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Graft transmission of Piper yellow mottle virus-like disease in black pepper (*Piper nigrum* L.)

Idi Hamizan bin Juharisam[1] & Intan Sakinah Mohd Anuar[1*]

[1]*Faculty of Plantation and Agrotechnology, Universiti Teknologi MARA, Jasin Campus, 77300 Melaka, Malaysia*

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ABSTRACT. Black pepper (*Piper nigrum* L.) is an economically important spice crop susceptible to Piper yellow mottle virus (PYMoV). This study evaluated the development of PYMoV-like symptoms following grafting between symptomatic and asymptomatic plants of the Kuching cultivar. The experiment was conducted in an insect-proof greenhouse in Jasin, Melaka, using a completely randomised design with four grafting combinations and five replicates, totalling 20 plants. Disease incidence, severity scores, disease severity index (DSI), plant height, and visible symptoms were monitored for three months, followed by histopathological examination of leaf tissues. Plants with asymptomatic scions and rootstocks remained symptom-free throughout the study. All plants in treatments containing symptomatic material exhibited symptoms by the second month. By the third month, DSI values reached 32% for symptomatic scions grafted onto asymptomatic rootstocks, 52% for asymptomatic scions grafted onto symptomatic rootstocks, and 72% when both components were symptomatic. Mean disease severity differed significantly among treatments at each monthly assessment, whereas plant height differences were not statistically significant. Histopathological examination revealed tissue discolouration and disruption, with the most pronounced changes occurring when both graft components were symptomatic. These findings are consistent with graft-associated development of PYMoV-like disease but do not establish virus identity or definitive transmission. Molecular testing of donor plants and initially asymptomatic recipient tissues is required for confirmation. The study highlights the importance of verified virus-free planting material and nursery sanitation.

Keywords: Black pepper, graft transmission, histopathology, disease severity, Piper yellow mottle virus

1. INTRODUCTION

Black pepper (*Piper nigrum* L.) is one of the most important spice crops in tropical agriculture. It has high economic value and is widely used in food, medicine, and other industries. Black pepper is commonly propagated vegetatively because this method can maintain useful cultivar characteristics such as yield, quality, and uniform growth. However, the use of vegetative planting materials can also spread systemic diseases from one generation of plants to another (Krishnamoorthy & Parthasarathy, 2010; Nair, 2020; Ventura et al., 2019).

Piper yellow mottle virus (PYMoV) causes an important disease of black pepper and other Piper species. Common symptoms include yellow mottling, chlorosis, vein clearing, leaf distortion, reduced leaf size, poor growth, and stunting. Symptom expression can change with temperature and relative humidity, so infected plants may not always show the same visible signs (Ahamedemujtaba et al., 2021). The virus has also been reported in several Piper hosts, showing that it can persist in different plants within pepper-growing areas (Ma et al., 2022; Sari et al., 2024).

The disease can spread through infected planting materials and insect vectors. Mealybugs are important in field

*Corresponding author:
E-mail address: *intan_sakinah@uitm.edu.my* (Intan Sakinah)

transmission, but human propagation practices can also move infected tissues between plants. Grafting is useful because it joins a scion and a rootstock and forms a direct vascular connection. This same connection may also allow a systemic virus to move between infected and healthy plant tissues (Bradamante et al., 2021; Buang et al., 2025; Sastry & Zitter, 2014).

Earlier studies have reported graft transmission of Piper yellow mottle virus in black pepper in Sri Lanka, Indonesia, and China (Alif et al., 2018; De Silva et al., 2002; Ma et al., 2022). However, there is still limited quantitative information on how disease severity develops after grafting and how visible symptoms relate to plant growth and internal leaf tissue changes, especially for the Malaysian Kuching cultivar. This information is useful for nurseries because infected scions or rootstocks may look acceptable at the time of propagation but later become a source of disease.

