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Humic acids induce the expression of nitrate transporters in passion-fruit seedlings

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Abstract: The management of organic fertilization in passion fruit has been carried out empirically based on the responses of the crops and the experience of farmers. Knowledge of the physiological responses of plants to organic fertilizers is essential to optimize fertilization programs. The objective of this study was to evaluate the differential expression of genes coding for nitrate transporters as well as plasma membrane H^+-ATPase in passion fruit seedlings treated with different organic fertilizers in the presence or not of humic acids. The equivalent of one gram of total nitrogen dissolved in water from cattle manure, castor oil cake and urea were added and the seedlings were treated or not with a suspension of humic acids (2 mmol/L). Differential expression of low and high affinity nitrate transporters (PeNRT2.2 and PeNRT1.1 PeNRT2.2e) and H^+-ATPase (PeMha) was performed by qRT-PCR. The use of cattle manure increased the differential expression of the high affinity transporter regarding to the control. The application of humic acids amplified the increase observed in the differential transcription of the high-affinity transporter with manure and urea fertilization, with no effect observed with the use of castor oil cake. Humic acids induced transcription of the gene encoding H^+-ATPases in all treatments compared to control. The application of biofertilizer based on humic acids in low concentrations directly on the plant can be used with the objective of optimizing the organic fertilization of passion fruit with cattle manure and urea.

Index Terms: organic fertilizers, physiological responses, NRT genes, H+-ATPase.

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Ácidos húmicos induzem a expressão dos transportadores de nitrato em mudas de maracujazeiro

Resumo: O manejo da adubação orgânica no maracujazeiro tem sido realizado de forma empírica, baseado nas respostas das lavouras e na experiência do produtor. O conhecimento das respostas fisiológicas da planta à adubação orgânica é fundamental para otimizar os programas de fertilização. O objetivo deste trabalho foi avaliar a expressão diferencial dos transportadores de nitrato bem como da H^+-ATPase de membrana plasmática, em mudas de maracujazeiro adubadas com diferentes fertilizantes orgânicos, na presença ou não de ácidos húmicos. Foram adicionados o equivalente a um grama de nitrogênio total dissolvido em água proveniente de esterco de curral, torta de mamona e ureia, e as mudas foram tratadas ou não com uma suspensão de ácidos húmicos (2 mmol/L). A expressão diferencial dos transportadores de nitrato, de baixa e de alta afinidade PeNRT2.2) e da H^+-ATPase, foi realizada por qRT-PCR. O uso de esterco de curral aumentou a expressão diferencial do transportador de alta afinidade em relação ao controle. A aplicação de ácidos húmicos ampliou o aumento observado na transcrição diferencial do transportador de alta afinidade com a adubação com esterco e também com ureia, não sendo observado qualquer efeito com o uso da torta de mamona. Os ácidos húmicos induziram a transcrição do gene que codifica a H^+-ATPases em todos os tratamentos, em relação ao controle. A aplicação de ácidos húmicos em baixas concentrações, diretamente sobre a planta, pode ser utilizada com o objetivo de otimizar a adubação orgânica do maracujazeiro quando realizada com esterco de curral e também com ureia.

Termos de Indexação: NTD, Fertilização orgânica, Genes NRT, ATPase.

Introduction

Brazil is the largest producer of passion fruit in the world. Brazilian production in 2019 reached 593,429 tons with an average productivity of 14 t/ha (IBGE, 2019). The organic fruit market is expanding and may become an attractive alternative for the passion fruit growers. The change from conventional production system to organic requires adaptation known as the transition period in which mineral nutrition with soluble and industrialized sources is replaced by the cycling of different sources of organic matter. This adaptation period is critical for the nutrients required in large quantities.

Nitrogen (N) is the nutrient most required by passion fruit (MATTAR et al., 2018). Organic fertilization for passion fruit follows the same criteria as conventional fertilization, in which the need of N to be applied is based on the amount extracted by plants and the expected productivity (BORGES, 2021). However, the efficiency of N fertilization from organic sources is influenced by several factors including the ready availability of N, the conditions that affect the mineralization process and the efficiency of uptake of mineralized N.

Nitrate (NO_3^-) is the principal source of N for the majority of plants (FERNANDES, 2006), including passion fruit. The uptake of NO_3^- occurs by an active process against electrochemical potential by 2:1 symport system, with simultaneous transport of 2 H^+ and 1 NO_3^- (FERNANDES; ROSSIELO, 1995) carried out by transmembrane transporters. NO_3^- transporters are classified in two types: high affinity transporters (HAT) that operate at low NO_3^- concentrations (from 0.2 mM to 1 mM) and low affinity transporters (LAT) that operate at concentrations above 1 mM of NO_3^-.

