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# Effect of inoculation with arbuscular mycorrhizal fungi on growth, nutrient uptake and curcumin production of turmeric (*Curcuma longa* L.)

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## ABSTRACT

**Profitable turmeric (*Curcuma longa* L.) production requires adequate nutrients. We have investigated the effect of inoculation with arbuscular mycorrhizal fungi (AMF) on growth, nutrient uptake, yield and curcumin production of turmeric under field and glasshouse conditions. Although AMF inoculation slightly increased plant height, leaf number and shoot N content, no statistical differences were observed in vegetative growth parameters, biomass production, nutrient uptake and curcumin content compared to control plants under field conditions. It was difficult to determine the exact effect of inoculated AMF on turmeric growth because of indigenous AMF. On the other hand, turmeric showed better response to AMF inoculation under greenhouse conditions. AMF inoculation resulted in higher biomass production and nutrient uptake of turmeric. Moreover the concentration of curcumin, contained in the rhizome of turmeric, increased in AMF treatment. These results indicate that AMF inoculation has beneficial effects on turmeric growth and curcumin production. AMF inoculation to turmeric field would be effective when indigenous soil populations of AMF are low or native AMF are no longer effective.**

**Keywords:** Arbuscular Mycorrhizal Fungi; Curcumin; Rhizome; Turmeric

## 1. INTRODUCTION

*Curcuma longa* L. belongs to the family Zingiberaceae, commonly known as turmeric, is an economically important plant cropped for its variety of uses such as condiment, dye, drug and cosmetic. Over the past few decades, a considerable number of studies have been conducted on turmeric. Curcumin, which is active yellow pigment found in turmeric rhizome, has been known as a natural antioxidant with antitumor activity [1], an inhibitor of arachidonic acid metabolism [2], and a good anti-inflammatory agent [3]. Previously, it was evaluated as a chemopreventive agent by the National Cancer Institute [4]. Due to increasing demand at medicinal properties, the social concern with turmeric has been growing for the last several years. Japan imports around 4000 tons of dried turmeric per year from India, China and other Asian countries. On the other hand, the domestic turmeric production is limited only about 100 tons of fresh one per year. Although turmeric growing area was limited in Okinawa islands and part of subtropical regions previously, it has been expanding gradually further to other regions in Japan.

Turmeric requires heavy nutrients for higher yields [5-7]. Especially, higher nitrogen (N) application is effective for rhizome production [8]. But intensive input of organic and inorganic fertilizers is harming the environment. In Okinawa, a major turmeric cultivation area, high fertilizers input has been a leading cause of water pollution in coral reef seas. Furthermore the excess use of pesticide and herbicide is not recommended since turmeric is used as medicinal matter. Therefore it is necessary to promptly establish a sustainable turmeric production system with a lowered chemical input.

Soil microorganisms and their activities play important roles in transformation of plant nutrients from unavailable to available forms and also have many metabolic qualities related to soil fertility improvement [9]. Arbuscular mycorrhizal fungi (AMF) are obligate symbionts that colonize the roots of about 80% extant terrestrial plant species. In this relationship, AMF improve the host plant growth by increasing the uptake of water and

 

minerals, especially the uptake of phosphorus (P) which is readily fixed in soil, and in return, they obtain photosynthates from the host plants [10-12]. Recently, the beneficial effect of AMF on nitrogen (N) uptake by crop has been extensively studied [13,14]. Consequently, effective AMF utilization would lead to low-input sustainable turmeric cultivation systems.

There are few reports on the interaction of AMF and the family Zingiberaceae. Several studies have made on the biotrophic interaction between AMF and turmeric [15-18]. To our knowledge, the influence of AMF inoculation on turmeric growth and yield has not been studied.

Production of curcumin, a secondary compound, is important factor for *C. longa*. The biosynthesis of curcumin begins with phenylalanine which is converted into feruloyldiketide-coenzyme A (CoA), a precursor of curcumin, by diketide-CoA synthase. The synthesized feruloyldiketide-CoA is condensed with feruloyl-CoA by actions of curcmin syntase 1, 2 and 3 to yield curcumin [19]. Some studies have demonstrated that AMF can influence secondary compounds such as phytohormone levels of jasmonate [20], terpenoids, carotenoids [21,22] and phenols [23] of host plant. Therefore, AMF inoculation offers the possibility of influence the concentration of the curcumin.