Therefore, this study had two main objectives. The first objective was to determine graft transmission from symptomatic donor plants to healthy black pepper plants. The second objective was to confirm disease establishment after grafting by comparing visible symptoms, disease severity, plant growth, and histopathological changes. The study used controlled greenhouse conditions to reduce the influence of insect vectors and to focus on the effect of infected grafting material.

2. METHODOLOGY

The experiment was conducted in an insect-proof greenhouse at Universiti Teknologi MARA Jasin, Melaka. The Kuching cultivar of black pepper was used. The seedlings were about three months old. A Completely Randomized Design was used with four grafting treatments and five replications, giving a total of 20 plants. One grafted plant in one pot was treated as one experimental unit. The grafting procedure was followed by wrapping the graft joint with grafting tape or parafilm and marking the graft joint with treatment, replicate, variety, and plant identification number. The grafted plants were kept thereafter in shaded high humidity conditions for 7 to 10 days until recovery and acclimatization of the plants took place followed by graft union formation. Following this period of healing, all plants were reared under similar greenhouse conditions with controlled watering and fertilization during the experiment. Also, during the experiment the greenhouse was kept as an insect-proof environment to remove alternative sources of transmission of PYMoV, as well as the insect vectors carrying it, thus attributing every observation of the treated plants solely to the grafting. Scions and rootstocks were obtained from the same healthy donor plants of cultivar.

Healthy plants showed no visible mottling, vein clearing, chlorosis, or leaf distortion. Infected donor plants were selected based on clear disease symptoms such as yellow mottling, chlorosis, leaf distortion, and poor vigour. Healthy and infected materials were kept separately to reduce cross-contamination. Cutting tools were sterilized with 70 percent alcohol during grafting. After grafting, the union was wrapped with grafting tape or parafilm. The plants were kept under shaded and high-humidity conditions for about seven to ten days before normal greenhouse management continued.

The four treatment combinations are shown in Table 1. T1 was the healthy control. T2 tested whether disease could move from an infected scion into a healthy rootstock or other healthy tissues. T3 tested possible movement from an infected rootstock into a healthy scion. T4 was the positive control because both plant parts were infected.

Table 1. Grafting treatments are used to evaluate disease transmission.

Treatment	Grafting combination	Purpose
T1	Asymptomatic scion × Healthy rootstock	Negative control
T2	Symptomatic scion × Healthy rootstock	Transmission treatment
T3	Asymptomatic scion × Infected rootstock	Transmission treatment
T4	Symptomatic scion × Infected rootstock	Positive control

Plants were observed weekly for yellow mottling, chlorosis, vein clearing, leaf distortion, discoloration, stunting, and poor plant vigour. Disease incidence was calculated from the percentage of symptomatic plants. Disease severity was scored from 0 to 5, where 0 represented a healthy plant and 5 represented very severe symptoms affecting 81 to 100 percent of the leaf area. Disease Severity Index was calculated from the number of plants in each severity class, the severity score, the total number of plants, and the maximum score of 5. The selected leaves represented the range of disease severity levels from 0 to 5 recorded based on disease severity level (Table 2).
Disease Severity Index Formula:

$$\text{DSI}\ (\%) = \frac{\sum(n \times v)}{N \times V} \times 100$$

Where:
n = Number of leaves or plants in each disease severity category
v = Numerical disease severity score, ranging from 0 to 5
N = Total number of leaves or plants evaluated
V = Highest disease severity score used in the study, which was 5

Table 2. Disease severity scoring criteria for black pepper leaves, adapted from Miftakhurohmah et al. (2023).

