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Effect of Arbuscular Mycorrhizal Fungi Combined with Exogenous Calcium on Papaya (*Carica papaya* L.) Plant Growth

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Authors' contributions

This work was carried out in collaboration among all authors. Author AFM designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. Author ELMN and CNT managed the analyses of the study. Author CNT managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

The pawpaw (*Carica papaya* L.) is a very productive fruit tree but is not widely exploited on the international market due to rapid deterioration of the fruits after harvest. This deterioration is due to a nutritional dysfunction related to the deficiency of nutrients, especially calcium, which is involved in the firmness of fruits in general and the papaya in particular. The symbiotic capacity of arbuscular mycorrhizal fungi (AMF) has been profiled to enhance uptake and regulation of exchangeable calcium ions in the soil. The aim of this study was to reduce losses and improve post-harvest quality of papaya fruits through the potential of arbuscular mycorrhizal fungi (AMF) in calcium uptake for international exploitation. A composite of AMF species identified of papaya rhizosphere in two study sites, combined with different calcium doses (0 µM, 100 µM, 200 µM, 300 µM and 1000 µM) was applied to two papaya varieties (calcium deficiency-resistant V1 *Calina papaya* IPB9 and calcium deficiency-sensitive V2 *Solo* N°8) and the effect was evaluated in greenhouse**.** The frequency and intensity of root colonization were evaluated. The AMF/calcium 1000 µM combination significantly influenced (P = .05) the number of plant height (44%, 68%), leaves (71%; 122%), fresh biomass of leaves (3,3%; 596.3%), roots (159.2%, 265.3%), and stems (910%;4075%) in both varieties V1 and V2 respectively compared to AMF/ calcium 0 µM. Increased absorption of calcium ions (150%) and phytochemicals were recorded in the leaves. The frequency (81%; 85%) and intensity (15%; 21%) of root colonization by AMF respectively was not significantly different (P>.05) in both two varieties (V1 and V2). However, the presence of AMF spores was observed on the roots of papaya plants mycorrhized with the calcium 1000 µM. Inoculation with AMF combined with the high calcium dose (1000 µM) promoted mycorrhizal infectivity of roots, colonization of mycorrhizal spores in the soil and increased roots absorption surface area.

Keywords: Arbuscular mycorrhizal fungi; calcium deficiency; Carica papaya L.

1. INTRODUCTION

Papaya is a very important fruit species from an economic point of view, providing a fairly considerable income per hectare alongside banana (Alara et al., 2020). It has very high nutritional and nutraceutical properties (Santana et al., 2019). The fruit is an important source of vitamin A, ascorbic acid, minerals (iron, calcium, potassium, etc.), polysaccharides and proteins (Daagema et al., 2020). The *Carica* genus comprises some 35 species, of which only papaya is cultivated for its edible fruits (Badillo, 2002). It is rich in phytochemical compounds like most tropical fruit pericarps (Santana et al., 2019) (and in various enzymatic compounds such as hymopapain, caricain, chymopapain, glycine endopeptidase, papain etc. Worldwide papaya production is estimated at around 13,708,400 tonnes/year (FAOSTAT, 2022). In Africa, Nigeria is the leading producer with 951,000 tonnes/year, followed by the Democratic Republic of Congo (DRC) with 220,480 tonnes/year (FAOSTAT, 2022). In Cameroon, papaya is grown mainly in rainforests with monomodal rainfall, more precisely in the Littoral zone (Njombe-Pendja and Loum), where ecological conditions are ideal (tropical climate, altitude 20 to 500 m, average temperature 30°C, rainfall 2,350 mm, volcanic soil). National production is estimated at around 700 tonnes/year. Exports are still very low (36 tonnes/year), mainly in dried forms. This low production, despite soaring demand on the international market (17,519 tonnes/year) which far exceeds national production (36 tonnes/year), is linked to a number of biotic and abiotic constraints. These include rapid ripening of the fruit (Zhu et al., 2020), lack of control over the right stage for harvesting the fruit (Greenwald et al., 1998), post-harvest handling (Elik et al., 2019), susceptibility to stress (hydric), parasitic and fungal diseases (Zhu et al., 2020), and soil mineral nutrition such as calcium (Hocking et al., 2016). In Cameroon, depending on the variety, some highly productive papaya trees unfortunately suffer from increased post-harvest dieback, affecting production by up to 50%, which is quite considerable for the grower in terms of income (Anonyme, 2020). Many authors (Ramakrisha & Haribabu, 2007; Singh et al., 2012; El Habbash et al., 2015; Paull & Chen, 2014; Kumar & Manivanna, 2011; Ahmad et al., 2006); Asghari & Aghdam, 2010) etc… have proposed two plausible explanations. In the first group, it is claimed to be a pathology caused by *Anthracnose*, *Oidium, Phytophthora, Pythium and Rhizoctonia* (fruit rot), while in the second group, it is a handicap that manifests itself during fruit ripening. Almost 80% of this physiological

explanation is based on the plant's calcium nutrition (Hocking et al., 2016). The post-harvest quality of papaya fruit is affected by a deficiency in exchangeable calcium ion (Ca^{2+}) in the soil solution, which, during the ripening process, causes diseases (viral, fungal and bacterial), softening of the fruit pulp (Rajput et al., 2008) etc... As far as the fruit is concerned, calcium ion deficiency has a major impact on its exploitation on the international market. As the ion is not very mobile in soil solution, it is adsorbed on the clay-humus complex and therefore cannot be absorbed by young papaya roots from the soil and transported to the plant via the xylem to the fruit. This adsorption is linked to nitrogen and potassium fertilization (Hocking et al., 2016), continuous papaya cultivation on the same plots without any crop rotation (Thapliyal, 2025), pressure on agricultural land from the growing human population and agro-industrialization (Li et al., 2006), particularly in Cameroon in the Njombe-Pendja locality. These factors are responsible for disruptions to the structural and functional diversity of soil microbial communities and enzyme synthesis (Constantin, 2011; Amiri et al., 2010; Wang et al., 2010). Fortunately, most plants more specifically papaya, have coped with limited amounts of available calcium via the establishment of symbiotic associations with rhizospheric microorganisms, more precisely arbuscular mycorrhizal fungi (AMF). Throughout the world, most papaya production comes from poorly structured units (home gardens, extensive fields, etc.) where the farmer pays little attention to the diversity of microorganisms found in the rhizosphere. However, knowledge of the activity of these living organisms in the soil, i.e. microbes, roots of living plants, etc., and their functional activities in the regulation, supply and absorption of calcium not only constitutes an important part of total biodiversity (Decaëns et al., 2006), but is also part of the solution to reducing post-harvest losses and a decisive step towards the development of papaya cultivation for international commercial purposes (Marcos et al., 2020). The use of AMF from the papaya rhizosphere (Maffo et al., 2022), combined with calcium, to reduce post-harvest losses of *C. papaya* L. fruit and the production of biological seedlings are still lacking in Cameroon. Developing biological strategies for supplying and regulating calcium through the potential of AMF to reduce post-harvest losses for international exploitation is part of the solution to the problem of post-harvest softening of papaya fruit.

2. MATERIALS AND METHODS

2.1 Study Site

Two study sites were selected for this work. They were geographically separated and one represents the most exploited site of papaya. (Table 1 and Fig. 1). The two sites were located in two different agro-ecological zones: (1) the forest zone with the monomodal rainfall, and (2) the bimodal rain forest zone (Fig.1).

