



Effects of Mycorrhizal Inoculant on the Quality of Macropropagated Seeds and the Agronomic Performance of Plantain (*Musa* spp.)

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Authors' contributions

This work was carried out in collaboration among all authors. Author DRRBV designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors JAA, CDT and IK managed the analyses of the study. All authors read and approved the final manuscript.

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ABSTRACT

The difficulties of nurserymen and producers of plantains in Côte d'Ivoire are the high mortality rate of seedlings and the delays of growth in the field. The aim of this study is to improve quality and agronomic performance of plantain seedlings produced by PIF technique (plantain seedlings production based on macropropagation). The plantain shoot bulbs were inoculated before being placed in the germinator and seedlings from germinator were inoculated again during the weaning phase at nursery with AMFs (*Rhizophagus intraradices*). Root mycorrhizal colonization, growth parameters such as girth, height, total leaf area, biomass and entry into production were measured in nursery and on the field. The results showed that the root colonization rate of the plants inoculated with *R. intraradices* inoculum was higher (30.59%) than that of the native arbuscular

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mycorrhizal fungi (2.78%). All inoculated plants survived while non-inoculated plants had 27.69% of mortality rate. Inoculated plants had higher growth than non-inoculated plants. The organs of inoculated plants also had higher biomass than non-inoculated plants. Eleven months after planting, nearly 50% of the inoculated plants had started production, while only 5.67% of the non-inoculated plants had started production. Mycorrhization improved the vigor and vegetative growth of plantain seedlings. It could therefore be used as a solution for a sustainable plantain culture.

Keywords: Plantain; seedlings; growth; Rhizophagus intraradices; mycorrhizal inoculation.

1. INTRODUCTION

Plantain (*Musa* spp.) is a widely consumed product in Central and West Africa [1], helping to alleviate the problems of famine and food insecurity in this part of the world. Its cultivation generates permanent income for a large number of farmers. In terms of world production, plantain remains a marginal crop, notably because of the unavailability of good quality seeds and the frequent use of poor rejects [2]. Particularly in Côte d'Ivoire, plantain is of great importance in terms of local consumption, but also in cropping systems [3] where it is an essential link in agroforestry cropping techniques because of it provides shade. With an estimated average annual production of 1,883,063 tonnes in 2018 [4], for a local consumption varying between 80 and 120 kg per year and per capita, plantain is the 3rd most important food crop in Côte d'Ivoire, after yam and cassava [5]. Despite its importance, production remains low to cover the needs of the ever-growing population. Indeed, plantain farms are insufficient due to the lack of good quality seeds, pest pressure, and high mortality of banana seedlings [6].

All these problems hamper the efforts and remuneration of farmers, leading to discouragement. To overcome these difficulties, the PIF technique is increasingly used by nurserymen and plantain producers in Côte d'Ivoire [7]. The PIF is a technique for plantain seedlings production based on macropropagation developed by CARBAP (African Center for Research on Bananas and Plantain). PIF acronym translates to "seedlings resulting from stem fragments" [8,9]. However, this technology, implemented in rural areas, is confronted with pest and disease attacks. This causes many difficulties, the most important of which are high rates of morbidity and mortality of seedlings, and delays in vegetative growth of seedlings in field. Combating these difficulties too often requires the use of synthetic nematicides or fungicides. These products are expensive and environmentally damaging [10]. Thus, in the

context of developing sustainable agriculture, it is necessary to consider environmentally friendly alternatives such as mycorrhizal inoculation. Indeed, described as bio-stimulators of plant growth and bio-protectors, mycorrhiza promote plant growth, particularly in soils lacking mineral elements [11]. In West Africa this inoculation technology is proving to be an alternative solution. Several countries, notably Senegal, Niger and Mali, have carried out pilot field trials. This technology has improved the productivity of soybean in Senegal [12]. In Côte d'Ivoire, mycorrhization technology has shown promising results on different food crops such as plantain [13], and cassava [14]. In this study, the improvement of plantain seedlings from PIF technique by mycorrhizal inoculation and their growth in the field were evaluated through the production of vigorous mycorrhized seedlings, the assessment of their growth performance under field conditions, the survival rate and their ability to be earlier in production.