The use of AMF inoculation for natural plant production is still in its infancy and demands basic researches. In our experiment, a commercial product was used as AMF inoculant in anticipation of use in actual turmeric fields. Here, we expected that establishment of symbiotic relationship between turmeric and AMF would increase not only turmeric yield by stimulation of nutrients uptake but also improve the quality of turmeric by enhancing the curcumin concentration. The objectives of this study were to verify the contribution of AMF inoculation on growth, nutrition uptake, yield and curcumin production of turmeric under field and greenhouse conditions.

## 2. MATERIALS AND METHODS

### 2.1. Field Experiment

Field experiment was conducted from 25 May 2009 to 4 Jan 2010 at the Experimental farm on Osaka Prefecture University, in Sakai, Osaka, Japan. The experimental field (12 $m^2$) was plowed well and then divided into 4 plots of 3 $m^2$ (3 m × 1 m) size leaving 20 cm spacing between each plot. The chemical characteristics of the soil were as follows: pH ($H_2O$) of 4.8, electrical conductivity (EC) of 0.07 $dS \cdot m^{-1}$, total C of 9.38 $g \cdot kg^{-1}$, total N of 1.33 $g \cdot kg^{-1}$, inorganic N of 12.8 $mg \cdot kg^{-1}$, total P of 0.76 $g \cdot kg^{-1}$, Truog-P of 71.4 $mg \cdot kg^{-1}$. Corn (*Zea maize* cv. Gold dent KD850, Kaneko Seeds, Japan) was grown as preceding crop.

For turmeric plantlet preparation, about 30 g rhizome of Okinawa cultivar (*Curcuma longa* L.) in fresh weight was planted in a paper pot containing vermiculite on 25 May 2010 and grown in the greenhouse. Total 80 pots were prepared for this experiment. Half of those pots were inoculated with 3 g AMF commercial inoculums containing about 100 spores, *Gigaspora margarita* (Central Glass Co. Ltd, Tokyo, Japan). Control (non-inoculation) pots were prepared by mixing the same amounts of sterilized AMF inoculums, which were autoclaved 20 min at 121˚C, in the half of pot. Pre-germinated plantlets in control or AMF treatment were planted manually at a depth of 10 cm in a 30 cm triangular pattern in two rows on a ridge (planting density of 20 plants 3 $m^{-2}$) in the field on 17 June 2010. Each treatment had two replications and 40 plantlets were planted respectively. Chemical fertilizer ($N:P_2O_5:K_2O$ = 8:8:8) at 83 $g \cdot m^{-2}$ was applied two times at 30 July 2010 and 29 August 2010. Overhead irrigation was given immediately after turmeric planting and fertilizer application. Weeds were removed manually.

At each sampling time, plants were separated into shoots and rhizomes. The plant parts were oven-dried at 80˚C for 48 hours. After measurement of dry weight, the samples were ground into a fine powder. Total N was analyzed by vario MAX CN (Elementar, Germany). For *phosphorus* content, samples were ashed in a muddle furnace at 550˚C and determined by the *vanado molybdate* colorimetric method. Curcumin concentration of rhizome was measured by the HPLC (LC-20$AD_{XR}$, Shimadzu, Japan). For mycorrhizal assessment, small parts of roots were sampled, washed, and stained by the technique of Phillips and Hayman [24]. Percentage of mycorrhizal colonization was determined using the grid-line intersection method [25].

### 2.2. Pot Experiment

Greenhouse experiment was conducted from 22 July 2011 to 19 November 2011 at the Experimental farm on Osaka Prefecture University, in Sakai, Osaka, Japan. About 20 g seed rhizome in fresh weight, surface sterilized by 2.5% sodium hypochlorite solution for 25 min, was planted 8 cm deep in a 1/2000 a Wagner pot filled with autoclaved (60 min at 121˚C) substrates: mix of 5 kg of Akadama soil (reddish soil; Heiwa, Japan), 1 kg of Kanuma soil (pale yellow soil; Heiwa, Japan) and 1 kg of fertilized granulated soil (0.4 g N $kg^{-1}$, 1.0 g $P_2O_5$ $kg^{-1}$ and 0.6 g $K_2O$ $kg^{-1}$; Kureha, Japan). The chemical characteristics of the soil were as follows: pH ($H_2O$) of 5.8, EC of 0.26 mS $m^{-1}$, total C of 15.7 $g \cdot kg^{-1}$, total N of 1.2 $g \cdot kg^{-1}$, $NO_3$-N of 290 $mg \cdot kg^{-1}$, total P of 0.65 $g \cdot kg^{-1}$, Truog-P of 32.5 $mg \cdot kg^{-1}$. Ten g of AMF inoculums mentioned above was inoculated into half of pots. The same amounts of sterilized AMF inoculums were mixed in the

