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Dependency of cassava (*Manihot esculanta* Crantz) on vesicular-arbuscular mycorrhizal fungi

M. Habte, M. N. Byappanahalli

Department of Agronomy and Soil Science, University of Hawaii, 1910 East West Road, Honolulu, HI 96822, USA

Abstract. Cassava plants were started in the greenhouse either from small cuttings (2.0 mg P/cutting) or large cuttings (20.2 mg P/cutting) in a subsurface Oxisol not inoculated or inoculated with *Glomus aggregatum* at target soil solution P concentrations of 0.003–0.2 mg l^{-1}. Vesicular-arbuscular mycorrhizal (VAM) fungal colonization levels in excess of 60% were attained on cassava roots irrespective of the size of cutting material used or target soil solution P status. However, plants started from large cuttings grew faster and better than those started from smaller ones. Cassava was found to be very highly dependent on VAM fungi if grown from small cutting but only marginally dependent if grown from large cuttings. The lower dependence of cassava on VAM fungi when started from larger cuttings appears to be related to the high P reserve in these cuttings and hence the low requirement of the plants for soil P until the P reserve in the cuttings is significantly depleted.

Key words: *Glomus aggregatum* – Cuttings – Dependency categories – *Manihot esculanta* – Soil solution P

Introduction

Cassava (*Manihot esculanta* Crantz) is an important root crop of the humid and subhumid tropics particularly noted for its adaptability to areas dominated by infertile soils. However, the plant exhibits a high demand for external P when grown in flowing solution cultures (Howeler et al. 1982; Kang et al. 1980).

The lower sensitivity of cassava to P deficiency in the field is explained by its slow growth habit or/and the tendency of the plant to form symbiotic association with vesicular-arbuscular mycorrhizal (VAM) fungi (Kang et al. 1980). Elimination of the fungi from soil by fumigation is known to reduce top growth of cassava and tissue P concentration (Kang et al. 1980; Van der Zaag et al. 1979; Yost and Fox 1979). The high external P requirement of cassava in flowing nutrient solution cultures is also known to be substantially lowered if the plant is pre-colonized by VAM fungi (Howeler et al. 1982). These observations have led some researchers to conclude that cassava is a highly mycotrophic species. On the other hand, there is evidence to suggest that the response of cassava to VAM colonization varies with soil type and with the size of the planting material used (Sieverding 1991). Consequently, the mycorrhizal dependency of the species has not been clearly established. The objective of the current investigation was to determine the mycorrhizal dependency of cassava and to see if its dependency falls within the categories of VAM dependency of host species recently proposed by Habte and Manjunath (1991).

Material and methods

The soil used in this study was a subsurface sample (10–15 cm) of an Oxisol (clayey, kaolinitic, isohyperthermic, Rhodic Eutrustox, Wahiawa series). It was crushed to pass through a 4-mm aperture sieve. The initial pH of the soil was 5.3 (1:2 soil-water suspension) and was adjusted to 6.2 with dolomite.

Portions of the 5 kg air-dried soil were transferred into 22.5-cm-wide by 25-cm-high plastic pots. The moisture content of the soil was adjusted to approximately 30% during an incubation period of 3 days. Pots containing the soil were then fumigated in a gas-tight chamber for 6 days and then left to stand on greenhouse benches for 20 days to allow the excess fumigants to dissipate. A week before planting, soil solution P concentration was either unadjusted or elevated to target concentrations of 0.02 and 0.2 mg l^{-1} based on a P sorption isotherm (Fox and Kamprath 1970). Mycorrhizal inoculation of soil in some of the pots was achieved by thoroughly mixing the contents of each pot with 50 g of a crude inoculum of *Glomus aggregatum* (Schenck and Smith emend Koske) (110 infective propagules/g). The inoculum consisted of sand, spores, hyphae, and pieces of infected roots.

