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Research Article

Spatial and Temporal Association of VAM in Soybean (*Glycine max* L. Merrill) Plants at Some Regions in Bali

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Abstract

Background and Objective: Arid soil region in Bali island are spatially located at Buleleng, Gianyar, Karang Asem, Tabanan and Bangli. The plant of soybean cultivated in poor soil is generally often associated with soil microorganisms, for example, VAM fungi. A study on the VAM fungi associated with the soybean plant has been undertaken for 6 months (March to August, 2017) to know the association of mycorrhizal fungi in the rhizosphere of soybean. **Materials and Methods:** The number of spores was counted by wet sieving and decanting method, root infection was observed by the grid-line intersect method. **Results:** The result showed that soybean plants in these regions have mutually symbiotic with VAM fungi. The spore density showed either temporal or spatial variation. The spore density was low in July and August, as the rainfall seasons compared to in March until June, as dry months. The variation of spore density in both areas is caused by soil properties in both seasons. The colonization was found as a vesicle, arbuscules, internal-external hyphae and spore forms. The colonization shows similar temporal variation at both regions, it is considerably related to the temporal variation of rainfall level. **Conclusion:** Overall, the soybean plant at either barren or fertile soil associates mutually with VAM during the dry season in particular. Consequently, VAM could be further observed as a bio-fertilizer for soybean farming under field study.

Key words: Fungi, spores, hypha, arbuscules, vesicle, Arid areas, spatial

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Vesicular-Arbuscular Mycorrhiza (VAM) is a symbiotic association between plant and fungus, in which the hypha of fungi can penetrate root cortex cells of vascular plants. This symbiosis is commonly known as the Vesicular-Arbuscular Mycorrhiza (VAM) owing to the formation of particular vesicles (bladder-like structures) and arbuscules (branched finger-like hyphae) after colonizing the root cortical cells[1]. More than 90% of plant species angiosperms and gymnosperms can form Vesicular-Arbuscular Mycorrhiza (VAM) association to get the benefit between plants and fungi[2]. The VAM fungi take a significant role in some aspects of plants living in arid regions e.g., increasing plant growth and productivity[3], maintaining the ecosystem processes by promoting plant fitness through a range of mechanisms[4], protecting the host plants from soil pathogens[5] and improving texture and structure of the soil, enhancing water and nutrient uptake[6,7].

Some northern regions of Bali island are classified as arid regions i.e., Western of Buleleng (i.e., Gerokgak) and Western of Karangasem suburb (i.e., Sukadana). Farmer at the regions planted mostly horticulture plant adapted to dry condition i.e., cassava, nevertheless, the farmer also planted soybeans particularly shortly after raining season. It is likely that such growing well of soybean plant at the regions because the plant lives mutually with VAM fungi[8,9]. This is evidenced by the finding of 13 species of VAM at the regions and the relatively high colonization of VAM fungi in cashew nut plants root[3,8]. The greenhouse study showed that inoculation of VAM spores can increase the growth and productivity of soybean in dried soil[10,11]. Despite VAM fungi seems to take an important role in the growth and survivorship of soybean in the regions, fewer exploration studies on to what extent the symbiotic association of endomycorrhizal fungi at rhizosphere of soybean. The cultivation of soybean plants is also widely found across Bali island regions known as fertile soil areas, such as Bangli, Tabanan and Gianyar suburbs. In the regions, soybean farming is particularly during the dry season, shortly after rice harvesting. Nevertheless less information on VAM association and soybean cultivation from these areas.

The objective of this study was to observe the spatial and temporal dynamic of VAM spore density in rhizosphere and colonization on to soybean plant of some regions in Bali known to have different environment condition.

MATERIALS AND METHODS

Study area: The field study was conducted at 5 soybean farming areas i.e., at Buleleng, Tabanan, Gianyar, Karang Asem and Bangli suburbs. The laboratory study was carried out at Mycology Laboratory, Program Study of Biology, Faculty of Math and the Natural Sciences University of Udayana Bali, Indonesia. The studies were carried out from March up to August, 2017.

Soil and plant root sampling: Soil samples were collected from the rhizosphere (±20-30 cm in depth)[3]. Soil samples from each farming area were randomly collected from 5 sites (at 5 sample bags, ca 2 kg per bag), the 5 samples from each site were then composite (5 bags composite soil sample per sampling period per farming area in total). soybean plant root sample was the tertiary root of young soybean plants 5 individual plants of soybean were sampled from the 5 sampling sites (25 individuals of soybean plants per sampling period per farming area in total)[12,13]. Sampling periods were monthly intervals from March up to August, 2017.