The NRT2 and NRT1 gene families (ASLAM et al., 1993) encode HAT and LAT, respectively. In addition to NO_3^- concentration in solution, other factors can influence the activity of the transporters including the presence of humified organic matter (QUAGGIOTTI et al., 2004; TAVARES et al., 2017).

Humic substances (HS) have been defined as a heterogeneous assembly of relatively small molecules held in a dynamic supramolecular arrangement by weak (non-covalent) interactions of a predominantly hydrophobic nature and by hydrogen bonds (PICCOLO, 2001). HS are very reactive chemically and, when used directly on the plant at low concentrations, regulate many physiological processes including nutrient uptake (CANELLAS et al., 2002; CANELLAS; OLIVARES, 2014).

HS effects on NO_3^- uptake and assimilation in different plants have already been reported in the scientific literature. The influence of humic acids (HA) on NO_3^- uptake in oat seedlings was studied by Dell'Anola and Nardi (1987), Nardi et al., (1991); Piccolo et al., (1992). The activity of NO_3^- assimilation-related enzymes was also monitored in different SH-treated plants such as barley, cucumber, oilseed rape, lettuce and maize (ALBUZIO et al., 1986; MORA et al., 2010; JANNIN et al., 2012; HAGHIGHI, 2012; VACCARO et al., 2015).

The induction of differential expression of high- and low-affinity transporters in maize (QUAGGIOTTI et al., 2004; ZANIN et al., 2018; AZEVEDO et al., 2019) and rice (TAVARES et al., 2017) by HS had been described. However, in longer cycle plants the studies are scarcer. A possible effect of SH on the transcription of genes involved in the uptake mechanism such as H^+-ATPases and NO_3^- transporters in passion fruit may contribute to the optimization of organic fertilization and assist in the transition process. The objective of this study was to evaluate the differential transcription of genes PeNRT2. 2 and PeNRT1.1 and PeMHA in passion fruit plants, cultivated in greenhouse using the superficial layer of an Oxisol submitted to nitrogen fertilization with different organic fertilizers in the presence or not of humic acids isolated from vermicompost.

Materials and Methods

The seedlings used were obtained from seeds of passion fruit hybrid H09-110/111 developed by EMBRAPA. The seedlings were grown in 290 cm^3 tubs with commercial Basaplant® substrate for 120 days. Before transplanting the seedlings into pots, the roots were washed in running water to remove the commercial substrate.

The soil used in the experiment is classified as Oxisol according US Soil Survey and typical "*Latossolo Amarelo distrocoeso*" according to the Brazilian Soil Classification System (SANTOS et al, 2006) with the following characteristics: pH= 4.6; C= 10.4 g/kg; N=1.1 g/kg; C:N= 9.54; total dissolved nitrogen (TDN)= 0.05 g/kg; dissolved organic carbon (DOC)= 0.49 g/kg; organic matter (OM) = 20.10 g dm^{-3}; P= 4.45 mg dm^{-3}, Al^{+3}= 0.10 $cmol_c$ dm^{-3}; H+Al= 3.18 $cmol_c$ dm^{-3}; Ca= 0.80 $cmol_c$ dm^{-3}; Mg=1.20 cmolc dm^{-3}; Sum of bases(SB) = 2.11 $cmol_c$ dm^{-3}; Saturation of bases (V)= 39,89 (%); Saturation by Al^{3+} (m)= 4,52 %; Cation Exchange Capacity (CEC)= 5.29 $cmol_c$ dm^{-3}. The soil collection was performed in the superficial layer (0-0.2 m) in the locality of Lagoa de Cima, Campos dos Goytacazes-State of Rio de Janeiro, Brazil, 21°44'24.6 "S 41°32'07.8 "W, in three distinct points of the same farm plot.

The experiment was conducted in an entirely randomized design, with a combination of three fertilizers (urea, cattle manure and castor bean cake), with and without the application of HA, humic acids (2 mmol of C/L) (CANELLAS et al., 2002). The treatments were: T1- Control; T2- Urea without HA; T3- Urea with HA; T4- Castor cake without HA; T5- Castor cake with HA; T6- Cattle Manure without HA; T7- Cattle Manure with HA. Once the TDN content in each of the fertilizers was quantified, 1g of TDN, of each fertilizer, was added per kg of soil. The characteristics of the fertilizers are described in Table 1.

Table 1 - Characteristics of organic fertilizers used in the experiment.