2.2 Preparation of Plant Materials

Seeds from two contrasting papaya varieties, namely the calcium deficiency-sensitive *Solo* N°8 (V2) pure line variety introduced from the Hawaiian Islands in the 1960s and safeguarded over generations by artificial self-fertilization, and the calcium deficiency-resistant *Calina papaya* IPB-9 (V1) variety from Indonesia, are the latest hermaphrodite (bi-sexual) hybrid papaya varieties developed by California Agriculture University, USA in 2013. Seed preparation followed the protocol developed by (Tchio et al., 2013) (modified).

2.3 Preparing Substrate and Nutrient Solution for Watering

The two papaya varieties (*Solo* N°8 and *Calina papaya* IPB-9) were grown in Sanaga sand. The sand was washed with tap water (five washes), autoclaved at 121°C for 1 hour and then left to cool for 24 hours. Approximately 20 kg of sand was then introduced into sterilized 25 kg perforated white bags. A hole about 10 cm deep was made in each bag to allow inoculation and transplanting of the seedlings. Stock solutions for 1000 ml: (g/l) Solution A- $MgSO_4$. $7H_2O$-120.02; Solution B- Ca $(NO_3)_2$. $4H_2O$-238.04; Solution C- KH_2PO_4. $3H_2O$-115.38; Solution D- Micro-elements: (g/l); Fe EDTA-12.500; $MnSO_4$. $4H_2O$-1.121; H_3BO_3-1.421; $(NH_4)6Mo_{24}.4H_2O$-0.093; $ZnSO_4.7H_2O$-0.220; $CuSO_4.5H_2O$-0.198 were prepared. From these stock solutions, the 10 ml spray solution: Stock solution 0 µM $Ca(NO_3)_2$ (10 ml of solution A, 0 ml of solution B, 10 ml of solution C, 10 ml of solution D) ; 100 µM $Ca(NO_3)_2$ (10 ml of solution A, 1 ml of solution B, 10 ml of solution C, 10 ml of solution D) ; 200 µM $Ca(NO_3)_2$ (10 ml of solution A, 2 ml of solution B, 10 ml of solution C, 10 ml of solution D) ; 300 µM $Ca(NO_3)_2$ (10 ml of solution A, 3 ml of solution B, 10 ml of solution C, 10 ml of solution D) ; 1000 µM $Ca(NO_3)_2$ (10 ml of solution A, 10 ml of solution B, 10 ml of solution C, 10 ml of solution D) with different concentrations of calcium (Ca),

supplied in the form of calcium nitrate ($Ca(NO_3)_2$ were prepared. These stock solutions were kept at room temperature. From the day of transplanting, 400 ml of Rorison watering solution was added every two days of the week, depending on the treatment, until harvest. Tap water was added on intermediate days. Morphological growth parameters were measured. Our experiment lasted six (06) months, during which mycorrhizal symbiosis was effective in papaya (Chebet et al., 2020).

Table 1. Characteristics of experimental sites where soil and root samples were obtained for culturing and molecular analyses of arbuscular mycorrhizal fungi

Sampling site	Climatic zone	Coordinates	Altitude (m)	Soil characteristics					
				PH	EC	Org C (%)	N (%)	C: N	P (mg/kg)
Njombe-Pendja	Equatorial	4°35'N; 9°39'E	200-500	6.3	31.2	8.8	0.29	30	59.95
Yaounde	Equatorial	3°50'N; 11°31'E	750	5.5	5.7	2.37	1.16	20	6.37

C:N: Carbon Nitrogen ratio; OrgC: Organic Carbon; EC: Conductivity; Total N: Total Nitrogen; P: Phosphorus.
Source: Author's results

Fig. 1. Sampled sites for arbuscular mycorrhizal fungi isolates of *C. papaya* L. in the monomodal rain forest and bimodal rain forest ecological zoned of Cameroon
Source: Institute of Agricultural Research for Development (IRAD, 2016)

2.4 Inoculum Preparation

The inoculum was prepared according to the protocol of (Ngonkeu, 2009). It contained sand and propagules (spores and mycelium) (Toh et al., 2018) derived from AMF spores isolated and purified from the rhizosphere of papaya (Maffo et al., 2022). In this experiment, a composite of eleven strains AMF (*Acaulospora rosa, Gigaspora magarita, Acaulospora délicatea, Funneliformis mosseae, Glomus* spp.*, Racocetra gregaria, Glomus manihotis, Scutellospora verrucosa, Entrophospora colombiana, Acaulospora tuberculata, Glomus etunicatum*) isolated from the papaya rhizosphere (Maffo et al., 2022) was used. Two hundred and thirty-five (235) g of composite containing around 400 spores per 100 g of dry soil (Ahmad et al., 2006) introduced into holes around 10 cm deep in each pot to facilitate mycorrhization of plant roots.

2.5 Assessment of Agronomic Parameters

The number of leaves was obtained by weekly counting of newly formed, fully expanded leaves. The last leaf to emerge was marked with a sign at the petiole, to facilitate detection of newly emerged leaves. Plant height was also measured. These parameters were taken from the first month after inoculation and transplanting of the plants through to harvest. Leaves, stems and roots were separated and weighed immediately after harvest using a sensitive balance (SCALTEC) to obtain fresh weight. After weighing, each part was oven-dried at 65°C for 96 h, then weighed using a sensitive balance to obtain the dry biomass. Water content (g water/DW plant) was obtained using the formula in (Smart & Bingham, 1974): Water content (WC) = (FW - DW) / DW where FW represents the fresh weight and DW the dry weight of the plant.

2.6 Extraction and analysis of Mineral Elements

The quantity of mineral elements (Ca, Mg and K) contained in 1g of plant leaves, stems and roots was analyzed at the Laboratory of soil analysis and environmental chemistry of the Faculty of Agronomy and Agricultural Sciences at the University of Dschang using the protocol developed by (Kumar et al., 2011).

2.7 Biochemical Analysis

Biochemical analysis were carried out on 0.5 g of leaves, stems, petioles and roots of papaya plants from six (6) months of growth. Extraction of phenolic compounds and flavonoids was carried out according to the method described by (Bahorun et al., 1996) (modified). Determination of total flavonoid content was carried out according to the method of (Boizot & Charpentier, 2006) (modified). All tests were carried out in triplicates.

2.8 Mycorrhizal Colonization of Papaya Roots

After six (6) months of greenhouse cultivation of the two papaya varieties, the small roots of Ca 1000 were collected and stained according to the method of (Phillips & Hayman, 1970). The roots were first rinsed thoroughly with tap water, then placed in test tubes containing a 10% KOH solution and boiled in a water bath at 90°C for 40 min. This operation empties the cells of their cytoplasmic contents. After rinsing with running water, the roots were dipped in a Trypan Blue solution (0.05%) and the tubes placed in a water bath at 90°C for 30 min. At the end of this operation, the dye was drained and the roots soaked in tap water. Trypan Blue staining makes it possible to observe and quantify the colonization of the plants root system by AMF. Frequency of mycorrhization ‘F’ was calculated by F% = 100 (N-no) / N, where ‘N’ is the number of fragments observed and ‘no’ the number of these fragments with no trace of mycorrhization. ‘F’ reflects the importance of the contamination. Intensity of mycorrhization ‘I’ was calculated by I% = (95n5 + 70n4 + 30n3 + 5n2 + n1) / N, where n5, n4 …n1 denote, respectively the numbers of fragments noted 5, 4 ….1 (Trouvelot et al., 1986). AMF spores were isolated from the trapping soil by wet sieving and settling (Ngonkeu, 2009).

