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Graft Union Formation Processes in Tomatoes as a Scion onto Eggplant Rootstocks: An Anatomical Study

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Abstract: Several researchers described physiological pathways in herbaceous or woody plants. There had not been much research on the process of tissue anatomy changes during the unification of tomato grafting using eggplant as a rootstock to determine the most suitable combination of scion and rootstock, and the detailed stages of connection during the first 15 days after grafting. The experiment was conducted in Kediri Regency, Indonesia, and anatomical observations were carried out in the agronomy laboratory of the Faculty of Agriculture, Brawijaya University. The study was conducted for two months in May-July 2018. The experiment resulted in 12 treatment combinations. The grafting combinations include Cervo/Cervo, Karina/Karina, Timoty/Timoty, Cervo/Gelatik, Karina/Gelatik, Timoty/Gelatik, Cervo/EG 203 line, Karina/EG 203 line, Timoty/EG 203 line, Cervo/S. *torvum*, Karina/S. *torvum*, and Timothy/S. *torvum*. The results obtained information that the unequal stem size between tomatoes and S. *torvum* as rootstock causes the grafting process to be slower than other rootstocks due to the anatomical closeness factor between tomatoes and S. *torvum*, which have different species compared to grafted tomatoes EG 203 and Gelatik eggplant. The EG 203 line originating from the AVRDC (World Vegetable Research Center) is the result of genetic manipulation prepared as a compatible rootstock of eggplant, while Gelatik is a local eggplant that has a similar description to the EG 203 line, so it is more compatible than S. *torvum*. At the age of 15 days after grafting (DAG), the self-grafting scions (Cervo-Cervo, Karina-Karina, and Timoty-Timoty) showed perfect connection compared to the other combination of tomatoes as scions with eggplant rootstocks that anatomically had not yet fully fused.

Keywords: graft union, scion, rootstocks, an anatomical study.

番茄作为茄子砧木接穗的嫁接结合形成过程：解剖学研究

摘要: 几位研究人员描述了草本或木本植物的生理途径。对于以茄子为砧木的番茄嫁接统一过程中组织解剖变化过程的研究，以确定最合适的接穗和砧木组合，以及嫁接后前 15 天的详细连接阶段，尚无太多研究。实验在印度尼西亚 凯迪里摄政 进行，解剖观察在布拉维贾亚大学农学院的农学实验室进行。该研究于 2018 年 5 月至 7 月进行了两个月。该实验产生了 12 种治疗组合。嫁接组合包括切尔沃/切尔沃,卡琳娜/卡琳娜,提摩太/提摩太,切尔沃/杰拉提克,卡琳娜/杰拉提克,提摩太/杰拉提克,切尔沃/例如 203 线,卡琳娜/例如 203 线,提摩太/例如 203 线,切尔沃/龙葵，卡琳娜/龙葵和 提摩太/S。旋转。结果得到的信息是，番茄和 龙葵作为砧木的茎粗大不等导致嫁接过程比其他砧木慢，原因是西红柿和 龙葵之间的解剖接近因子，与嫁接番茄例如 203 相比具有不同的物种和杰拉提克茄子。源自 AVRDC（世界蔬菜研究中心）的例如 203 系是基因操作的结果，作为茄子的相容砧木，而杰拉提克是当地的茄子，与例如 203 系的描述相似，因此更相容比 龙葵。在 15 天大时，自嫁接穗（切尔沃-沃,卡琳

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娜-卡琳娜 和 提摩太-提摩太）与其他番茄组合相比表现出完美的连接，因为它们的接穗与茄子砧木在解剖学上尚未完全融合。

关键词: 嫁接结合,接穗,砧木,解剖学研究。

1. Introduction

Plants are very important plants worldwide because they can ensure a balanced diet and effective nutrition for human health. Vegetables such as tomatoes are increasingly important for a country. Globally, these vegetables, an important component of the diet and have been widely consumed and recommended [1]. Projected national tomato demand between 2017-2021 ranges from 855-1053 tons, while tomato production until 2016 only reaches 157.1 tons. Tomato production centers in Indonesia are spread in several provinces, including West Java, North Sumatra, Central Java, East Java, and Bali [2]. Climate change is expected to cause environmental stresses that disrupt vegetable productivity and significantly have an impact on food systems around the world) [3]. Environmental constraints lead to the limited productivity of vegetables. Biotic and abiotic stresses are the main causes of crop losses worldwide, as they can reduce the yield of most major crops by more than 50% [4].