2. MATERIALS AND METHODS

2.1 Climatological Description of the Study Area

The field experiment was carried out in Gonate, located 22 kilometres from the department of Daloa in the Haut-Sassandra region (center-west of Côte d'Ivoire), The department of Daloa is located between 6°53'58" N latitude and 6°26'32"W longitude. The climate is humid equatorial, with two wet and two dry seasons. The dry and wet seasons alternate with temperatures ranging from 24.65°C to 27.75°C on average [15]. Average annual rainfall is 1120.4 mm per year, [16].

2.2 Construction of Germinators for the Production of Plantain Seedlings

Ten germinators, each measuring 10 m × 1.5 m × 0.23 m (length × width × depth), were used. Five of the germinators were used for the

production of inoculated seedlings and the other five for the production of non-inoculated seedlings. The inside of the germinators was lined with cement and each germinator was filled with substrate (sawdust). In the nursery, the substrate used was white sawdust mixed with decomposed coffee parchment at the ratio of 1:1.

2.3 Sowing of Germinators and Seedlings Production Process

Each germinator was sown with 300 explants (plantain shoots) of the Big-Ebanga variety. The plantlets were obtained using the PIF technique [8] modified at the incision stage. The plantain shoots were inoculated, after the cross-incision step, by coating the bulbs with an inoculum of mycorrhizal fungi before being sown into the germinators. The mycorrhizal fungus inoculum consisted of propagules of the mycorrhizal fungus (*Rhizophagus intraradices*) contained in a mixture of vermiculite + clay (1:1). The number of propagules was estimated at 220 spores per 10 g of inoculum. This *R. intraradices* was isolated from the rhizosphere of plantain in Bouafle region (middle-west, Côte d'Ivoire) and then multiplied on sterile substrate using cowpea (*Vigna unguiculata*) plants as trap plant. This treatment constitutes the first phase of mycorrhizal inoculation. In contrast for non-inoculated germinators, the plantain shoot bulbs were directly sown into the germinators after incision without inoculation with any other product. Watering of the germinator took place every two days, and from the second week the first seedlings started to appear until the end of the germinator production cycle, three months later.

2.4 Nursery Placement of Seedlings

Seedlings with height of 5 to 7 cm at 3-leave stage were sprouting out from the germinators and placed in bagged nurseries under shade. In the nursery, the substrate used was white sawdust mixed with decomposed coffee parchment in the proportion of 1:1. The seedlings were kept in the nursery for 120 days.

2.5 Inoculation Process of Seedlings in Nursery

The second phase of inoculation consisted of adding inoculum (*Rhizophagus intraradices* at a rate of 10 g per plant in the nursery from the already inoculated germinators. The seedlings from the non-inoculated germinators did not

receive any inoculum. After 120 days in the nursery seedlings was taken to the field for planting.

2.6 Setting Up the Experimental Plots

The field experiment was set up on a plot of a two-year fallow land. This fallow land was mainly colonised by *Chromolaena odorata* (Asteraceae), *Pueraria* spp. (Fabaceae) and *Panicum* spp. (Poaceae). After clearing with a machete and drying the plant cover on site, a hoe ploughing was carried out. Two experimental plots of 1000 m² each and 50 m apart were established. Staking (2 m x 2 m) and digging (50 cm x 50 cm x 50 cm) according to the method of [17] were carried out. In order to obtain an experimental surface centered in the defined plot (1000 m²) with a staking density of 2mX2m, one of the plots received 200 mycorrhizal plants and the other 200 non-mycorrhizal plants. The maintenance of the plots throughout the experiment consisted of manual weeding without the use of phytochemicals.

2.7 Assessment of Mycorrhizal Development

For assessment of root colonization by AMFs, fine plantain roots were sampled at the end of nursery then 14 weeks after planting, each time with three replicates per treatment. Each treatment contained three plants. Indeed, these 14 weeks were observed in order to evaluate the capacity of the inoculated fungus to develop in the field's agroecological constraints. Roots were rinsed and cut into 1–2 cm fragments. These roots fragments were cleared by boiling in 10% (w/v) KOH and stained with 0.05% (v/v) trypan blue in lactoglycerol [18]. Ten pieces of roots per plant were placed in glycerol (50%) between slide and coverslip [19] and observed under an optical microscope and colonized roots were evaluated [20].

