic of nutrients in the several parts of the plant, throughout cultivation, aiming to provide subsidies to better adequate fertilization programs for the crop (Ordóñez, 2011).

Conclusions

Nitrate reductase activity in 'Palmer' mango plants is influenced by environmental factors, especially solar radiation, and potassium/nitrate spraying.

To obtain a maximum nitrate reductase activity in the leaf and root tissues, the collection of the vegetal material around 10 a.m. is recommended.

2nd flush leaves and roots constitute the main activity sites, whereas in 1st flush leaves there is minimum variation in the nitrate reductase enzyme.

The enzyme activity is lower in the vegetative phase than in the reproductive phase.

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Conflict of interest

On behalf of all authors, the corresponding author declares that there is no financial and personal relationships that could inappropriately have influenced our work.

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ORCID
Alana Juliete da Silva Santos https://orcid.org/0000-0001-9725-9120
Vespasiano Borges de Paiva Neto https://orcid.org/0000-0003-3347-7043
Luciana Guimarães Sanches https://orcid.org/0000-0003-2319-009X
Daniel de Almeida Carreiro https://orcid.org/0000-0003-1048-7038
Maria Poliana Martins Pereira https://orcid.org/0000-0001-8744-1930
M.C. Rezende Zuffo-Borges https://orcid.org/0000-0002-1013-4710
Stefany E. Rodrigues dos Santos https://orcid.org/0000-0003-2700-0337
Ítalo Herbert Lucena Cavalcante https://orcid.org/0000-0003-1610-1546

Address of the corresponding author
Department of Agricultural Engineering (CCA), Universidade Federal do Vale do São Francisco, Petrolina-PE, Brazil.
E-mail: vespasiano.paiva@univasf.edu.br