Fertilizers	pH	C (g/kg)	N (g/kg)	C:N	TDN (g/kg)	DOC (g/kg)
Catle manure	7.24	106	10.9	9.72	1.37	3.13
Castor bean cake	7.84	480.9	44.7	10.75	27.07	57.19

TDN and DOC were quantified after extraction in water (1:20; v:v) followed by filtration (0.45 µm) (CHANTIGNY et al., 2014) using the Shimadzu Total Organic Carbon TOC-L CSH / CSN analyzer equipped with an ASI-L autosampler (Shimadzu, Tokyo, Japan).

The HA were isolated from vermicompost produced with cattle manure using NaOH 0.1 mol L^{-1} for 4 h followed by centrifugation (3000 ×*g*) (CANELLAS et al., 2002). The extraction procedure was repeated until the extracts presented absorbance near zero at 465 nm. The separation of the HA from the alkaline extract was obtained by acidification to pH 1 with 6 mol L^{-1} HCl. The dissolution and precipitation was repeated three times. After centrifugation, the HA fraction was washed with water until negative test with $AgNO_3$ and dialyzed on membrane with pore size smaller than 1 kDa (Spectrapor, USA) and lyophilized. The elemental composition of HA was obtained in a CHN analyzer (Perkin-Elmer 1483; Perkin-Elmer, Norwalk, CT, USA) and the ash content obtained after incineration of the sample in a muffle furnace at 750 °C for 8 h. The molecular characterization of HA was performed by solid-state ^{13}CNMR spectroscopy cross-polarization magic angle spinning (CP/MAS) with a Bruker AVANCE 300 NMR spectrometer equipped with a 4-mm-wide bore MAS probe, operating at a ^{13}C resonating frequency of 75.475 MHz. Four thousand scans were collected over an acquisition time of 25 ms, and a recycle delay of 2.0 s. All the free induction decays (FID) was transformed by applying a 4K zero filling and a line broadening of 100 Hz spectra were integrated into the chemical shift resonance intervals (ppm): 187-162 (carbonyls of ketones, quinines, aldehydes and carboxyls), 162-112 (aromatic and olefinic carbons), 112-93 (anomeric carbons), 93-46 (C-O systems, such as alcohols and ethers, C-N groups and aliphatic carbon complexes), and 46-0 ppm (sp^3 carbon, mainly methylene and methyl). The hydrophobic C/C hydrophilic ratio of HA was estimated by a dimensionless structural index calculation derived from the relative C distribution of NMR spectra: the combined relative areas of C-alkyl (46-0 ppm) and aromatic components (162-112 ppm), as representative of hydrophobic C (HB) were compared with the areas in intervals related to polar groups (187-162pp, 112-46 ppm) indicating the proportion of hydrophilic carbon (HI) and calculated the hydrophobicity index.

The application of HA (2 mmol and C/L) in the treatments was at 7, 11 and 14 days after planting with 100 mL of solution, during the morning period, directly to the substrate around the roots. In the treatments without HA and in the control, 100 mL of water was applied. The plants were not irrigated on the days of the treatments application. At 15 days after transplanting the seedlings, three plants of each treatment were collected for analysis of NRT expression and MHA genes. When collected, the roots were washed in running water to remove the substrate and then with Tween 20 before extraction of the RNA for analysis of gene expression by qRT-PCR.

The sequences of genes NRT 1.1; NRT 2.2; MHA. ERS and TUBβ from different plant species were compared with the information from the genome of *Passiflora edulis* L, using the BLAST tool of the NCBI database to verify which sequences could generate significant alignment with the base pair sequences provided. After researching the homology between the studied species and the genes of interest, the possible oligonucleotide sequences susceptible of expression were established with the aid of three primer designer programs: Primer3Plus, NCBI Primer-Blast and OligoExplorer. The data generated

in each software were crossed with each other to define the primers with the best characteristics. The selected primer pairs were submitted to the verification of possible dimers and folding using the NetPrimer program (*http://www.premierbiosoft.com/NetPrimer/AnalyzePrimerServlet*).

Specialized supplier with lyophilized formulation produced the designed sequences. The primers were then diluted in TE buffer solution as instructed by the supplier, and then bench tests were applied before performing RT-qPCR. The bench tests included the following steps: primer dilution to 100 µM (stock solution); primer dilution to 10 µM (working solution); ringing temperature and amplification checks (tested in conventional PCR and agarose gel); primer dilution test (tested in RT-qPCR). The design of the primers are described in Table 2.

