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## Research Article

# Sugarcane Roots Dynamics Inoculated with Arbuscular Mycorrhizal Fungi on Dry Land

<sup>1</sup>Wawan Sulistiono, <sup>2</sup>Taryono, <sup>2</sup>Prapto Yudono and <sup>2</sup>Irham

<sup>1</sup>Assessment Institute for Agricultural Technology of North Maluku, Sofifi, Indonesia

<sup>2</sup>Faculty of Agriculture, Universitas Gadjah Mada, Yogyakarta Indonesia, Jl. Flora Bulaksumur Yogyakarta, Indonesia

## Abstract

**Background and Objective:** A conclusive technical solution is necessary to enhance roots ability to support the growth and yield of sugarcane on dry land because of limitations of water and nutrients. This study aimed to determine the effect of timing of mycorrhizal application to infection and root growth dynamics. **Materials and Methods:** Seedlings of five clones were transplanted in the field and applied with mycorrhiza at different timing consisting of the nursery, planting time and without mycorrhiza. The parameters of infection percentage, total root length, root surface area, root diameter, root dry weight, root:shoot dry weight ratio and the weight of millable cane were evaluated using analysis of variance based on  $5 \times 3$  factorials completely randomized design followed by Duncan's multiple range test with  $p < 0.01$  as the *post hoc*. The relationship between millable cane weight and root parameters was analyzed using step-wise regression. **Results:** The application of mycorrhiza in the nursery significantly increased ( $p < 0.01$ ) the highest infection percentage by reaching 86.3% compared with the controls. The application of mycorrhiza in nurseries significantly increased ( $p < 0.01$ ) the length of roots, root surface area, root diameter, root dry weight and significantly increased ( $p < 0.05$ ) the root:shoots dry weight ratio except for KK clones. The root surface area, root diameter and root:shoots dry weight ratio positively determined the weight of millable cane. **Conclusion:** The application of mycorrhizal inoculum in the nursery has a higher root infection percentage at the early growth on dry land and it improves the root traits and weight of millable cane.

**Key words:** *Saccharum officinarum* L., arbuscular mycorrhiza fungi, transplanted seedlings, root traits, millable cane

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**Corresponding Author:** Wawan Sulistiono, Assessment Institute for Agricultural Technology of North Maluku, Sofifi, Indonesia Tel: +6282196773678

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**Competing Interest:** The authors have declared that no competing interest exists.

**Data Availability:** All relevant data are within the paper and its supporting information files.

## INTRODUCTION

Roots are essential for different functions during plant development including plant anchorage<sup>1</sup>, water and mineral nutrient uptake and synthesis of various essential compounds<sup>2,3</sup> and improve the physical conditions of the soil<sup>4</sup>. Vigorous root growth which is depicted by diameter, density and length is essential to support vigorous shoot growth and development of healthy plants<sup>5</sup>. Therefore vigorous root growth is probably correlated with better yield.

Sugarcane is one of the most valuable global crops. The yield potential is considered very high due to the distinct anatomical and biochemical features associated with the C4 plant which include photosynthesis, leaf weight, leaf area index and total cane biomass of dry matter which reaches 22.9-39.0 t ha<sup>-1</sup> year<sup>-1</sup><sup>6,7</sup>. Indonesian sugarcane production is 69.68 t fresh cane ha<sup>-1</sup><sup>8</sup>, however such high potential yield has not been reached due to several field constraints. The constraint of growing sugarcane in dry land is limitations of soil water<sup>9</sup>. Soil water deficit can decrease both stomatal conductance, leaf water potential and increase proline<sup>10,11</sup>. Water deficit can reduce the net photosynthesis by causing stomatal closure which reduces the CO<sub>2</sub> diffusion inside the leaf and in turn the net photosynthesis<sup>12,13</sup>. Water stress condition reduces cane yield (tons of cane per hectare) and total dry matter by 17-52 and 20-56%, respectively<sup>14</sup>.

The growth and development of the root probably plays an important role in improving the productivity of sugarcane. Sugarcane root growth in the soil is influenced by cultural practices, irrigation and fertilizer application as well as species and cultivar<sup>15-18</sup>, whereas its root distribution is affected by physical, chemical and biological factors<sup>19</sup>. Early seedling root growth and development determined the optimum root system throughout the entirely life of plant, consequently affecting growth and potentially leading to optimization of yield<sup>20,21</sup>. Early rapid root growth and branching refer to more efficient uptake of soil water and nutrients<sup>5</sup>. Indeed, there is a need for preparing seedlings with a good rooting system because it is essential for the absorption of organic materials and nutrient dynamics of sugarcane.

One of the greatest important factors of root study is the root architecture and distribution as well as the root growth dynamics<sup>22</sup>. According to Smith *et al.*<sup>16</sup> and Ohashi *et al.*<sup>23</sup> the growth of sugarcane shoot is directly determined by the capacity of the rooting system. Furthermore, Arbuscular Mycorrhizal Fungi (AMF) would increase the sink capacity in the rooting system, such as an increase in soluble sugars in the roots<sup>24</sup>, soluble protein, the concentration of N and P on

rooting cane<sup>25</sup> and increased the sugarcane biomass 20 days after inoculation<sup>26</sup>. Therefore a study on root development of sugarcane with the application of AMF inoculum must be carried out. The timing of AMF application is expected to give a difference in root growth and its positive effect on millable cane. The treatment of the inoculation time of AMF will result in the development of mycorrhizal infection and the roots growth among 5 sugarcane clones namely root surface area, root length, root diameter, root dry weight and root dry weight ratio, thus contributing new knowledge about the dynamic of early roots growth of 5 sugarcane clones by the influence of timing of AMF inoculation. From some traits of the roots it can be ascertained the root traits that determine the weight of millable cane, thus contributing new knowledge about the traits of the roots that best characterize sugarcane seedlings to increase the weight of millable cane on dry land. The aim of this study was to gain insight on the influence of the timing of AMF application into the transplanted sugarcane single bud chips seedling from the viewpoints of (1) Development of mycorrhizal infection in the rooting system, (2) The dynamics of roots growth after mycorrhizal inoculation and (3) The roots' characteristics that significantly affect millable cane weight.

