

Original Research Article

<https://doi.org/10.20546/ijcmas.2023.1207.019>

Root and Leaf Phenomenons of C3 Plants Infected with Vesicular Arbuscula Mycorrhizal

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ABSTRACT

The utilization of VAM in plants generally uses powder dosage forms and not yet used dosage forms of sachet and tablet. The research aimed to examine the phenomenon of the roots and leaves of C3 plants infected with VAM in different dosage forms at the screen house scale. The research was conducted at the Agrotechnology Laboratory and Screen House, Faculty of Agriculture, Animal Husbandry and Fisheries, Muhammadiyah University of Parepare, using factorial design. The first factor was the VAM dosage forms, namely powder/control, sachet, and tablet dosage forms. While, other factors were the C3 plant species, namely Pueru (*Pueraria javanica*)/control, mung bean (*Vigna radiata*), and red spinach (*Amaranthus tricolor*). The parameter observations on the percentage of infected roots, root volume, leaf area, and stomata density. Data were analyzed using variance analysis. The results showed that the VAM dosage form had a significant effect on all parameters observed with various root and leaf phenomena so VAM in a sachet and tablet dosage form can be recommended as a new dosage form for VAM application in the field.

Keywords

Colonization, leguminosae, mycorrhiza, root nodule, stomata

Article Info

Received:
04 June 2023
Accepted:
06 July 2023
Available Online:
10 July 2023

Introduction

The based on the photosynthetic pathway, plants are grouped into C3, C4, and CAM plants (Cousins *et al.*, 2020; Kumar *et al.*, 2017). C3 plants use the

Calvin cycle as their photosynthetic pathway, the name C3 is obtained from the first molecule produced, namely the three-carbon (3C) molecule 3-phosphoglyceric acid in the first stage of carbon fixation (Muhie, 2022; Upadhyay *et al.*, 2020). C3

plants are the most common plant species on earth, with an estimated 85 percent of plant species being C3 plants (Kumar *et al.*, 2017; Sivaram *et al.*, 2020), including puero (*Pueraria javanica*), mung bean or green gram (*Vigna radiata*), and red spinach (*Amaranthus tricolor*). C3 plants have an adaptive ability in environments with high atmospheric CO₂ content (Bhargava and Mitra, 2021; Wang *et al.*, 2020), however, the growth process of these plants is also influenced by climate, weeds, pest attacks, plant diseases, nutrient availability, and water (Anwar *et al.*, 2021; Skendzi *et al.*, 2021).

High availability of nutrients can be found in the topsoil layer containing dark-colored organic matter up to 25-35 cm (Gerke, 2022; Hoffland *et al.*, 2020). However, this topsoil layer has been lost a lot due to unwise tillage (Krauss *et al.*, 2022; Krauss *et al.*, 2020), excessive use of herbicides (Khangura *et al.*, 2023; Theint Theint Win *et al.*, 2020), land clearing without regard to conservation (Abdelrahman, 2023; Marpaung *et al.*, 2021), and eroded due to erosion (Feeney *et al.*, 2023; Halecki *et al.*, 2018), so an environmentally friendly technology is needed that can help plants to absorb bound nutrients and water in the ground.

Vesicles Arbuscular Mycorrhizal (VAM) is an environmentally friendly biological technology that can be in mutualistic symbiosis with plant roots (Anand *et al.*, 2022; Weng *et al.*, 2022) and has received attention from agronomists.

VAM has been proven to be used as an alternative technology to encourage plant growth (Begum *et al.*, 2019; Huey *et al.*, 2020), increase crop productivity and quality (Hristozkova and Orfanoudakis, 2023; Wu *et al.*, 2022) especially on dry land, acid soil, and land contaminated with heavy metals.

The utilization of VAM in plants generally uses powder dosage forms and has not used sachet and tablet dosage forms. Until now, evaluation of the phenomenon of the roots and leaves of C3 plants infected with VAM in different dosage forms at the screen house scale has not been carried out. In this

regard, it is necessary to conduct a more in-depth study of the phenomena of the roots and leaves of C3 plants infected with VAM in different dosage forms under tropical conditions. The research aimed to examine the phenomenon of roots and leaves of C3 plants infected with VAM in different dosage forms at the screen house scale.

Materials and Methods

The research was conducted at the Agrotechnology Laboratory and Screen House, Faculty of Agriculture, Animal Husbandry and Fisheries, Muhammadiyah University of Parepare. This research uses a factorial design. Factor 1 was the dosage form of VAM, namely powder dosage form (D1)/control, sachet dosage form (D2), and tablet dosage form (D3), While other factors were C3 plant species, namely puero (*Pueraria javanica*) (P1)/control, mung bean (*Vigna radiata*) (P2), and red spinach (*Amaranthus tricolor*) (P3).