 

control treatment. The pots were arranged in a completely randomized block design with 12 replications. Liquid fertilizer, including 1.7 g N, 0.34 g $P_2O_5$ and 2.59 g $K_2O$, was applied at five-day intervals from 75 days after planting (DAP) to 95 DAP.

Plant height, number of leaf and number of stem were recorded from each treatment from 30 DAP to 120 DAP. SPAD value was measured on the top of two leaves by using SPAD-502 plus (Konica Minolta Optics, Japan) after liquid fertilizer application. Four plants of uniform size from both treatments were sampled two times (60 and 120 DAP). At each sampling time, plants were separated into shoots, roots and rhizomes, and oven-dried. Dry weight, N contents, P contents and curcumin concentration and contents, mycorrhizal colonization were investigated as mentioned above.

### 2.3. Statistical Analysis

The statistical difference was determined using paired or unpaired *t*-test. Mycorrhizal colonization data were arcsin transformed before analysis using *t*-test. Difference with $P < 0.05$ was considered significant. Statistical analysis was performed using Excel Tokei 2008 software version 1.05 (SSRI Co Ltd, Tokyo, Japan).

## 3. RESULTS AND DISCUSSION

As shown in **Table 1**, biomass production, N and P content of shoot and rhizome with AMF treatment tended to be higher than in the control under field conditions. But there were no significances statistically throughout the experiment. AMF inoculation also had no effects on curcumin concentration and content of rhizome. Even though AMF inoculation was done, mycorrhizal colonization was not statistically different between treatments, where as a about 19% - 55% colonization level was observed.

The effectiveness of AMF inoculation is affected by various environmental and biological factors, especially P availability in soil and the inoculums potential of indigenous AMF. It is suggested that enhancement of mycorrhiza inoculum potential by a given preceding crop improves the mycorrhizal activity of a subsequent crop [26]. Corn, which was cultivated as preceding crop, is known to increase the population of indigenous AMF well. Other possibility is that AMF already infected rhizome. The AMF form obligate symbiotic association with not only the roots and but also other underground parts of most of the plants. There are findings on the natural colonization of underground parts of turmeric [27,28]. Consequently, an inoculants product is best used when there is reason to believe that indigenous soil populations of AMF are low or native AMF are no longer effective.

In greenhouse experiment, there were no differences in plant height, number of leaf and number of stem until fertilization (**Figures 1(a)-(c)**). After fertilization, however, these parameters were increased with AMF inoculation. Appearance of stem was observed soon in AMF treatment. SPAD value was also significantly higher in AMF treatment than that in control after fertilization (**Figure 1(d)**). Dry weight of shoot and rhizome in AMF was higher than those in control at 120 DAP (**Table 2**). Mycorrhizal colonization was found in only roots of plants inoculated with AMF. Although AMF infected turmeric roots at 60 DAP, there was no growth differences between treatments until 75 DAP.

Needless to say, the mere presence of AMF does not imply benefits to turmeric because the initial soil nutriaents were very low in pot experiment. After fertilization,

**Table 1.** Effect of arbuscular mycorrhizal fungi (AMF) on dry weight, N content and P content of shoot (ST), rhizome (RM), curcumin concentration (conc.) and curcumin content of *Curcuma longa* 60 days, 120 days and 201 days after transplanting (DAT) under field conditions.