## MATERIALS AND METHODS

**Site description and field management:** The experiments were conducted in Harjobinangun (600 m a.s.l., S: 07°40'16" and E: 110°24'14") Sleman district, Special region of Yogyakarta, Indonesia and according to Koppen's classification, belongs to tropical monsoon climate<sup>27</sup>. The soil in the experimental station is classified as Regosol<sup>28</sup>. The main chemical characteristics of the soil at the start of the experiment show that soil pH, cation exchange capacity, organic material concentration, soil moisture at field capacity, N total, available P and available K are 5.80, 11.87 cmol (+) kg<sup>-1</sup>, 3.38, 8.93, 0.16 and 3.95 mg g<sup>-1</sup> and 0.71 cmol (+) kg<sup>-1</sup>, respectively.

**Plant material and treatments:** This study used seeds of single bud chips from 5 sugarcane clones: PS864, Kidang kencana (KK), PS881, Bululawang (BL) and VMC. The seed of single bud chips were germinated in the nursery, using polybags (8×12 cm). Seeds from each clone were subjected to one of the following treatments: no inoculation (M0), inoculation of Arbuscular Mycorrhizal Fungi (AMF) at the nursery (MN) and inoculation when transplanting to the field (MF). The experiments were set up using 15 combinations.

The experiment was conducted by raising the seedlings of bud chips in polybags of 8×12 cm consisting of five sugarcane clones, i.e., 40 seeds per combination treatments. Each experimental treatment was replicated 4 times. Inoculation treatments in the nursery began shortly after the bud chips were placed in polybags in the nursery.

**Strains of Arbuscular Mycorrhizal Fungi (AMF) and doses:**

The AMF inoculum was in the form of zeolite granular media. The doses of AMF inoculum was 1 g (3.6 spore)/seed. The AMF was obtained by collecting the fungi from various regions in Java island of Indonesia. Some genera of AMF obtained from isolation by a different experimental team were *Glomus* sp., *Funneliformis* sp., *Acaulospora* sp., *Gigaspora* sp. and *Scutellospora* sp.

**Experimental design and planting:**

The experiment had a Completely Randomized Design (CRD) with four replicates. The first factor corresponded to 5 sugarcane clones (PS864, Kidang Kencana (KK), PS881, Bululawang (BL) and VMC), while the second consisted of three treatments of mycorrhizal inoculum application times (M0, MN and MF). There were 15 combination treatments. At the age of 40 days after sowing in the nursery, the seedlings were transplanted in large polybags (45×45 cm). Each combination treatments consisted of 40 seedlings and its was replicated four times arranging the polybag (2400 polybag) according to a randomized completely scheme. The space between rows was 1 m between seedling inter row was 0.45 m and the total experimental area was 2500 m<sup>2</sup>. In the MF treatment, inoculation began on the same day with seedlings transplantation into larger polybags (45×45 cm). In the M0 treatment, each clone had no inoculation of mycorrhizal inoculum. The transplanting was conducted in April 2014.

Fertilization was conducted in the large polybags. The fertilizers used were ZA (*Zwavelzure Ammoniak*: (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>) with 21% of nitrogen content for N, SP-36 (Super Fosfat: P<sub>2</sub>O<sub>5</sub>) with 36% of phosphate content for P, KC1 (Kalium Klorida: K<sub>2</sub>O) with 60% of potassium content for K sources which produced by Indonesian Fertilizer Company (PT Pupuk Indonesia), each large polybags received 2 g ZA given during transplantation as much as 0.67 and 1.3 g at the age of 60 days and P and K doses of 1 g were given during seedling transplantation. In accordance with the standard procedures for sugarcane cultivation, the plants were treated with pre-emergence herbicides and post emergence weeding but were not irrigated.

**Measurements:** The parameters observed were: infection percentage, total root length; root surface area, root diameter, root dry weight, root:shoot dry weight ratio and the weight of millable cane. The infection percentage was measured by using modified clearing and staining<sup>29</sup>. Sixty root cuttings (1 cm) per combination treatment were randomly chosen from 9 sample plants. Observation was done from the 1st-11th week after transplantation.

Measurement of the total root length and root surface area was done using area meter. Measurement of the total root length, root surface area and root diameter was done using the method of line intersection perfected by Indradewa<sup>30</sup>. The result was compared with the calculation of length according to Tennant<sup>31</sup>. In order to obtain the root surface area, roots were assumed to be cylindrical so that the root projection area = 2 RP with R was the radius, P was the root length. The root surface area was the area of the cylinder bark without cover at both the root edges, i.e., circumference multiplied with root length = 2πRP. The root diameter was obtained from the formula of root surface area, i.e., 2R. Root dry weight was measured from all roots after they were constantly dried. The root:shoot ratio was calculated by dividing the value of root with the shoot dry weight at each time of observation. The sample of each root parameters such as total root length, root surface area, root diameter, root dry weight, root:shoot dry weight ratio were harvested from plants from the 1st-11th weeks (1 weeks interval) after seedlings transplant and MF treatment. Each root parameters was measured from 6 whole rooting system per combinations treatments which were collected.

The weight of millable cane was measured at the age of 10 months from 12 samples of millable cane per combination treatments. The weight of millable cane (individual cane) was measured at the first internode from the bottom until the end of internodes.

**Data analysis:** Data were submitted to analysis of variance of CRD using SAS 9 program for Windows. If there was an interaction between treatment, a comparison of the interaction effects was made. Otherwise, treatment mean effects were compared based on Duncan's multiple range test at p<0.05. To determine the important root parameters for millable cane weight, root parameter data were analyzed by step-wise regression analysis through zero<sup>32</sup>.

## RESULTS

**Mycorrhizal infection percentage:** The results of the variance analysis demonstrated that there were significant

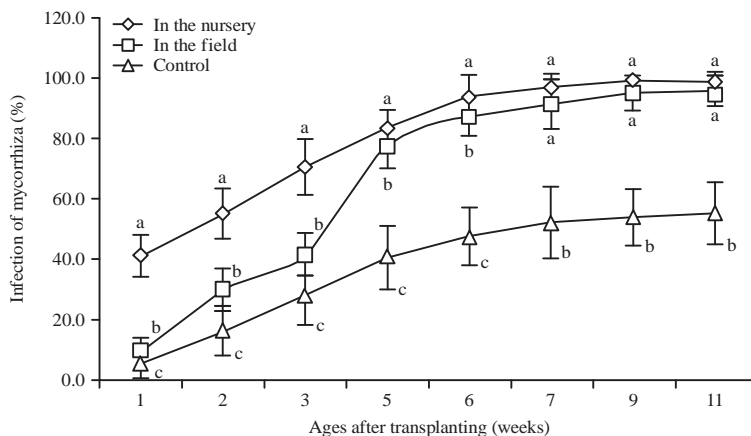


Fig. 1: Effect of timing of mycorrhizal inoculum application on infection of mycorrhiza (%)

The same letters in each root parameters at the same ages indicated no significant differences between treatments at  $p < 0.05$  according to Duncan's multiple range test. Data are averages of sixty root cuttings (1 cm) from nine plants  $\pm$  SD

differences ( $p < 0.01$ ) among the time of AMF inoculum application effected on infection percentage. The application of AMF inoculum in the nursery significantly increased ( $p < 0.01$ ) the infection percentage in early transplanting in the field at the age of 1-6 weeks after transplanting (Fig. 1). These results indicated the importance of inoculating the sugarcane during the germination phase in the nursery before transplanting in the field.