2.9 Data Analysis

Data on calcium levels in the various plant organs, mycorrhization rate were subjected to analysis of variance (ANOVA). Means were compared using the Tukey, Ducan and SNK (Student and Newman-Keuls) tests at the 5% threshold (p=.05). R studio version 4.0.5, IBM SPSS version 20.0 and XLSTAT 2010. Ink version 10.0 were used. Results, presented as curves and histograms, were produced using Microsoft Excel 2013. Correlations between

variables were highlighted using Pearson's correlation test.

3. RESULTS AND DISCUSSION

3.1 Results

3.1.1 Arbuscular mycorrhizal fungi/calcium interaction on plant height of two papaya varieties as a function of time

Arbuscular mycorrhizal fungi combined with calcium from Rorison's nutrient solution had a very significant effect (P<.001) on plant height from the first week after transplanting (S1AR) to the fourth week after (S4AR) in V1 and V2 (Fig.2).

3.1.2 Arbuscular mycorrhizal fungi /calcium interaction on the number of leaves of two papaya varieties as a function of time

After six (6) months of greenhouse cultivation of the two papaya varieties, arbuscular mycorrhizal fungi associated with calcium in Rorison's nutrient solution had a significant (P = .05) influence on the evolution of leaves number from the first week after transplanting (S1AR) to the fifth week after transplanting (S5AR). From S6AR to S19AR, a highly significant increase (P < .01) in the number of leaves was observed (Fig. 3). Calcium combined with AMF has a positive influence on papaya plant growth (Fig. 4).

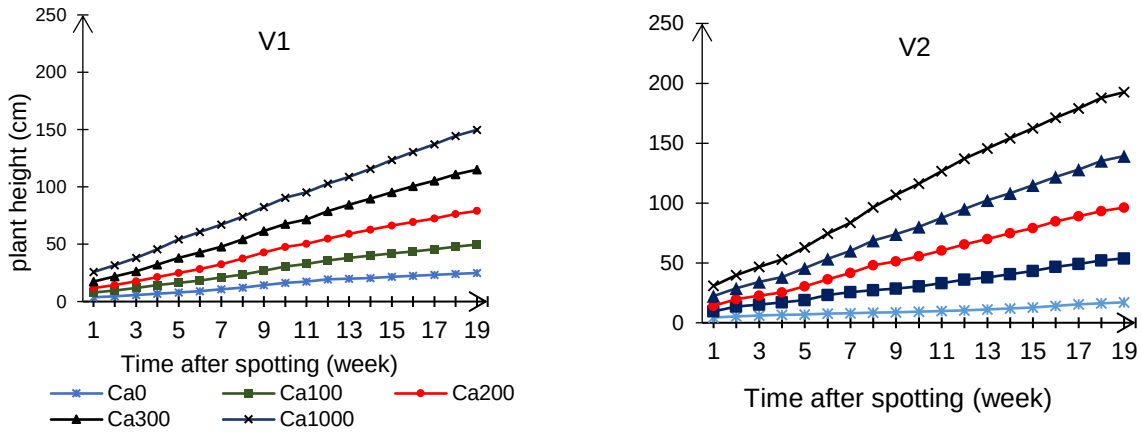

Fig. 2. Effect of arbuscular mycorrhizal fungi (AMF) combined with calcium on the height of papaya plants V1: *Calina papaya* IBP9 with AMF, V2: *Solo N°8* with AMF; Ca0, Ca100, Ca200, Ca300, Ca1000: Calcium concentrations in µM

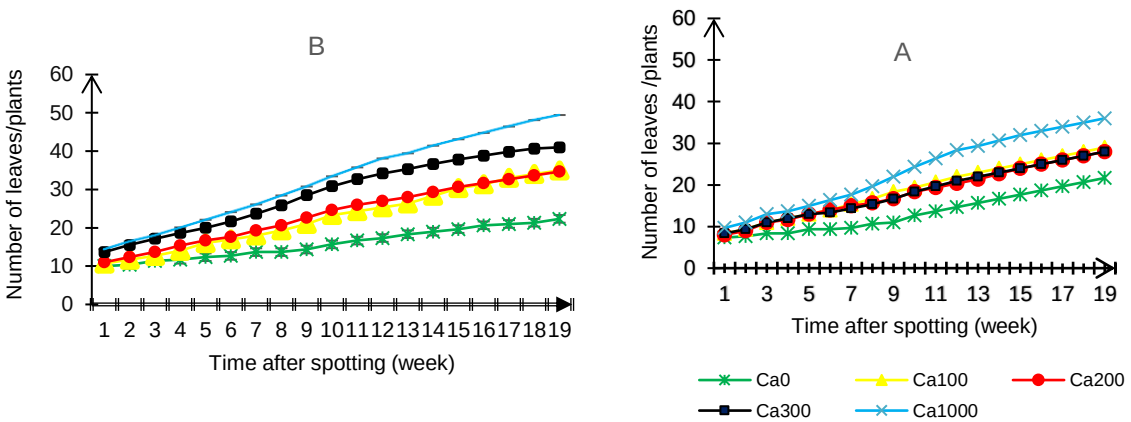

Fig. 3. Effect of arbuscular mycorrhizal fungi on the evolution of the number of leaves on papaya plants. A: V1 *Calina papaya* IBP9; B: V2 *Solo N°8*. Ca0, Ca100, Ca200, Ca300, Ca1000: Calcium concentrations in µM

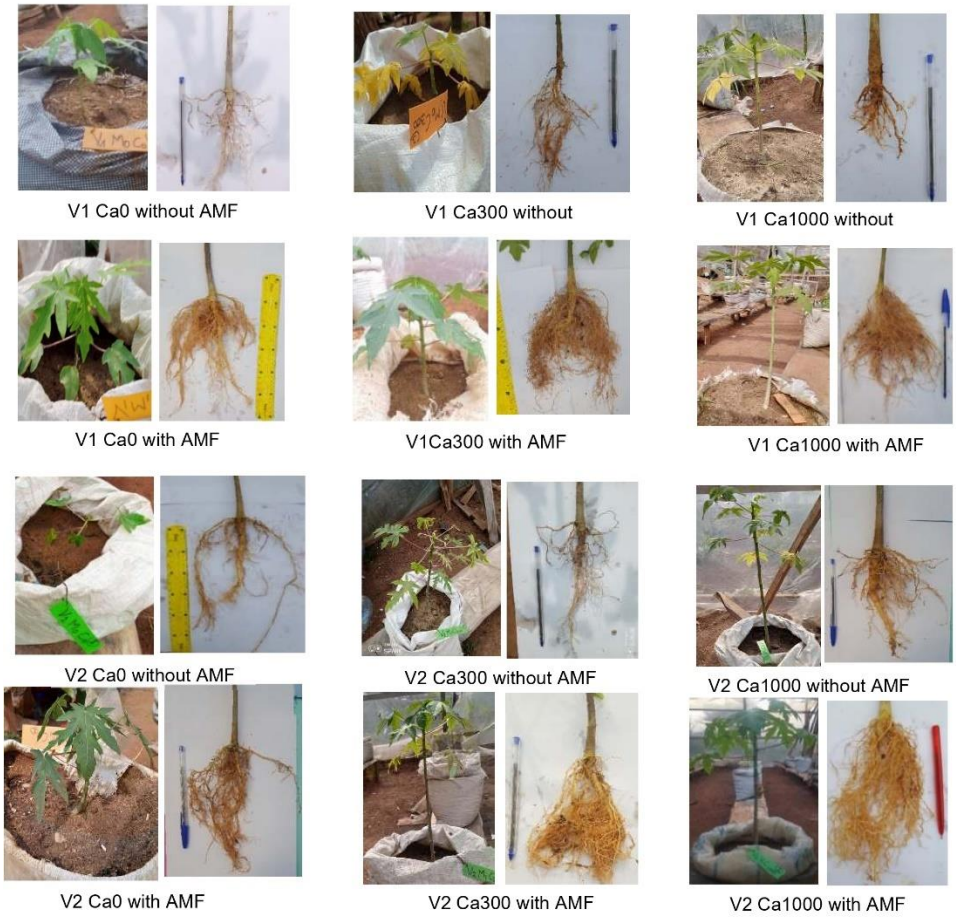

Fig. 4. Papaya plants mycorhized or not with or without soluble calcium from Rorison's solution after six (6) months of greenhouse cultivation. AMF: Arbuscular mycorrhizal fungi; V1: resistant variety *Calina papaya* IBP9; V2: sensitive variety *Solo* n°8; Ca 0: calcium 0 µM; Ca300: calcium 300 µM, Ca1000: calcium 1000 µM

