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**Research Article**

# Responses of Tomatoes Grafting Using Variation of Rootstock against Virus Infection and Tomato Yields

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## ABSTRACT

Grafting methods on tomato have been done to reduce the infection rate of various pathogens. *Begomovirus* and *Crinivirus* are important viruses in tomato plants. The research aimed to determine the resistance response of tomato plants to viral infection, and tomato production. Field research was conducted in Harjobinangun, Pakem, Sleman, Yogyakarta in the endemic area of the viral diseases transmitted by *Bemisia tabaci*. This experiment used a Completely Randomized Design non-factorial with "Servo" as scion and "Amelia", "H-7996", "Mawar" as rootstock. The disease development, presence of viral diseases, and tomato yields were observed. PCR detection using Krusty & Hommer primer successfully amplified *Begomovirus* DNA bands with an approximate size of 580 bp in tomato plant with interveinal chlorosis, curling, thick, rigid, and stunt symptoms. Chlorotic spots and yellowing symptoms successfully amplified using ToCV-CF/ToCV-CR specific primer for the amplification of *Tomato chlorosis virus* with DNA band approximately size of 360 bp, whereas using TICV-CF/TICV-CR specific primer could not amplify the virus cDNA. The leaves roll upward with purple interveinal symptoms that were not infected by both viruses. Both viral infections affected the quality of the fruit which indicated by a higher number of abnormal fruits. "Servo" grafted onto "Amelia" and non-grafted Servo were tolerant to viral infection, "Servo" grafted onto "H–7996" or to "Mawar variety were susceptible to viral infection, self-grafted Servo were very susceptible to viral infection.

Keywords: *Begomovirus*; defense responses; PCR; *Tomato chlorosis virus*; tomato grafting

## INTRODUCTION

One obstacle in the cultivation of tomato plants is the soil-borne disease. According to Nunez (2012), soil-borne diseases are difficult to control because not many effective fungicides and harmful fumigants to farmers and the environment so that they need an integrated approach. Louws *et al.* (2010) stated that grafting is an Integrated Pest Management (IPM) strategy that can control soil-borne pathogens such as *Verticillium dahliae*, *Fusarium oxysporum*, *F. solani*, *Pythium* spp., *Rhizoctonia solani*, *Phytophthora* spp., *Pyrenochaeta lycopersici*, *Ralstonia solanacearum,* root-knot nematodes, and some viral diseases. Besides, to control soil-borne diseases, grafting also has a positive impact on leaf disease control.

Farmers in the Yogyakarta area tend to do monoculture for tomato and chili on adjacent land for long periods. This factor can contribute to the emergence of viral disease epidemics that spread through insects as the vector. Sulandari *et al.* (2006) reported that cropping patterns supported by the environment and the presence of insects as a vector can cause geminivirus infections. It also allows the occurrence of multiple viral infections, thus causing an increase in the intensity of the virus disease. Adkins *et al.* (2012) stated that viruses infect tomato plants and spread through insects are *Crinivirus* and *Begomovirus* (whiteflies), *Tospovirus* (thrips), *Potyvirus* and *Cucumovirus* (aphids), *Curtovirus* (leafhoppers). According to Kusumaningrum *et al.* (2015), tomato plants in the high altitude of 1300 m above sea level with symptomatic of curling and yellowing showed multiple infections. Multiple infections were caused by two different groups of viruses, *Begomovirus* (*Geminiviridae* family) and *Crinivirus* (*Closteroviridae* family).

The grafting between commercial tomato cultivars (Permata, Lentana, Fortuna) with resistant rootstock (H-7996 or Eg-203) could suppress the development of bacterial and increase tomato production (Arwiyanto *et al*., 2015). Grafting tomatoes between susceptible commercial and resistant rootstock may suppress the development of viral infections in the endemic

areas. Therefore, this research aimed to determine the resistance response of the grafted tomato plants to viral infections (*Begomovirus* and *Crinivirus*) and tomato production. By grafting tomato plants, it is expected to show resistance response to viral infection and high production, thus it can be recommended for breeders, agribusiness entrepreneurs, and farmers.

## MATERIALS AND METHODS

This research was designed using a Completely Randomized Design Non-Factorial with "Servo" cultivars as scion and "Amelia", "Mawar", "H-7996" cultivars as rootstocks. The grafting was carried out by modifying the method by Black *et al*. (2003). Disease development (incidence and intensity of disease) due to viruses, the presence of viral diseases (Begomovirus and Crinivirus), and yields (number of fruits and fruit weights) were observed. Virus inoculation occurred naturally in the field. The virus attack was categorized by scoring based on the symptoms level of viral infection by Friedmann *et al*. (1998). The resistance level of tomato plants to viral infections based on symptoms, disease incidence and normal vs. abnormal fruit by Taufiq *et al*. (2007) with modification (Table 1).

