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# New Host Record of *Geminivirus* on Bambara Groundnut (*Vigna subterranea* (L.) Verdc. (Fabaceae)) in East Java, Indonesia

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**ABSTRACT**

Bambara groundnut (*Vigna subterranea* L. Verdc.) is a legume plant originating from Africa. In Indonesia, Since 2006, the Bambara groundnut has been classified as a food crop based on the Decree of the Minister of Agriculture No:511/kpts/PD/310/9/2006. In East Java, bambara groundnut was planted in Gresik and Bangkalan regencies. In 2021, we recorded symptoms of virus infections, such as mosaic, stripes, dwarf, yellowing, and leaf malformations. However, information about the virus in Bambara groundnut (*Vigna subterranea* L. Verdc.) in Indonesia has not been reported. This study aims to detect the virus that infects the Bambara groundnut in East Java. This research was conducted using a survey method of Bambara groundnut in Gresik district and Bangkalan district. Certain symptoms such as mosaic, striped, stunted, yellow, and malformation were counted for the incidence of the disease. Samples of symptomatic plants were taken and brought to the laboratory for genomic extraction and analysis. DNA amplification was performed by RT-PCR using a pair of degenerative primers SPG1 and SPG2. The results of this study showed that the sample from the Gresik district was positively infected with the Geminivirus. Geminivirus infections were associated with yellowing and malformation of the leaves, and dwarf. This result record that Bambara is a new host of Geminivirus. But the species of the virus are not yet identified. The disease incidence of this disease caused by *Geminivirus* was 5.49%.



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## 1. INTRODUCTION

Bambara groundnut (*Vigna subterranea* L. Verdc.) is a legume plant originating from Africa[1]. The bambara groundnut has spread to South America [2], [3], Philippines [4], India [5], Malaysia [6], Sri Lanka [7], and Indonesia [5]. This Bambara groundnut plant in Indonesia is cultivated in East Java, Central Java, West Java, and East Nusa Tenggara. Bambara groundnut can grow and develop well on sub-optimal, marginal, and dry land [8]. This Bambara bean plant is resistant to drought stress and is also able to grow well in soils that have low nutrients such as sandy soils [9]. Based on the Decree of the Minister of Agriculture No:511/kpts/PD/310/9/2006, Bambara beans have been classified as food crops. The Bambara bean plant in its dry seeds contains 50-60% carbohydrates, 16-21% protein, and 4.5-6.5% fat. This Bambara bean plant also

contains B1 vitamins, potassium, iron, and phosphorus [8]. The Bambara bean plant can be recommended as a healthy food that is important for body health [10].

Decreased production can be caused by infection of pathogens. Several diseases in Bambara groundnut have been reported, including leaf blight disease caused by *Rhizoctonia solani* [11], brown blotch disease by *Colletotrichum capsici*[12], leaf spot disease caused by *Cercospora* [13], mottle disease caused by Potyvirus [14], the yellowing disease caused by *Cucumber mosaic virus* [15], and others disease.

In 2002 in Zimbabwe, mosaic disease on Bambara groundnut is infected by the genus potyvirus, the family Potyviridae [16]. In 1999 in Balangoda, Sri Lanka, a disease was reported with symptoms of bright yellow mosaic pattern on the leaves, rugosity, reduced leaf size, and stunting of all plants, but the genus was not found [17]. In our previous study, we recorded that the vegetative phase of the Bambara groundnut has a mosaic and rosette, but this information has not been verified. So, further research is needed to identify the virus that causes the disease molecularly. In this article, we recorded that *Geminivirus* was detected and associated with yellowing and malformation of the leaves base on molecular detection using reverse transcription polymerase chain reaction (RT-PCR).

## 2. METHODS

The survey and collecting sampling were carried out on the Bambara bean field in Kamal and Socah sub-districts, Bangkalan district, East Java in November 2021. The samples were brought to the laboratory and stored at -80ºC until used. The genome extraction was carried out by Total RNA Mini Kit (Plant) (Geneaid). RT-PCR Using degenerative primer SPG1 (5'–CCCKGTGCGWRAATCCAT-3') and SPG2 (5'–ATCCVAAYWTYCAGGGAGCTAA-3') as specific primer *Geminivirus* with One-Step RT-PCR methods [18], [19]. The visualization of DNA was conducted by 1,5% agarose electrophoresis. The positive amplicon had a size of ±900 bp. Calculation of the incidence of disease caused by viruses is determined based on positive PCR results. The calculation of disease incidence follows the method with the formula: $VF = n/N\ x100\%$ (note: FV = Virus Frequency, n = Number of positive plants detected by the virus, N = Total number of plants tested [20].

## 3. RESULTS AND DISCUSSION

Symptoms of disease on Bambara groundnut (*Vigna subterranea* (L.) Verdc) conducted by the survey have various disease symptoms. The symptoms found are generally mosaic, striped, stunted/dwarf, yellowing, and malformations, as shown in Fig. 1.

