

## **EFFECT OF LIGHT-EMITTING DIODES ON ARBUSCULAR MYCORRHIZAL FUNGI ASSOCIATED WITH BAHIAGRASS (*Paspalum notatum* Flügge) AND MILLET [*Pennisetum glaucum* (L.) R.Br]**

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### **ABSTRACT**

This research was conducted to verify the *in vivo* effects of light-emitting diodes (LED) on propagule formation of arbuscular mycorrhizal fungi (AMF) in pots cultivated with bahiagrass and millet. A glass pot was separated into two sections, inside and outside, and placed in a paper box covered with aluminum foil. In the inner section, spores of AMF, bahiagrass, and millet seedlings were inserted, while the outer section was filled with glass beads (1 mm diameter). Spores of the AMF, *Gigaspora margarita* Becker and Hall, from commercial inoculums and two kinds of LED, red and blue, were utilized for this experiment with different combinations: dark (the control pot), blue, red, red+blue, red/blue (alternated every 12 h) and red-blue (red for 60 days followed by blue for 30 days). The experiment was conducted for a total of 90 days. Among LED treatments the red-blue induced higher plant growth in millet seedlings although the growth did not surpass the one of the control. This response was less notorious in bahiagrass. On the other hand, the plant growth under blue LED was always lower than the control. The red-blue and red alone treatments stimulated the formation of AMF spores in glass beads, whereas the blue treatment alone inhibited it. In summary, based on AMF colonization and sporulation, red-blue and red alone treatments were the most efficient.

**Additional key words:** *Gigaspora margarita*, mycorrhizal colonization, Poaceae, sporulation

### **RESUMEN**

#### **Efecto de diodos emisores de luz (LED) sobre hongos micorrízicos arbusculares asociados a pasto bahía (*Paspalum notatum* Flügge) y mijo perla [*Pennisetum glaucum* (L.) R.Br]**

Esta investigación se llevó a cabo para comprobar los efectos *in vivo* de los diodos emisores de luz (LED) en la formación de propágulos de hongos micorrízicos arbusculares (HMA) dentro de contenedores con pasto bahía y mijo perla. Vasos de vidrio separados en dos secciones, dentro y fuera, fueron colocados en cajas de cartón cubiertas con papel de aluminio. En la sección interna se inoculó con las esporas de HMA junto con las plántulas de pasto bahía y mijo, mientras que la sección exterior contenía perlas de vidrio (1 mm de diámetro). Las esporas de la HMA, *Gigaspora margarita* Becker y Hall, provenientes de inóculos comerciales y dos tipos de LED (rojo y azul), se utilizaron en este experimento con diferentes combinaciones: oscuridad (tratamiento testigo), azul, rojo, rojo+azul, rojo/azul (alternado cada 12 h) y rojo-azul (rojo durante 60 días, seguido de azul por 30 días). El experimento se llevó a cabo durante de 90 días. Entre los tratamientos con LED, el rojo-azul indujo mayor crecimiento de las plantas de mijo aunque este crecimiento no superó al del testigo. Los tratamientos rojo-azul y solo rojo estimularon la formación de esporas de HMA en las perlas de vidrio, mientras que el tratamiento solo azul inhibió dicha formación. En resumen, basados en la colonización y la esporulación de los HMA, los tratamientos rojo-azul y solo rojo fueron los más eficientes.

**Palabras clave adicionales:** Colonización micorrízica, *Gigaspora margarita*, Poaceae, producción de esporas

### **INTRODUCTION**

The obligate biotrophic nature of arbuscular mycorrhizal fungi (AMF) has been well documented wherein their association with plant roots provides benefits for both plant and fungi (Mehrotra, 2005). The first interaction between fungus and its host is the fungal reaction to signals

from the exudates of the host root (Buee et al., 2000). The pre-symbiosis of AMF is induced by the production of specific plant root exudates and/or volatile compounds, and the growth response is generally characterized by hyphal elongation and branching (Tahat and Sijam, 2012).