In the first experiment, cuttings of cassava (*Manihot esculanta* Crantz) were used that were 18 cm long and 1.8 cm in diameter (large). These cuttings had an average P content of 20.2 mg/cutting and were planted in a vertical position with nodes facing up-

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Correspondence to: M. Habte

ward. There were three cuttings per pot, subsequently thinned to one plant per pot.

In the second experiment, the soil used was as above but with an initial P content of 0.007 mg l^{-1}. The cassava cuttings used were 2.1 cm long and 0.92 cm in diameter (small). These cuttings had an average P content of 2.0 mg/cutting. They were planted horizontally in depressions made on the surface of the potted soil and were covered with a thin layer of soil. Five cuttings were planted in each pot and were thinned 30 days later to obtain one plant per pot.

Basal nutrients (Aziz and Habte 1987) were added to all the pots 30 days after cuttings were planted. A second blanket nutrient application was made 25 days later.

The 3 × 2 factorial experiments consisting of 3 levels of phosphorus and 2 levels of VAM fungus inoculation were arranged on greenhouse benches in a randomized complete block design with three replicates per treatment. Plants were grown for 69 or 103 days under natural light (21°10′ N, 157°58′ W) during 7 February to 14 April 1992 or 10 September to 22 December in 1992. Pots were watered as needed to maintain the soil at approximately maximum water holding capacity.

The development of VAM effectiveness was monitored as a function of time by determining the concentration of phosphorus in leaf disks (Aziz and Habte 1987). At harvest, VAM colonization of roots, the shoot and root dry matter yields, and shoot P contents were determined.

The proportion of root length colonized by the VAM fungus was determined by the grid line intersect method (Giovannetti and Mosse 1980) after clearing roots with 10% KOH and staining with acid fuchsin (0.15% in a lactic acid-glycerol solution) (Kormanick et al. 1980). The dry weights of shoot and root were determined after drying samples at 70° C for 96 h. Tissue P content was determined colorimetrically (Murphy and Riley 1962) after dry-ashing samples in a muffle furnace at 500 ° C for 3 h.

Data were analyzed using the SAS (Statistical Analysis System) procedure (SAS Institute 1991). VAM dependency of cassava was calculated according to the procedure proposed by Plenchette et al. (1983). The category of VAM dependency to which cassava belonged was then determined according to the schema proposed by Habte and Manjunath (1991).

Results

First experiment

Inoculation of soil with *G. aggregatum* at the first two concentrations of soil P led to high levels of VAM colonization of roots (Fig. 1). Colonization levels declined significantly when soil P was increased to 0.2 mg l^{-1} but never went below 60%. Roots of plants grown in the uninoculated soil did not exhibit evidence of VAM colonization.

Mycorrhizal inoculation stimulated growth of cassava at target soil solution P concentrations of 0.003 and 0.02 mg l^{-1}, but reduced shoot dry weight at a soil P concentration of 0.2 mg l^{-1} (Fig. 2A). The trend observed for root dry weight was similar (Fig. 2B).

Shoot P concentrations determined at the time of harvest were generally low but plants colonized by *G. aggregatum* had higher tissue P concentrations than those not colonized by the fungus (Fig. 3). However, the effect of VAM colonization on tissue P status was most pronounced in the soil with a P concentration of 0.02 mg l^{-1}.

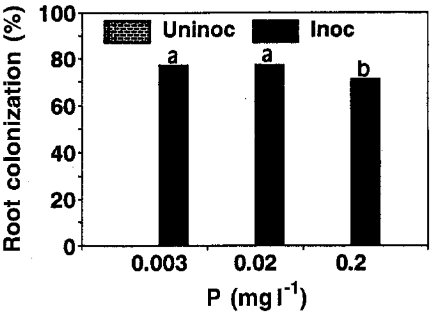

Fig. 1. Changes in the level of vesicular-arbuscular mycorrhizal (VAM) colonization of roots of cassava raised from large cuttings in response to VAM inoculation and soil solution P concentration. Means followed by the same letter are not statistically different at the 5% level. Abbreviations in all figures: *Inoc* inoculated, *uninoc* not inoculated