**Root length:** The clones that interacted with the AMF application significantly increased ( $p < 0.01$ ) the root length at the age of 2-11 weeks after transplanting. The inoculum application of AMF in the nursery caused higher total root length than application in the field and was significantly different ( $p < 0.01$ ) from the control for PS881 and BL (Fig. 2). These results showed that the rooting of PS881 and BL clones with AMF inoculation was more responsive in the form for increased root length.

**Root surface area:** The clones that interacted with the AMF application significantly increased ( $p < 0.01$ ) the root surface area 2-11 weeks after transplanting. There are variations between sugarcane clones on the difference of times of mycorrhizal application to the root surface area. PS881 and BL clones have the highest root surface area with mycorrhizal application in the nursery (Fig. 3). The results indicated that the inoculation of sugarcane seedlings with AMF in the nursery positively influenced the root surface area in some clones of sugarcane, for example on PS881 and BL, indicating the genetic variability.

**Root diameter:** The root diameter was influenced by the interaction of the timing of application of mycorrhizal inoculum and clones. The interactions occurred during observations at the ages of 1-11 weeks after transplanting. Root diameter was significantly higher ( $p < 0.01$ ) in AMF application than the control except for PS864 and KK clones. Increased root diameter reached 50-96% compared with the controls. Meanwhile, the combination of PS881 clones with mycorrhizal application at the nursery produced the highest root diameter (Fig. 4). The general trend for root diameter of the clones and the inoculation of the sugarcane with AMF inoculum positively influenced the root diameter.

**Root dry weight:** The clones interacted with the timing of AMF application significantly ( $p < 0.01$ ) and increased the dry weight of roots at the age of 2-11 weeks after transplantation. The AMF inoculation was preferably given in the nursery for PS864, BL and VMC clones and in the field for PS881 in increasing the dry weight of roots significantly. On KK clone, the timing of AMF application did not significantly affect the dry weight of roots compared with the control (Fig. 5). These results indicated that each clone had a different root biomass accumulation capacity due to the influence of mycorrhizal inoculation.

**Root dry weight: shoot dry weight (RDW:SDW) ratio:** The clones interacted with the timing of AMF application significantly increased ( $p < 0.01$ ) the RDW:SDW ratio at the age of 4-10 weeks after transplanting and significantly increased ( $p < 0.01$ ) at the age of 11 weeks after transplanting. However, at the beginning of transplanting, RDW:SDW ratio was

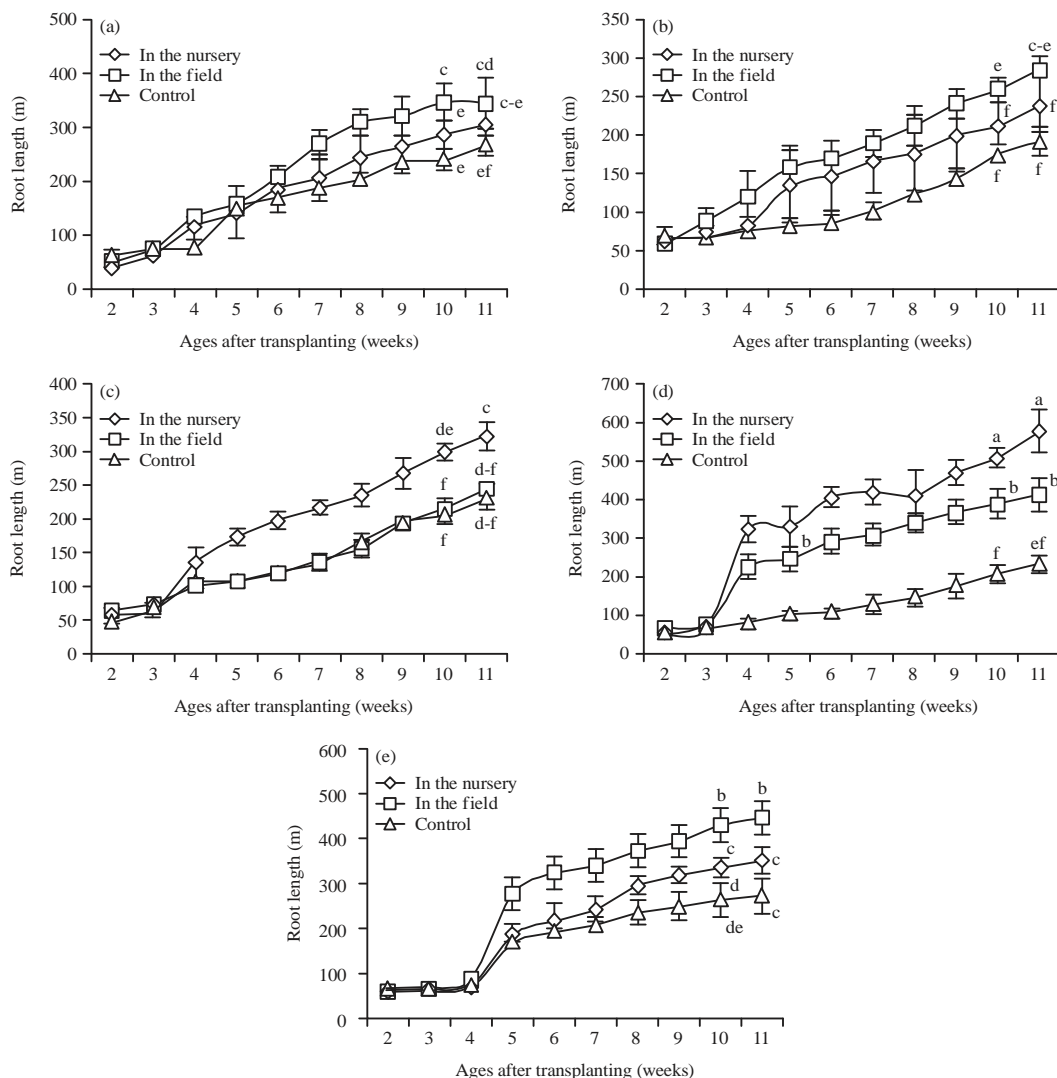


Fig. 2(a-e): Effect of mycorrhizal inoculation treatment on root length (a) PS864, (b) KK, (c) PS881, (d) BL and (e) VMC