The powder dosage form is a standard propagule that consists of zeolite, sand, biochar, root pieces of a host plant, and VAM spores. Standard propagule is required for the manufacture of sachet dosage forms using the sachet packaging, and for the manufacture of tablet dosage forms by adding organic or inorganic adhesives. The planting medium used consisted of soil, biochar, and compost with a ratio of 1:1:1.

The percentage of roots infected is calculated based on the formula from Phillips and Hayman (1970), and root morphology includes root length, secondary root number per 1 cm of primary roots, secondary root diameter, and root volume. Observational data were analyzed using Factorial Variance Analysis followed by Duncan's Test (Akib, 2014).

Calculating the percentage level of infected roots begins with making semi-permanent root preparations. Calculate the percent infection from the roots infected number from 10 observed root sections. Characterized by infected roots are the presence of hyphae and arbuscules in root tissue.

The infections percentage of VAM was calculated using the formula from Phillips and Hayman (1970), which can be seen in equation 1:

$$IP\ VAM = \frac{JAT}{JSP} \times 100 \dots\dots(1)$$

Note: JAT: Σ infectionroots, JSP : Σ totalobservation

The percentage of infected roots was determined based on the criteria of Rajapakse and Miller (1992) as follows:

- <5% = very low (grade 1)
- 6 – 25% = low (grade 2)
- 26 – 50% = moderate (grade 3)
- 51 – 75% = high (grade 4)
- >75% = very high (grade 5)

The root volume (RV) was determined by cutting the cleaned of plant roots. Plant roots were insert to a 1000 ml measuring glass containing 250 ml of water (V1) to obtain an additional volume (V2) (Sattelmacher, 1987). The formula for root volume can be seen in equation 2:

$$RV = V2 - V1 \dots\dots(2)$$

Leaf area (LA) is determined using the Gravimetric method. For the implementation of the gravimetric method, as follows: use leaf patterns (leaf replicas) drawn on plain paper. The leaf replicas were weighed using an analytical balance (Chaudhary *et al.*, 2012). Calculate leaf area using the formulain equation 3:

$$LA = \frac{\text{Leaf replica weight}}{\text{Total paper weight}} \times \text{total paper area} \dots\dots(3)$$

Stomata density was measured using the replica method. The steps for the replicated method are as follows: Coat the leaf surface with a thin layer of

nail polish and let it dry for about 5-10 minutes. After the nail polish has dried, the scotch tape is applied, and immediately unsealed, and the nail polish is removed immediately as a pore impression.

Place the mold on the glass object and observe the preparation under a microscope with 400x magnification (Brown and Rosenberg, 1970; Paul *et al.*, 2017; Wolf *et al.*, 1979).

Results and Discussion

The variance analysis showed that the VAM application in different dosage forms affects the root phenomenon of C3 plants, especially at infected root percentage, nodules number, and root volume. Furthermore, Duncan's analysis showed that infected roots percentage of VAM and root volume of C3 plants at the application of VAM in sachet dosage forms had significantly different effects from other dosage forms on the three C3 plants. Whereas variable nodule number is shown at the application of VAM in powder dosage form (Table 1).

Dosage forms that increase VAM activity in infecting C3 plant roots are carried out on hyphae organs with moderate infection rates and other organs, namely arbuscules with infection rates low to moderate (Table 1 and Figure 1). Even though the infection class was not optimal, the infection percentage increased from 46.02% to 63.51% compared to powder and tablet dosage forms. Eldina *et al.*, (2021) stated that efficiency of VAM infection in host plants depends on the host plant characteristicsand abiotic factors (Morkunas *et al.*, 2018; Sabrina *et al.*, 2021) such as temperature, pH, humidity, and soil microorganisms.

In addition to these factors, the presence of VAM propagule in sachet dosage forms causes VAM spores to always be around the roots so that they can infect the roots properly as a form of maximum effectiveness.

The number of root nodules formed on C3 plants differs between VAM dosage forms. VAM in a powder dosage form can increase the number of root

nodules by 15.02% to 56.22% compared to other dosage forms. Possibly caused by application of VAM in powder form cannot optimally induce plant root infection, because the number of spores lost by water and wind, provides an opportunity for rhizobium bacteria in the planting medium to initiate infection which eventually forms root nodules.

According to Goyal and Habtewold (2023); Damanhuri *et al.*, (2020), plant roots that symbiosis with Rhizobium sp bacteria can fix nitrogen (N₂) from the air.

The free nitrogen is fixation and then stored in the form of nodules that contain nitrogen which is very important because it increases soil fertility (Cheng *et al.*, 2023; Santi *et al.*, 2013)

Vesicular AM in sachet dosage form, besides increasing infection, can also increase the root volume of C3 plants. The increase in root volume as a performance of root growth is inseparable from the role of VAM in absorbing water and nutrients through hyphae which in turn increases the

production of assimilate which is translocated to roots for root growth and fulfills VAM energy needs through the role of arbuscules.