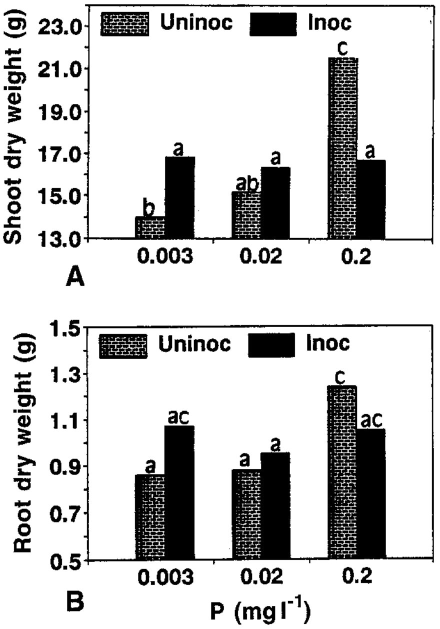

Fig. 2A, B. Changes in shoot and root dry weight of cassava grown from large cuttings in response to VAM colonization and soil P concentration. Means followed by the same letter are not significantly different at the 5% level

Leaf P declined as a function of time in the soil with a target P concentration of 0.02 mg l^{-1} irrespective of VAM colonization until day 39 (Fig. 4B). After this time, the leaf P content of inoculated plants increased to a peak value and then declined gradually, while that of the uninoculated plants stabilized around the concentration attained on day 39. The changes in the leaf P status of plants grown at the lowest and the highest soil P concentrations followed patterns similar to that observed above, except that the effect of VAM inoculation was less pronounced (Fig. 4A, C).

The dependency of cassava on *G. aggregatum* decreased with increase in soil solution P concentration, becoming negative at the highest concentration of P tested (Fig. 5). The maximum degree of dependence of the plant on the fungus for growth was less than 20% and was observed at a soil solution P concentration of 0.003 mg l^{-1}.

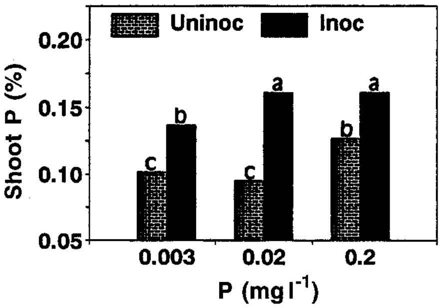

Fig. 3. Changes in shoot P concentration of cassava started from large cuttings in response to VAM colonization and soil solution P concentration. Means followed by the same letter are not significantly different at the 5% level