The same letters in each root parameters at the ages of 10-11 weeks after transplanting indicated no significant differences between treatments at  $p < 0.05$  according to Duncan's multiple range test. Data are averages of eight plants  $\pm$  SD

determined significantly by the clone and the application of mycorrhiza. There were variations between sugarcane clones at the timing of AMF application for RDW:SDW ratio (Table 1). The PS864, BL and VMC clones were responsive to AMF application in increasing the RDW:SDW ratio, while the KK and PS881 clones were not responsive. The results of Table 1 demonstrated that clones played a role in determining the ratio of root to shoots dry weight due to the influence of mycorrhizal inoculation and inoculated seedlings showed better performance than without inoculation (Fig. 6).

**Weight of millable cane (individual cane):** The weight of millable cane at the age of 10 months after transplanting was significantly increased ( $p < 0.01$ ) by the interaction of clones and time of mycorrhizal application. The inoculation treatment

of AMF inoculum when transplanting in the field resulted in the highest weight of millable cane and it significantly differs ( $p < 0.01$ ) from treatment without inoculation (control) of BL, VMC and PS881 clones, except for KK clone, which resulted in the highest weight of millable cane and its significantly differs ( $p < 0.01$ ) from control with AMF inoculation in the nursery (Fig. 7). In general, except clone PS864, mycorrhizal inoculation for all treatments positively influenced the weight of millable cane than treatment without inoculation. These results indicated that mycorrhizal inoculation is required in the system of transplanting seedlings to increase the weight of millable cane.

**Root parameters determinant of weight of millable cane:** The roots parameters with the most decisive of weight of

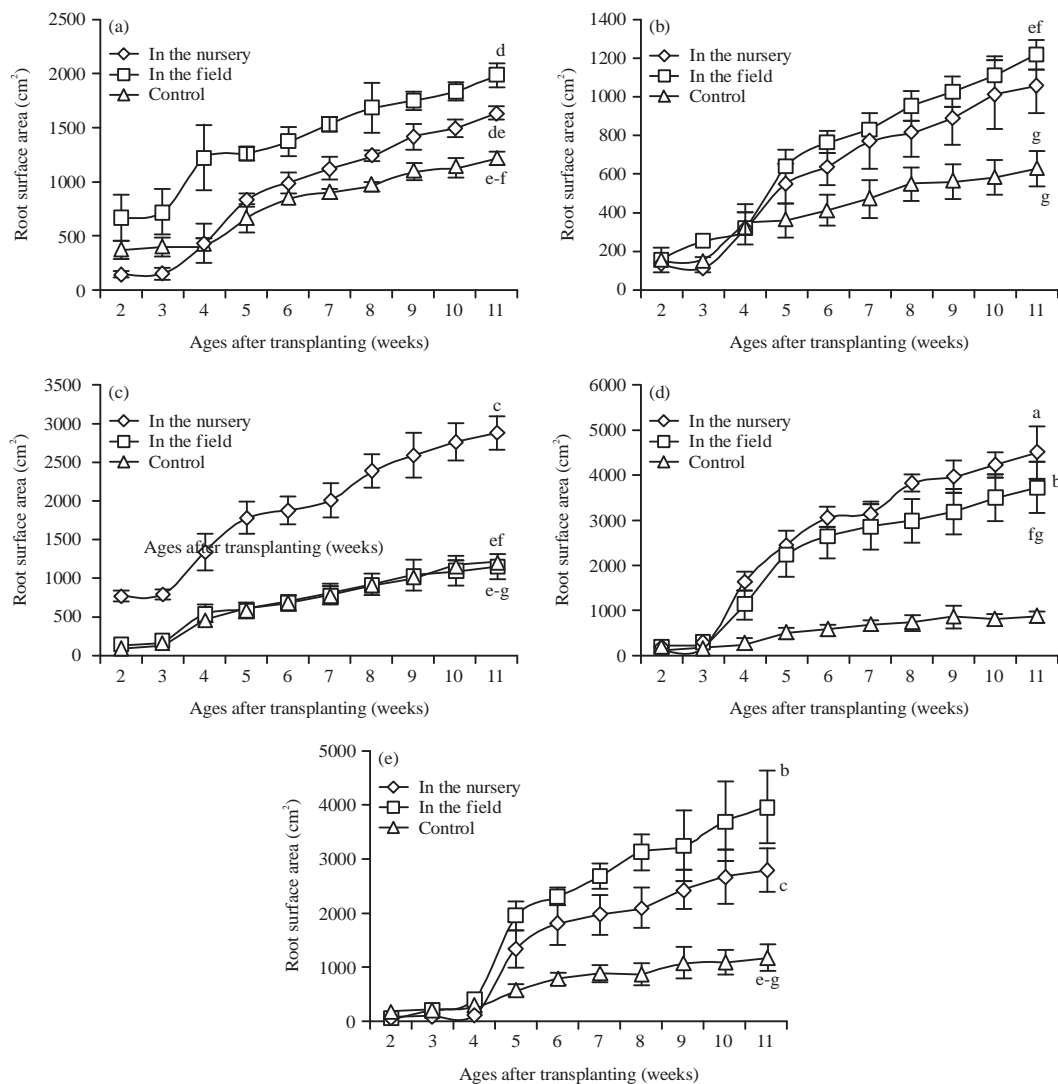


Fig. 3(a-e): Effect of mycorrhizal inoculation treatment on root surface area (a) PS864, (b) KK, (c) PS881, (d) BL and (e) VMC  
 The same letters in each root parameters at the age of 11 weeks after transplanting indicated no significant differences between treatments at  $p < 0.05$  according to Duncan's multiple range test. Data are averages of eight plants  $\pm$  SD

millable cane were root surface area, root diameters and RDW:SDW ratio (Table 2). Root surface area, root diameter and RDW:SDW ratio played a positive role in increasing the weight of millable cane of 52.09, 40.84 and 7.06% respectively. The results (Table 2) suggested that root parameters related to the weight of millable cane are root surface area, root diameter and RDW:SDW ratio and those root parameters can be improved by application with AMF when the seedlings were sown in the nursery.

