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Combination of C3 and C4 host plants for the reproduction of the Sorowako indigenous arbuscular mycorrhizal fungi

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Abstract

The selection of the right host plant needs attention because of the interaction between the host plant, the type of AMF, the composition of the media, and the climate during its growth. It has been reported that in the C4 group, the best type of host plant used in producing AMF spores is *Zea mays*, while in the C3 group, *Pueraria javanica* is able to produce optimum AMF development, so a study is needed to combine C4 and C3 groups as hosts plant in AMF spore propagation. The study was arranged using a completely randomized design with 5 combination treatments of host plant groups C4 and C3, to determine the productivity of the host plant combination, the parameters observed were percentage of infected roots, abundance of mycorrhizal spores, and diameter of spore. The results showed that good host plant productivity was obtained from host plant combinations of *Zea mays* and *Vigna angularis* or *Vigna unguiculata* or *Phaseolus vulgaris*, where mycorrhizae can infect roots above 90% and produce spores that have a diameter of 300-400 µm, so it can be recommended that a host plant combination that has a fast life cycle is better used to stimulate the growth and regeneration of mycorrhizal spores, as a novelty in this study.

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Introduction

Arbuscular mycorrhizal fungi (AMF) in their associations have a host plant range of 80 to 90% (Diagne et al., 2020) with terrestrial plants, both food crops, horticulture, plantations, and forestry. The distribution of mycorrhizae is very wide and can be found in various cropping areas in the world, from mountainous areas to coastal areas.

Plants commonly used as hosts plant include *Sorghum vulgare* (Wang et al., 2021), *Zea mays* (Halim et al., 2020), *Panicum maximum* (Rezacova et al., 2018), *Paspalum notatum flugge* (Junior et al., 2021), *Peanuts* (Hasid et al., 2019), and *Pueraria javanica* (Mahulette et al., 2021). However, the level of effectiveness of each

host plant is different, because certain types of AMF show specifications to select and associate with certain types of host plants (Torrecillas et al., 2012), so the selection of compatible host plants with AMF isolates is important.

The type of host plant and environmental conditions will greatly determine the level of root colonization, the number of spores, and the diversity of spore types (Furrazola et al., 2015). Therefore, the host plants commonly used for AMF propagation are plants that have a the best root system (Huang et al., 2020), are drought-tolerant (Begum et al., 2019) can grow in almost every type of soil (Sukmawati et al., 2020), can form twice as many secondary roots like other plants (Johnson and Gibson, 2021), deep-rooted and fibrous so that it is more effective in absorbing nutrients and water (Begum et al., 2019; Gao et al., 2020).

The selection of the right host plant needs attention because of the interaction between the host plant, the type of AMF, the composition of the media, and the climate during its growth. In group C4 plants, the best type of host plant used in producing AMF spores is corn (*Zea mays*) (Nuridayati et al., 2019; Sukmawati et al., 2021), while in group C3 plants, *Pueraria javanica* species are able to produce optimum MA development (Rini et al., 2017; Rini and Rozalinda, 2010). However, from several research activities, the utilization of this plant is still used separately in the propagation of AMF spores, and secondary products from plant hosts have not been focused on. Therefore, research is needed to combine C4 and C3 plants as host plants in AMF spore propagation which also has economic value. So that, the research objective to be achieved is to obtain a combination of C4 and C3 plants as good host plants for the reproduction of mycorrhizal spores.

Materials and methods

This research was carried out at the Agroplastidfarm experimental location in Parepare City and the Microbiology Laboratory of the Makassar Environmental and Forestry Research and Development Center and complied with the health protocol rules to avoid transmission of the Covid-19 virus during the Covid-19 pandemic.

The study was arranged using a completely randomized design with combination of host plant treatments, namely a combination of *Zea mays* and *Vigna angularis* (HP1), *Zea mays* and *Puraria javanica* (HP2), *Zea mays* and *Vigna unguiculata* (HP3), *Zea mays* and *Vigna radiata* (HP4), *Zea mays* and *Phaseolus vulgaris* (HP5).