RNA extraction was performed in the Molecular Biology room of the Laboratory of Cell and Tissue Biology - LBCT of Universidade Estadual do Norte Fluminense Darcy Ribeiro. The RNA of the roots of passion fruit plants was isolated following the procedures of the extraction protocol with TRIZOL® (BAÍA et al., 2020). The cDNA synthesis was performed using the SuperScript™ III Reverse Transcriptase kit (Invitrogen). The reaction for RT-qPCR was performed 7.5 µL of SYBR Green PCR Master Mix, 5 µL of H_2O for PCR, 0.75 µL of reverse primer, 0.75 forward primer and 1 µL of cDNA 1.0 µL of template cDNA at dilution of 1: 8 in a final reaction volume of 15 µL, RT-qPCR was performed in the Step One Fast Real Timer PCR thermocycler (Applied Biosystems) with the following conditions: 2 minutes at 95°C, followed by 40 cycles of 20 seconds at 95°C and 30 seconds at 58°C. Three technical replicates as well as three independent cDNA syntheses were used in all RT-qPCR assays. The differential expression of passion fruit genes was evaluated by the 2-ΔΔCq (Livak) method for relative gene expression analysis (LIVAK; SCHMITTGEN, 2001).

At 60 days after the treatments application, a collection of plants was performed for the evaluation of seedling height, dry mass of the aerial part and roots, root volume and root: aerial part ratio. The evaluation of plant growth was submitted to analysis of variance (ANOVA) in GraphPad Prism 7 program and the means were compared by Student's t, $p \leq 0.05$. The R-Studio program was used for orthogonal contrasts and F test that compared contrasts are presented in Table 3.

Table 2 - Primers used in the experiment.

GENE	GenBank	Function	Fowerd (F) e Reverse (R) (5 '→ 3')	size	TEMP
PeMHA (1)	MUZT01105592.1	Plasma membrabe H+ ATPase	TACCAGTTGCCGTCAGAATC GCTCGCTCAAACATCACAG	95	61.1
PeNRT2.2	MUZT01123395.1	high affinity nitrate transporter	AAGACAGCGGCAATGATACC GTCTGATGACTTACGGTTCTCTG	84	62.4
PeNRT1.1 N2	MUZT01131525.1	Low affinity nitrate transporter	CCCTCAGTTTTTCTTTGTCG CTGTGCTCATAGTCTTCATTCC	104	59.7
PeTubB	AC278210.1	control	TCTTCCCACTAGCACAACTC ATGACCAAGCAACCAGTAAG	82	57.8
PeERS	AAX84670	control	TTATTTCTGACCAAGGGAGC CCATCTCCCTGTCAAGTTC	95	58

Table 3 - Orthogonal contrasts used in the experiment.

Contrast	Description
C1	Control vs Urea, Urea+HA, CC, CC+AH, CM, CM+HA
C2	Urea, CC, CM vs Urea+HA, CC+HA, CM+HA
C3	Urea vs Urea+HA
C4	CC vs CC+HA
C5	CM vs CM +HS

Results

HA characterization

The elemental composition analysis of the HA revealed the presence of 470 g kg^{-1} of C, 55 g kg^{-1} of N, 451 g kg^{-1} of O and 5 g kg^{-1} ash. The CP/MAS ^{13}C NMR spectrum is showed in Figure 1. Resonance signals were observed at chemical shifts (d^{13}_{C}) typical of alkyl groups (0-45 ppm), associated with the presence of aliphatic chains (-CH_2- groups) belonging to various lipid compounds, such as fatty acids, vegetable waxes and biopolyesters. Sharp signals centered at 56 ppm are typical of the methoxyl groups presence on aromatic rings of guaiacyl and syringyl units in lignin components, as well as C-N bonds in amino acids. The different ^{13}C signals between 60-110 ppm are typical C-O-alkyl assigned to monomeric units of polysaccharide chains such as cellulose and hemicellulose from plant tissue. The intense signals around 73 ppm are formed by the overlapping resonances of carbon 2, 3 and 5 in the pyranoside structure. The shoulders at 82 ppm are derived from carbon 4 involved in the glycosidic bond with the more unprotected di-O-alkyl anomeric carbon centered at 105 ppm in glucose units. The resonance signals along the aryl-C interval (116-140 ppm) involve unsubstituted and C-substituted units of different aromatic components, while the signals shown in the phenolic region (140-160 ppm) are indicative of O-containing carbon 3, 4 and 5 in aromatic ring of lignin derivatives, with carbons 3 and 5 being coupled to the methoxyl substituent. Finally, the sharp signal at 174 ppm includes all the carbonyl and carboxyl groups present in the sample and mainly responsible for the exchangeable acidity.