### DISCUSSION

**Effect of inoculation on the percentage of mycorrhizal infection and root growth:** The application of mycorrhizal

inoculum in the nursery of single bud chips seedlings resulted increased the infection percentage (41.30%) at the early further growth in the field (1 week after transplanting). This indicated that the ability of AMF to colonize the roots occurs early since mycorrhizal application in the nursery. This is in accordance with the finding reported by Javot *et al.*<sup>33</sup> that the development of infection was already seen 6 days after inoculation in the form of arbuscular population. Shaul-Keinan *et al.*<sup>34</sup> also reported that the infection percentage of mycorrhiza reached 40-50% at the age of 5 weeks after inoculation in tobacco. In this study the ability of AMF to colonize the roots (Fig. 1) has reached the optimum at the age of 7-11 weeks after transplanting. This finding is in line with

Table 1: Effect of mycorrhizal inoculation treatment on the root:shoot dry weight ratio

Clones	Time of mycorrhiza application	Root:shoot dry weight ratio at weeks after transplanting						
		4	6	7	8	9	10	11
PS864	Nursery	3.03 <sup>a</sup>	1.14 <sup>bc</sup>	1.07 <sup>c</sup>	1.06 <sup>c</sup>	1.05 <sup>c</sup>	1.04 <sup>cd</sup>	1.03 <sup>bc</sup>
	Field	1.60 <sup>b-d</sup>	0.57 <sup>ef</sup>	0.58 <sup>ef</sup>	0.57 <sup>g</sup>	0.58 <sup>ef</sup>	0.57 <sup>g</sup>	0.57 <sup>cd</sup>
	Control	1.05 <sup>c-e</sup>	0.54 <sup>f</sup>	0.54 <sup>ef</sup>	0.53 <sup>g</sup>	0.53 <sup>g</sup>	0.52 <sup>g</sup>	0.55 <sup>cd</sup>
KK	Nursery	2.06 <sup>b</sup>	1.11 <sup>bc</sup>	1.09 <sup>c</sup>	1.06 <sup>c</sup>	1.09 <sup>c</sup>	1.08 <sup>c</sup>	1.04 <sup>bc</sup>
	Field	1.06 <sup>c-e</sup>	0.98 <sup>c</sup>	0.99 <sup>c</sup>	0.98 <sup>c</sup>	0.97 <sup>cd</sup>	0.99 <sup>cd</sup>	1.06 <sup>bc</sup>
	Control	1.30 <sup>b-d</sup>	0.99 <sup>c</sup>	1.02 <sup>c</sup>	1.00 <sup>c</sup>	1.01 <sup>cd</sup>	1.01 <sup>cd</sup>	1.01 <sup>bc</sup>
PS881	Nursery	1.12 <sup>b-e</sup>	0.77 <sup>de</sup>	0.74 <sup>d</sup>	0.75 <sup>e</sup>	0.76 <sup>e</sup>	0.81 <sup>e</sup>	0.79 <sup>b-d</sup>
	Field	0.69 <sup>de</sup>	0.50 <sup>f</sup>	0.48 <sup>f</sup>	0.39 <sup>h</sup>	0.51 <sup>g</sup>	0.53 <sup>g</sup>	0.58 <sup>b-d</sup>
	Control	1.10 <sup>b-e</sup>	0.64 <sup>ef</sup>	0.64 <sup>de</sup>	0.61 <sup>fg</sup>	0.62 <sup>eg</sup>	0.60 <sup>fg</sup>	0.69 <sup>b-d</sup>
BL	Nursery	1.72 <sup>bc</sup>	1.26 <sup>b</sup>	1.27 <sup>b</sup>	1.25 <sup>b</sup>	1.27 <sup>b</sup>	1.19 <sup>b</sup>	1.09 <sup>b</sup>
	Field	1.90 <sup>bc</sup>	0.97 <sup>cd</sup>	0.99 <sup>c</sup>	0.98 <sup>c</sup>	1.03 <sup>cd</sup>	1.00 <sup>cd</sup>	1.06 <sup>bc</sup>
	Control	1.92 <sup>bc</sup>	0.77 <sup>de</sup>	0.73 <sup>d</sup>	0.67 <sup>ef</sup>	0.69 <sup>ef</sup>	0.70 <sup>f</sup>	0.51 <sup>d</sup>
VMC	Nursery	1.33 <sup>b-d</sup>	1.74 <sup>a</sup>	1.77 <sup>a</sup>	1.77 <sup>a</sup>	1.81 <sup>a</sup>	1.83 <sup>a</sup>	1.94 <sup>a</sup>
	Field	0.32 <sup>e</sup>	1.06 <sup>bc</sup>	1.03 <sup>c</sup>	1.05 <sup>c</sup>	1.06 <sup>c</sup>	1.06 <sup>c</sup>	1.04 <sup>bc</sup>
	Control	2.02 <sup>bc</sup>	0.94 <sup>cd</sup>	0.95 <sup>c</sup>	0.89 <sup>d</sup>	0.91 <sup>d</sup>	0.93 <sup>d</sup>	0.95 <sup>b-d</sup>

Numbers followed by same letters in the same columns did not differ significantly at p<0.05 according to Duncan's multiple range test

Table 2: Factorwise of roots parameters at the age of 11 weeks after transplanting on the weight of millable cane at the age of 10 months

Root parameters	Parameter estimate	Type II SS	Effect (%)	p-value
Roots surface area	0.00007239±0.00001463	0.42576	52.09	<0.0001
Roots diameter	0.00531±0.00121	0.33383	40.84	<0.0001
RDW:SDW ratio	0.10959±0.06017	0.05771	7.06	0.0738
Sum of residuals -0.000863				
Sum of squared residuals 0.988289				
Sum of squared residuals error SS -0.000000				
First order autocorrelation 0.39069				
Durbin-watson D 1.20767				
R <sup>2</sup> 0.94				

Parameter ±SD. The Sum of Square Residuals-Error, SS: Sum of Square Residuals, Sum of Square Error in ANOVA table

Sieverding<sup>35</sup> that the exponential phase reaches a maximum of 40 days after infection.

In general the application of AMF inoculum in the nursery, besides resulting in speed and extent of infection percentage on roots (Fig. 1) has a positive effect on root length, root surface area, root diameter, root dry weight and the RDW:SDW ratio. This indicated that the developing roots in these clones were attributed to the ability of symbiosis (percentage of infection) since the beginning in the nursery (Fig. 1). The effect of mycorrhiza on root growth may be due to the mycorrhiza which formed vesicles that facilitated the carbon exchange between the two organisms. Likewise, it formed arbuscules as organs of reproduction<sup>36,37</sup> and the mycorrhiza improved absorption and transfer of nutrients such as P<sup>38</sup>. According to Yao *et al.*<sup>39</sup>, Smith and Smith<sup>40</sup> and Abdel-Fattah *et al.*<sup>25</sup> the root colonization was associated with the ability of P, N absorption capability on the roots as well as higher concentrations of total soluble proteins in root tissues or

phosphatase activities. This might explained that the application of mycorrhiza in the nursery improves the root parameters on some clones.