Percentage of plant root colonization: The method of counting the percentage of VAM colonization was put into practice to calculate the plant root colonization percentage[8,13]. Plant roots samples were cleared, stained and destained progressively. The observation of VAM colonization in the root cortex and endomycorrhizal hifa was carried out by utilizing dissecting and binocular microscopes and the percentage of mycorrhizal colonization were calculated by using the formula as below[14]:

$$\text{Root colonization (\%)} = \frac{\sum \text{vertical cross} + \text{horizontal cross hypha}}{\text{Total root length examined}} \times 100$$

Isolation and spore density: The spore isolation from rhizosphere soil was conducted by method[9,10] i.e., wet decantation method. Soil sample i.e., 250 g is diluted and stirred in 1 L of water with ratio water: The soil is 1:4. The supernatant was strained into 5 consecutive sieves (Analysensieb Eckhardt 5657 Haan W. Germany) with the top down mesh size 500, 300, 200, 63 and 45 μm consecutively. The spores found were observed under a dissecting microscope. after the number of spores was counted it was then fixed in a glass bottle filled containing sterilized water.

Physical and chemical properties of soil: Rhizosphere soil sample (2 kg) was collected from the 5 sampling sites at each farming area and the samples were then composite

1 composite sample bag per sampling period per farming area in total). The physical and chemical properties of soil were analyzed at the Laboratory of Soil, Faculty of the Agriculture University of Udayana and Technical Service of the Analytical Laboratory University of Udayana Bali.

RESULTS

Endomycorrhizal spores density: The soil samples from 5 regions were examined. Direct microscopic counting showed a massive number of endomycorrhizal spores at these regions over the sampling period is presented in Fig. 1. According to Fig. 1, the spore density showed convincingly variation either spatially or temporally among 5 regions of sampling. The spores number sampled from Karangasem showed the highest spores meanwhile the lowest number of spores was in the Bangli region. Interestingly, the number of spores in all regions indicated the lowest density in August, meanwhile on April, indicated that the number of spores was high.

Percentage of root colonization: The total of root samples examined showed clearly the colonization of endomycorrhizal hyphae in the root of soybean varied from 38.9 until 68.3% and convincingly temporal dynamic but not spatially. The highest colonization among the 5 regions was in August, meanwhile in May and July, the colonization showed less (Fig. 2).

Number of endomycorrhizal spores: According to Fig. 3, seem that the number of spores among 5 genera of endomycorrhizal fungi was dominated by a genus of

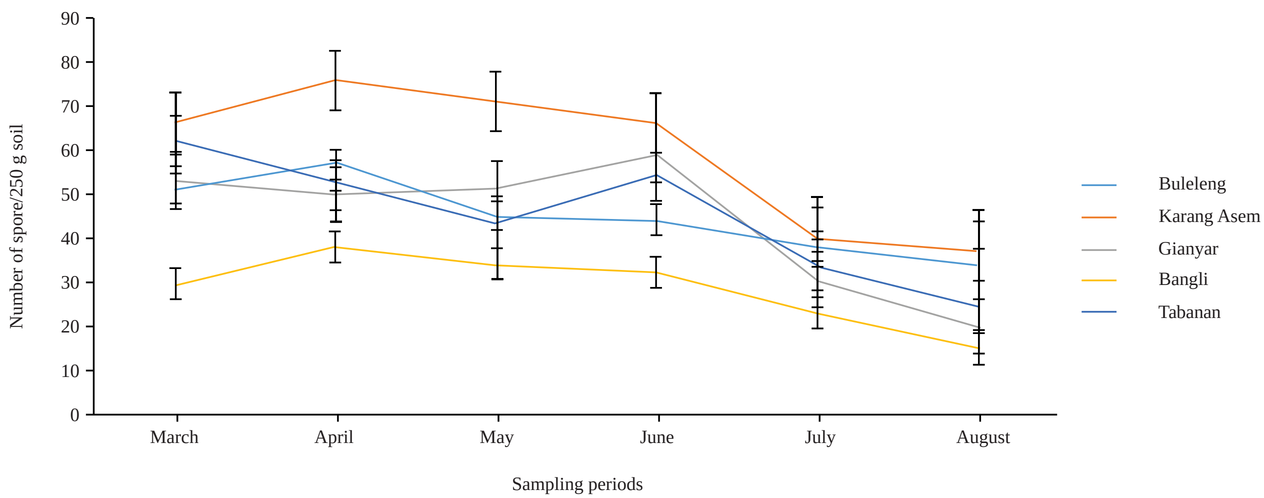

Fig. 1: Temporal and spatial of spore density in the rhizosphere of soybean plants