The fungal hyphae are able to respond to host-

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derived signals by directing their growth toward roots to perceive chemotropic signals (Sbrana and Giovannetti, 2005). The increase of hyphal branching in the presence of root exudates indicates host recognition (Bais et al., 2006). For a better understanding of *in vivo* conditions, several studies have identified the chemical nature of signals released by host roots, which act as triggers of fungal development switches, leading to the establishment of AMF symbiosis (Nagahashi and Douds, 2000), and tested their effects on the hyphal growth *in vitro* (Ishii et al., 2003). These authors and others (Kuwada et al., 2005; Kuwada et al., 2006) succeeded in identifying fractions of bahiagrass, root exudates, some peptides, oligosaccharide, and some flavonoids, all of which function as stimulatory compounds for AMF.

The production of AMF spores in pot cultures often requires manual removal of contamination before proceeding with any ultrastructural or molecular study; thus, the recovery of intact hyphae requires laborious procedures (Horn et al., 1993). For massive spore production with pure characteristics with regard to the purity, quantity, and viability of the recovered material, it may be beneficial to use a 'glass beads' system (Redecker et al., 1995), because the spores probably retain their natural microbiome, such as helper bacteria, that are necessary for their survival and development. This system in combination with light-emitting diodes (LED), as described in this study, would lead to the production of cleaner and higher number of spores. The new spores produced in the glass beads section could be cleaned easily and had similar surface characteristics to the original ones.

Light can indirectly affect soil microorganisms through its effects on plants, whose photosynthetic products are released from the roots (Dehlin et al., 2008). The penetration of light through soil is important because of its effects on factors of ecological significance, such as spore germination, root growth, fungal growth, and formation of mycorrhizal and leguminous nodules. Light penetration can be affected by soil moisture content, soil type, cover material, and particle size (Tester and Morris, 1987). Phytochromes that are biliprotein photoreceptors enable some microorganisms to adapt to the light regime in the

soil (Rottwinkel et al., 2010).

Fungi are unable to use light for photosynthesis; however, radiation plays a role in the biochemical and morphological responses of some fungi such as *Phycomyces blakesleeana* (Corrochano and Cerda, 1991), including their growth and differentiation (Horwitz and Berrocal, 1997). Physiologically and ecologically, a significant amount of light penetrates the soil approximately 4-5 mm from the surface, eliciting some phototrophic responses in plant roots (Tester and Morris, 1987). This information has led some AMF experts to hypothesize the function of LED on AMF formation. The induction of hyphal growth by light and chemicals, for example, the effect of blue light on hyphal branching, has been reported (Nagahashi and Douds, 2004). These authors demonstrated that blue light and some exudate components effectively stimulate hyphal branching, suggesting the involvement of a second messenger responsible for this synergism. The photo-induction caused by photomimetic compounds has been studied in many other fungi as well (He et al., 2002).

It is important to assess some environmental factors that stimulate hyphal growth and sporulation, such as root exudates, and LEDs applied individually and in combination. Lighting from red LED or red+blue LED could stimulate hyphal growth in *G. margarita* and *Glomus* spp. (R-10) *in vitro* (Ishii et al., 2003). Moreover, AMF colonization of corn roots was improved when the rhizosphere was exposed to light (Nagahashi et al., 2000). The marginal AMF colonization of chalk false-brome [*Brachypodium pinnatum* (L.) P.B.)] under shade conditions could show that when low light limits photosynthesis and thus growth of the plants, they dispense with the colonization of AMF in order to save the expenditure of organic carbon (Fuzy et al., 2014).

Previous studies have reported the effects of blue light on hyphae (Akiyama et al., 2005; Nagahashi and Douds, 2004); however, its synergistic effect with root exudates on the production of new spores with minimum soil residues is still unclear. Several attempts have been made to produce AMF spores axenically, but pot culture methods have often obtained contaminated material along with plant and other debris. In this study, we aimed to determine