DNA extraction followed the Geneaid Genomic DNA Mini Kit (Plant) protocol. RNA extraction was performed by following the Geneaid Total RNA Mini Kit (Plant) protocol. PCR used the KAPA Taq ReadyMix PCR Kit (KAPABiosystems). Revill *et al*. (2003), stated that *Begomovirus* DNA amplification using Krusty universal primer (5'-CCNMRDGGHTGTGARGGNCC-3') and Hommer (5'-SVDGCRTGVGTRCANGCCAT-3') amplified the coat protein (CP) gene with 580 bp DNA bands. The amplification consisted of initial denaturation at 95°C for 5 minutes, 35 cycles of denaturation at 95°C for 30 seconds, annealing at 54.5°C for 30 seconds, the extension at 72°C for 1 minute, and the final stage at 72°C for 7 minutes.

Detection of TICV and ToCV using TICV-CF (5'-AATC GGTAGTGACACGAGTAGCATC-3') and TICV-CR (5'-CTTCAAACATCCTCCAT CTGCC-3') primers which amplified divergent genes of a protein coat (CPd) and ToCV-CF (5'-GTGTCAGGC CATTGTAAACCAAG-3') and ToCV-CR (5'-CAC AAAGCGTTTCTTTTCATAAGCAGG-3') which amplifies parts of the coat protein (CP) gene. According to Hartono *et al.* (2003), RT-PCR products from plants infected by TICV showed DNA bands of 416 bp and according to Hirota *et al.* (2010), tomato plants infected by ToCV showed DNA bands of 360 bp. The amplification consisted of predenaturation at 95°C for 2 minutes, 30 cycles of denaturation at 95°C for 30 seconds, annealing at 51°C for 30 seconds and extension at 72°C for 1 minute, followed by the final extension stage at 72°C for 5 minutes.

## RESULT AND DISCUSSION

In the 4$^{th}$ week, some plants have been in the generative phase, which showed by the emerge of flowers. In the third week, Servo grafted onto H-7996 and the self–grafted Servo showed symptoms of virus infection while Servo grafted onto Amelia showed a viral infection in the 5$^{th}$ week. Viral infection at the beginning of growth inhibited plant growth and the virus developed faster than uninfected plants during the vegetative phase. Viral infections occur at the young stage of the plants or early growth (3$^{rd}$ week) cause high symptom scores which indicated by severity reaching 60% on self-grafted Servo. When the virus infection occurs slower (6$^{th}$ weeks), the severity of the disease was

Table 1. The resistance level of tomato plants to viral infections based on symptoms, disease incidence and normal vs. abnormal fruit

| Level of resistance* | Symptoms | Disease incidence (%) | Weight or number of normal vs abnormal fruit |
|---|---|---|---|
| Immune | Asymptomatic | 0 | No abnormal weight |
| Resistant | Mild | 0<x<10 | Normal > abnormal |
| Tolerant | Moderate | 10<x<30 | Normal > abnormal |
| Susceptible | Severe | 30<x<50 | Normal = abnormal |
| Very susceptible | Very severe | >50 | Normal < abnormal |

Remark: *Taufiq *et al.* (2007 with modification

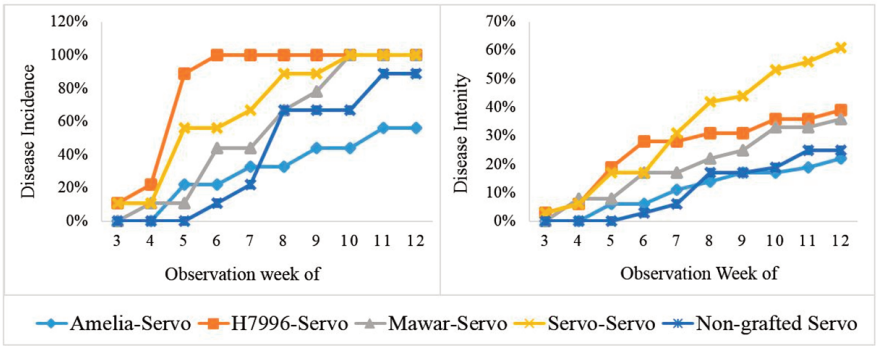


Figure 1. Development of viral diseases in tomato plants infected by *Begomovirus* and *Crinivirus*


