Characterization of Peanut Stripe Virus from West Nusa Tenggara

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Abstract. Peanut Stripe Virus (PStV) infection on peanut plants causes a reduction in the yield of peanuts. This study was aimed to characterize PStV that infected peanut (Arachis hypogaea) plants in the West Nusa Tenggara province. Peanut plants that showed symptoms of stripes on leaves were collected from West Nusa Tenggara province peanut fields. Stripes symptoms on leaves indicated that the plant was infected with PStV. PStV mechanical inoculation to the healthy Chenopodium amaranticolor plant with sap from PStV infected peanut leaf showed local lesions symptoms on the leaf. Molecular characterization was carried out using RT-PCR with specific primers produced fragments of 1071 nt, consists of NIb, coat protein (CP) gene, and 3’UTR. Based on nucleotide sequences of coat protein gene, PStV from West Nusa Tenggara was similar with others PStV isolated reference in Genbank.

1. Introduction
Peanut Stripe Virus (PStV) is an RNA virus of the potyvirus group that is often found to infect peanut plants. PStV infection in peanuts causes chlorosis in the leaves, thus showing symptoms of green striped leaves with a yellowish colour around them. This causes the process of photosynthesis is not optimal, thus reducing peanut production [1, 2]. Some studies suggest that PStV infection can cause a decrease in peanut yield by up to 60% [2, 3, 4]. In addition to peanut plants, PStV can also infect Chenopodium amaranticolor. The typical symptom of PStV infection on C. amaranticolor is the appearance of local lesions on the leaves. In addition to observing the symptoms that appear in plants, molecular detection is also mostly done to identify and characterize the virus base on the coat protein. Data on nucleotide sequences can reveal variations and relationship between viruses at the genetic level. Virus genome regions that are typically used to characterize potyvirus is a coat protein [5, 6, 7, 8, 9, 10].

PStV was first reported in 1984, discovered a PStV attack on peanut plants in the United States, but the seeds of these plants were originated from China [2, 4, 11]. PStV spreads in several countries such as China, Japan, Thailand, the Philippines and Indonesia. Distribution of PStV can be influenced by human intervention such as through agricultural practices, trade, and traffic in various regions and continents. The spread of the virus is also by insects as vectors [2, 12, 13, 14]. PStV can also be transmitted through infected plant seeds, which when planted will generate diseased plants [1, 4].
Several studies have shown that PStV is found in various provinces in Indonesia [7, 15]. Based on the preliminary survey, several peanut fields in the province of West Nusa Tenggara were found to be infected with PStV.

The purpose of this study was to determine the biological and molecular character of PStV in peanut plants in West Nusa Tenggara Province. Biological characterization was done by mechanically transmitting PStV to the test plants. Molecular characterization was carried out using the RT-PCR method to obtain the coat protein virus nucleotide data and then compared with the PStV data stored on genebank. Information about the character of a virus is needed as basic data to determine preventive measures against the virus.

2. Material and methods

2.1. Sample collecting

The samples were taken from the peanut field area in Lombok and Sumbawa Islands, Province of West Nusa Tenggara. Sampling was done on the second cropping season in 2016, using purposive sampling methods with consideration of peanut plants infected at a planting area. Samples of infected leaves were observed by morphological symptoms, based on a green striped pattern on the leaves.

The environmental factors measured at the time of sampling were the altitude, point coordinates, temperature, humidity, and light intensity. Altitude and coordinate points were determined using a Global Positioning System (GPS). Air temperature and humidity were measured using Thermo hygrometer.

2.2. Mechanical inoculation on C. amaranticolor

The test plant used was C. amaranticolor. Inoculum solution was prepared by extracting the peanut leaves that showed positive symptoms of PStV using mortar with phosphat buffer pH 7 added in a ratio of 1: 5 (w / v). C. amaranticolor leaf surface was scratched slowly with the addition of carborundum to create injury on the leaf surface. Furthermore inoculum extract solution was applied to the injured leaf. After a while, the leaf was rinsed using distilled water. The test plants were cultivated in the screen house and the symptoms were observed.