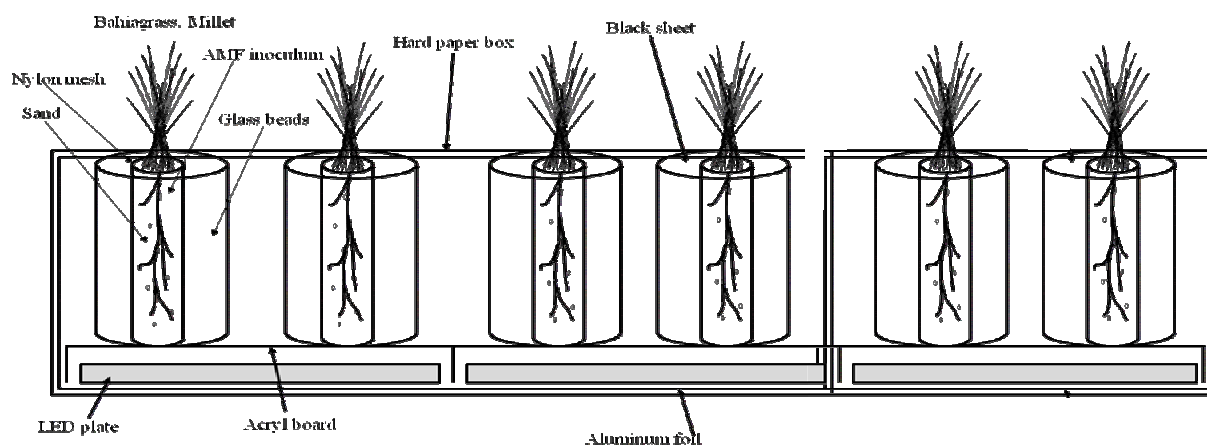
whether different LED combinations could induce AMF colonization in bahiagrass and millet roots, and form new spores in the rhizosphere by using a 'glass beads' pot method.

## MATERIALS AND METHODS

The growth system consisted of special paper boxes (76 x 46 x 25 cm). These boxes were internally covered with aluminum foil, and the LED plates were placed at the bottom of each box and protected from water seeps from the plants. An acrylic board (70 x 40 x 5 cm) was placed between the LED and the glass pots (Figure 1).

Additionally, 900-mL glass pots divided into two compartments were prepared. The internal and external compartments were separated by nylon mesh screens with pore sizes of 40  $\mu$ m, allowing hyphae, but not roots, to cross. The internal compartment, with 4 cm diameter and 15 cm depth, were filled with sterilized sand and the external compartment were filled with glass beads

(1 mm diameter). Then, 5 g of commercial inoculant (Serakinkon) (Idemitsu Co., Japan.) containing 100 spores of the AM fungal *G. margarita* were added into the internal compartment. And then 3 seedlings of bahiagrass (*P. notatum* Flügge) (Takii seeds, Co., Japan) and millet (*P. glaucum* (L.) R. Br. subspecies *monodii* (Maire) Brunken) (Takii seeds, Co.) were transplanted. To avoid penetration of external light the external compartment of the glass pots (glass beads) was completely covered with black sheets, leaving open only the internal part with plants and sand. Also the entire box was internally covered with aluminum foil, whose system allows only the light from LED to effect the pot. The system was maintained under greenhouse conditions at 25 °C with 16 h light and 8 h dark cycle. The plants were watered with 400 mL of Hoagland solution (25 %) with phosphorous at 10 % of the original recommendation, once a week to avoid anaerobiosis. This amount could maintain the plant growth without disturbing the AMF activity.



**Figure 1.** Box design with six glass pots divided in two sections each one (three pots with bahiagrass and three with millet)

The treatments involved five different LED systems: blue, red, red+blue, red/blue and red-blue. A completely dark box was prepared as control. For the first three treatments, each box was equipped with two LED plates (31.5 x 31.5 x 4.7 cm) with the following specific conditions: red light (LEDwholesalers, 2501RD, 13.8 W, 630 nm), blue light (LEDwholesalers 2501BU, 13.8

W, 450 nm), red+blue light (Plant Growth red and blue LED plate, a145, 15 W). For the last two systems, red and blue LEDs (31 cm length, 15 W) were alternately placed at the bottom of the boxes. The red/blue treatment consisted of 12 h red light and 12 h blue light, and the red-blue treatment involved exposure to red light for 60 days followed by exposure to blue light for 30

days. All LEDs were applied in flashes system consisted of 15 min of light and 15 min in dark. The internal temperature in the boxes was 27 °C and light intensity of LEDs was 1000 lux throughout the experiment which was conducted for 90 days.