Differential genes expression

Figure 2 shows the results of the differential expression of low (Figure 2 A) and high (Figure 2 B) affinity NO_3^- transporters in passion-fruit seedlings. As expected, due to the high concentration of N added to the soil (1 g TDN kg^{-1} soil), differences were observed in the expression of the LAT that was repressed with the addition of the fertilizers (Fig 2 A).

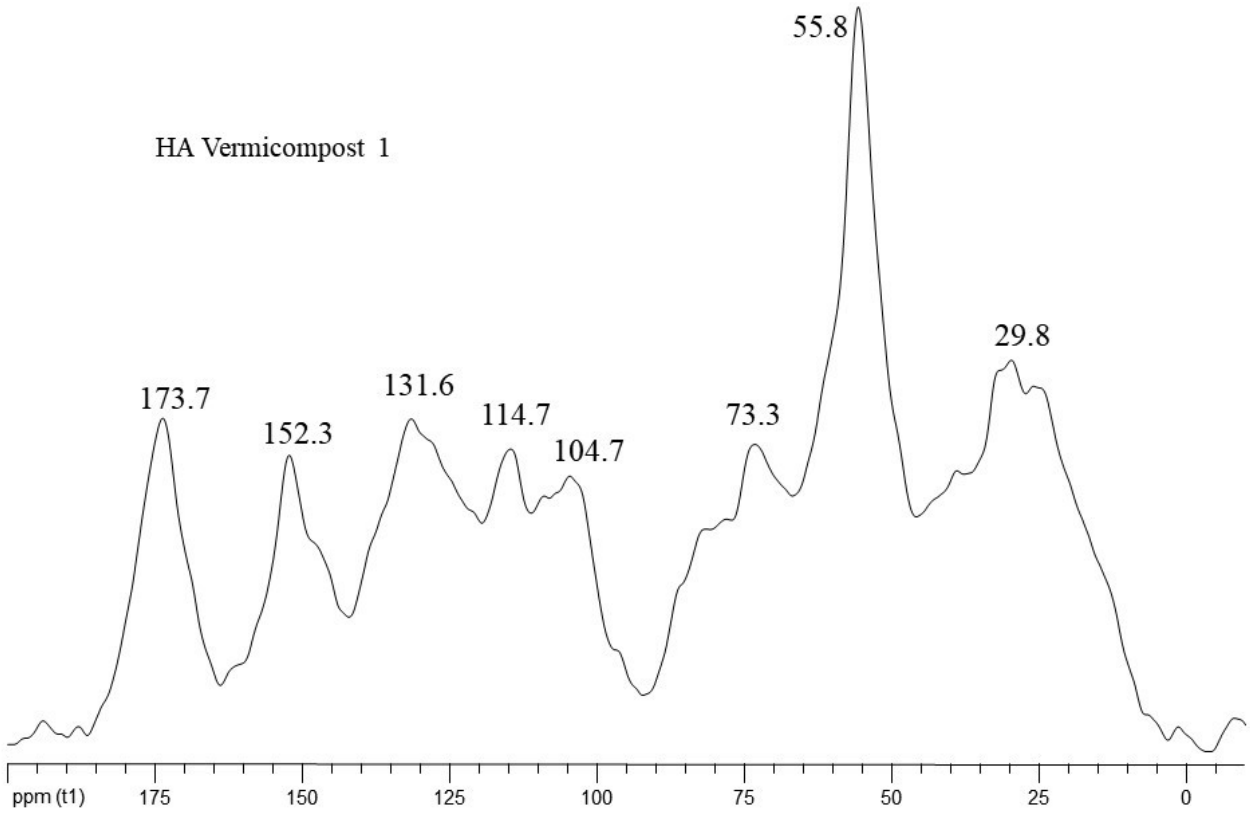

Figure 1 - ^{13}C NMR spectrum of humic acids isolate from vermicompost.