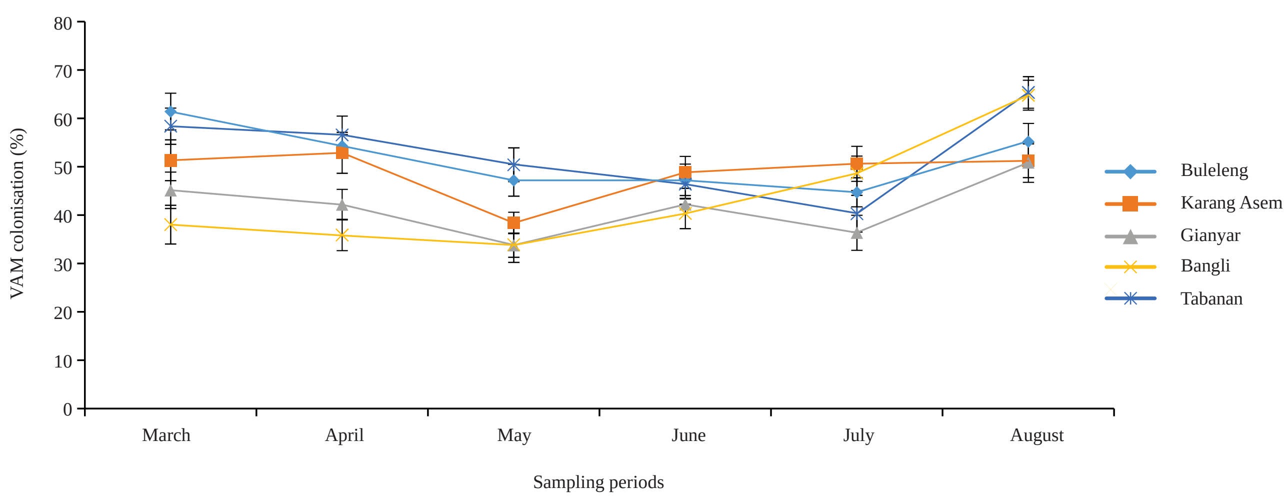

Fig. 2: Spatial and temporal variation of the average percentage of endomycorrhizae

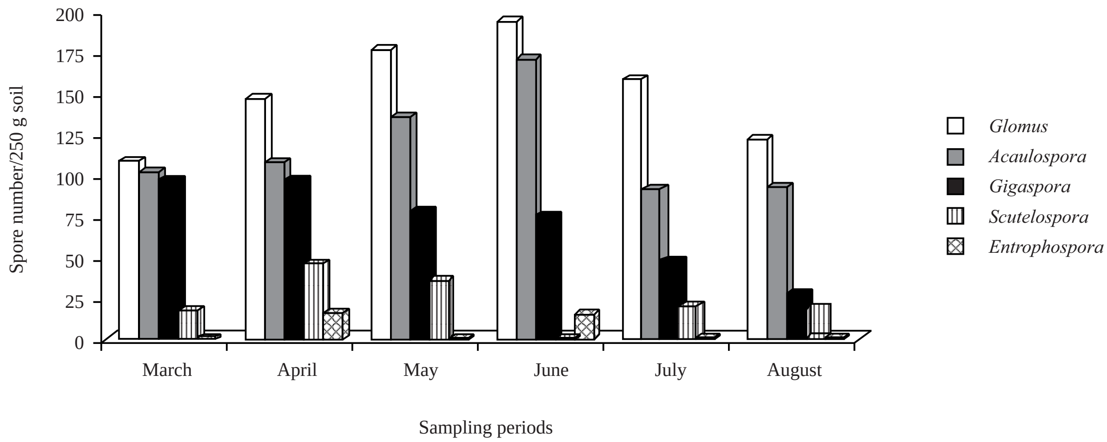

Fig. 3: Number of spores from five genera of endomycorrhizal fungi

Glomus spatially and temporally indeed however the Entrosphospora was the lowest spores among the 5 regions along the temporally sampling. The number of *Glomus* spores was more than 100 overall in these regions and seasons.