This results are consistent with the finding by Chatzistathis *et al.*<sup>41</sup> that the application of AMF improved the rooting system such as root branches, root dry weight and root:shoots dry weight ratio of Greek olive cultivars. Another report also showed that the application of AMF increased the number of lateral roots per cm of primary roots, the total root area, the number of roots as well as root length in the seedlings of bamboo<sup>42</sup>.

In this experiment on the root length and root surface we found that these root traits increased sharply at the age of 4 weeks after transplanting (PS864, KK, PS881 and BL). It is caused by many primary roots that died at the age of 1-3 weeks after planting (47-61 days after seedling) and secondary root development occurred afterward (Fig. 2, 3). This finding is in line with Chopart *et al.*<sup>43</sup> and Smith *et al.*<sup>16</sup>



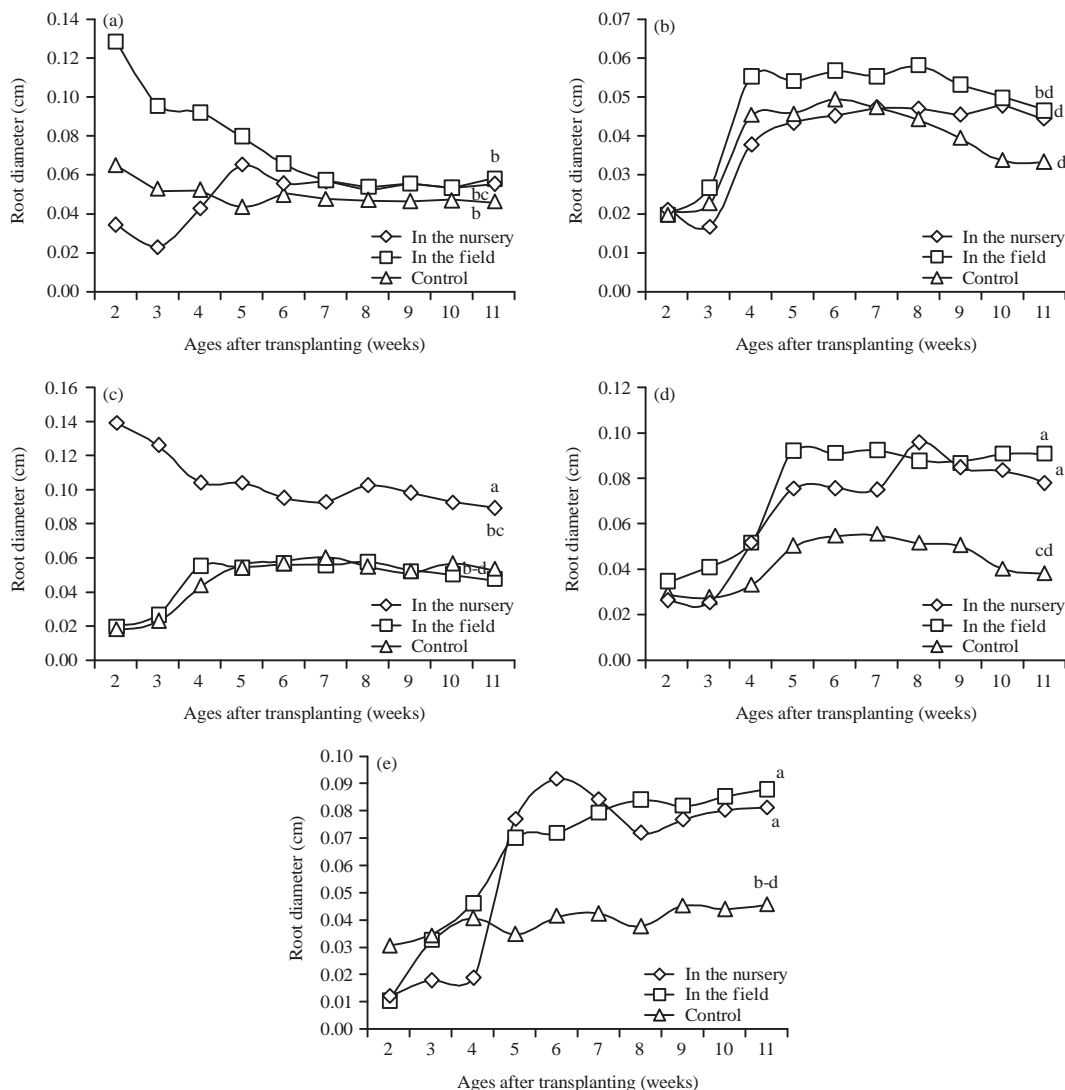


Fig. 4(a-e): Effect of mycorrhizal inoculation treatment on root diameter (a) PS864, (b) KK, (c) PS881, (d) BL and (e) VMC

The same letters in each root parameters at the age of 11 weeks after transplanting indicated no significant differences between treatments at  $p < 0.05$  according to Duncan's multiple range test. Data are averages of eight plants

suggested that the death of the primary root of sugarcane occurred at the age of 60-90 days. However, VMC clones showed a slower growth of roots. These was possibly caused by the genotypic effect on roots growth.

The increase in root parameters was also shown by the change from the RDW:SDW ratio among the clones (Table 1). This is caused by the difference in the growth of sugarcane shoot that is directly determined by the capacity of the rooting system<sup>16,23</sup>. At the age of 4 weeks after transplanting, sugarcane root growth was higher than shoots. According to Smith *et al.*<sup>16</sup> higher root growth in sugarcane at the early of growth is required to support the growth of shoots.

The increased RDW:SDW ratio in the mycorrhiza application in the nursery was caused by the increased root

diameter, root surface area and root dry weight. These results are consistent with the finding of research by Muniyamma *et al.*<sup>44</sup> that the application of AMF spores of the *Glomus* type on the growth media of the tissue culture increases in the formation of the rooting system and the root length of sugarcane seedlings and Chen *et al.*<sup>45</sup> that inoculation of AMF spores increased the fresh weight root:shoots ratio of *Poncirus trifoliata*. Besides, the sugarcane inoculated with mycorrhiza produced a high root:shoots ratio when sugarcane was stressed with drought<sup>46</sup>.