| Date | Treatment | Dry weight ($g \cdot m^{-2}$) | | N content ($g \cdot m^{-2}$) | | P content ($g \cdot m^{-2}$) | | Curcumin conc. (%) | Curcumin content ($g \cdot m^{-2}$) | Mycorrhizal colonization (%) |
|---|---|---|---|---|---|---|---|---|---|---|
| | | ST | RM | ST | RM | ST | RM | | | |
| 16 August | Control | 121.3 | - | 3.1 | - | 0.6 | - | - | - | 54.1 |
| (60 DAT) | AMF | 128.2 | - | 3.4 | - | 0.5 | - | - | - | 55.3 |
| | Significance[z] | ns | - | ns | - | ns | - | - | - | ns |
| 15 October | Control | 272.0 | 243.8 | 3.9 | 2.1 | 0.8 | 1.0 | 0.36 | 0.9 | 33.7 |
| (120 DAT) | AMF | 263.6 | 266.0 | 3.8 | 2.7 | 0.9 | 1.2 | 0.33 | 0.9 | 18.9 |
| | Significance | ns | ns | ns | ns | ns | ns | ns | ns | ns |
| 4 January | Control | 247.5 | 515.7 | 1.9 | 8.4 | 0.3 | 2.1 | 0.20 | 1.0 | - |
| (201 DAT) | AMF | 270.7 | 594.6 | 2.2 | 9.8 | 0.3 | 2.4 | 0.20 | 1.2 | - |
| | Significance | ns | ns | ns | ns | ns | ns | ns | ns | - |

[z] ns represent non-significance at 5% level by *t*-test.

 

**Table 2.** Effect of arbuscular mycorrhizal fungi (AMF) on dry weight, N content and P content of shoot (ST), rhizome (RM), curcumin concentration (conc.) and curcumin content of *Curcuma longa* 60 days and 120 days after planting (DAP) under greenhouse conditions.

| Date | Treatment | Dry weight (g·plant$^{-1}$) | | | N content (mg·plant$^{-1}$) | | | P content (mg·plant$^{-1}$) | | | Curcumin conc. (%) | Curcumin content (mg·plant$^{-1}$) | Mycorrhizal colonization (%) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | ST | RM | RT | ST | RM | RT | ST | RM | RT | | | |
| 60 DAP | Control | 6.0 | - | 2.8 | 148.2 | - | 28.8 | 9.5 | - | 7.4 | - | - | nd [y] |
| | AMF | 5.9 | - | 2.3 | 155.0 | - | 27.8 | 13.5 | - | 6.1 | - | - | 30.6 |
| | Significance[z] | ns | - | ns | ns | - | ns | ns | - | ns | - | - | - |
| 120 DAP | Control | 8.4 | 12.0 | 5.2 | 195.6 | 211.7 | 41.0 | 5.6 | 8.9 | 7.7 | 0.12 | 14.9 | nd |
| | AMF | 11.2 | 20.2 | 5.8 | 296.6 | 327.4 | 52.8 | 20.9 | 39.8 | 9.8 | 0.16 | 31.7 | 87.2 |
| | Significance | * | * | ns | * | * | ns | * | * | ns | * | * | - |

[z] ns and * represent non-significance and significance at 5% level by *t*-test; [y] nd indicates not detected.