2.8 Assessment Vegetative Growth Parameters

The seedlings were brought to the field in November and growth measurements were taken in November, December, January, February and March which correspond to a dry season in Daloa region. The different growth parameters, i.e. plant height, pseudo-trunk circumference, leaf area and dry biomass, were assessed when the seedlings were taken out of

the germinators (when they were placed in the nursery) and then every 15 days for the first 14 weeks of their transport to the field. The seedlings were transported to the field after 120 days in the nursery. The number of plants in production at 11 months of cultivation was then recorded. For the measurements, plants in the first two rows on the sides of each plot were avoided. From the third row onwards, 20 plants were randomly selected for the measurements. The height was estimated from the base of the bulb to the point of intersection of the last two unrolled leaves. The circumference was taken at the mid-length of the pseudo-stem, while the leaf area (S) was calculated according to the formula $S = L \times l \times 0.82 \times N \times 0.662$, where L and l are the length and width of the last leaf emitted, respectively, 0.80 is the leaf area index, N is the total number of leaves and 0.662 is the coefficient proposed by Kumar [21].

The fresh and dry biomass of above-ground and below-ground parts were also determined at week 14 by the destructive method. For plants per treatment were removed from the soil with their roots. Weighing of the underground stem and attached roots, pseudostem and leaves was carried out first in the fresh state and then after 10 days of oven drying at 50°C (i.e. until the measured dry weight did not change).

2.9 Assessment of Mycorrhization Impact on the Ability of Plantain Plants to Initiate Production

The number of plants in production was estimated in relation to the number of plants still alive. From the installation of the fields, the number of dead plants per treatment was noted. Thus, the survival rate at 11 months of experimentation was determined according to the following formula:

$$\text{Survival rate} = \frac{\text{Number of growing plants}}{\text{Initial number of seedlings}} * 100$$

Then the rate of plants in production was determined according to the following formula:

$$\text{Rate of plants in production} = \frac{\text{Number of plants in production}}{\text{Number of growing plants}} * 100$$

2.10 Statistical Analysis of the Data

The vegetative growth parameters (height, circumference of pseudo-stem, leaf area) data were subjected R-4.0.3. at 5% level. Then the Welch's t-test was used to evaluate the effect of

mycorrhization on the agronomic parameters of the studied plants. The Welch's t-test (which is a variant of Student's t-test) was used here for two reasons. First, because we have two independent sample groups and second, because these groups do not follow a normal distribution and have different variances.

Mycorrhizal colonization and biomass data were subjected to ANOVA with STATISTICA 8.0.550. Tukey HSD test was used for post-hoc comparisons to determine differences between means within and among treatments. Differences were considered significant at $p < .05$.

3. RESULTS AND DISCUSSION

3.1 Plantain Root Colonization

Mycorrhizal colonization of plantain root at the end of nursery and 14 weeks after planting is shown in Table 1. The data presented revealed that after 120 days in nursery, the seedlings from the germinators that received mycorrhizal inoculum showed 33.27% mycorrhizal colonization. No inoculated structure was noted in the plants from the non-inoculated germinators. After 14 weeks in plantation, the inoculated plants showed 30.59% mycorrhizal colonization compared to 2.78% for the non-inoculated plants.

Controlled inoculation in the germinators and then in the nursery favoured an important colonization of the roots of the plantain seedlings, first in the nursery and then after 14 weeks in the plantation. This confirms that plantain plants are highly mycotrophic [22,23]. The roots of inoculated plants contain abundant and essentially the structures of the strain constituting the inoculum (*Rhizophagus intraradices* isolated from the rhizosphere of plantain). Which confirms the efficiency of this strain on plantains. Whereas non-inoculated plants with roots free of mycorrhizal structures at the nursery exit were colonized in planting by the native mycorrhizal strains. However, these colonization levels are low because of adversity from other soil organisms that struggle to penetrate the roots as well. Indeed, native AMFs whose propagules are in lower concentrations relative to the inoculum would compete with other soil fungi and nematodes for root colonization [24]. This competition would have negative effects on colonization rates when mycorrhization is not previously installed [14]. Mycorrhizal inoculation under controlled nursery

conditions of plantain seedlings therefore favours high colonization rates with the corollary of a better impact on seedling development in plantation [25].