DISCUSSION

Spatially, the spore density in the rhizosphere of the soybean plant (*Soya max* L.) sampled from the Karangasem region showed consistently more abundant than those sampled from the Bangli region. Taking into account the soil physical-chemical properties, such spore density variation confirms the spatial variation of the soil properties i.e., texture, nutrient and water level. The soil water level is likely to be the most determinant factor affecting such spatial variation of spore density, water stimulates spore to germinate therefore spore density tends to decrease during the rainy season because the spore will germinate to form hypha[8,10] and try to colonize the root of plants as a mutualism symbioses[12]. This study also indicated that the level of Phosphorous (P) and Nitrogen (N) positively linear to the spore density, therefore this finding was in contrast to other researchers[13,14], who showed negative linear of phosphorous availability and spore density. While, the finding on nitrogen level-spore density correlation agrees with the study of previous authors[15,16], who showed the VAM spore density in the region was supported by the availability of nitrogen levels in the soil. However, this finding does not fit the study done[17,18], who revealed that nitrogen soil content does not have any influence on spore density. The spore density is found high in the Karangasem region having high pores soil texture i.e., sandy clay. This is in contrast to study[19] that showed the clay-sand mixture (2:1) to have more spore density. Nevertheless, soil pore is likely to exert a positive role in spore formation.

Temporally, the dynamic of spore density at both regions are fixed to the temporal dynamics of rainfall frequency and water level. The figure showed the opposite trend between spore density and the rainfall frequency and water level, the spore number tends to increase when rainfall frequency and water level decrease (June up September, 2017) and *vice versa* (April up to June, 2017).

Spore is resulted by hypha of VAM fungi and spore formation is one of the mechanisms of some microorganism self-defence when the environmental condition does not support the development of hypha. This finding fit[20,21] and the existence of VAM spores in nature is season-related[22], the spore number tends to decrease during the rainy season because the spore will germinate to form hypha and the hypha will grow to find out soil or supply water over the dry season.

In this study, based on spatial observation of vesicle, arbuscular and mycelia in *Glicine max* root, the root colonization at both regions varies from 38.38% up to 52.94. This finding confirms the high-level symbiosis between endomycorrhiza and soybean plants. The finding of this study also showed that despite the difference in soil properties in the regions studied, no significant difference in root colonisation. High root colonisation at the Buleleng region can be addressed to the low phosphorous availability. One adaptation of the plant to low phosphorous availability is a symbiosis with endomycorrhiza[23], the fungi re-mineralise phosphorous and deliver Phosphate (P) and other minerals in exchange for carbohydrates[2]. Though Phosphate (P) availability is very high in the Sukadana region that could suppress the VAM colonisation, the low level of nitrogen likely overruled phosphate suppressive[11,12].

The result of the temporal dynamic of root colonisation showed a similar trend at Sukadana and Gerokgak regions, the trend fits the dynamic of rail fall frequency and rainwater level. The root colonisation tends to be high over the rainy season (April up to June, 2011 and December up to February, 2012) and decrease gradually over the dry season (June up to December, 2011). This dynamic of root colonisation is likely related to again soil water level, water stimulates VAM spores to germinate and form hypha that immediately colonizes plant roots[20].

The result also agrees with the study were done by authors[14,2], hypha colonization is season-related. Such colonization facilitates endomycorrhiza gets to benefit from the host plant i.e., glucose. While the host plant gets some benefits from endomycorrhiza colonization i.e., the endomycorrhiza takes a role in re-mineralization of phosphorous and providing Phosphate (P) in soil[2], on water absorption and mineral uptake[24] and on producing plant growth hormone-like similar to auxin, cytokine and Gyberelin[15,16]. This study showed that the soybean plant shows mutual symbiosis with VAM in particular during the dry season at either barren or fertile areas. Such an important VAM role is related to the capability of the hypha of VAM in absorbing water and minerals from dry oil. The finding on various VAM species over the regions is promising to support the biofertilizer program and the organic soybean farming development across Bali island.

CONCLUSION

Nowadays, eco-friendly strategies for organic farming is prioritized in many farming research project in Bali. VAM possesses plant growth supporting potential and can be applied for supporting organic farming. Although, VAM is mainly known to support plant growing at the bare region, VAM should be further observed for biofertilizer in soybean. Therefore, our findings underline VAM has potential for soybean organic farming.

SIGNIFICANCE STATEMENT

This study discovers the association of soybeans and VAM fungi at either bare or fertile regions. This study will help the researcher to uncover the critical areas of study on bio-fertilizer for soybean farming that many researchers were not able to explore. Thus a new theory on VAM bio-fertilizer for soybean may be arrived at.

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