Score	Severity Level	Leaf Area Affected (%)	Visual Symptoms
0	Healthy	0%	Leaf appeared green and normal with no visible yellowing, mottling, vein clearing, or distortion.
1	Very mild	1-20%	Very small yellow spots or slight mottling appeared on a small part of the leaf
2	Mild	21-40%	Clear mottling pattern, slight yellowing, or mild vein banding

			was observed
3	Moderate	41-60%	Obvious chlorosis, mottling, and prominent vein clearing appeared on the leaf.
4	Severe	61-80%	Large yellowing patches, strong mottling, leaf malformation, or necrotic areas were observed.
5	Very severe	81-100%	Tissue collapse, severe chlorosis, stunting, crumpled leaf, or very poor leaf condition was observed.

Plant height was measured during the three observation months. Leaf samples from all four treatments were collected at the final observation period for histopathological examination. The tissues were fixed in formalin-acetic acid-alcohol solution, dehydrated through graded ethanol, embedded in paraffin, and sectioned at about 8 micrometres. Sections were stained with 0.05% Toluidine Blue O and observed under a compound light microscope at 10 times and 40 times magnification. Tissue organization, mesophyll condition, vascular tissue, discoloration, cell disruption, and tissue collapse were compared among treatments.

Quantitative data were analysed using the Statistical Package for the Social Sciences. Descriptive statistics were used for disease incidence and other summary data. One-way analysis of variance was used to compare disease severity and plant height among treatments at a significance level of p less than or equal to 0.05. Tukey's HSD test was used to compare treatment means when appropriate.

3. RESULTS AND DISCUSSION

The treatments showed a clear difference in disease development. T1 remained asymptomatic during all three months and recorded zero disease incidence and zero Disease Severity Index. This result shows that the grafting process itself did not produce the observed disease symptoms when both scion and rootstock were healthy. In contrast, T2, T3, and T4 developed Piper yellow mottle virus-like symptoms after grafting.

Disease incidence increased quickly in treatments that contained infected grafting material. One month after grafting, disease incidence was 60% in T2, 80% in T3, and 100% in T4. By the second month, all plants in T2, T3, and T4 were symptomatic and this incidence persisted through the third month. The Disease Severity Index also increased over time. By the third month, it had reached 32% in T2, 52% in T3, and 72% in T4 (Table 3). This pattern shows that disease expression became stronger as the grafted plants developed.

Table 3. Disease incidence and Disease Severity Index during three months after grafting.

Treatment	Incidence M1 (%)	Incidence M2 (%)	Incidence M3 (%)	DSI M1 (%)	DSI M2 (%)	DSI M3 (%)
T1	0	0	0	0	0	0
T2	60	100	100	12	20	32
T3	80	100	100	20	36	52
T4	100	100	100	32	52	72

Mean disease severity followed the same trend. T1 stayed at 0.00 ± 0.00 for all three months. T2 increased from 0.60 ± 0.55 in the first month to 1.60 ± 0.55 in the third month. T3 increased from 1.00 ± 0.71 to 2.60 ± 0.55, while T4 increased from 1.60 ± 0.55 to 3.60 ± 0.55. One-way analysis of variance showed significant treatment differences at every observation month. The F-value increased from 8.242 in the first month to 52.370 in the third month, showing that treatment differences became clearer as disease developed (Table 4).

Table 4. Mean disease severity and statistical comparison among treatments.

Treatment	M1 mean ± SD	M2 mean ± SD	M3 mean ± SD
T1	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
T2	0.60 ± 0.55^{ab}	1.00 ± 0.00^{b}	1.60 ± 0.55^{b}
T3	1.00 ± 0.71^{bc}	1.80 ± 0.45^{c}	2.60 ± 0.55^{c}
T4	1.60 ± 0.55^{c}	2.60 ± 0.55^{d}	3.60 ± 0.55^{d}

Note: ***M1 = Month 1, M2 =Month 2, M3 = Month 3, SD = Standard deviation***
Analysis of variance: Month 1, $F = 8.242$, $p = 0.002$; Month 2, $F = 49.467$, $p < 0.001$; Month 3, $F = 52.370$, $p < 0.001$.