Combination of planting media used was rice husk charcoal, sand, and cocopeat (the best combination of media in phase 1 research), while the inoculants used were spores and propagule *Gigaspora* sp. indigenous Sorowako. Parameters observed to determine the productivity of the host plant combination as detailed below.

Percentage of infected roots

Calculating the percent root infection begins with the preparation of semi-permanent root preparat. The percentage of mycorrhizal infection was calculated from the number of infected roots from 10 observed root pieces. Infected roots are characterized by the presence of vesicles or arbuscules in the root cortex. The percentage of mycorrhizal infections was calculated based on the method provided by (Giovannetti et al, 1980).

$$\% \, infected = \frac{\sum infected \, roots}{\sum total \, root \, observed} \times 100\%$$

Abundance of indigenous mycorrhizal spores

Spores with a size between 50–120 m are expected to be filtered in the mess (size 350 µm) at the bottom. The spores were then transferred to filter paper that had been given vertical and horizontal lines with a box size of 0.5 x 0.5 cm to facilitate observation. These spores were observed and counted under a microscope with a magnification of 40x (Pacioni, 1991).

Spore diameter

Spores are first placed on a glass object and covered with the glass, then measured and observed under a microscope with 40x magnification, the measurement results are then calibrated to get the actual diameter size. The data of observation were calculated using an analysis of variance. The results of analysis variance that significance followed by Duncan's test.

Results and discussion

Analysis of variance for percentage of roots infected, showed that the host plant treatment had significant

effect on the percentage of roots infected by *Gigaspora* sp. and Duncan's test results showed that the host plsnt combination of *Zea mays* and *Vigna angularis* (HP1), *Zea mays* and *Vigna unguiculata* (HP3), as well as *Zea mays* and *Phaseolus vulgaris* L (HP5) resulted in relatively the same percentage of infected roots, and different with the combination *Zea mays* and *Puraria javanica* (HP2) as well as *Zea mays* and *Vigna radiata* (HP4), as shown in Fig. 1.

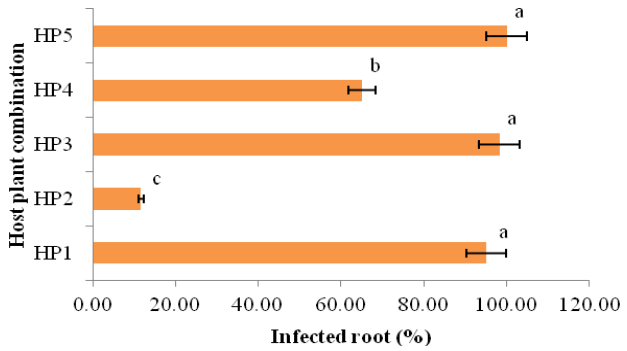

Fig. 1: The percentage of *Gigaspora* infection in various combinations of host plants [*Zea mays* and *Vigna angularis* (HP1), *Zea mays* and *Puraria javanica* (HP2), *Zea mays* and *Vigna unguiculata* (HP3), *Zea mays* and *Vigna radiata* (HP4), *Zea mays* and *Phaseolus vulgaris* L (HP5)].

This phenomenon is possible because the C3 host plants (*Vigna angularis, Vigna unguiculata, Vigna radiata* and *Phaseolus vulgaris*) have a shorter life cycle (generally approximately 60 days) than the life cycle of *Puraria japanica*, so seeds that fall on the media can germinate and produce a new hosts plant that has young roots so that the next root infection occurs again, as stated by Canarini et al. (2019), Monther and Kamaruzaman (2012), Pantigoso et al. (2020) and Zhou et al. (2020) that infection is more common in young roots that produce the large amount exudate, so mycorrhizae are more likely to infect young plant roots to obtain nutrition from plants. Canarini et al. (2019) and Monther and Kamaruzaman (2012) added that mycorrhizal infection in host plants is also determined by root exudates released in the form of sugars, organic acids, and amino acids which are food for mycorrhizae. Each plant secretes a different root exudate so that the mycorrhizal response to the host plant is also not the same (Sun et al., 2012).