2.3. Molecular detection

Molecular characterization was carried out by observing the coat protein (CP) gene using RT-PCR methods. Viral RNA was extracted from PStV infected peanut leaves using Plant virus RNA extraction kit by Geneaid and following its procedures. cDNA was synthesized using superscript II and following its procedures. The CP gene of PstV was amplified using specific primers, PST1 and PST 4 [8]. PCR products were electrophoresed on 1% agarose media then visualized using UV transilluminator and documented by photograph. The further amplification product was sequenced to obtain the nucleotide sequences of data. Data of nucleotide sequences were analyzed using bio edit software. The phylogeny tree was constructed using MEGA 7 software [16]. Reference isolates used from gene bank were by accession code #Z21700.1 (PstV Indonesia Ib), #AJ132156.1 (PstV Indonesia I13), #AJ132151.1 (PstV Indonesia I7), #U34972.1 (PstV USA 1), #U05771.1 (PstV USA 2).

3. Result and Discussion

Environmental factors have been reported to affect viral infections. Viral infections in a plant are influenced by interactions between viruses, the host and the environment [13]. Most strains PStV cause a typical symptom. There are some variations of PStV symptoms in peanut leaf [17,18]. Types of symptoms of two isolates PStV found in West Nusa Tenggara was blotch pattern symptoms (Figure 1).
PStV symptom was similar in several different areas likely due to plant seeds which are used both infected with the same virus strain. Plant viruses can be transmitted through seeds and can be distributed widely through human activity [4, 13]. The presence of viral diseases in new plantation areas and the transmigration area indicates that the virus is spread through infected seedlings brought by migrants [2].

![Image of peanut leaves showing symptoms of PStV infection](image1)

**Figure 1.** Peanut leaves showing symptoms of PStV infection (A) PStV infected peanut leaf; (B) Healthy peanut leaf

### 3.1. Mechanical inoculation on Chenopodium amaranticolor.

PStV infection in *C. amaranticolor* causing symptoms such as local lesions on its leaf [8, 11, 19]. Mechanical inoculated of PStV on *C. amaranticolor* in this study showed positive results with the appearance of local lesions on leaves with smaller than 2.0 mm diameter in size (Figure 2). Symptom begins to appear on the seventh day after inoculation on young leaves that are not inoculated. The appearing symptom on young not inoculated leaves indicating a systemic infection.

Wongkaew and Dollet's [19] mentioned that latent period of some PStV isolates on *C. amaranticolor* was in the range of 4-7 days, and the latent period of PStV isolate I1 originating from Indonesia on *C. amaranticolor* was 7 days. The latent period of PStV on *C. amaranticolor* in this study was similar to the latent period of PStV isolate I1 that originating from Indonesia. However, the clustering of viruses is more dependent on the viral symptoms of the virus than the geographical origin of the viruses [19].

![Image of local lesion symptom on leaf of PStV infected Chenopodium amaranticolor](image2)

**Figure 2.** Local lesion symptom on leaf of PStV infected *Chenopodium amaranticolor*
3.2. Molecular detection

In this study, we characterized PStV Lombok and PStV Sumbawa from West Nusa Tenggara based on the coat protein gene using primer PST1 and PST4. The amplification product was specific fragment of 1200 bp (Figure 3). It is according with [8] that mentions that the PCR product using these primers is 1200 bp containing coat protein gene of PStV. The BLAST result of the two samples showed a high similarity with the PStV in genebank database. This confirmed that the sample obtained was correct PStV. There were obtained 1071 nt of good quality nucleotide sequences, consisting of part of NIb, complete cp gene, and part of UTR

![Figure 3. PStV genome amplification using PST1 and PST4 primers. (L) 100 bp DNA ladder; (1-2) CP gen of PStV from West Nusa Tenggara](image)