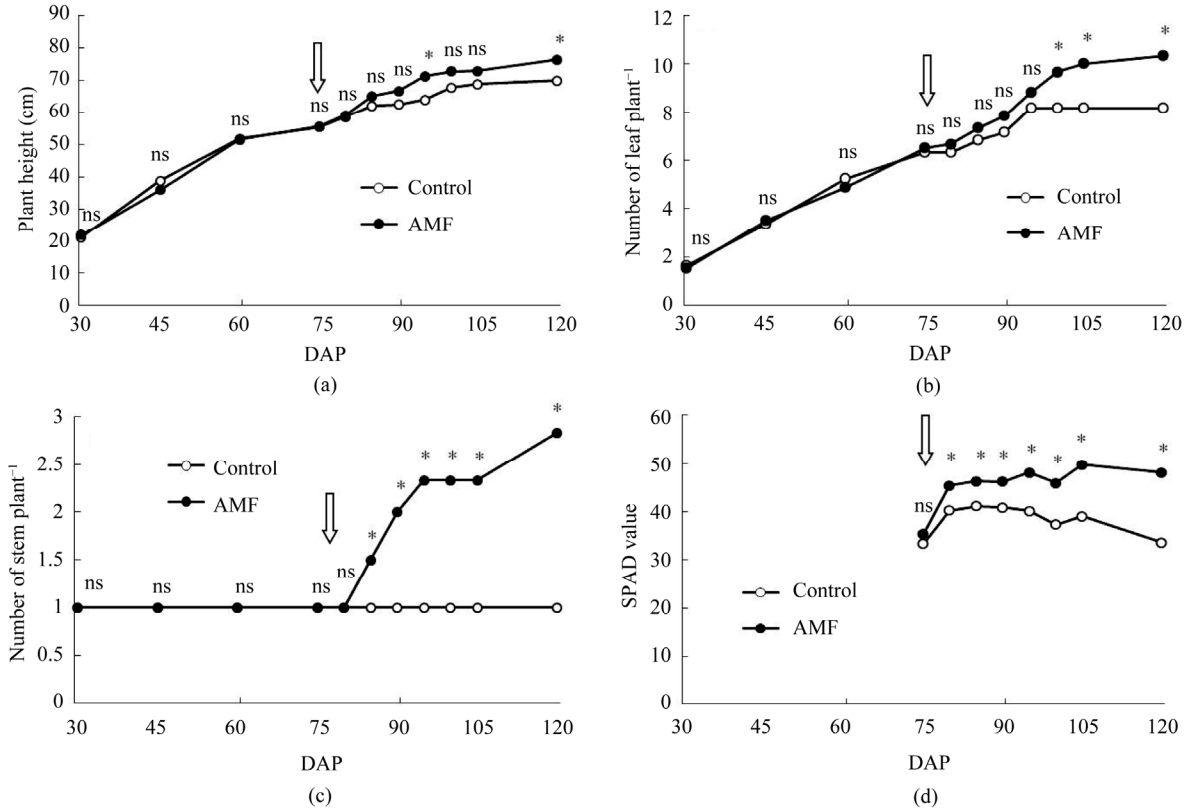


**Figure 1.** Effects of arbuscular mycorrhizal fungi (AMF) inoculation on plant height (a), number of leaf (b), number of stem (c) and SPAD value (d) of turmeric in pot experiment. Arrow indicates the date when liquid fertilizer was applied. Data are means of 8 replications from 30 DAP to 60 DAP and 6 replications from 75 DAP to 120 DAP, respectively. ns and * represent non-significance and significance at 5% level by *t*-test.

the growth of AMF inoculated turmeric was extremely stimulated. N content was significantly increased at 120 DAP, approximately 1.5-fold. P content followed similar patterns as N content. P content was higher in AMF treatment than control, approximately 2-fold. AMF scavenged supplied nutrients, mainly phosphorus from the soil into the root system through extraradical hyphae.

The mean curcumin concentration of AMF treatment was 0.16%, which was significantly higher ($P < 0.05$) than that of control (0.13%). The curcumin content was also higher in AMF treatment. Our hypothesis that AMF would influence curcumin concentration and content was supported by the data obtained. P is one of the main nutrients involved in the synthesis of secondary metabolites as their production demands ATP [29]. The increase in availability of P through mycorrhizal association would

 

probably underlie the increase of curcumin. Similarly, Silva *et al*. [30] in their work on ginger inoculated AMF found oil concentration was modulated according to AMF. At the moment we are unable to propose a mechanism explaining how AMF influence turmeric curcumin production. Although turmeric varieties may have a great effect on the curcumin concentration, the increase of curcumin, which determines quality and international market price of turmeric, by AMF inoculation is an important finding.

Mycorrhizal associations with plants have evolved in complex and relatively stable natural environments. Now we seek to manage this symbiosis in agricultural systems. In Japan, most turmeric is cultivated by the avoidance of chemical biocides and soluble inorganic fertilizers. It is often assumed that AMF will play a more pivotal role in alternative systems than in conventional systems. Our results indicate that the use of mycorrhizal inoculation is a feasible approach for turmeric production. The greenhouse experiment suggests that AMF inoculation can increase turmeric growth and rhizome production significantly. In our experiment *G. margarita*, included in commercial inoculums, was used. But the genus *Glomus* is more frequently found in the fertile agricultural soils [31]. On the other hand, the genus *Gigaspora* is more abundant in low-nutrient or nutrient-binding soils [32,33]. It is suggested that different AMF isolates influence the host plant growth and qualitative differences in the secondary metabolites. Therefore further studies are needed for selection of effective AMF for turmeric growth and curcumin production.

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