The beginning of root infection by mycorrhizae starts from plant produces of hormone strigolactone which functions as signal for mycorrhizae to penetrate the hyphae into root epidermis of host plant (Maclean et al., 2017; Sun et al., 2012). As a result of chemical and mechanical stimulation of these compounds, mycorrhizae then form hyphae that function as a means of penetrating the host plant roots (Berruti et al., 2016; Bobbu, 2016; Bonfante and Genre, 2010). The hyphae will continue to elongate until they enter the cortical cells after the appressorium penetrates the root epidermis (Chifetete and Dames, 2020; Ganugi et al., 2019). Furthermore, mycorrhizal hyphae will enter the cortical cells and then branch to form arbuscules (Luginbuehl and Oldroyd, 2017; Vergara et al., 2019). After the arbuscules get nutrients in the form of carbohydrates from their host plants, mycorrhizae will develop extra radical mycelium (external hyphae) which then eventually form new spores (Begum et al., 2019; Rai et al., 2019).

Analysis of variance showed that the combination of host plant treatment gave a significant effect on the density of spores of *Gigaspora* sp., then Duncan's test results showed that host plant combination *Zea mays* and *Vigna unguiculata* (HP3) produced the highest number of spores compared to other media combinations, but not significantly different from the host plant combination *Zea mays* and *Vigna angularis* (HP1) as well as *Zea mays* and *Phaseolus vulgaris* (HP5) (Fig. 2).

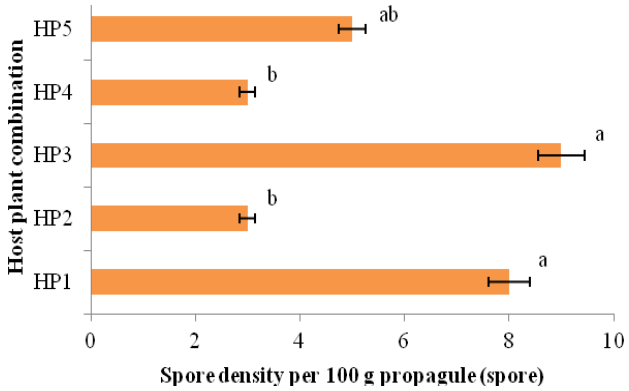

Fig. 2: The density of *Gigaspora* sp. spores in various combinations of host plants [*Zea mays* and *Vigna angularis* (HP1), *Zea mays* and *Puraria javanica* (HP2), *Zea mays* and *Vigna unguiculata* (HP3), *Zea mays* and *Vigna radiata* (HP4), *Zea mays* and *Phaseolus vulgaris* (HP5)]. The displayed data has been transformed into a logarithmic form.

The rhizosphere environment from the hosts plant combination *Zea mays* and *Vigna unguiculata* (HP3) is

thought to support the development and is compatible with *Gigaspora* sp. spores. According to Pulungan and Nasution (2021), Rai et al. (2019) and Yuwati et al. (2020) one of the factors that influence the development of mycorrhizal spores is the type of host plant. In addition, another hypothesis is that the production of photosynthate produced by the host plant combination *Zea mays* and *Vigna unguiculata* (HP3) is used by mycorrhizae for hyphae development so that a high percentage of root infection is followed by high spore formation. However, according to Costa et al. (2013); Furrazola et al., (2015), that spore formation is a dynamic process so that some spores are formed and some others germinate at the same time.

Furthermore, Delvian and Rambey (2019) and Diagne et al. (2020) suggested that one way to increase the production of mycorrhizal spores is to apply stress conditions to the culture pot. The results of the study Rini et al. (2020) showed that with more host plants in pots, this triggered a stress condition for plants in the form of competition for space for roots to grow and get water and nutrients. This stress condition is thought to trigger the production of spores so that the number of spores produced will also increase.

Gigaspora spores are formed from the rounded ends of hyphae (bulbous suspensor), then small spheres appear that get bigger and reach their maximum size which eventually becomes spores. The suspension is attached to the outer surface of the spore wall. A distinctive feature is the presence of a bulbous suspensor without a germination shield (INVAM, 2020) (Fig. 3).