Analysis of the coat protein gene among two PStV isolates from West Nusa Tenggara with other PStV isolates from other regions showed a high degree of conservative sequence. However, there were genetic variability in these all PStV isolates. From 864 nucleotides of coat protein gene, there were 821 invariable (monomorphic) sites and 43 variable (polymorphic) sites. The polymorphic sites consist of 22 singleton variable sites and 21 parsimony informative sites. Analysis of nucleotide of the coat protein gene between PStV Lombok and PStV Sumbawa also showed variability. There were 11 variable sites, at the nucleotide site 8, 43, 52, 121, 145, 162, 354, 567, 684, 687, and 819.

Analysis of the similarity of the coat protein gene between PStV isolates from West Nusa Tenggara with several isolate references showed a close relationship with each other (Table 1). The genetic similarity between PStV Lombok and PStV Sumbawa was 99%. The genetic similarity value among two PStV isolates from West Nusa Tenggara with reference isolates were 98-99%. However the highest similarity of PStV from West Nusa Tenggara was with PStV from Indonesia. Based on the genetic similarity value, it can be concluded that PStV isolates from West Nusa Tenggara are closer to others PStV from Indonesia than PStV from USA. This confirms that both PStV isolates from West Nusa Tenggara and other PStV isolates from Indonesia are one strain [22].

| No. | Isolat           | 1  | 2  | 3  | 4  | 5  | 6  | 7  |
|-----|------------------|----|----|----|----|----|----|----|
| 1.  | PStV Lombok      |    |    |    |    |    |    |    |
| 2.  | PStV Sumbawa     |    |    |    |    |    |    | 99.2|
| 3.  | PStV Indonesia Ia|    |    |    |    |    | 98.8| 98.5|

Table 1. Genetic Similarity (%) based on nucleotide sequence of coat protein gene of PStV isolates studied.
4. PStV Indonesia I13 99.6 99.3 99.1
5. PStV Indonesia I7  99.6 99.3 99.1 99.9
6. PStV USA1  98.2 97.8 98.1 98.3 98.3
7. PStV USA2  98.3 97.8 98.2 98.4 99.9 99.9

Genetic relationship based on the nucleotide sequences of coat protein gene of the two PStV isolates from West Nusa Tenggara with PStV isolates from data on the genbank were observed through phylogeny trees constructed by Neighbor Joining (NJ) using the MEGA 7 program [20, 21]. The phylogeny tree showed two main groups (Figure 4). The first group consisted of PStV isolates from Indonesia. PStV Lombok and PStV Sumbawa, the two isolates from West Nusa Tenggara were also in the first group. The second group consisted of PStV isolates from the USA. Based on the phylogeny tree in figure 4, the two PStV isolates from West Nusa Tenggara may have a common ancestor with the others Indonesian isolates, however to ensure this presumption required more PStV isolates studied.

Figure 4. Neighbour-Joining (NJ) phylogeny tree of PStV based on the coat protein gene of two isolates from west nusa tenggara and PStV isolates from genbank.

Information about the character of the virus is useful in determine efforts to control the virus. High genetic similarity between PStV isolates from West Nusa Tenggara with other isolates originating from Indonesia, simplify to control the virus. The efforts were currently being developed to overcome plant viruses by assembling GMO plants that resistant to certain viruses. These GMO plants were assembled by inserting the viral coat protein gene into the plant genome. Transgenic plants that carry the coat protein gene of a virus will have resistance to viruses that have genetic similarity to the sequence of the nucleotide coat protein gene [23, 24].
4. Conclusion
Mechanical inoculated of PSTV to C. amaranticolor causes a typical symptom of local lesions on the leaves, average at 7 days after inoculation. Based on coat protein gene, PSTV isolated from West Nusa Tenggara had high genetic similarity with others PSTV isolates originating from Indonesia.

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