3.2 Effects of Mycorrhizal Inoculation on Plantain Seedlings Growth

The inoculated plants showed the largest sizes compared to the non-inoculated plants (Fig. 1). Rapid growth in height of mycorrhized plants was noted during the 120-day nursery while for non-mycorrhized plants, the growth in height was slower. In the field, this agronomic parameter continued to increase quite rapidly with inoculated plants until the fourteenth week, while for non-inoculated plants this growth is rather slow.

The leaves of the mycorrhized plants were larger than those of the non-mycorrhized

plants (Fig. 2). A significant increase in leaf area of the mychorized plants is noted during 120 days of nursery, while for non-mycorized plants the leaf area evolved weakly. Also in the field, the leaf area continued to increase rapidly with inoculated plants until the fourteenth week, while for non-inoculated plants the increase in leaf area is slower.

Inoculated plants showed larger pseudo-stem circumference compared to non-inoculated plants (Fig. 3). A drastic increase in seedling circumference, which is much more remarkable in inoculated seedlings, was observed in the nursery (120 days). In plantation, the pseudo-stem circumference of plantain plants increased continuously for these plants until the fourteenth week, while in non-inoculated plants, a constant decrease was noticed after a slight peak at the fourth week.

Table 1. Mycorrhizal colonization of plantain root at the end of nursery and 14 weeks after planting

Traitments	Mycorrhizal colonization at end of Nursery (%)	Mycorrhizal colonization Week 14 at field (%)
Non-inoculated plants	-	2.78 ^b ± 0.33
Inoculated plants	33.27 ^a ± 4.3	30.59 ^a ± 5.66
p	0.024	
F	4.544	