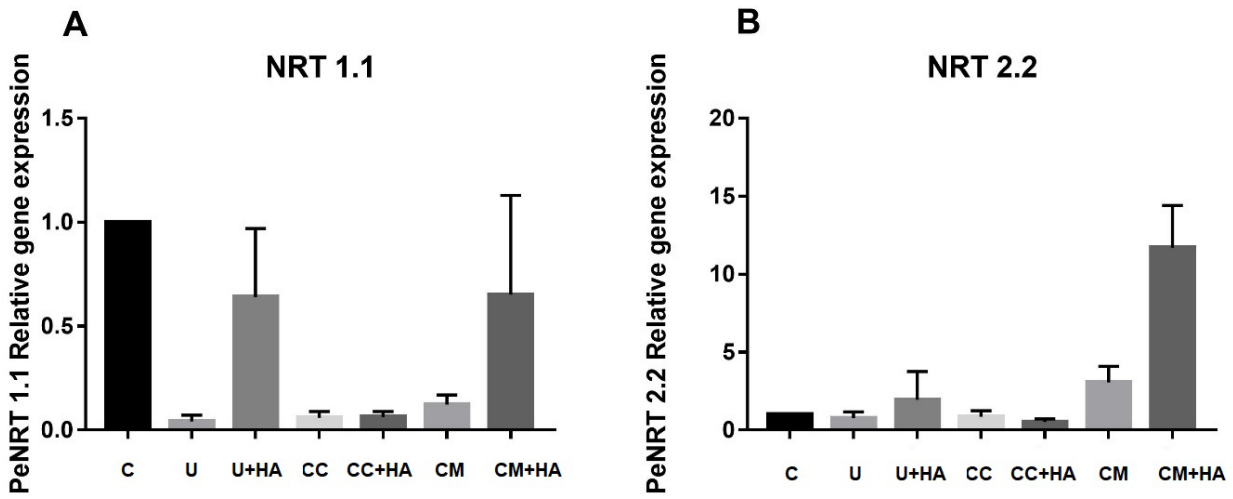

Figure 2 - Relative expression of PeNRT 1.1 (A) and PeNRT 2.2 (B) genes in roots of passion fruit seedlings. C: control; U: urea; CC: castor oil cake; CM: cattle manure; HA: humic acids

The application of HA decreased the inhibition of NRT1.1 expression in the urea and manure fertilization. HA had no effect on the inhibition of the expression of the low-activity NO_3^- transporter with castor cake fertilization. High-affinity transporters (NRT2.2) were induced by cattle manure compared to control and by urea and cattle manure in the presence of HA compared to control (Fig 2 B).

Figure 3 showed the differential expression of PeMHA gene that encodes different isoforms of plasma membrane H^+-ATPases. It was observed that all fertilizers promoted the expression of Mha compared to the control. The stimulation was higher in the presence of HA (Figure 3).

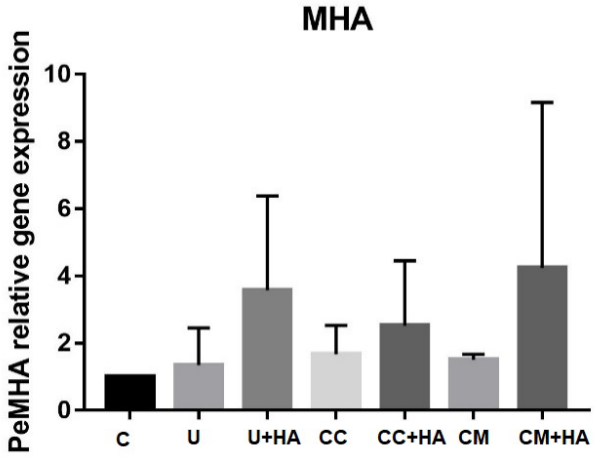

Figure 3 - Relative expression of PeMHA genes in roots of passion fruit seedlings. C: control; U: urea; CC: castor oil cake; CM: cattle manure; HA: humic acids

Treatments with HA application (U+HA, CC+HA and CM+HA), positively influenced the expression of genes compared to the treatments without HA, with 166%, 47% and 179% increase, respectively, compared to the controls without HA application.

Plant biomass

Figure 04 shows the biometric results for passion fruit plants at 60 days after planting. A significant difference was observed among the treatments evaluated in all variables (Figure 04: A, B, C, D).

The plant height (PH) at 60 days, showed significant difference from the treatments applications. All treatments with fertilizer application, with and without HA, promoted significant increase in PH at 60 days compared to the control (Figure 4 A). The plants with CC and CC+HA applications obtained significantly higher averages compared to the other treatments, and the control. HA application promoted a significant increase in CM+AH, compared to CM, with an average 18% higher with HA application. HA application did not significantly influence PH at 60 days in the other treatments.

HA application significantly influenced shoot dry weight (Figure 4 B) of passion fruit plants. All treatments with HA application, independent of the fertilizer applied, increased the shoot dry weight of the plants at 60 days.