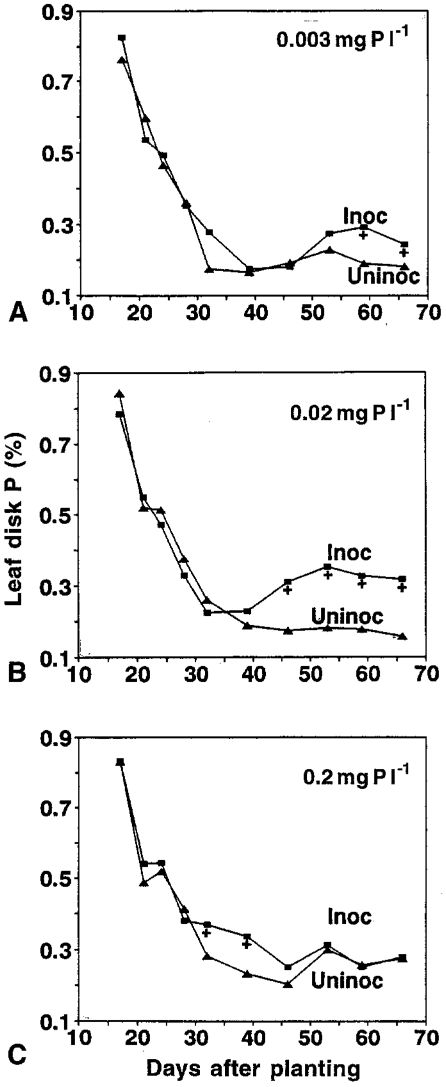

Fig. 4A–C. Changes in leaf P status of mycorrhizal and nonmycorrhizal cassava raised from large cuttings in response to soil solution P concentration. Mean pairs not marked by a plus sign are not significantly different at the 5% level

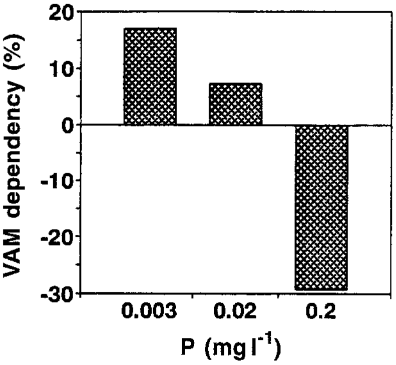

Fig. 5. Changes in the VAM dependency of cassava raised from large cuttings in response to soil solution P concentration

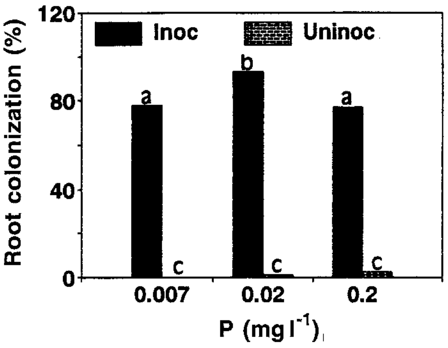

Fig. 6. VAM colonization of roots of cassava started from small cuttings. Means followed by a common letter are not significantly different at the 5% level

Second experiment

In the soil inoculated with *G. aggregatum*, roots were highly colonized by the fungus at all soil P levels, although the maximum level of VAM colonization was observed at 0.02 mg P l^{-1} (Fig. 6). Mycorrhizal inoculation increased shoot dry weight of cassava at all concentrations of soil solution P, although the effect of mycorrhizal colonization at the highest soil P concentration was significantly lower than the effects observed at the lower soil P concentrations (Fig. 7A). The response of root dry weight to mycorrhizal colonization and soil solution P status was similar to that observed for shoot dry weight (Fig. 7B).

At the lowest two concentrations of soil P, mycorrhizal inoculation had little or no effect on shoot P status of cassava (Fig. 8). At the highest soil P concentration, VAM colonization had a positive effect on shoot P concentration. Changes in leaf P status determined as a function of time during the last 90 days of the experiment revealed that mycorrhizal-mediated P accumulation had taken place during the earlier phases of growth (Fig. 9).

Calculation of VAM dependency according to the formula of Plenchette revealed that cassava depended on *G. aggregatum* to the extent of 80% if the soil P concentration was 0.007 or 0.02 mg l^{-1} (Fig. 10). How-

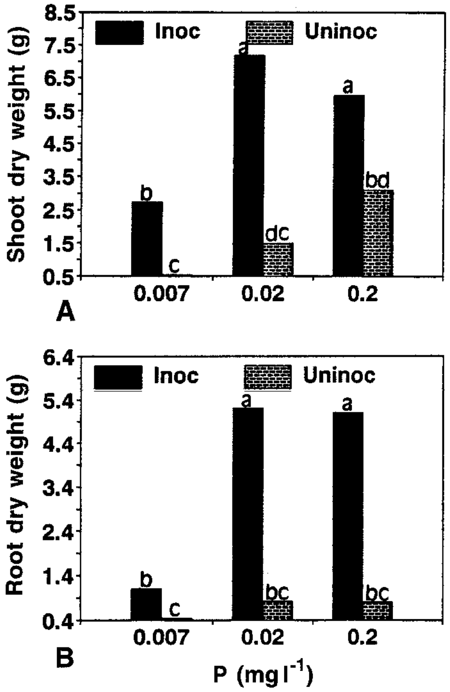

Fig. 7A, B. Changes in shoot and root dry weight of cassava started from small cuttings in response to VAM inoculation and soil solution P concentration. Means followed by the same letter are not significantly different at the 5% level