± indicate the standard deviation

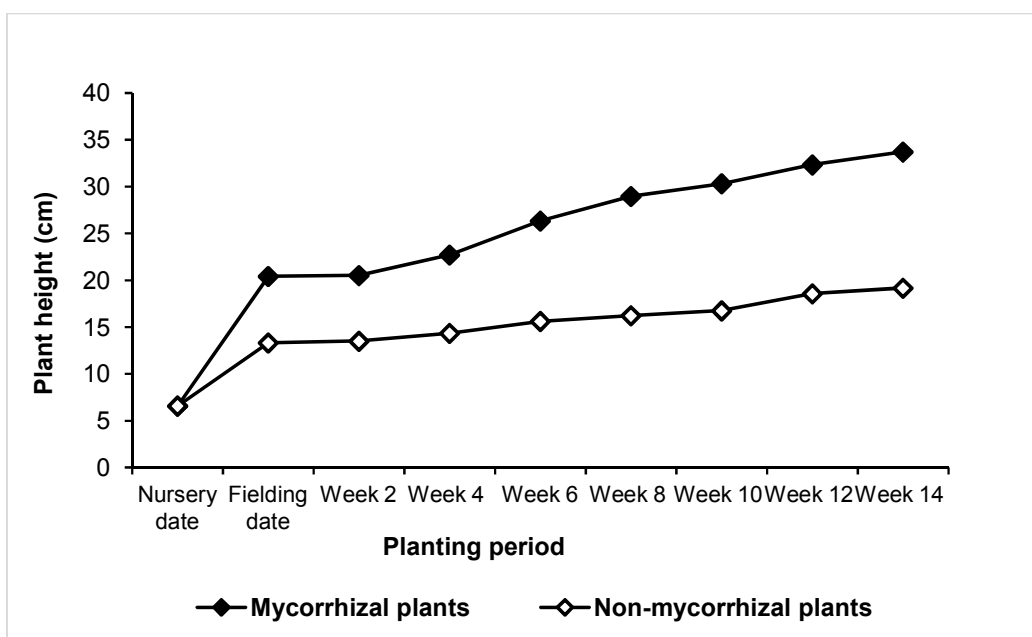


Fig. 1. Effects of mycorrhizal inoculation on plantain height

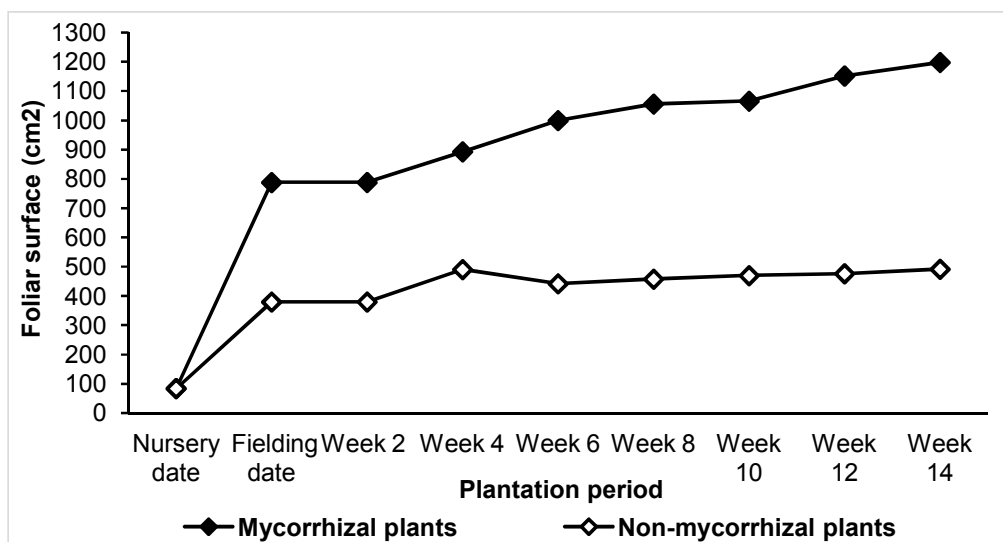


Fig. 2. Effects of mycorrhizal inoculation on plantain foliar surface

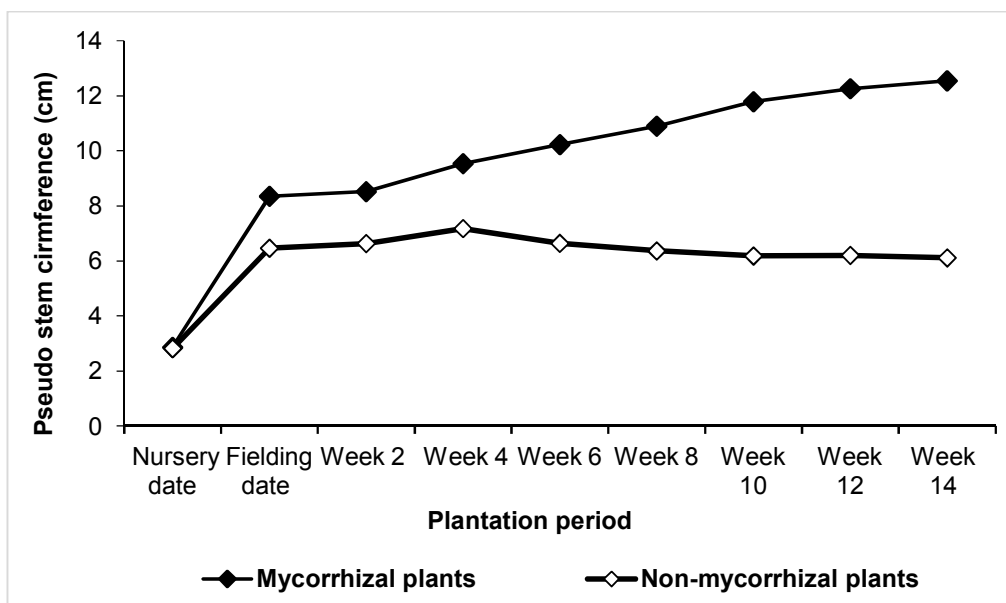


Fig. 3. Effects of mycorrhizal inoculation on plantain seedlings circumference

Table 2. Effects of mycorrhizal inoculation on plantain organ fresh weight

Treatment	Leaves	Pseudo-stem	Underground stem + roots
Inoculated plants fresh weight (g)	382.84 ^a ± 2,36	460.70 ^a ± 33.10	231.31 ^a ± 8.32
Non inoculated plants fresh weight (g)	108.01 ^b ± 22,69	120.35 ^b ± 26.16	76.98 ^b ± 13.62
F	1599.5	676.20	1078.7
P	.000	.000	.000