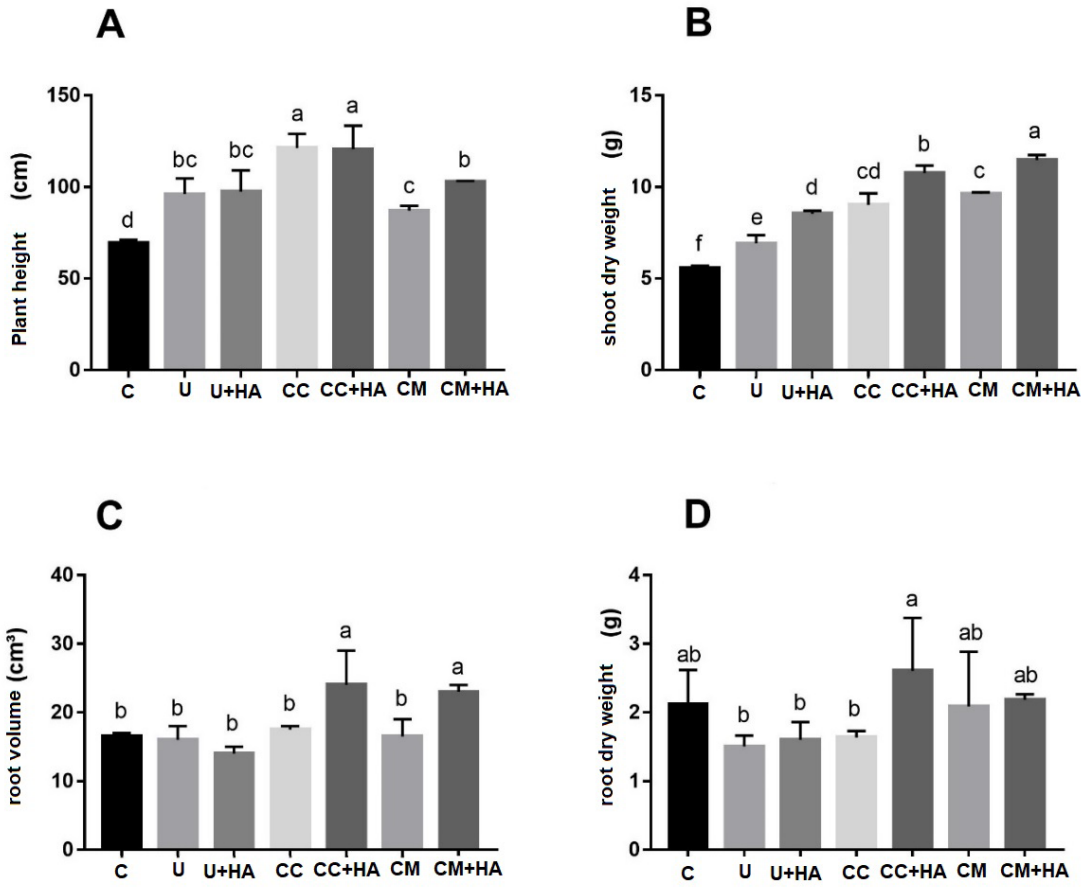

Figure 4 - Height (cm), shoot dry mass (g), root volume (cm³) and root dry mass (g) of passion fruit plants at 60 days after planting. Averages followed by the same letter do not differ statistically, by t test (p <0.05). C: control; U: urea; CC: castor oil cake; CM: cattle manure; HA: humic acids.

The root volume showed significant difference among the treatments in 60 days (Figures 4 C). Higher means were observed for plants in the treatments that received HA application (CC+AH and CM+AH), compared to the treatments without HA and the control. However, no significant difference was observed for the treatment with U+HA, and no significant difference of fertilizer application on root volume was observed.

No significant effect of HA application was observed for root dry weight at 60 days, nor the significant effect of fertilizer application compared to the control. Plants treated with CC+AH showed higher means for root dry weight at 60 days, in comparison, with plants treated with CC, Urea, U+HA. As showed in table 4, only regarding to the shoot dry weight a significant effect can be observed in the orthogonal contrasts evaluated. Regarding to plant height at 60 days, the significant effect of the treatments application compared to the control can be observed.

Discussion

The meta-analysis gathering different experiments with plant stimulation by HS performed by ROSE et al., (2014) showed an average increase of 20% in biomass production of both roots and shoots of plants different types. This increase is close to that observed in passion fruit in this experiment (18%). Plant growth promotion by HS is related to the source of isolation, the application mode, and the plants type and age (NARDI et al., 2021). The HA used in the stimulation of passion fruit presented chemical characteristics typical of humified organic matter with high bioactivity (Figure 1), such as the presence of methoxy groups, substituted aromatics and

Table 4 - F values and contrasts among treatments.