The stronger response in T3 compared with T2 suggests that infected rootstock had an important role in disease establishment. Once a graft union forms, scion and rootstock become connected through vascular tissues. Systemic viruses can use plant transport pathways to move into newly connected tissues. Earlier black pepper studies also reported graft transmission of Piper yellow mottle virus and development of typical symptoms after grafting (Alif et al., 2018a; De Silva et al., 2002a).

T4 had the highest disease incidence, Disease Severity Index, and mean severity score because both the scion and the rootstock were infected. This treatment acted as the positive control and had the greatest infection pressure. The symptoms observed in infected treatments, including yellow mottling, chlorosis, discoloration, leaf distortion, and poor growth, were similar to symptoms described in earlier black pepper studies (Ahamedemujtaba et al., 2021; Bhat et al., 2012).

Plant height increased with time in all treatments, showing that the plants continued to grow after grafting. However, the initial plant height was not recorded systematically before inoculation in the present experiment. The absence of baseline measurements limits comparisons of growth among treatments. The healthy control had the highest

mean height at all three monthly assessments. At the third month, T1 recorded 68.96 ± 4.58 centimetres, compared with 61.56 ± 14.90 centimetres in T2, 55.84 ± 6.11 centimetres in T3, and 57.76 ± 7.54 centimetres in T4. Even though infected treatments generally had lower mean height, the differences were not statistically significant. The p-values were 0.363, 0.228, and 0.156 for the first, second, and third months, respectively. Therefore, plant height was a useful supporting growth parameter but was less sensitive than disease severity during the short three-month study (Table 5).

Table 5. Mean plant height and statistical comparison among treatments.

Treatment	M1 (cm)	M2 (cm)	M3 (cm)
T1	56.98 ± 7.65	62.90 ± 5.24	68.96 ± 4.58
T2	52.26 ± 10.93	56.90 ± 12.48	61.56 ± 14.90
T3	47.90 ± 6.21	52.08 ± 6.00	55.84 ± 6.11
T4	47.86 ± 10.70	53.38 ± 8.51	57.76 ± 7.54

Analysis of variance: Month 1, F = 1.139, p = 0.363; Month 2, F = 1.605, p = 0.228; Month 3, F = 1.993, p = 0.156. All plant height differences were not significant.

Visual observation at the third month supported the quantitative disease results. T1 had green leaves with no visible disease symptoms. T2 showed mild yellow mottling and slight chlorosis. T3 showed clearer mottling, chlorosis, and discoloration. T4 had the strongest yellowing, mottling, discoloration, and poor leaf appearance. The order of visual symptoms matched the order of mean disease severity and Disease Severity Index.

Histopathological examination showed a similar pattern. T1 had a healthier tissue structure with no obvious severe disruption. T2 showed mild discoloration and slight tissue disruption. T3 showed clearer discoloration and more visible disruption, while T4 showed the strongest tissue changes (Figure 1). The histopathological observations at the final assessment are summarised in Table 6. Plant viral infection can alter tissue organization, chloroplast-related structures, and cell integrity, so these changes support the visible disease observations (Majumdar et al., 2025; Taufik et al., 2024).

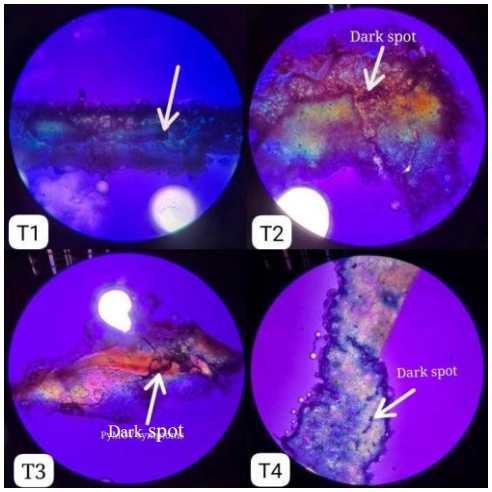

Figure 1. T1 showed relatively intact tissue structure with no obvious discoloration. T2 showed mild tissue discoloration and slight disruption. T3 showed clearer discoloration and tissue disruption. T4 showed the strongest tissue discoloration and disruption, supporting severe disease establishment.