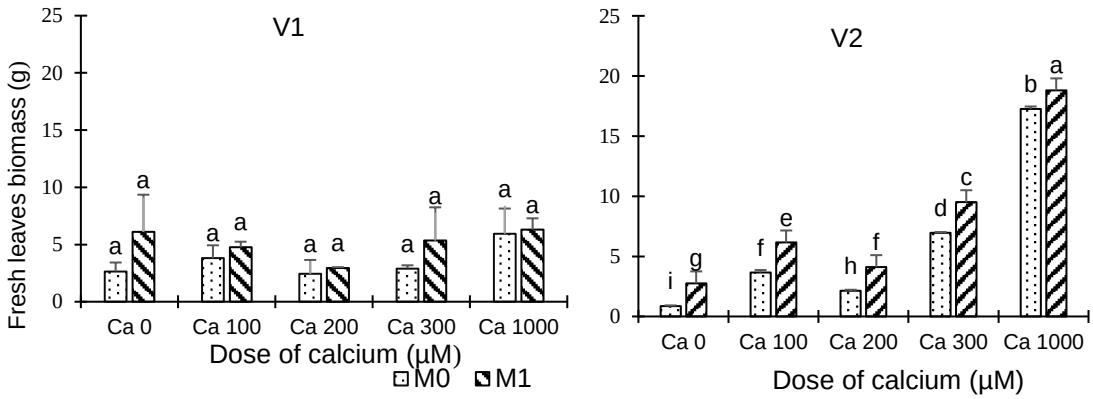

Fig. 5. Changes in fresh papaya leaves biomass as a function of calcium concentration. V1: *Calina papaya* IPB9; V2: *Solo* N°8; Ca0, Ca100, Ca200, Ca300, and Ca1000: calcium concentrations in µM; M0: without AMF, M1: with AMF. Bars bearing the same letter are not significantly different at the 5% threshold

3.1.3 Fresh leaves biomass

Calcium deficiency (0 µM) does not significantly affect (P >.05) the evolution of the number of leaves produced by the resistant papaya variety V2 (*Calina papaya* IPB9), whether mycorrhized or not, over time. On the other hand, a significant difference (P = .05) was observed in the sensitive variety V2 (*Solo* N°8) with an increase in calcium concentration, especially in the mycorrhized plants (1000 µM). (Fig. 5).

3.1.4 Fresh roots and stems biomass

The fresh biomass of papaya stems and roots increased significantly (P= .05) with increasing calcium concentration in the Rorison nutrient solution of mycorrhized plants, in contrast to no mycorrhized plants in V1 and V2 (Fig. 6). Calcium deficiency (0 µM) significantly reduced (P< .001) the fresh roots and stems biomass of no mycorrhized plants in V2.

3.1.5 Total calcium content

Calcium content increased significantly (P < .001) in papaya as a function of variation in the calcium dose of the Rorison nutrient solution (Fig. 7). Calcium content was higher (1592.667 mg/100 g) in plants treated with 1000 µM calcium and lower (570 mg/100 g) in plants treated with 0 µM calcium. Calcium has a positive influence on papaya growth.

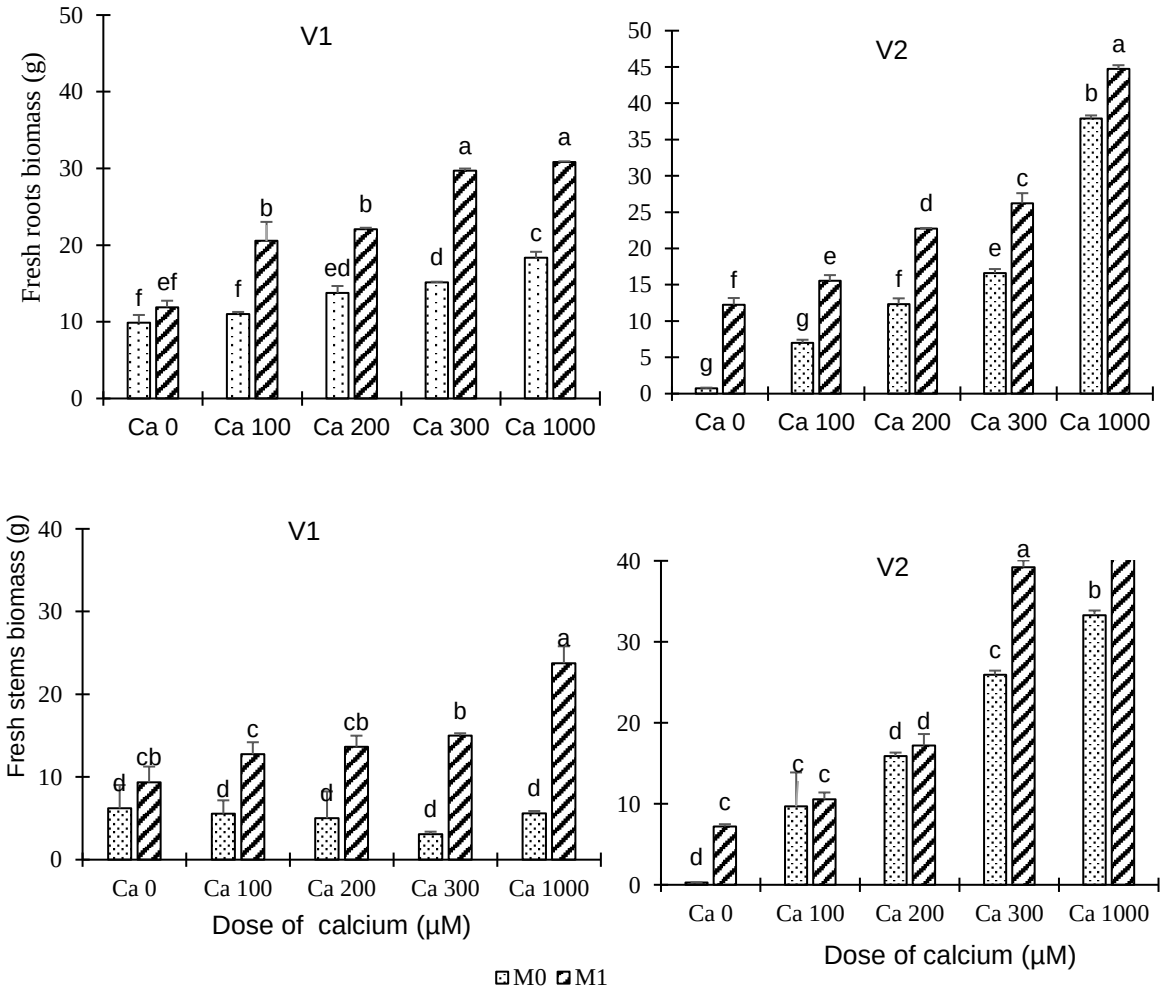

Fig. 6. Effect of calcium concentrations on fresh biomass production of papaya stems and roots after six (6) months growth in the greenhouse. V1: *Calina papaya* IPB9; V2: *Solo* N°8; Ca0, Ca100, Ca200, Ca300, and Ca1000: calcium concentrations in µM; M0: without AMF, M1: with AMF. Bars bearing the same letter are not significantly different at the 5% threshold