Plant biomass, shoot fresh weight, and root fresh weight were determined. The glass beads were collected and new spores were extracted by adding them to a 70 % glucose solution; the new spores were harvested from the surface of this solution. Sand samples (10 g) were also used to count the number of spores. From the bahiagrass and millet plants, root samples were also taken, washed, and stained to observe the AMF colonization (Phillips and Hayman, 1970). Briefly, segments of the stained roots were placed on glass slides and observed by light microscope and the percentage of AMF colonization (% AMF) was calculated by the following equation:

% AMF = (number of observed areas colonized by AMF / total areas) x 100 (Cruz et al., 2014).

Each box contained one LED system and six glass pots (three with bahiagrass and three with millet). The experimental design consisted of five LED treatments, and two kinds of plants with three replicates, composing in total 30 experimental units. The statistical analysis was carried out by the software SPSS 20 where the means were compared using the Scott-Knot test with the significance at 5 %.

## RESULTS

The plant biomass, represented by shoot fresh weight, indicated higher growth of millet seedlings under red-blue treatment as compared to the other LED treatments. However, the growth did not surpass the one of the control (Table 1). For the root fresh weight in millet the red+blue treatment had higher values than the rest of the LED treatments and similar to the growth of the control (Table 1). The response was less notorious in bahiagrass where the shoot growth only showed differences for the blue treatment (lower figures), while the root growth was higher for red+blue, red/blue and red-blue treatments.

Both in bahiagrass and millet AMF colonization was higher in the red alone and red-blue treatments than that of the rest of treatments where

the blue alone showed the lowest figures. Also, significant difference in the effect of red alone and red-blue in comparison to the rest of treatments was observed in both plants, where the blue alone showed the lowest result (Table 2).

The number of spores in the glass beads displayed the highest value from the red-blue treatment, similar to the data from the sand. In addition, the inhibitory effect of blue light was stronger than stimulatory effect due to the fact that all LED treatments including the control led to higher number of spores than that obtained on exposure to blue light alone (Table 2).

The amount of spores produced in the sand was higher under the red-blue treatment followed by the red alone in both plants as compared to the rest of treatments. This effect was more expressive when compared to blue alone treatment. In general, the pots kept in dark and those subjected to some LED treatments (red+blue and red/blue) showed no significant difference from each other, and had more spores than those in pots exposed to blue light only and less spores than those observed in pots exposed to red-blue light (Table 2).

## DISCUSSION

In this study we showed that LED inhibited the plant growth in millet seedlings, but just slightly in Bahiagrass where only the blue light affected the shoot growth. Interestingly, the effects of LED were mostly observed on the *in vivo* formation of AMF propagules in plant rhizosphere and outside, as in the glass beads section. Red-blue and red LED treatment resulted in a better AMF colonization or a higher number of new spores, depending on their kind and respective combinations. This finding is further supported by the data from this study, where the red-blue and red LED could affect sporulation and AMF colonization. The proliferation of hyphal branches increases the frequency of fungal contact with the root surface (Harris, 2008).

The use of blue light after red light (red-blue treatment) produced no effect on the AMF hyphal growth when compared to red light. As shown in Table 2, there were no differences between both treatments on colonization of bahiagrass (75 vs. 82 %) or millet (82 vs. 79 %). However, both plants had higher number of spores under red-blue

than under red alone, either in glass beads or sand.

The previous findings indicate that although blue light alone showed the lowest response for colonization or sporulation of AMF, it produced good results when used after the red light alone.

Even more, the blue LED following the red LED functioned more as a sporulation stimulator than as a hyphal grower. In fact, Yachi et al. (2001) and Ishii et al. (2003) found that the blue LED alone may inhibit hyphal growth.