Contrasts	Plant height (cm)	shoot dry weight (g)	rppt dry weight (g)	root volume (cm^3)
C1	71.79*	363.93 *	0.55 ns	1.58 ns
C2	2.47 ns	86.54 *	3.37 ns	24.78 ns
C3	0.04 ns	35.73 *	0.10 ns	1.91 ns
C4	0.02 ns	25.55 *	5.52 ns	10.28 ns
C5	7.16 ns	25.81 *	0.27 ns	13.02 ns
Total	19.75*	112.76 *	1.61 ns	10.32 ns

ns = no significant; ** significant (p<1%); * significant (p<5%)

carboxylic acids at 56, 130 to 150 and 174 ppm as observed in the ^{13}C-CP/MAS NMR spectrum (Figure 1) (CANELLAS et al., 2012; AGUIAR et al., 2013, AQUINO et al., 2019).

One of the main mechanisms related to the increase in production provided by the use of soluble HS directly on plants is related to the increased efficiency of nutrient uptake (DU JARDIN, 2015; CANELLAS et al., 2015). The H^+ gradient generated by the activity of H^+-ATPase regulates the mechanism of ion uptake by energizing the active transport through transporters and/or controlling the opening and closing of channels responsible for the passive ions transport (FERNANDES; SOUZA, 2006). The main role of plasma membrane H^+-ATPase in plant physiology is to activate the secondary transport of ions (SONDERGARD et al., 2004). This enzyme functions as a proton (H^+) pump activated by the hydrolysis of ATP, it is responsible for the primary transport of H^+ from the interior of the cell to the apoplasm and, consequently, for the formation of the H^+ gradient generated across the plasma membrane. This H^+ gradient energizes the secondary transport of ions and other metabolites against a concentration gradient. NO_3^- needs to be actively transported to the cell interior, where they are more concentrated. For this, there are several NO_3^- transporter proteins in the plasma membrane capable of coupling the dissipation of the electrical and, or, chemical component of the H^+ gradient generated by the pumps to the co-transport of H^+ from NO_3^-. One of the phenomena that have been most related to the HS bioactivity corresponds to the activation of plasma membrane H^+-ATPase (ZANDONADI et al., 2016) associated with the action of auxin, a plant hormone that activates the H^+-ATPase by several mechanisms, among them the induction of H^+-ATPase synthesis modulated by Mha1 and Mha2 genes (FRIAS et al., 1996). These genes have been induced by HA isolated from several sources and in several plants (CANELLAS et al., 2002; QUAGGIOTI et al., 2004; TAVARES et al., 2017; TREVISAN et al., 2011; AZEVEDO et al., 2019).

The increase in the concentration of macronutrients (CAVALCANTE et al., 2012) and micronutrients (CAVALCANTE et al., 2008) in passion fruit plants treated with HS has been observed previously. As well as the significant improvement in the general nutritional state of passion fruit trees treated with biofertilizers (SILVA JÚNIOR et al., 2013). The maintenance of production levels of passion fruit with a smaller amount of N-urea in the presence of HS has also been observed (SILVA et al., 2016) without significant changes in the industrial quality of the fruit (SILVA et al., 2015). Therefore, the effect of HS on the activity of the H^+-ATPases is compatible with the results of improvement in the nutritional state of passion fruit observed previously.

The study of N uptake and transporters in HS-treated plants is normally carried out using inorganic N sources, such as NH_4^+ or NO_3^- or chemically synthesized organic N as N-urea (QUAGGIOTI et al., 2004). Studies using organic matter as a source of N are scarcer, but have a relevant role for organic agriculture and fruit growing. Organic passion fruit pro-

duction plays an important role in generating employment and income and the success of the activity depends partly on the management of organic fertilizers. In this study it was observed that manure increases the differential expression of high affinity NO_3^- transporters (NTR2.2) and that the application of HA amplifies this response not only for manure, but also for urea but without any effect for castor cake This effect can be justified in part by differences in the organic matter of each fertilizer (CARNIER et al., 2019) directly influencing the concentration of NO_3^- in the medium and consequently the expression of transporter genes (ASLAM et al., 1993).

The activation of proton pumps and the induction of high affinity NO_3^- transporters was promoted in passion fruit roots with the addition of manure and N-urea and significantly amplified with the treatment with soluble HA (Figure 3).

Plant growth analysis carried out at 60 days after the treatments revealed that CC and CC+HA significantly promoted the increase in the dry mass of the shoot and root volume. The release of TDN in CC is different from that of manure and urea, which readily release N with a linear decrease in availability. On the contrary, CC provides TDN to the soil solution in increasing quantities as a function of time. The castor oil plant cake has a high quantity of organic N in its composition, making a good quantity of nutrients available to the plants throughout the experiment.

Conclusion

The application of humic acids promoted the expression of PeNRT2.2 and PeMHA genes indicating the possibility of improving the efficiency of nitrogen absorption in the initial growth phase of passion fruit fertilized with organic fertilizers.

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