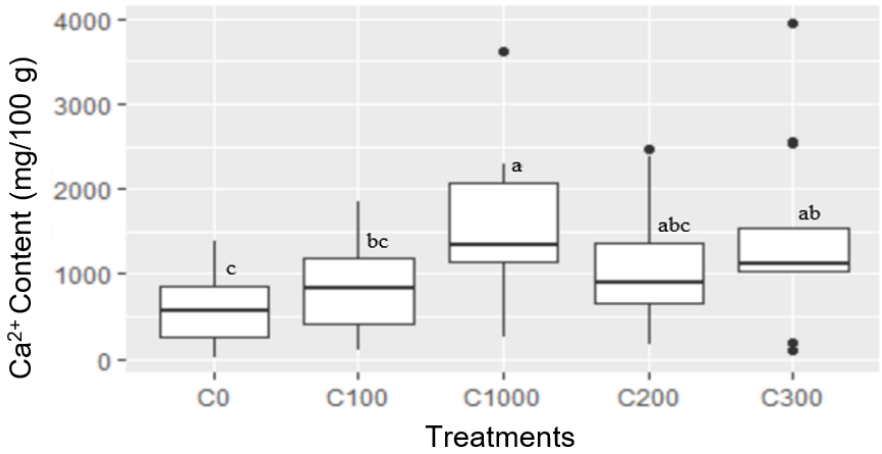

Fig. 7. Potential of arbuscular mycorrhizal fungi in calcium uptake and regulation in papaya seedlings after six (6) months of growth on sand: Ca0, Ca100, Ca200, Ca300, and Ca1000: calcium concentrations in µM

Box-plots followed by the same alphabetical letter are not significantly different at the 5% threshold

3.1.6 Effect of arbuscular mycorrhizal fungi on calcium uptake

Six (6) months after inoculation with AMF strains extracted from the papaya rhizosphere (composite of 11 strains: 400 to 700 spores/100 g dry soil), papaya significantly (P< .001) increases the total Ca^{2+} ion content in the leaves of mycorrhized plants, in contrast to no mycorrhized plants (Fig. 8). Calcium content was higher (1964 mg/100g) in the leaves of mycorrhized plants and lower (668 mg /100g) in the leaves of no mycorrhized plants. A highly significant difference (P<.001) in calcium content in the various organs (leaves, stems, roots) of mycorrhized and no mycorrhized plants was observed.

AMF appear to have a positive effect on calcium uptake and regulation in the various papaya organs. The leaves of mycorrhized plants remain the organ where calcium is stored.

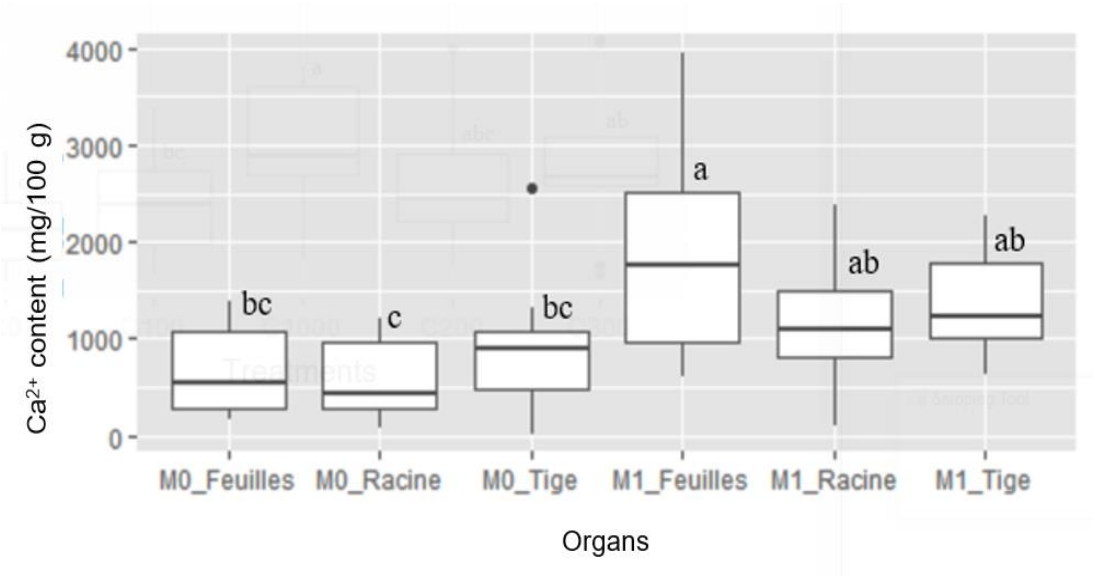

Fig.8. Potential of arbuscular mycorrhizal fungi for calcium uptake in leaves, stems and roots of papaya plants after six (6) months growth on sterilized sand. M0: No AMF; M1: AMF

Box-plots followed by the same alphabetical letter are not significantly different at the 5% threshold

3.1.7 Arbuscular mycorrhizal fungi /calcium interaction in magnesium ion uptake and regulation

Six (6) months after inoculation, the Mg^{2+} ion content of papaya plants did not increase significantly (P <.001) in mycorrhizal and no mycorrhizal plants treated with Ca 0, Ca 100, Ca 200, Ca 300 calcium from Rorison's watering solution (Fig. 9). However, a highly significant difference was observed in mycorrhizal plants treated with Ca 1000 µM calcium at leaf level compared with no mycorrhizal plants. AMF appear to have no effect on the uptake and regulation of magnesium ions in papaya. Nevertheless, when the calcium concentration of the watering solution is high (1000 µM), papaya plants store the maximum amount of magnesium ions in their leaves. And when the calcium concentration of the watering solution is low (Ca0, Ca100 and Ca200 µM), papaya stores magnesium ions in the stem. AMF influence magnesium uptake when soil calcium ion.

