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## **Influence of Arbuscular Mycorrhizae on Callusing and root colonization of Tea (*Camellia sinensis*) Clones in Kenya**

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### **Authors' contributions**

*This work was carried out in collaboration among all the authors. Authors AC and JPGO designed the study and wrote the first draft of the manuscript. Authors NKK and JPGO managed the analyses of the study. Authors AC and MM managed the literature searches. All authors read and approved the final manuscript.*

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### **ABSTRACT**

Mycorrhizal fungi are a major component of the soil micro flora in many ecosystems, but usually have limited saprophytic abilities. Arbuscular mycorrhizal fungi (AMF) are an important component of soil life and soil chemistry. In soil, phosphorus may be present in relatively large amounts, but much of it is poorly available because of the very low solubility of phosphates by formation of complexes with iron, aluminum, and calcium, leading to soil solution concentrations of 10µm or less and very low mobility. Tea is a major income earner in the country, but yields are declining since high yielding tea varieties have a major problem with rooting and take so long in the nursery. The current study was initiated to investigate the role between Mycorrhizae and plants to explain rooting and growth rates during early stages of tea establishment. It was conducted at James Finlay in Kericho County, Kenya. The experiment was laid out in a Randomized Complete Block Design (RCBD) with factorial arrangements. Phosphorus treatments consisted of a standard rate of

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107.66kg ha<sup>-1</sup>, two clones of the tea (S15/10 and SC 12/28) and two mycorrhizal strains (*Glomus mosseae* and *Glomus intraradices*) plus one control without mycorrhizae. Data was collected on rate of callusing, chlorophyll content and rate of root infection by mycorrhizal fungus. Application of 50kg Mycorrhizae ha<sup>-1</sup> exhibited the highest callusing rate on clone SC 15/10 with significant differences (P≤0.05) observed on the chlorophyll content from week 1 to week 30 where the standard application of phosphorus plus 50kg Mycorrhizae ha<sup>-1</sup> on clone S 15/10 had the highest content consistently throughout the trial. The highest frequency of mycorrhizae colonization in the rhizosphere was observed when 70kg ha<sup>-1</sup> was added under clone SC 12/28. AMF strains are recommended for use on tea propagation in improving callusing rate and the chlorophyll content at a rate of 50kg Mycorrhizae ha<sup>-1</sup>.

**Keywords:** Mycorrhizal fungi; arbuscular; soil life; callusing, tea.

## 1. INTRODUCTION

Tea (*Camellia sinensis* L.) is a perennial shrub belonging to the *Camellia* genus of the *Theaceae* family. Management practices such as crop rotation, fertilization, and residue management affect the organic matter, nitrogen content and microbial population of soils [1]. Since tea is a perennial crop, soils under it have different characteristics from layer to layer Pandey and Palni [2]. Hayatsu and Kosuge [3] studied variations in nitrification activity, soil pH and inorganic nitrogen content at different depths and distances from a tea stem.

Tea is a major income earner in the country, but yields are declining since high yielding tea varieties have a major problem with rooting and take so long in the nursery. Most soils have a limiting P and so Mycorrhiza association play a key role in assisting the plant roots to acquire the P [4]. Diffusion of phosphate ions in soil solution is slow compared to the rapid absorption of phosphate by the root resulting in a depletion zone around them. The mycelium of the Mycorrhizal fungus can, however, access these phosphorus sources, and make them available to the plants they colonize. It is also able to create a vast connection between the roots of a plant and with the soil around them, which allows for the fungus to uptake nutrients and particularly phosphorus for the plant and increase the surface area of the roots [5]. Thus, mycorrhizal plants are advantaged for hyphae extend beyond the depletion zone into unexploited soil. Colonization by different AM fungi does not result in the same growth responses in a single AM plant species [6,7,8], and colonization by the same AM fungus does not necessarily result in the same growth responses in different plant species (or even varieties). This diversity of responses was

nice shown with naturally co-occurring plants and AM fungi from the same site [6].

It is clear, therefore, that there is considerable functional diversity among plant-AM fungal symbioses in terms of benefits (P supply to the plant, in this context) and costs (C supply to the fungus). Different plant species in the field will be colonized by many AM fungal taxa, and the sum total of benefits and costs contributes to success, in terms of growth and reproduction specific to the crop in most cases [9]. The outcomes of the symbioses are determined by interactions between plant and AM fungal genomes as well as environmental conditions (e.g. soil pH and P chemistry). Overall, this means that mycorrhizae interactions can lead to changes in the plant composition of an area. This benefit can significantly boost tea growth as this can enhance rooting of tea seedlings, especially the clones that seem to be very difficult to root [10]. The current research will dissect this interactive role between Mycorrhizae and plants to explain the rooting and growth rates during early stages of tea establishment at the study area.