(n = 4) Values within the same column with different letters are significantly different; ± indicate the standard deviation

Table 3. Effects of mycorrhizal inoculation on plantain organ dry weight

Treatment	Leaves	Pseudo-stem	Underground stem + roots
Inoculated plants dry weight (g)	35.12 ^a ± 2,27	58.52 ^a ± 10.88	52.44 ^a ± 10.22
Non inoculated plants dry weight (g)	10.31 ^b ± 2,28	17.37 ^b ± 4.84	10.86 ^b ± 4.38
F	956.28	96.352	98.802
P	.000	.0000	0.000

(n = 4) Values within the same column with different letters are significantly different; ± indicate the standard deviation

Table 4. Survival and production rates of mycorrhized plantains

Traitments	Survival rate at the end of nursery (%)	Survival rate after 11 month in plantation (%)	Rate of plants in production after 11 month in plantation (%)
Inoculated plants	100	100	46,67
Uninoculated plants	100	72.31	5.67

Differences between organ biomass of inoculated and non-inoculated plantain seedlings after 14 weeks of planting were demonstrated. Inoculation with mycorrhiza increased plants organ fresh weight (Table 2). Similarly, a gain in dry weight of inoculated plants compared to uninoculated plants is observed (Table 3).

Growth of mycorrhized plantain plants revealed that inoculated plants had a higher vegetative growth than non-inoculated plants, regardless of the parameter considered (girth, height and leaf area). Mycorrhizal inoculation would therefore positively influence the development, growth and photosynthetic activity of plantain plants. This resulted in higher fresh and dry weights of plant organ of inoculated plantain. These results showed that *R. intraradices* strains isolated from Côte d'Ivoire plantain fields have good mycorrhizal efficacy as well as significant effect on the vegetative growth and biomass parameters on macropropagated plantain seedlings. These results on growth and biomass production are similar to those obtained in several authors' work [13,26]. These authors reported that inoculation of plantain plants with mycorrhizal fungi leads to better plant development via improved hydromineral nutrition, but also due to the reduction of deleterious effects caused by phytopathogenic soil microorganisms [27]. Particularly in this study, a significant difference in weight was observed between the fresh biomass of inoculated and non-inoculated plants. This means that inoculation increased the water uptake of plantain plants.

3.3 Effects of Mycorrhizal Inoculation on the Ability of Plantain Plants to Initiate Production

The survival percentage of inoculated plants is 100% (Table 4). For non-inoculated plants, a survival rate of 72.31% was observed. After 11 months of planting, 46.67% of inoculated plants have started to produce flowers or banana bunches while only 5.67% of the non-inoculated plants were at the same physiological stage (Table 4).

It was shown that inoculated plants were able to survive by showing a high capacity of resistance to environmental stresses (water stress) [28,29] and biotic stresses such as nematode attacks [30,31]. The faster growth of inoculated plants favored an earlier onset of production than in non-inoculated plants. Indeed, at 11 months of planting, almost half of the inoculated plants were in production. These plants would have reached this physiological stage more quickly thanks to the mycorrhizal symbiosis established with this strain of *Rhizophagus intraradices*.

4. CONCLUSION

Controlled inoculation in germinators and then in the nursery promoted significant root colonization of plantain seedlings, first in the nursery and then in the field. This symbiosis established with an inoculum based on a local strain of *Rhizophagus intraradices* from the banana rhizosphere improved the vigor, growth and survival rate of plantain seedlings in the plantation. By the same

token, mycorrhizal inoculation also positively influenced the ability of plantain plants to enter production. In sum, mycorrhizal inoculation is a non-polluting approach capable of mitigating damage due to biotic and abiotic stresses on plantlets resulting from the PIF technique. In the perspective of sustainable plantain agriculture, the practice of mycorrhizal inoculation appears to be a promising alternative in tropical areas.

SUPPLEMENTARY MATERIALS

Supplementary materials available in this link:

<https://www.journalijpss.com/index.php/ijpss/libraryfiles/downloadpublic/18>

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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