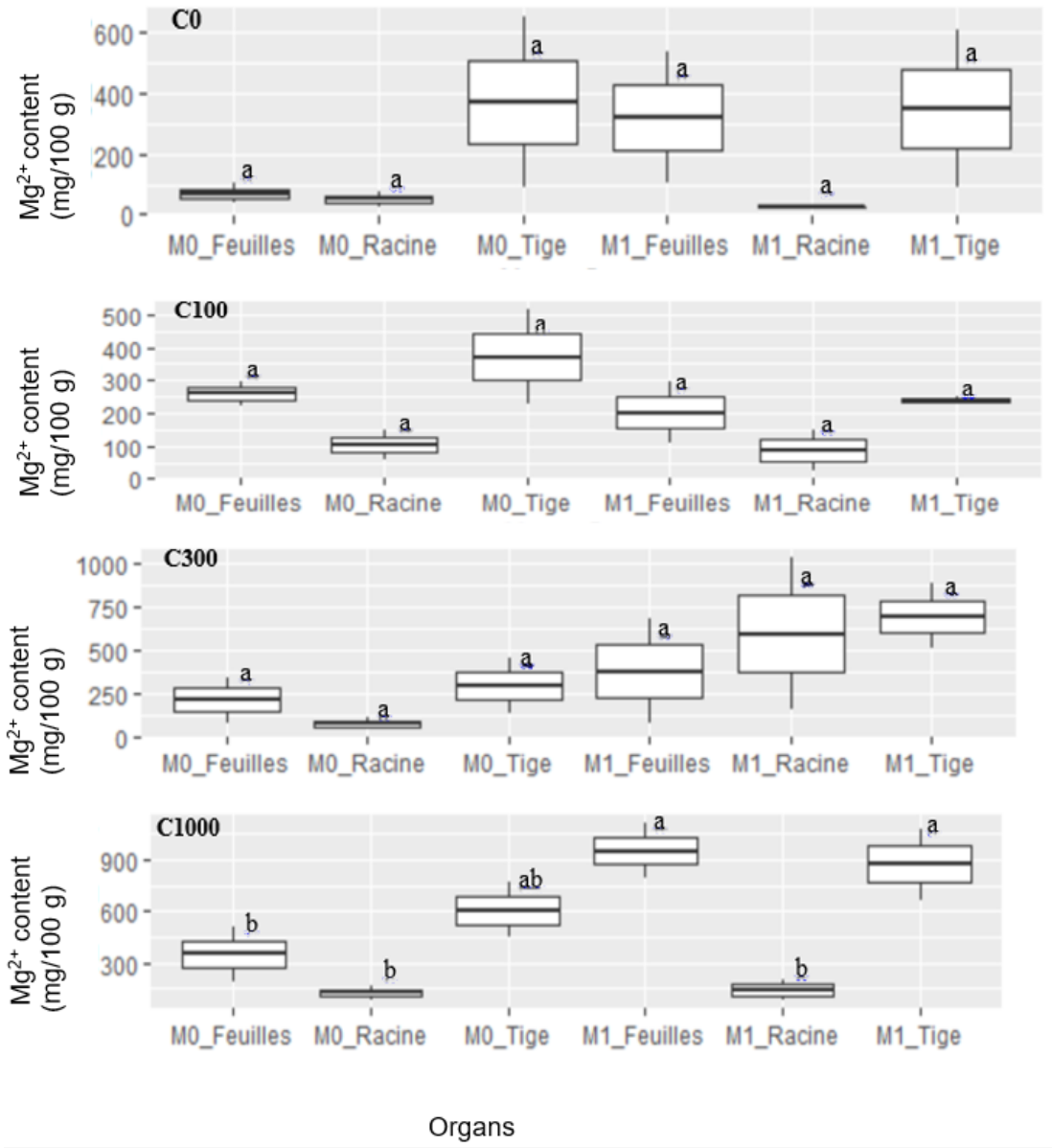

Fig. 9. Effect of calcium concentrations on magnesium ion uptake in leaves, stems and roots of papaya plants after six (6) months of growth on sterilized sand. M0: No AMF; M1: AMF

Box-plots followed by the same letter are not significantly different at the 5% threshold.

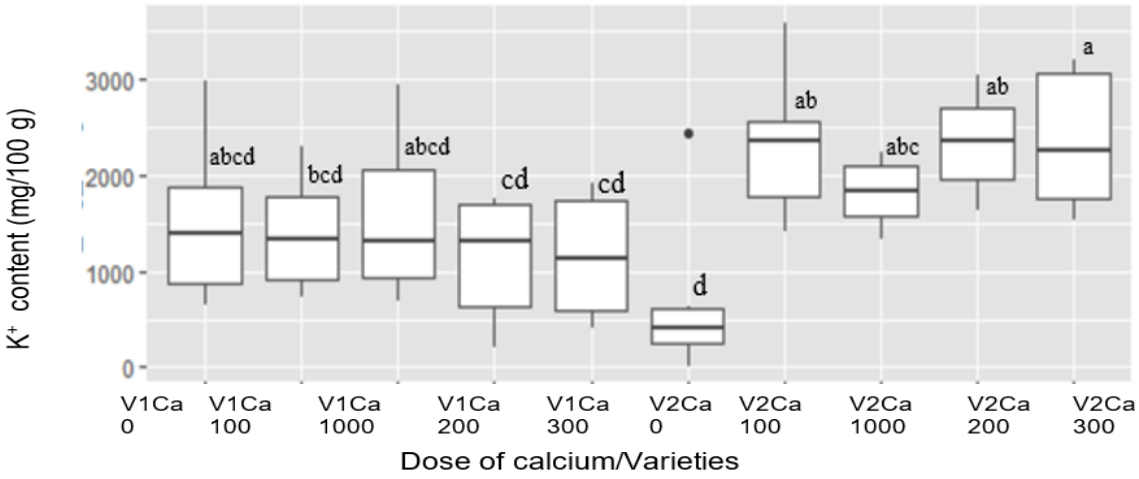

Fig.10. Effect of calcium on potassium ion uptake in two papaya plant varieties V1: *Calina Papaya*; V2: *Solo* N°8; Ca (0, 100, 200, 300, 1000 µM): applied calcium dose of Rorison's solution

Box-plots followed by the same alphabetical letter are not significantly different at the 5% threshold.

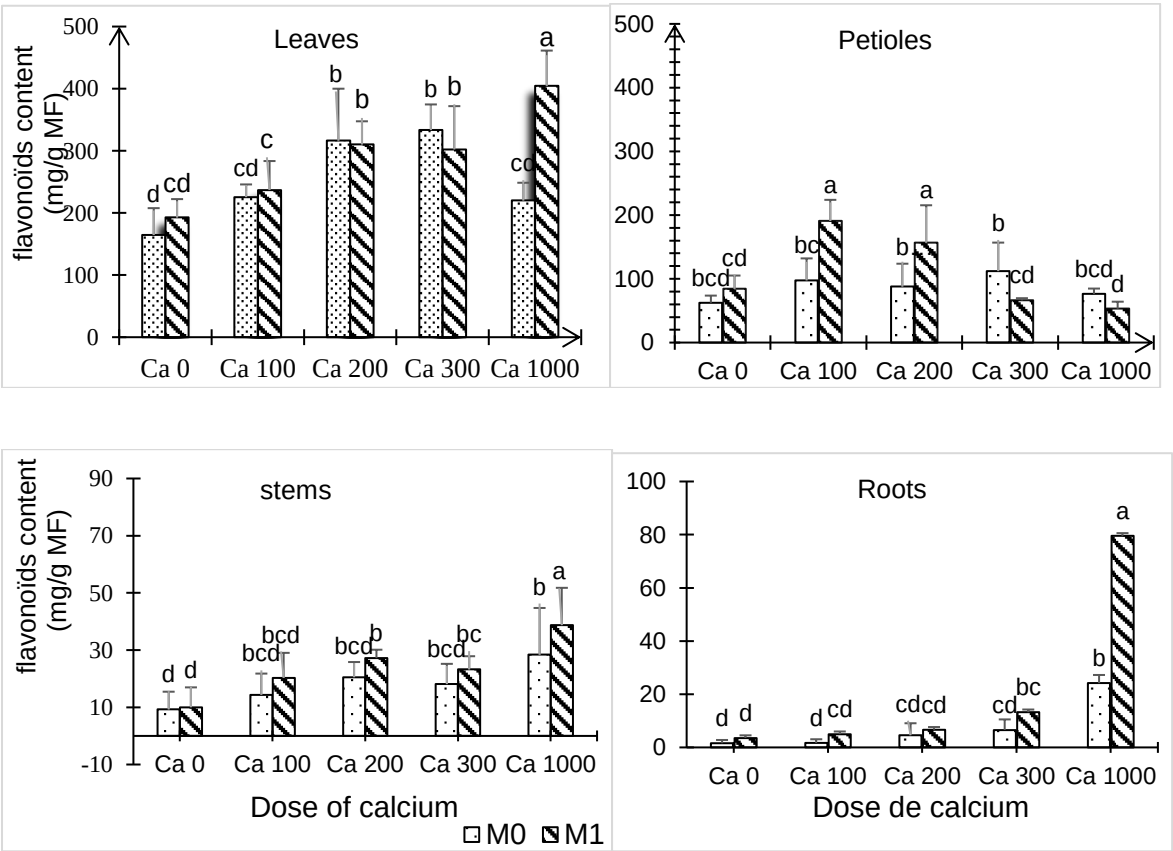

Fig. 11. Effect of calcium combined with arbuscular mycorrhizal fungi on the total flavonoid content of different organs. Ca (0, 100, 200, 300, 1000 µM): Applied calcium dose of Rorison solution. Values followed by the same letter are not significantly different at the 5% threshold