Figure 2. Symptoms variation of viral infections in grafted and non-grafted tomato plants in the study area: (a) chlorotic spots; (b) interveinal chlorosis, bright yellow, necrotic, and flat-leaf edges; (c) interveinal chlorosis, curly, thick, rigid and small-sized leaves; (d) the leaves curl upward, purple interveinal

28% in non-grafted Servo (Figure 1). This result similar to Lapidot (2007) that plants infected by viruses at an older age might have milder symptoms than at a young age. Infected plants in the generative phase would have slower disease development because plants are more resistant (Hull, 2002).

Servo grafted onto H-7996 revealed disease develops rapidly. In the 6$^{th}$ week after planting the disease incidence has reached 100%, while in non-grafted Servo the disease incidence was 10%. Non-grafted Servo at the beginning of growth showed the most resistant response among all treatments, however at the end of the observation of disease incidence in non-grafted Servo was not the lowest. Servo grafted onto Amelia showed a slow rate of disease development (Figure 1). High rates of the disease might be caused by vector preferences. As the plant morphology in the field, the grafting with H-7996 showed the highest number of branches, thus the plants become dense with more leaves than the other grafts that could attract vector insects to land on the plant. Navas-Castillo *et al.* (2000) reported that the level of the vector insect population could influence the incidence rate of disease due to *Begomovirus* and *Crinivirus* in the field.

Variations in symptoms with the type of interveinal chlorosis, thick, and curly leaves, were found in all cultivars with a mild level in the Servo grafted onto Amelia and non-grafted Servo (Figure 2). In the self-grafted (Servo-Servo), these symptoms continue until the leaves were rigid and smaller. Symptoms of interveinal chlorosis, bright yellow, necrotic, flat-leaf edges were almost found in all cultivars with dominant symptoms in the scions. The symptoms of chlorotic spots were only found in a few plants, especially in Servo grafted onto H-7996 and Mawar. The leaves curl upward and the purple interveinal were only found in Servo grafted onto H-7996. According to Matthews (1992), variations in symptoms were influenced by plant factors, i.e. cultivars, age, and plant genotypes. Bos (1994) stated that variations in symptoms are expressions of the virus's development

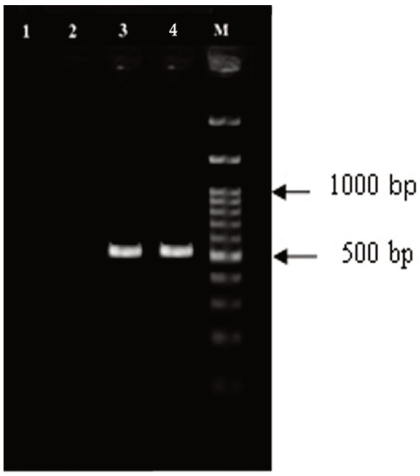


Figure 3. Begomovirus DNA detection results using Krusty & Hommer primers on symptomatic tomato plants with visualization of agarose gel 1%: (1) leaves curl upward and purple interveinal; (2) chlorotic spots; (3) interveinal chlorosis, curly, thick, rigid, and small leaves; (4) Positive control; (M) marker 100 bp DNA ladder; DNA band is 580 bp

whose replication depends on host plant cells. Variations in these symptoms were molecularly detected to determine the type of infecting virus.

Molecular detection of *Begomovirus* using Krusty & Hommer primers succeeded in amplifying the *Begomovirus* genome in tomato plants have symptoms of interveinal chlorosis, curly, thick, rigid, and smaller, whereas in leaves with chlorotic spots and leaves curling upward with purple interveinal was not amplified hence the symptoms were not caused by *Begomovirus*. In samples with symptoms of chlorotic spots showing positive results with a DNA band 360 bp on RT-PCR amplification using ToCV-CF/ToCV-CR specific primers, while using TICV-CF/TICV-CR specific primer there was no amplification. Samples with interveinal chlorosis, curly, thick, rigid, small leaves, curled upward leaves and purple interveinal were not amplified by those specific primer pair so that the virus that infected the plant was not TICV or ToCV. Based on Figure 4, chlorotic tomato plants were infected by the ToCV virus. According to Wisler *et al*. (1998b), ToCV is transmitted by *Aleyrodidae* which includes two genera, namely *Bemisia* (*B. tabaci* or *B. argentifolii*) and *Trialeurodes* (*T. vaporariorum* and *T. abutiloneus*). Wisler *et al.* (1998a) stated that TICV is different from ToCV which could be transmitted by four whiteflies because TICV is only transmitted by green-house whitefly (*T. vaporariorum*).