In general on the plants inoculated mycorrhizal, the carbon allocation to the rooting system tended to be high in comparison with treatment without AMF inoculation<sup>47,48</sup>. The amount of carbon transferred to the rooting system positively

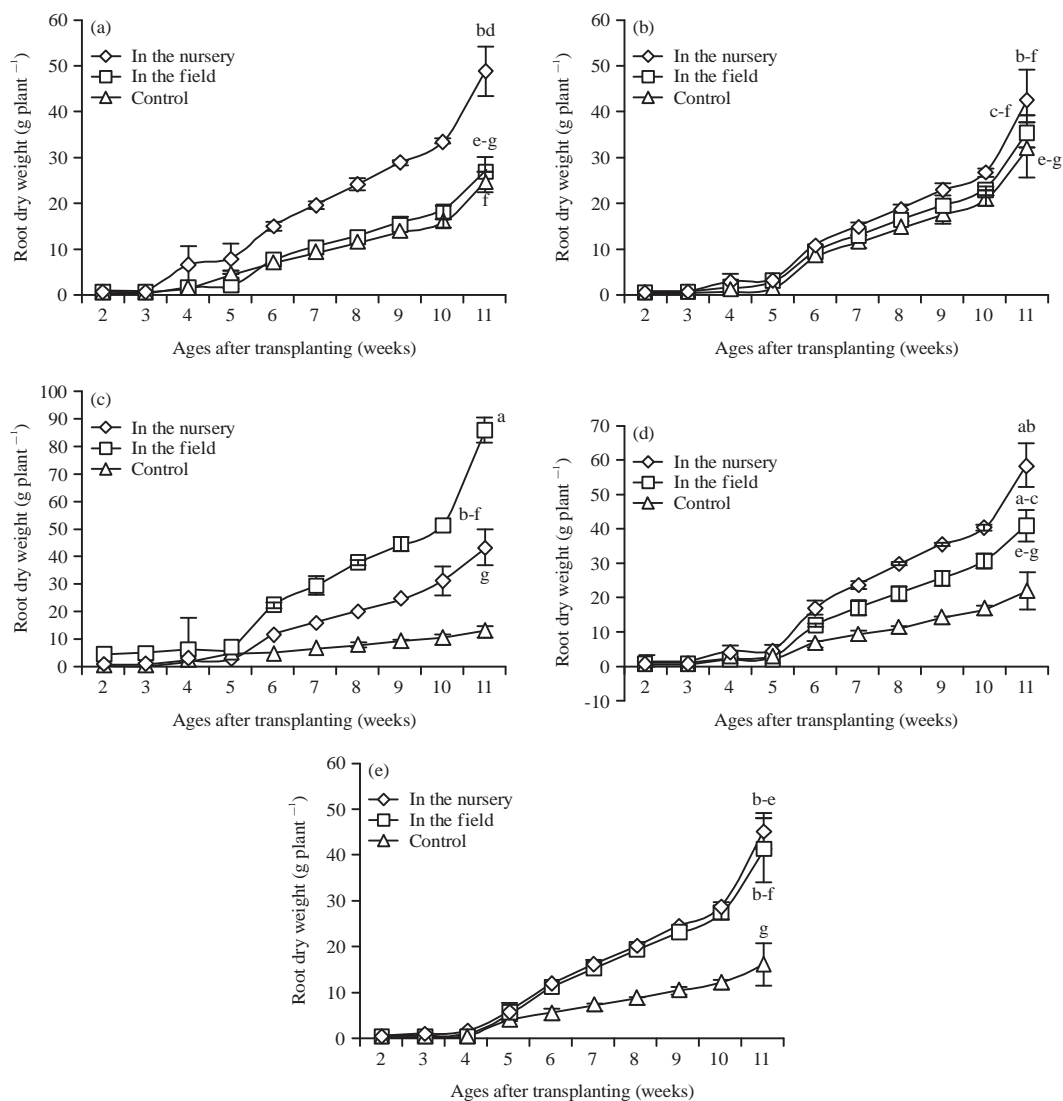


Fig. 5(a-e): Effect of mycorrhizal inoculation treatment on root dry weight (a) PS864, (b) KK, (c) PS881, (d) BL and (e) VMC  
 The same letters in each root parameters at the age of 11 weeks after transplanting indicated no significant differences between treatments at  $p < 0.05$  according to Duncan's multiple range test. Data are averages of eight plants  $\pm$  SD

correlated with the presence of vesicle on the rooting system. Vesicle was used for the structure of the development of mycorrhizal fungi storage<sup>49</sup>. Brunner *et al.*<sup>50</sup> reported that mycorrhiza seem to play important protective roles. This might explain the increased root growth by the effect of mycorrhiza.

The interaction effect of AMF application and clones on the root parameters was consistent for all clones except the KK clone. The result showed the genetic variation in root parameters of sugarcane. Different responsiveness of root development was also shown in the research by Morris and Tai<sup>51</sup> who showed a very significant difference in root dry weight ( $p < 0.01$ ), root length ( $p < 0.01$ ), root diameter ( $p < 0.05$ ) in the treatment of water provision.

There were un-responsive clones to mycorrhiza which is related to the level of mycorrhizal responsiveness. The mycorrhizal responsiveness is one unit result (dry weight for example) arising from the interaction between plants and mycorrhizal genus<sup>52,53</sup>. It is assumed that the difference in the responsiveness of clone in AMF application is related to the level of colonization<sup>54,55</sup>. The ability of plants colonized by mycorrhiza is determined by the ability of plants to produce "Small inducible secreted protein mycorrhizal 7" (MiSS 7) as a protein symbiosis<sup>56</sup> or *Glomus intraradices* secretes protein (SP7) is an effectors that contribute to the status of colonization of AM fungi in the roots<sup>57</sup>. Nagata *et al.*<sup>58</sup> reported that AM colonization increased because of the greater amount