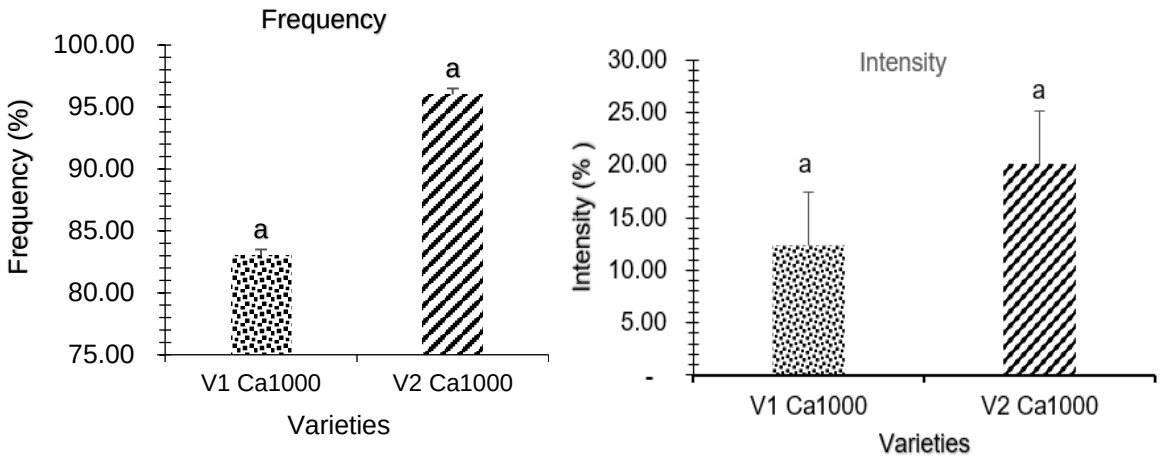

Fig. 12. Mycorrhizations frequencies and intensities of roots of two varieties of papaya
V1 Ca 1000 µM: Calina papaya IPB9 calcium 1000 µM; V2 Ca 1000 µM: Solo N°8 Calcium 1000 µM

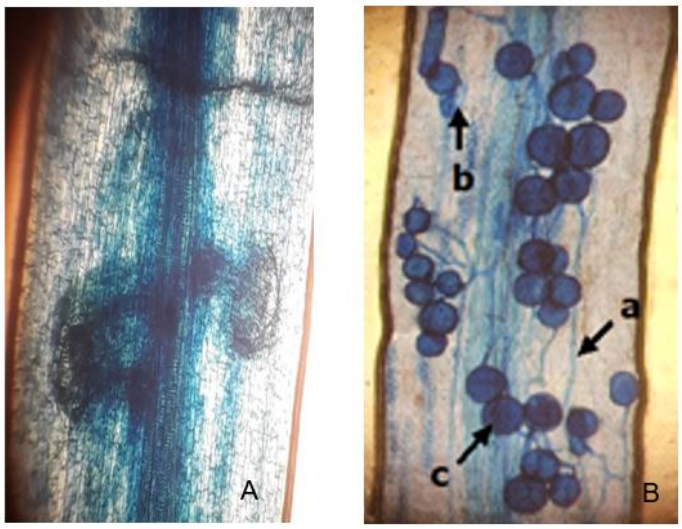

Fig. 13. Mycorrhizal structures of papaya roots fragments stained with Trypan blue. a: hyphae; b: vesicles; c: spores. A: No mycorrhizal roots; B: Mycorrhizal roots

3.1.8 Arbuscular mycorrhizal fungi/calcium interaction in potassium ion uptake and regulation

After six (6) months of papaya growth on sterilized sand, calcium deficiency (Ca0) does not significantly affect potassium content in V1 (Fig. 10). On the other hand, a highly significant (P=.05) increase in potassium content is observed when the calcium concentration (300 µM) of the Rorison solution is raised in V2. The absence of calcium (Ca 0) increased potassium ion content in V1. A high concentration of potassium ions (2358.63 mg/100g) is observed in V2 when plants are treated with calcium Ca 300 from Rorison's solution. A high potassium content was observed in V1 in the absence of Ca. Increasing the calcium concentration in the growing medium reduces the potassium ion content in plants of the Solo N°8 (V2) papaya variety.

3.1.9 Effect of calcium/mycorrhizal arbuscular fungi on the flavonoid content of different parts of papaya plants

After six (6) months cultivation of papaya plants in the greenhouse, the application of Ca 1000

µM from Rorison's nutrient solution to mycorhized plants significantly (P=.05) increased flavonoid content in the leaves and roots of papaya plants, in contrast to no mycorhized plants (Fig. 11). Flavonoid content is more concentrated in the leaves of mycorrhized plants when the calcium dose is high (Ca 1000) than in other plant organs. Increased calcium reduces the beneficial action of AMF in the petioles of papaya plants. This is not conducive to increasing flavonoid content. Thus, calcium increases root colonization and beneficial action on the leaves and stems of the plants. This implies an increase in flavonoid content, essential for regulating the establishment of mycorrhizal symbiosis in these organs. Pawpaw leaves and stems could be used as a natural source of antioxidants.

3.1.10 Mycorrhizal colonization of papaya roots

After six (6) months of greenhouse cultivation, the frequency and intensity of roots mycorrhization varied between 81% - 85% and 15% - 21% respectively in the two varieties (V1,V2) (Fig. 12). No significant difference was observed at the threshold of P>.05. The root colonization of AMF was observed in the two papaya varieties.

The roots of *C. papaya* L. showed mycorrhizal structures in the form of hyphal vesicles and spores (Fig. 13).

3.2 Discussion

3.2.1 Combined effect of arbuscular mycorrhizal fungi and calcium on the fresh and dry biomass of different parts of papaya plants in greenhouse

Results on calcium nutrition in papaya showed that calcium supplementation improved papaya growth three weeks after transplanting. This improvement is still very significant from the first week after transplanting when calcium supplementation is combined with AMF. The improvement occurred through increases in leaf number, plant height, fresh and dry biomass accumulation, and calcium levels. Many researchers have also reported the benefits of the combined effect of AMF and calcium on the above growth parameters (Cui et al., 2019) on peanut cultivation in China. Similar observations were made by (Chebet et al., 2020) on papaya cultivation in Kenya. The improved performance of mycorrhizal seedlings could be attributed to a greater efficiency of phosphorus uptake, evidenced by increased calcium accumulation in the leaves. In a study of papaya in India, leaf petioles of mycorrhized seedlings showed higher levels of total phosphorus (0.42 - 0.63%) compared with control plants (0.35%) (Mohammed et al., 2021).