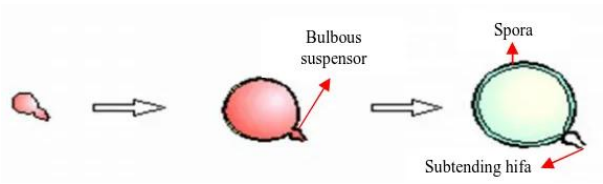

Fig. 3: The process of spore development in *Gigaspora* sp. (INVAM, 2020).

Spores are one of the stages in the mycorrhizal life cycle, Garg and Chandel (2010) stated that the mycorrhizal life cycle consists of three stages, namely, the first stage of symbiosis, involving propagule activity, host plant, the appressorium, root penetration. and arbuscular. At this stage, the involvement of the inoculum and plant species is key, because the energy used for mycorrhizal growth and development comes from plant photosynthesis, and conversely, mycorrhizae provide nutrients and water as the basic materials of photosynthesis (Giovannetti et al., 1994; Vierheilig and Bago, 2005). The second stage of growth and development involves external, internal, and extraradical hyphae as a whole increasing mycorrhizal biomass, forming hyphal structures, and expanding mycorrhizae outside and between plants. The development of hyphae serves as a channel that expands radically, then the development of arbuscular structures plays a role in taking up nutrients and the formation of vesicles that function as lipid storage (Bonfante and Genre, 2010; Diagne et al., 2020). The third stage is the propagation stage which involves the reproductive structure, namely the formation of spores which are the main inoculum for mycorrhizae (Bobbu, 2016; Selvakumar et al., 2018; Vilcatoma-Medina et al., 2018).

Analysis of variance for the spore diameter variable showed that treatment of host plant had a significant effect on the growth of spore diameter of *Gigaspora* sp., and then results of the Duncan test showed that host plants combination *Zea mays* and *Vigna angularis* (HP1), *Zea mays* and *Vigna unguiculata* (HP3), as well as *Zea mays* and *Vigna radiata* (HP4) produced spore diameters that were relatively the same, and different from the spores diameter in the host plant combination *Zea mays* and *Pueraria javanica* (HP2) along with *Zea mays* and *Phaseolus vulgaris* (HP5) (Fig. 4).

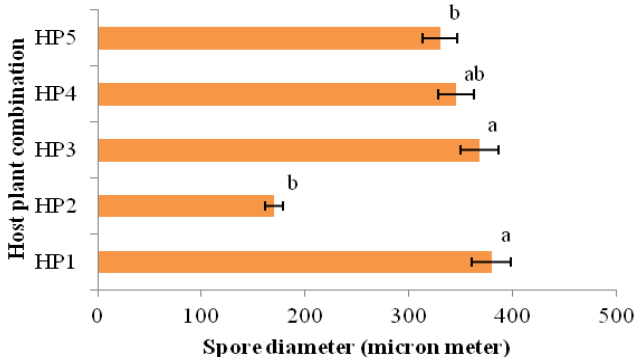

Fig. 4: Diameter of *Gigaspora* spores in various combinations of host plants [*Zea mays* and *Vigna angularis* (HP1), *Zea mays* and *Puraria javanica* (HP2), *Zea mays* and *Vigna unguiculata* (HP3), *Zea mays* and *Vigna radiata* (HP4), *Zea mays* and *Phaseolus vulgaris* (HP5)].

Mycorrhizae genus *Gigaspora* has the largest spore size (205-600 m diameter) of all mycorrhizal spores

(INVAM, 2020). Spore diameter is one variable to see the growth of spores. The growth of spores is strongly influenced by the energy supply for mycorrhizal growth obtained from the photosynthesis of the host plant (Balestrini et al., 2020; Becklin et al., 2016). So host plants compatible with mycorrhizal species greatly affect sporulation and spore growth.

Conclusions

Good host plant combination productivity was obtained from *Zea mays* and *Vigna angularis*, *Zea mays* and *Vigna unguiculata*, and *Zea mays* and *Phaseolus vulgaris*, so that can be recommended that the combination of host plant which has a short/fast life cycle is better used to stimulate spore growth and regeneration mycorrhizae.

Conflict of interest statement

Authors declare that they have no conflict of interest.

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