**Table 1.** LED effects on shoot fresh weight (SFW) and root fresh weight (RFW) in pots cultivated with bahiagrass and millet (grams per pot)

	Plant	Treatment					
		Dark	LED				
			Blue	Red	Red+Blue	Red/Blue	Red-Blue
SFW	Bahiagrass	2.9 a	1.3 b	2.1 a	2.6 a	2.7 a	2.3 a
	Millet	5.7 a	0.8 d	1.2 d	2.7 c	2.9 c	4.4 b
RFW	Bahiagrass	3.6 b	3.5 b	4.0 b	6.9 a	6.6 a	4.4 b
	Millet	3.4 a	1.2 c	1.4 c	3.9 a	2.5 b	1.9 b

Means followed by the same letters in each line are not significant different according to the Scott-Knott test ( $P \leq 0.05$ ).

**Table 2.** LED effects on mycorrhizal colonization and sporulation in glass beads and sand sections in pots cultivated with bahiagrass and millet

	Plant	Treatment					
		Dark	LED				
			Blue	Red	Red+Blue	Red/Blue	Red-Blue
Colonization (%)	Bahiagrass	47.6 b	38.0 c	82.0 a	47.9 b	52.0 b	75.0 a
	Millet	52.7 b	39.0 c	79.0 a	56.2 b	48.0 b	82.0 a
Glass beads (spores per pot)	Bahiagrass	112.0 c	20.0 e	153.0 b	83.0 d	75.0 d	215.0 a
	Millet	142.0 b	65.0 d	159.0 b	86.0 c	95.0 c	198.0 a
Sand (spores per 10 g)	Bahiagrass	147.3 c	108.6 d	185.0 b	157.2 c	142.0 c	220.0 a
	Millet	116.0 d	102.2 d	150.8 b	127.6 c	135.0 c	185.6 a

Means followed by the same letters in each line are not significant different according to the Scott-Knott test ( $P \leq 0.05$ )

The number of spores was not altered by the blue LED in the sand as much as it was altered in the glass beads. It is possible that the inhibition by blue light was alleviated in glass beads section near the rhizosphere (in contact with nylon mesh), due to the involvement of the root exudates. Some branching might occur at the sites not exposed to the light, suggesting the involvement of a second messenger responsible for such synergism (Nagahashi and Douds, 2000). If this is the case, other environment factors such as host root exudates could also induce hyphal branching,

germination, or growth (Horii et al., 2009).

Although LED affected AMF propagation, the effects of root exudates should be considered. Previous studies have shown that LED (Nagahashi et al., 2000; Nagahashi and Douds, 2004) and root exudates (Buee et al., 2000; Cruz et al., 2000) could stimulate hyphal growth of AMF spores. From the root exudates, some active compounds for AMF stimulation have been isolated (Horii et al., 2009; Kuwada et al., 2006). Individual treatments of blue light and diluted compounds that induce a response to light have been

ineffective in stimulating hyphal branches, but they became effective when combined (Nagahashi and Douds, 2004). Moreover, the inhibitory effect on growth by blue LED was observed in other cultured microorganisms, especially pathogens (Giusti et al., 2008; Maclean et al., 2009).

The red LED may function as a hyphal growth stimulator *in vitro* (Ishii et al., 2003; Yachi et al., 2001). These researches suggested that the wavelength of the LED could determine whether the AMF hyphal growth or branching can be stimulated or inhibited, in this case high wavelength light, such as the red one, would be able to stimulate AMF hyphal growth and spore germination.

If we standardize the volume occupied by glass beads and sand, the amount of spores in sand is higher. Nonetheless, it should be emphasized that the advantage to produce spores in glass beads is the purity of these for further studies at molecular level, for example, saving laboring on clean.

Little is known about the response of AMF to light induction. Several studies have reported the most efficient hyphal branching at 390 and 430 nm (Nagahashi and Douds, 2003), similar to the cryptochrome region, which is defined by their action spectra with a typical broad band in the blue/UV-A region and a second band in the blue region (Lin, 2002). This research has shown that physical signals, here represented by the light-induced factors, are involved in the mechanism wherein AMF can colonize host roots.

## CONCLUSIONS

This research found that LED had, depending on their type, some effects on AMF propagation *in vivo*.

The red-blue and red alone were the most efficient treatments that favored colonization and sporulation in glass beads

The blue itself inhibits the spore formation in glass beads.

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