Based on observations of in the field, the scion tomato plants showed interveinal chlorosis, curly, thick, rigid, and small leaves, while the rootstock showed yellow and chlorotic spots. Observation of symptoms in the field and molecular revealed that tomato plants in Harjobinangun, Pakem, Sleman, Yogyakarta areas had a double infection with Begomovirus and Tomato Chlorosis Virus (ToCV). Both viruses are transmitted by *B. tabaci*. The altitude of the studied area is around 400 m above sea level (asl) which is suitable for the ecology of *B. tabaci*. Tomato infectious chlorosis virus does not infect the observed tomato plants that might be caused the ecological of *T. vaporariorum* was less suitable. According to Fitriasari (2010), tomato planted in the altitude of 0–1000 m asl was dominated by *B. tabaci*, 1000–1200 m asl was dominated by *B.*

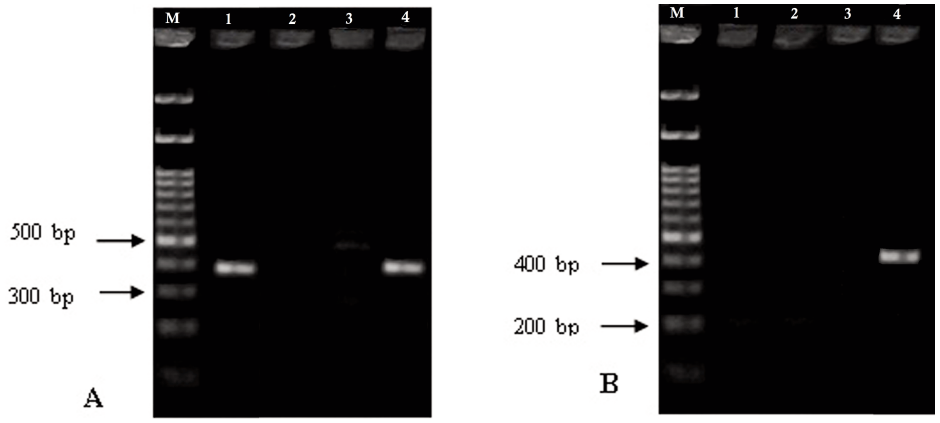


Figure 4. Results of virus detection in symptomatic tomato plants by RT-PCR visualized with 1% agarose gel: (A) using ToCV-CF/ToCV-CR primer; (B) TICV-CF/TICV-CR primer; (1) chlorotic spots (2) interveinal chlorosis, curly, thick, rigid, and small leaves; (3) leaves curl upward and purple interveinal; (4) Positive control; (M) marker 100 bp DNA ladder; DNA band is 360 bp

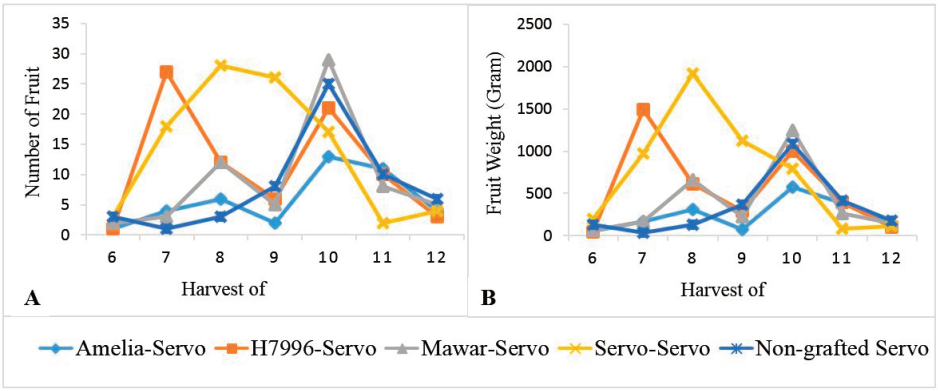


Figure 5. Development of c yields in grafted and non-grafted tomato plants: (A) the development of the abnormal number of tomato fruit; (B) abnormal weight development of tomatoes

*tabac*i and *T. vaporariorum*, and more than 1200 m asl was dominated by *T. vaporariorum.*