3.2.2 Combined effect of arbuscular mycorrhizal fungi and calcium on the fresh leaves biomass in greenhouse

The fresh leaves biomass evaluated in this study varied according to variety and the applied calcium concentration of the Rorison solution, as well as according to the AMF treatments. AMF application and increasing calcium concentration (1000 µM) significantly influenced leaves biomass production in the susceptible V2 variety (*Solo* N°8). Similar results were obtained by (Chebet et al., 2020) on papaya and by (Cui et al., 2019) on peanut cultivation. (Mohammed et al., 2021) justify this increase in fresh biomass as the product of improved nutrient and water uptake directly involved in the process of photosynthesis; (Mitra et al., 2019) also demonstrate that AMF increase the concentration of various macro and micro-nutrients significantly, leading to increased production of photosynthates and thus increased biomass. The fresh biomass of papaya stems and roots increases significantly (P= .05) with increasing calcium concentration in the Rorison nutrient solution of mycorrhized plants in contrast to non-mycorrhized plants in V1 and V2. On the other hand, calcium deficiency (0 µM) significantly (P< .001) reduces the fresh roots and stems biomass of no mycorrhized plants in V2. In the absence of calcium at root level, lignification enzymes such as phenylalanine ammonia-lyase and peroxidases are activated (Finger et al., 2006), leading to root necrosis. These results were observed in the work of (Duan et al., 2022) on grapevine. The addition of AMF and a high calcium concentration (1000 µM) in the V2 variety promoted root system growth and stem elongation. In fact, AMF promoted mycorrhizal infectivity of roots and colonization of mycorrhizal spores in the soil. They improved the plants' ability to absorb nutrients, possibly by increasing the effective root surface area from which the available form of nutrients is absorbed, and also by increasing roots access by filling in depletion zones (Chebet et al., 2020).

3.2.3 Combined effect of arbuscular mycorrhizal fungi /calcium in the uptake and regulation of certain mineral elements (Ca, Mg, K) in different parts of papaya plants in greenhouse

Macro and micronutrient uptake varies considerably between different plant organs. (Duan et al., 2022) demonstrated this in their study of grapevine (*Vitis vinifera*). Similar results were observed in papaya. Papaya significantly (P< .001) increases total Ca^{2+} ion content at leaf level in mycorrhized plants, in contrast to no mycorrhized plants. Calcium content is higher (1964 mg/100g) in leaves, especially in mycorrhized plants, than in other organs such as stems and roots. Papaya leaves remain the most important calcium storage organ. The abundance of Ca in leaves is explained by the formation of calcium pectate in the middle lamella of cells (El Habbash et al., 2015). This result is similar to the work of (Chebet et al., 2020) on papaya, (Cui et al., 2019) on peanut and (Duan et al., 2022) on grapevine. On the other hand, calcium deficiency in papaya does not affect the absorption of magnesium ions, which are stored in the stems. In fact, in the absence of calcium, a compensation of cation ions is created in order to maintain the electrical and chemical balance of the cells (Finger et al., 2006). Cation ions (Ca^{2+}, Mg^{2+}, K^{+}) substitute for each other in the event of a lack or excess of one of them (Ramalho et al., 1995). If accumulated in excess, they interfere with the physiological process (Schulte-Baukloh & Fromm, 1993). Calcium deficiency (0 µM) does not significantly (P< .001) affect potassium content in papaya. This result is similar to the work of (Awada & Long, 1980), who demonstrated in their studies that potassium ions reduced calcium concentration in the leaves of certain plant species. Furthermore, increasing the calcium concentration of the Rorison solution increases the potassium content in papaya plants. (Chebet et al., 2020) btained similar results on papaya in Kenya. This is also consistent with a study on papaya in India, which showed that total leaf petiole potassium content was higher in mycorrhized plants and ranged from 2.68 to 4.39% compared with no mycorrhized plants (2.26%) (Khade & Rodrigues, 2023). Potassium uptake was also increased by AMF inoculation in cowpea and sorghum (Awada & Long, 1980). This may be attributed to greater soil exploration and increased supply to host roots. A further increase in K levels in mycorrhized plants can be attributed to the fact that AMF bind soil particles to each other and to roots, which is beneficial for nutrient uptake (Awada & Long, 1980).

3.2.4 Combined effect of arbuscular mycorrhizal fungi and calcium on phytochemicals in different parts of papaya plants in greenhouse

The results obtained from phytochemical analysis show that calcium supplementation combined with AMF increases flavonoid content, particularly in leaves compared with other plant organs. This is justified by the fact that leaves are the organs of synthesis and distribution of synthetates (Cui et al., 2019). (Dhalaria et al., 2024) obtained similar results on AMF effect on mineral nutrient (N, P, Ca, Mg and K) absorption in *Gomphrena globosa*. The observed increase could be directly associated with the presence of the different AMF strains used and the calcium supplied. Phenolic compounds are higher in plants inoculated with AMF (Mohammed et al., 2021). Indeed, AMF increase water and nutrient uptake, recalibrate plant metabolic pathways and affect the concentration of primary and secondary metabolites (Machiani et al., 2022). Furthermore, AMF combined with calcium (1000 µM) stimulate the synthesis of genes involved in flavonoid biosynthesis. These include the genes coding for chalcone synthase, involved in the early stages of biosynthesis, the BGI-novel-G001027 gene coding for shikimate O-hydroxycinnamoyltransferase and the gene (Araip.6PA6C) coding for flavonone synthase responsible for flavonoid biosynthesis (Cui et al., 2019).

3.2.5 Roots colonization

After six (06) months of greenhouse cultivation, the frequency and intensity of roots mycorrhization varied between 81%; 85% and 15%; 21% respectively in the two varieties (V1 and V2). The frequency and intensity of colonization of papaya roots combined with the best calcium dose were not significantly different in the two varieties. However, the presence of AMF spores was observed on the roots of papaya plants mycorrhized with the calcium 1000 µM. These results are similar to the findings of (Khade & Rodrigues, 2023) who reported high roots colonization (78%) in papaya roots. Much colonization of the roots could be justified by the fact that the papaya crop has a root system characterized by the presence of a taproot and adventitious roots which allow them to draw their nutrients only from the superficial horizons. Thus,

AMF allow roots development so that they can improve the uptake of water and nutrients from the soil. This result is contrary to that of (Chebet et al., 2020) who had obtained a low rate of mycorrhizal infection (43% and 15%), in papaya. He also obtained a relatively low rate of colonization of papaya roots.

4. CONCLUSION

Functional differences were observed within the AMF as a function of varying calcium concentrations (0, 100, 200, 300, 1000 µM). The difference was highly significant (p=.05) with calcium dose (Ca 1000 µM). A composite of eleven strains AMF combined with calcium strongly stimulated plant growth and conferred on the plants a capacity for absorption and regulation. The positive effects were particularly noticeable in the uptake of Ca, Mg and K, through the secondary roots and preferentially stored in the leaves (an essential organ during calcium nutrition in plants). Positive effects have also been seen in the synthesis of secondary metabolites (flavonoids) which contribute to the body's defense against all forms of attack (bacterial, viral and fungal).

Overall, all growth parameters were improved in mycorrhized plants of both papaya varieties, in contrast to no mycorrhized plants. Inoculation with AMF combined with the high calcium dose (1000 µM) favoured mycorrhizal infectivity of the roots, colonization of mycorrhizal spores in the soil and increased root surface uptake. This led to increased accumulation of calcium ions and phytochemicals such as flavonoids in the xylem of the leaves (storage organ). These results clearly show that AMF can be used as useful biofertilizers to improve the post-harvest quality of papaya fruit.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of this manuscript.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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