According to Carstensen *et al.*, (2018) and Kayoumu *et al.*, (2023) that VAM plays a significant role in the absorption of nutrients, especially the element P for the formation of assimilates through the process of photosynthesis that occurs in plant leaves.

The construction of ATP and NADPH using the help of sunlight which is absorbed by plant leaves and then converted into chemical energy in the light reaction. Furthermore, in the dark reaction, ATP and NADPH are used to reduce CO₂ to produce assimilates in the stroma (Li *et al.*, 2023; Wasilewska-d, 2022)

The results of variance analysis showed that the dosage form of VAM had a significant effect on the number of leaves, leaf area, and number of stomata of C3 plants. While the Duncan test showed a different phenomenon in C3 plants due to the treatment of VAM dosage forms (Figure 2).

Table.1 Phenomenon of C3 plant roots infected with vesiculararbuscular mycorrhizal

No	Treatment	VAM infected roots (%)		Number of Nodules (unit)	Root volume (cm ³)
		Hyphae	Arbuscular		
1	D1P1	3,33 ⁱ	0,00 ^e	79,00 ^a	4,36 ^b
2	D1P2	21,67 ^f	10,00 ^b	48,33 ^{ab}	3,60 ^b
3	D1P3	41,67 ^a	3,33 ^d	79,00 ^a	4,57 ^b
4	D2P1	33,33 ^c	7,41 ^c	46,00 ^{ab}	15,05 ^a
5	D2P2	31,67 ^d	11,67 ^a	11,00 ^b	13,10 ^a
6	D2P3	40,00 ^b	10,00 ^b	59,00 ^{ab}	11,01 ^a
7	D3P1	6,67 ^h	0,00 ^e	0,00 ^b	2,77 ^b
8	D3P2	15,00 ^g	0,00 ^e	24,67 ^{ab}	2,82 ^b
9	D3P3	26,67 ^e	0,00 ^e	6,33 ^b	2,74 ^b

Note: Percentage of root colonization by VAM in the combination of VAM dosage forms and C3 plant species (D1, powder dosage forms; D2 sachet dosage forms; D3 tablet dosage forms; P1, *Puraria javanica*; P2, *Vigna radiata*; P3, *Amarantus gangeticus*)

Fig.1 Infection of vesiculararbuscular mycorrhizal (VAM) on the C3 plants roots (Magnification 10x 40)