Besides vectors that play a role in spreading the virus, the presence of host plants also to be a source of inoculum. In the research field, besides tomato plants, were also planted chili. Chilli could be an alternative host for the virus. ToCV had a wide range of hosts. According to Wintermantel & Wisler (2006), ToCV infects 24 plant species from 7 families. In tomato plants, ToCV is difficult to distinguish from other *Crinivirus* symptoms such as TICV, but the virus could be easily distinguished through differential hosts (different hosts have different responses when inoculated with different virus strains). ToCV infects *N. glutinosa* and New Zealand spinach (*Tetragonia expansa*), while TICV does not infect these plants. TICV infects shepherds-purse (*Capsella bursa-pastoris*), lettuce (*Lactuca sativa*), zinnia (*Zinnia elegans*), and sowthistle (*Sonchus oleraceous*), while ToCV did not infect these plants. Tomato plant samples that had curled upward and purple interveinal were not infected with *Begomovirus*, Tomato infectious chlorosis virus, and Tomato chlorosis *virus* (Figures 3 and 4). These symptoms might be caused by other viruses. Adkins *et al*., (2012) stated that viruses that infect tomato plants generally consist of 7 genera i.e. *Begomovirus, Crinivirus, Cucumovirus, Curtovirus, Potyvirus, Tobamovirus,* and *Tospovirus*.

Servo grafted onto H-7996 at the 7$^{th}$ harvest showed the highest fruits weight and abnormal ones, followed by Servo-Servo in 8$^{th}$ harvest, Mawar-Servo, non-grafted Servo, and Amelia-Servo at 10$^{th}$ harvest. Virus infection at the beginning of development affected the weight and number of abnormal fruit (Figure 5). The earlier a virus infection occurs, the faster the fruit deviates from normal. In the 3$^{rd}$ week, H-7996-Servo and Servo-Servo showed symptoms of a viral infection thus in the 7$^{th}$ harvest, the abnormal fruit was higher. In contrast to Amelia-Servo, the lowest level of disease intensity showed the lowest weight and number of abnormal fruit at the 10$^{th}$ harvest that showed inhibition of abnormal fruit over 3 weeks. The spread of the virus in plants was increasingly limited when infections occur in older plants so that the abnormal fruit produced is low.

Viral infections affect the quality of the fruit, the higher the intensity of the disease, the more abnormal fruits were produced thus it has a low economic value. The consumption level of Servo tomatoes as fresh fruit or vegetables will reduce when the fruit is abnormal. Self-grafted Servo was more susceptible than non-grafted Servo. This was different from Servo grafted onto Amelia which showed a low disease intensity level (22%), although this treatment was not significantly different from non-grafted Servo. This cause Amelia-Servo and non-grafted Servo to contribute more as a source of inoculum because plants with a moderate susceptible level were highly more able to survive and not deteriorate as in susceptible ones. Thus, moderate susceptible plants could be in the field for a long time to become a source of inoculum. However, at the beginning of the infection which had a higher risk as a source of inoculum was the self-grafted (very susceptible) (Table 2).

Table 2. Resistance levels of grafted and non-grafted tomato plants against double infections by *Begomovirus* and *Crinivirus*

| Variety | Symptom | Disease Incidence | Weight or number of abnormal | Level of fruit resistance* |
|---|---|---|---|---|
| Amelia-Servo | Moderate | 56% | Normal>abnormal | Tolerant |
| H7996-Servo | Severe | 100% | Normal>abnormal | Susceptible |
| Mawar-Servo | Severe | 100% | Normal>abnormal | Susceptible |
| Servo-Servo | Very severe | 100% | Normal=abnormal | Very susceptible |
| Non-grafted Servo | Moderate | 89% | Normal>abnormal | Tolerant |

Remark: *Taufiq *et al.* (2007 with modification

## CONCLUSIONS

Tomato plants with chlorotic between leaf bone were infected by the Tomato chlorosis virus, while plants with interveinal chlorosis, curly, rigid, thick, and smaller leaf were infected by *Begomovirus.* Curled upward with purple interveinal did not indicate the presence of infection by both types of the virus. *Begomovirus* and Tomato chlorosis virus infections affect fruit quality as indicated by the high number of malformed and small-sized fruits. The resistance level of grafted tomatoes to the virus, namely "Servo" grafted onto "Amelia" and non-grafted "Servo" was tolerant to the virus, "Servo" grafted onto "H-7996" and "Servo" grafted onto "Mawar" was susceptible, and self-grafted "Servo" was indicated very susceptible to viral infections.

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