(a) (b) (c) (d)

**Fig. 1.** Variation of symptoms, (a) dwarf and mosaic, (b) yellowing leaves and malformations, (c) dwarfs and malformations, (d) malformation

Symptoms of disease are very important to know the cause of the disease. Symptoms of diseased plants can be divided based on the nature of the symptoms that arise, and direct, and indirect effects based on the size of the symptoms morphologically and anatomically [21]. Diseases can cause different or the same symptoms in different plants. If several diseases together attack one plant, it will be very difficult to separate or determine the main cause because the symptoms that arise are a mixture [22]. The research survey in Gresik (field 1) collected 673 samples and 37 samples had symptoms. In another field, (sample 2) collected 842 samples and 13 samples had symptoms. Bangkalan district (sub-district Socah) had 346 samples and 9 samples were disease. The calculation results in the Gresik (Field 1) have a disease incidence level is 5.49% and in Gresik (field 2), the disease incidence is 1.54%. In the Bangkalan district had a disease incidence of 2.94%, as shown in Table 1. Environmental factors could affect various symptoms and disease severity [23][24].

**Table 1**. Disease incidence of *Geminivirus* in East Java, Indonesia

| No. | Location | Disease Incidence |
|---|---|---|
| 1. | Sedayu, Gresik (Field 1) 6°59'52.2''S 112°33'50.0''E | 5.49% |
| 2. | Sedayu, Gresik (Field 2) 6°59'52.2''S 112°33'50.0''E | 1.54% |
| 3. | Socah, Bangkalan 7°05'12.8''S 112°42'34.3''E | 2.94% |

Based on molecular detection results showed that a composite sample from Sedayu, Gresik (Field 2) was positively infected by *Geminivirus* with a band length of ±900 bp. But another sample showed negative as in Fig. 2. Sample Gresik (Field 2) had 13 plants that experienced disease symptoms, and the 13 samples detected showed positive results in samples 5 to 12. Samples 1 to 4 and 13 showed negative results as in Fig. 3.

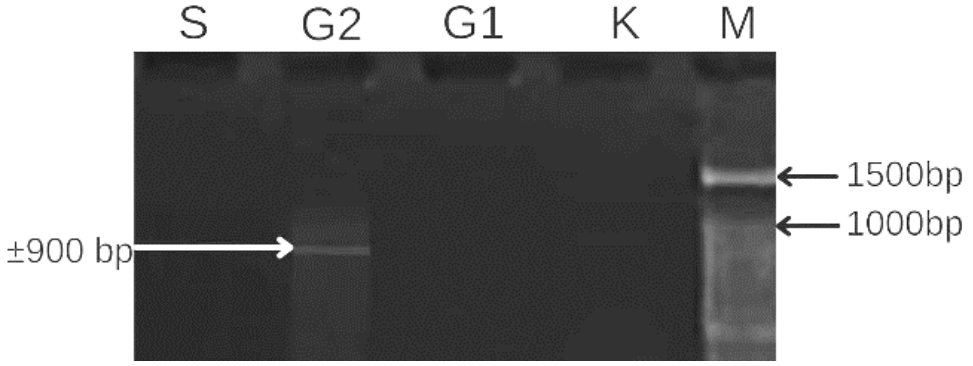


**Fig. 2.** Visualization of DNA band that amplified with *Geminivirus* degenerative-primer had ±900 bp (G1. Sample from Gresik field 1, G2. Sample from Gresik field 2, S. Sample from Bangkalan)

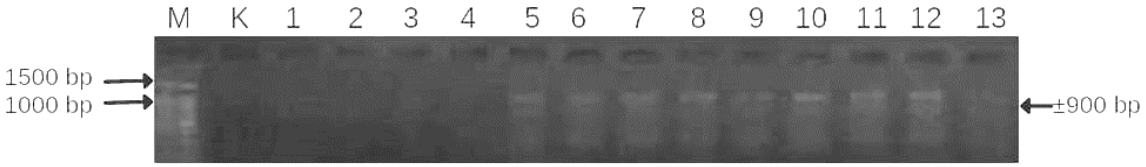


**Fig. 3.** DNA bands amplified with Geminivirus primers ±900 bp, detection from Gresik 2 (G2) sample with 13 plant samples experiencing disease symptoms. Detection shows positive results in samples 5 to 12

The detected *Geminivirus* that infects Bambara groundnut is the first record. This virus correlated with mosaic, yellowing, and malformation symptoms seen in Fig. 2. Various symptoms are also reported in ornamental plants [25]. So far, *Geminiviruses* that have been reported to attack legumes include *Bean dwarf mosaic virus* (BDMV) and *Bean golden mosaic virus* (BGMV) on chickpeas [26], [27], [28]. *Geminivirus* is classified as an isometric plant virus and is always in pairs which have an incubation period of 10-15 days in plants [29][30]. Geminiviruses are transmitted by whitefly (*Bemisia tabaci*). The leaf-sucking insect plays a role in the spread of the *Geminivirus* in the field. One vector can transmit viruses and make plants sick [31].

## 4. CONCLUSION

The field record of symptoms of disease, such as yellowing, mosaic, malformation, and dwarf. Based on the results of molecular detection, *Geminivirus* was detected to infect Bambara groundnut in Gresik Regency, East Java, Indonesia. These results are the first record that Bambara is a *Geminivirus* host. The disease incidence of this disease caused by *Geminivirus* was 5.49%.

**Author Contribution**

All authors contributed equally to the main contributor to this paper. All authors have read and agreed to the published version of the manuscript.

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**Conflict of Interest**
The authors declare no conflict of interest.

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