Table 6. Summary of histopathological observations at the final observation period.

Treatment	Histopathological observation	Relation with disease severity
T1	Tissue appeared normal with no clear discoloration or severe disruption	Healthy control; score 0
T2	Mild tissue discoloration and slight disruption	Mild disease severity
T3	Clearer tissue discoloration and more visible disruption	Moderate disease severity
T4	Strong tissue discoloration and severe disruption	Highest disease severity

The combination of disease incidence, Disease Severity Index, severity scores, visual symptoms, and histopathological changes strongly supports graft-mediated disease establishment in treatments containing symptomatic material. However, this study did not include molecular detection. Visual symptoms and tissue changes alone cannot provide definitive virus identification because some viral infections may be latent or show similar symptoms. Molecular methods such as polymerase chain reaction or reverse transcription polymerase chain reaction should therefore be included in future work for specific confirmation (Wang et al., 2022).

A limitation of the present study is the relatively small sample size of five plants per treatment. Although the experiment was conducted under controlled conditions and each plant was treated as an experimental unit, the limited number of plants may have reduced the statistical power to detect relatively small treatment effects and may constrain the generalizability of the findings. Future studies should include larger sample sizes and independent experimental repetitions to provide stronger statistical support and improve the reliability and generalizability of the findings.

4. CONCLUSION

This study documented the development of Piper yellow mottle virus (PYMoV)-like disease following grafting between symptomatic and asymptomatic black pepper plants of the Kuching cultivar under controlled greenhouse conditions. Plants with asymptomatic scions and rootstocks remained symptom-free, whereas treatments containing symptomatic material showed increasing disease incidence and severity. The greatest disease severity occurred when both graft components were symptomatic. Disease severity differed significantly among treatments at all three monthly assessments, while plant height differences were not statistically significant. Histopathological examination revealed tissue discolouration and disruption, with the most pronounced changes in plants with symptomatic scions and rootstocks. These observations are consistent with graft-associated disease development; however, the absence of molecular testing prevents definitive identification of PYMoV and confirmation of its transmission into initially asymptomatic tissues. Nurseries should use verified virus-free planting material, maintain strict sanitation, and avoid symptomatic propagation material. Future studies should incorporate PYMoV-specific molecular testing of donor and recipient tissues, larger sample sizes, longer observation periods, and additional cultivars. This study provides comparative evidence of disease progression and associated tissue changes across reciprocal graft combinations in the Kuching cultivar.

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AUTHOR CONTRIBUTIONS

Idi Hamizan bin Juharisam: conceptualization, methodology, investigation, data collection, formal analysis, interpretation, and writing of the original manuscript. **Intan Sakinah Mohd Anuar:** The supervisor contributed to the conceptualization and overall design of the study, provided guidance on the research methodology and experimental design, supervised data collection and analysis, and provided critical review and intellectual input throughout the research process. The supervisor also contributed to the interpretation of results and critically reviewed and revised the manuscript for important intellectual content. All authors approved the final version of the manuscript.

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DECLARATION OF GENERATIVE AI IN THE WRITING PROCESS

ChatGPT and QuillBot were used to improve language clarity and writing quality. The authors reviewed the revised text and take responsibility for the final manuscript.

DATA AVAILABILITY

The data are available from the corresponding author upon reasonable request.

COMPETING INTEREST

The authors declare that there are no competing interests.

COMPLIANCE OF ETHICAL STANDARDS

Not applicable. The study involved black pepper plants and did not involve human participants or experimental animals.

SUPPLEMENTARY MATERIAL

No supplementary material is provided with this article.

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