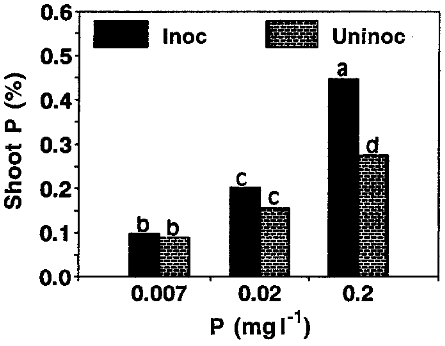

Fig. 8. Changes in shoot P status of cassava started from small cuttings in response to VAM inoculation and soil solution P concentration. Means followed by the same letter are not significantly different at the 5% level

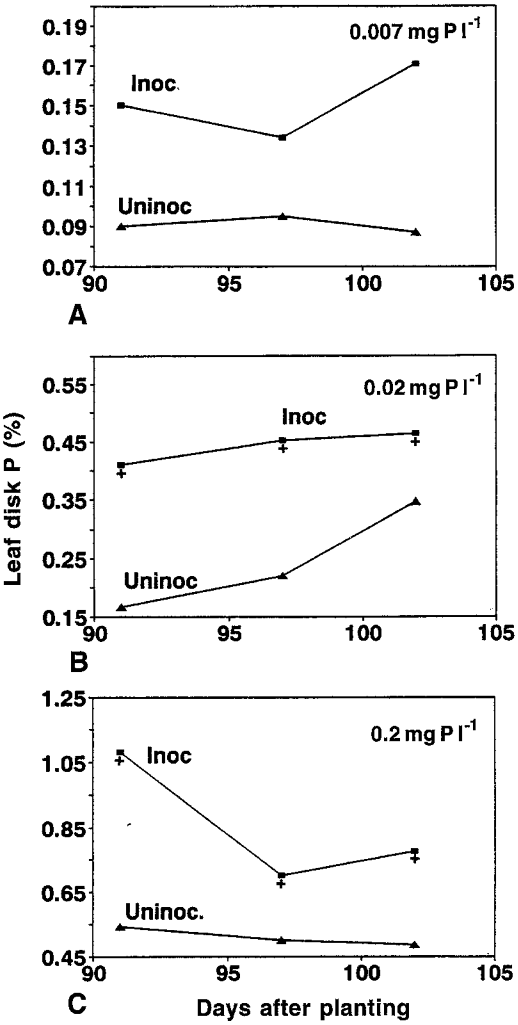

Fig. 9A–C. Leaf P status of mycorrhizal and nonmycorrhizal cassava raised from small cuttings during the last 12 days of growth. Mean pairs not marked by a plus sign are not significantly different at the 5% level

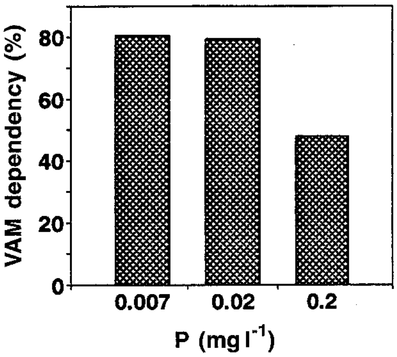

Fig. 10. Changes in the VAM dependency of cassava raised from small cuttings in response to soil solution P concentration

ever, the dependency of cassava on the fungus dropped to less than 50% if the soil was amended to a concentration of 0.2 mg P l^{-1}.