## 2. MATERIALS AND METHODS

### 2.1 Sites Description

The study was conducted at the Applied Research Department of James Finlay which is located between Kericho and Bomet Counties of Rift Valley, Kenya. The location altitude is 2157 above sea level with average annual rainfall of about 2000mm. It lies on the Equator, at latitude 0° 24'21.09"S and longitude 35° 19'26.73"E [11].

### 2.2 Experimental Design and Treatments

The experiment was laid out in a Randomized Complete Block Design (RCBD) with factorial arrangements. Phosphorus treatments consisted

of one standard rate of phosphorus at  $107.66 \text{ Kg P ha}^{-1}$ , two clones of the tea (S15/10 and SC 12/28) and two mycorrhizal strains (*Glomus mosseae* and *Glomus intraradices*) plus one control without mycorrhizae. The treatments were replicated three times.

### 2.3 Data Collection and Analysis

Data was collected at establishment for initial callusing and on leaf chlorophyll content after every one week. The root infection by mycorrhizal fungus (%) was measured in the Kenya National Museum Laboratory. Two-way analysis of variance (ANOVA) was used to determine treatment effects on dependent variables at  $P \leq 0.05$  probability level using GenStat Version 15.1 statistical package. In case where there were significant differences, separation of means was done using Fischer's Protected LSD test at 95% confidence level.

## 3. RESULTS AND DISCUSSION

### 3.1 Callusing

There were significant differences observed between the different rates of Mycorrhizae with application of Arbuscular mycorrhizae strains on callusing rate of tea clones (Fig. 1). The application rate of  $50 \text{ kg Mycorrhizae ha}^{-1}$  showed the highest callusing rate on clone S 15/10 while no significant differences were observed on clone SC 12/28 between the different rates of Arbuscular mycorrhizae. On clone S 15/10 the control had significantly the lowest callusing rate.

Clone S15/10 responded positively to callusing compared to clone SC 12/28 where application of  $50 \text{ kg glomus}$  enhanced callusing. The overall interactions between tea roots, microbes and environmental conditions prevailing in the tea rhizosphere seem to favor the growth of microbes. Since very limited and isolated efforts were made for tapping of microbial diversity, identification, evaluation and preserving them for different applications, thus the selection and inoculation of specific microbial strains or by simply promoting naturally existing microbes hold great promise in sustainable agricultural systems. Improvements in phosphorus acquisition have significant impact on plants growth and health [12]. The most recognized AMF potential to mobilize plant nutrients; especially phosphorous is one among the many functional attributes that qualify them to be the

plant growth promoting microorganisms par excellence [7].

### 3.2 Chlorophyll Content

Significant differences ( $P < 0.05$ ) were observed between the treatments on the chlorophyll content from week 1 to week 14 and only week 15 showed non-significant differences between the treatments (Table 1). Clone S 15/10 had the highest chlorophyll content compared to clone SC 12/18 under the  $50 \text{ kg ha}^{-1}$  Glomus strains with the control having the lowest on most of the weeks under observation.

The chlorophyll content differed significantly ( $P < 0.05$ ) between the Arbuscular mycorrhizae strains treatments on tea clones from week 16 to week 30 except at week 18 and 27 (Table 2). Standard P +  $50 \text{ Kg Mycorrhizae ha}^{-1}$  on clone S 15/10 had the highest chlorophyll content consistently at all the weeks. The inconsistency on the trends could be due to unpredictable weather patterns experienced during the trial period.

Arbuscular mycorrhizal fungi (AMF) enhanced plant nutrient and water uptake, increase plant tolerance to cultural and environmental stresses, and play an important role at the plant-rhizosphere interface and increases photosynthetic rate through increased chlorophyll content which corroborate with the findings by Tanwar et al. [13].

### 3.3 Mycorrhizal Root Colonization

The highest frequency of mycorrhizae colonization in the rhizosphere was observed when  $70 \text{ kg Mycorrhizae ha}^{-1}$  was added under clone SC 12/28 with 30% (Table 3). Modification of plant-pathogen relations: mycorrhizae influence the colonization of roots by other microorganisms, reduce the susceptibility (or increase the tolerance) of roots to soil-borne pathogens such as nematodes or phytopathogenic fungi. Secretion of antibiotics and support of a community that competes or antagonizes pathogenic microorganisms, thus aiding in disease suppression and increasing the number of beneficial soil organisms in the soil.

The degree of dependence varies with plant species, particularly the root morphology, and conditions of soil and climate [14]. Plants with thick roots, poorly branched and with few root hairs, are usually more dependent on

mycorrhizae for normal growth and development. The fungi do not disperse with the wind like mold fungi, but instead move by growing from root to root, or by moving with quantities of soil [15].