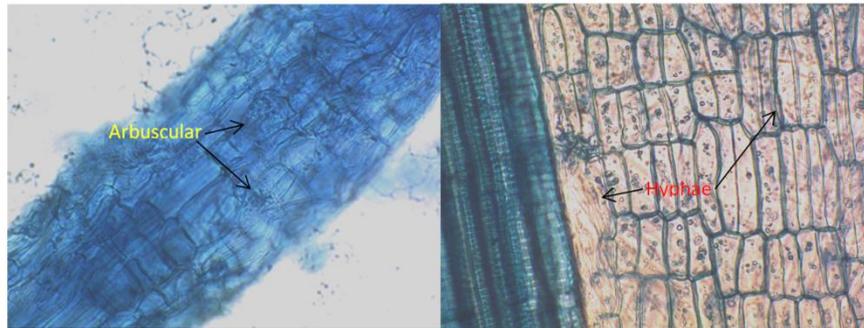
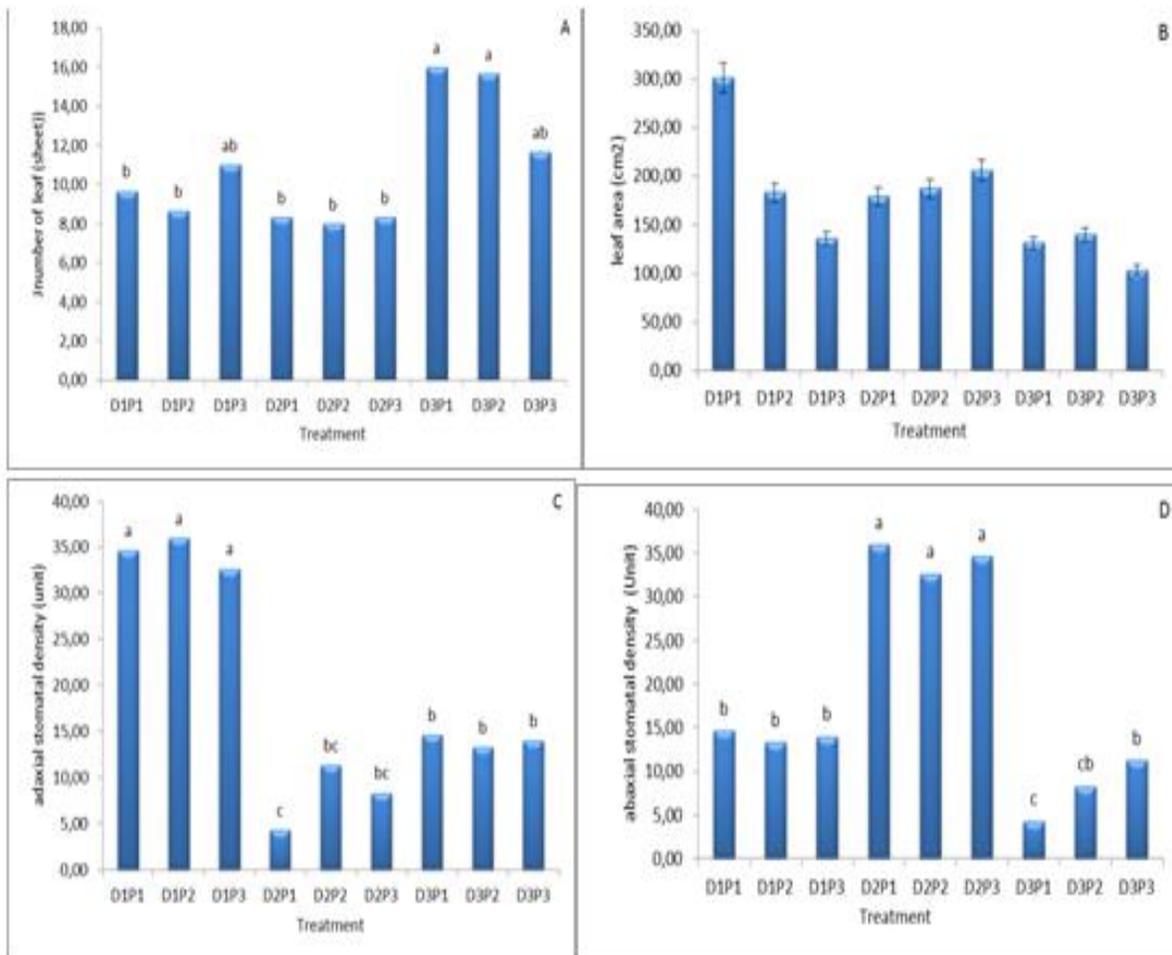


Fig.2 Phenomenon of C3 plant leaf infected with vesicular arbuscular mycorrhiza



Vesicular AM in tablet dosage forms promotes C3 plants to form more leaves than other dosage forms, although not followed by wide leaf size. Organic materials that function as binders in tablet dosage

forms can be a hypothesis for the occurrence of this phenomenon. According to Albano *et al.*, (2023) and Gerke (2022) that the organic matter can increase the availability of nutrients in the soil.

This phenomenon also shows that leaf numbers of more can cause smaller leaf sizes because of internal competition between leaves in a plant in the assimilate use. The different phenomena in C3 plants that received VAM in the sachet dosage form show the leaf number and leaf area were relatively balanced.

The stomatal density phenomenon on the adaxial and abaxial of the C3 plant's leaf was also unique. The C3 plants that received VAM in powder dosage form generally had more congested stomata density on the leaf adaxial parts compared to other VAM dosage form treatments. Meanwhile, the stomata density on the leaf abaxial tended to be higher in C3 plants that received VAM in the sachet dosage form.

Whether this phenomenon is caused by the effects of the VAM dosage form or the genetic factors of the three C3 plants is not yet well clarified. Cui *et al.*, (2023) explain that stomata cells have formed by two cells, namely guard cells and subsidiary cells.

The movement and function of guard cells are supported by subsidiary cells that provide mechanical support or act as reservoirs for ions and water.

Vesicular AM dosage form has a significant effect on root infection, number of nodules, root volume, number of leaves, leaf area, and stomata density with various phenomena. VAM in the dosage form of sachets and tablets can be a new dosage form for VAM applications in the field.

Acknowledgement

Authors thanks to Ministry of Education, Culture, Research, and Technology which has provided support through the competition of Penelitian Dasar Unggulan Perguruan Tinggi (PDUPT) in 2023-2024.

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How to cite this article:

Muh. Akhsan Akib, Syatrawati and Retno Prayudyaningsih. 2023. Root and Leaf Phenomenons of C₃ Plants Infected with Vesicular Arbuscula Mycorrhizal. *Int.J.Curr.Microbiol.App.Sci*. 12(07): 172-180. doi: <https://doi.org/10.20546/ijcmas.2023.1207.019>