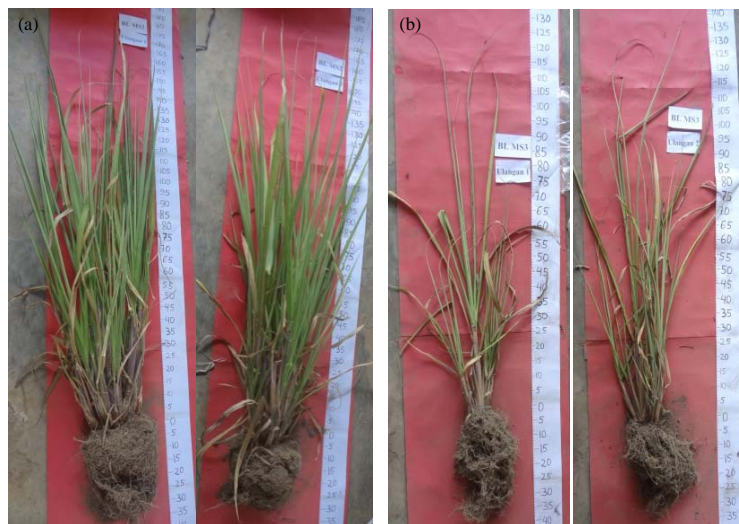


Fig. 6(a-b): Performance of root: shoot ratio of sugarcane from bud chips seedling due to mycorrhizal inoculation at the nursery (a) Control and (b) BL clones

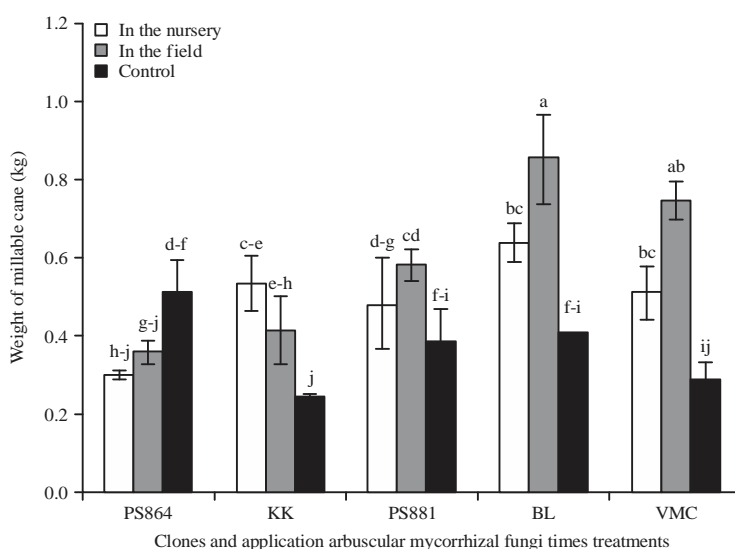


Fig. 7: Effect of mycorrhizal inoculation times on weight of millable cane

The same letters in each weight of millable cane among clones indicated no significant differences between treatments at  $p < 0.05$  according to Duncan's multiple range test. Data are averages of eight millable cane  $\pm$ SD

of plant hormones such as jasmonic acid and strigolactone in AM-colonized roots of host plants and hormones are secreted to the rhizosphere. Foo *et al.*<sup>59</sup> reported that gibberellic acids signal interacts with symbiosis signaling pathways, directed AMF colonization in host roots. These results indicated that the inoculation of the sugarcane seedlings with AMF positively influenced root parameters.

**Roots parameter that significantly affects the weight of millable cane:** The root parameters that determine the

increase in the weight of millable cane were the root surface area, root diameters and RDW:SDW ratio (Table 2). The role of root surface area to increase the weight of millable cane was possibly related to expansion the field of nutrient absorption. According to Finlay<sup>60</sup>, the extra radical mycelium provides increased surface area for nutrient uptake, bridging nutrient depletion zones. Miyasaka and Hobte<sup>61</sup> argued that the increase of P absorption is usually attributed to increased root surface area and soil exploration by the root-AMF association. This finding is in line with Smith *et al.*<sup>16</sup> and Ohashi *et al.*<sup>23</sup>

suggested that higher root growth in sugarcane at the early growth is required to support the growth of shoots. Therefore, the results of this study indicated that the role of the root surface area reached the highest in determining the weight of millable cane per clump, reaching 52.09%.

Higher root diameter increased the weight of millable cane (Table 2). Root diameter that increases in the application of mycorrhizal than control was due to the increase in the root length. According to Gomathi *et al.*<sup>15</sup>, a large diameter root has higher O<sub>2</sub> diffusion ability than the small diameter root. Shiotsu *et al.*<sup>62</sup> reported that the diameter of nodal roots of *Eriarthus arundinaceus* correlates with the number of large and small xylem vessels with coefficient of determination (R<sup>2</sup>) as big as 0.762\*\* and 0.764\*\*, respectively. Root diameters of sugarcane in dry land are smaller than those in irrigated land which have larger diameter, as a form of adaptation<sup>15,52,63</sup>. Based on that, it is suspected that the sugarcane with large root diameter in dry land indicates a better ability to utilize soil O<sub>2</sub>, water and nutrient absorption which in turn increases the weight of millable cane. Therefore, in this study this is shown by the fact that the role of the root diameters determine the weight of millable cane per clump, reaching 40.84%.

Higher RDW: SDW ratio also increased the weight of millable cane per clump (Table 2). Smith *et al.*<sup>16</sup> reported that sugarcane has more roots than are needed for maximum growth under optimal soil conditions. Sugarcane root:shoot ratio increased in the vegetative growth phase, then decreased and was constant at the age of 211 days until harvest<sup>64</sup>. The root:shoot ratio was determined by the varieties and growth phase<sup>65</sup>. According to Smith *et al.*<sup>66</sup> sugarcane growth was consistent with functional equilibrium between roots and shoots. Shiotsu *et al.*<sup>67</sup> reported that root: shoot ratio relationships with the number of root and root length was closely related to the number and diameter of the stem, respectively. Therefore, the root:shoots dry weight ratio determines the weight of the millable cane, reaching 7.06%.

## CONCLUSION

The application of AMF inoculum in the nursery provides better infection percentage reaching 86.3% of 5 sugarcane clones on dry land. The most appropriate treatment of AMF application on clones BL and PS881 is in the nursery because it produces the highest roots length, root surface area and root diameter in comparison with other clones. The BL clone also produces the highest root dry weight and root: shoots dry weight ratio on application of AMF in the nursery. Root traits, i.e., root surface area, root diameter and root: shoot dry weight ratio positively determine the weight of millable cane.

## SIGNIFICANCE STATEMENTS

This study discovers the application of arbuscular mycorrhiza fungi in the nursery which reaches the highest infection of 86.3% in sugarcane transplanted seedlings and the effect of the time of arbuscular mycorrhiza fungi application differs among the clones on root traits that can be beneficial to generate the best root traits of seedling to be transplanted on dry land. This study will help the researcher to uncover the critical areas of sugarcane root dynamic on single bud chips transplanted seedlings on dry land and root traits determinant of weight of millable cane that many researchers were not able to explore. Thus, a new theory on sugarcane root dynamic and mycorrhiza infection may be developed.

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