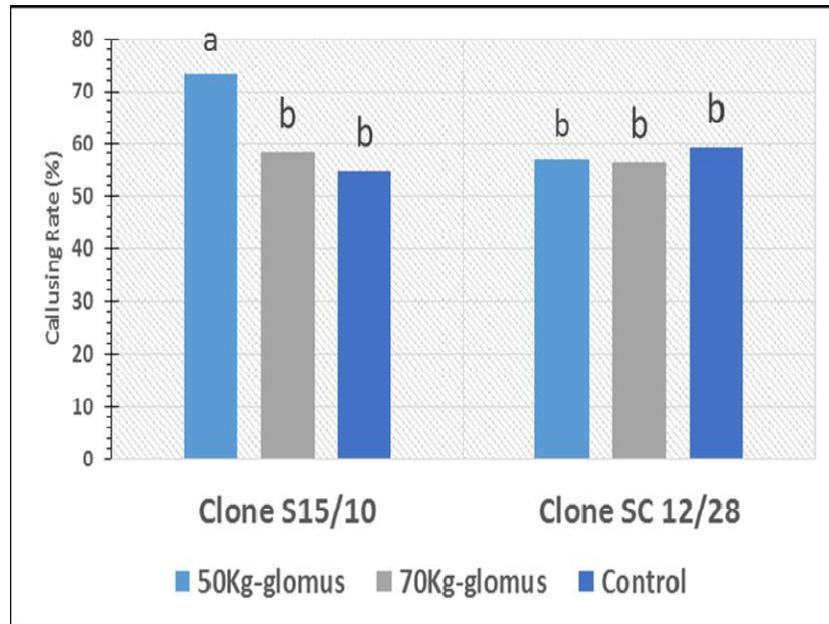


Fig. 1. Effect of arbuscular mycorrhizal strains on callusing in different tea clones

Table 1. Effect of arbuscular mycorrhizae on chlorophyll content of tea strains from week 1 to week 15

Clone	Treatment	Week 1	Week 5	Week 10	Week 15
S 15/10	50kg-glomus	295.0a	315.0ab	283.2a	355.8a
	70kg-glomus	247.2ab	260.0abc	303.7a	354.7a
	Control	214.1bc	350.2a	166.9b	343.7a
SC 12/28	50kg-glomus	183.7c	214.7bc	205.9b	360.9a
	70kg-glomus	181.8c	188.8c	270.5a	304.7a
	Control	170.2c	226.2bc	162.2b	295.3a
	P-Value	<.001	0.002	0.027	0.094
	LSD	51.65	103.7	58.46	63.69
	CV%	20.2	33.7	21.2	15.9

Means followed by different letters in the same column are significantly different at  $P \leq 0.05$

Table 2. Effect of arbuscular mycorrhizae on chlorophyll content of tea strains from week 16 to week 30

Clone	Treatment	Week 16	Week 20	Week 25	Week 30
S 15/10	50kg-glomus	489.8a	419a	274.8b	344.8a
	70kg-glomus	435.3a	355bc	391a	362.8a
	Control	325.8b	391.6ab	303.2b	353.2a
SC 12/28	50kg-glomus	309.9b	326.2c	275.8b	283.8b
	70kg-glomus	434a	304.6c	312.2b	283.7b
	Control	258.5b	353.5bc	292b	267.8b
	P-Value	<.001	<.001	0.003	<.001
	LSD	71.4	48.79	58.66	42.71
	CV%	16	11.5	16	11.4

Means followed by different letters in the same column are significantly different at  $P \leq 0.05$

**Table 3. Effect of mycorrhizae strains on mycorrhizal activities in the root rhizosphere under different levels of two tea clones**

Clone	Treatment	%F	M%	m%	a%	A%
SC 12/28	50kg/ha	26.7b	12.4a	46.4b	83.8a	10.4a
	70kg/ha	30.0a	9.0b	30.1c	63.1b	5.7b
	Control	6.7e	0.3d	5.1e	0d	0e
S 15/10	50kg/ha	10.0d	8.7b	86.7a	13.5c	1.2d
	70kg/ha	17.2c	4.4c	25.6d	85.9a	3.8c
	Control	0f	0d	5.0e	0d	0e

Values in columns followed by the same letter are not statistically significant ( $p < 0.05$ ),

\*%F-The frequency of mycorrhizae in the root system\* M%-The intensity (Total) of the mycorrhizal colonization in the root system\* m%-The intensity of AMF colonization in the root fragments\*a%-Arbuscular abundance in mycorrhizal parts of root fragments\*A%-Arbuscular abundance in the root system

#### 4. CONCLUSION

Arbuscular mycorrhizae application significantly increased the callusing rate on tea clones where the highest was on clone S 15/10 at the Mycorrhizae rate 50 kg P ha<sup>-1</sup>. The same treatment led to the highest chlorophyll content in both clones but higher on clone S 15/10.

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#### COMPETING INTERESTS

Authors have declared that no competing interests exist.

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