Discussion

In both experiments, a negative effect of high P on VAM colonization was evident but relatively high levels of colonization were attained, particularly in the second experiment. However, maximum VAM colonization of cassava roots was observed at a soil solution P concentration of 0.02 mg l^{-1}, a concentration previously determined to be optimum for VAM activity in *Leucaena leucocephala,* a very highly mycorrhizal dependent species (Habte and Manjunath 1987, 1991).

Shoot and root dry matter yields observed in the two experiments diverged significantly. In general, plants developed faster and grew better if started from large cuttings than if started from smaller ones. In the first experiment, the response of cassava to VAM colonization was minimal even at soil P concentrations known to be optimal for VAM activity in highly VAM-dependent species. This suggests that the bulk of the P demand for growth was satisfied by the P reserve in the planting material. The conclusion is supported by the prolonged leaf P decline phase observed (Fig. 4). Other studies have generally shown a short initial decline phase in leaf P content, reflecting the rapid depletion of P in seed reserves, followed by a reversal of this decline as mycorrhizal roots developed and efficiently took up P from the soil (Aziz and Habte 1987; Habte and Manjunath 1987).

In the second experiment, where an effort was made to minimize the impact of P reserves by using the smallest planting material possible without compromising rooting ability, cassava shoot dry weight increased approximately fivefold in response to VAM colonization at the lowest two concentrations of soil P and roughly twofold at the highest soil P concentration (Fig. 7). The best response was observed at a soil P concentration of 0.02 $\mathrm{mg\,l^{-1}}$. However, even though the mycorrhizal inoculation effect (MIE) observed at this P level was comparable to that observed at the initial unadjusted P concentration, dry matter yield at the latter soil P concentration was substantially lower. Apparently, this P level was not sufficient for optimum VAM activity in cassava. The decrease in MIE as P was increased from 0.02 to 0.2 $\mathrm{mg\,l^{-1}}$ reflects the fact that the unaided root was beginning to take up appreciable quantities of P at the higher soil P concentration. It could also reflect the adverse effect of high P on VAM activity.

Although the P status of cassava shoots at harvest was very low in the first experiment, the data suggest that by the time of harvest some VAM-mediated P uptake had taken place, particularly at a soil P concentration of 0.02 $\mathrm{mg\,l^{-1}}$. During the initial 39 days of growth, the high P content of the cuttings apparently precluded appreciable inflow of P from soil, supporting the earlier suggestion of Habte and Manjunath (1987) that P movement into the plant is regulated by the plant. This is probably why shoot P status determined at the time of harvest is not generally a reliable indicator of VAM effectiveness. Tissue P content usually tends to decline after a certain level has been attained in the plant (Habte and Manjunath 1987), and is further influenced by the the dilution/concentration effect of growth/lack of growth (see Soedarjo and Habte 1993).

On the basis of the VAM dependency values observed in the current investigation, it can be concluded that the dependency of cassava on VAM fungi for growth is variable. In the first experiment using large cuttings, VAM dependency ranged from less than 20 to negative, the dependency values decreasing with increasing soil solution P. On the basis of these data cassava would be classified as a marginally VAM-dependent species.

The second experiment was conducted to test the hypothesis that cassava is inherently highly mycotrophic and that if it is started from the smallest cutting compatible with rooting ability its dependency on VAM colonization would increase substantially. The hypothesis was confirmed by the data collected. In this experiment, cassava exhibited a VAM dependency of 80% at the lowest two soil solution P concentrations and slightly less than 50% at the highest soil P concentration tested. The lower dependency of the plant on the fungus at the highest soil P concentration suggests that the unaided cassava root was capable of taking up P at this P concentration to satisfy a portion of the plant's P demand. On the basis of these data, cassava would be categorized as a species that is very highly dependent on VAM fungi. This categorization holds where the P content of cassava cuttings does not increase much above 2.0 mg and VAM dependency is determined as prescribed in the current study.

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