Until recently, scientific information in Asia about the influence of environmental stress on vegetables was emphasized on tomatoes [5]. Biotic and abiotic stresses in real terms reduce productivity and become a severe constraint on growth. The pressure demands attention and urges horticulturists both through breeding and with proper agronomic management. One of the safest and most effective solutions for plants to be stress-resistant is to use grafting technology. Grafting is one of the methods of artificial vegetative propagation that has long been known and used by the public to improve the nature of plants, both quality-related properties or those related to quantity. In Europe and Asia, grafted tomatoes currently account for a large proportion of total tomato production. For example, Spain already uses ~50 to 70 million grafted plants per year, accounting for about 40% of the country's tomato production [5].

The compatibility of the scion and rootstocks depends on anatomical, physiological, and genetic variables [6], but molecular mechanisms for the development of grafting unification are still unknown [7]. Very little data is available on the mechanism of grafting unification in vegetables. Compatible or incompatible mechanisms are not yet fully understood. Many authors describe the sequence of physiological pathways during grafting healing in both woody and herbaceous plants.

The difference between compatibility and incompatibility in the grafting process, there are no studies that define it clearly. There is a healing process at the point where the stem is cut during grafting, which over time can develop symptoms of growth disorders that cause abnormal growth [8]. Grafting compatibility can be assessed through physiological characteristics scion [9] and stem diameter at the grafting point [9]. However, in non-woody or herbaceous plants, not much is known about rootstock affecting scion physiological behavior [8], as was the case for several Solanaceae species. [10] reported that there are three important steps in the grafting process: callus formation, cambium differentiation, and continuity of vascular vessel formation. These three things affect the success or failure of the grafting process. The success of grafting is reflected in the structure and the physiological function of the vascular junction of the exchange of substances between the scion and rootstock, where the exchange of these substances is reciprocal from the scion to the rootstock and from the rootstock to the scion [11].

Mineral assimilation and transport processes are commonly used to study vascular development in grafting and graft fusion processes. According to [11] that the factors that affect the success of grafting and incompatibility are influenced by: plant species and type of grafting, environmental conditions after and during grafting, anatomical incompatibility, expertise for grafting, anatomical abnormalities of vascular tissue in the callus bridge. Viruses and phytoplasmas can also cause incompatibility. Some researchers describe the physiological pathways in herbaceous or woody plants. Compatibility occurs if the first phase of the upper and lower stem tissues are cut and then touched, forming parenchymal cells (calluses) capable of connecting each other between the upper and lower stems, the second phase of which occurs differentiation of cells taking place together with the continuous formation of the cambium between the upper and lower stems, the third phase in which the relationship of vascular vessels between the lower and upper stems is manifested by the formation of a new cambium layer in the callus bridge [6]. Thus, it is necessary to find out (a) the process for detail the stages of grafting during the first 15 days after grafting, (b) the process of tissue anatomy changes during the process of unification of tomato and eggplant grafts to get the most suitable combination of scion and rootstock and (c) the most anatomically suitable eggplant rootstock for tomato plants.

2. Methodology

This study was conducted to determine the anatomy of joining tissue during tomato grafting with eggplant bottom stems to be known as compatible upper and lower stem combinations. After grafting, it is immediately put in a grafting chamber, with a temperature of 25-32°C. Grafting experiments were conducted in Bangsongan Village, Kayen Kidul District, Kediri Regency, East Java Province, Indonesia, and in the agronomy laboratory of the Faculty of Agriculture, Brawijaya University Malang. The study was conducted for two months in May-July 2018. Tools used included: Olympus microscopes, digital cameras, stationery writing, razor blades, cutters, plastic trays, and wooden boxes for grafting. Experimental materials for grafting between the bottom stem of eggplant and tomatoes, nipples, small polybags. This research was conducted using the descriptive method in comparing tissue anatomy between combinations. The experiment resulted in 12 combinations of treatments. The combination of treatments is shown in Table 1.

Table 1 Experimental treatment

Code Treatments	Treatments	
	Tomato scion with high yielding	eggplant rootstocks which resistant to stress
Control		
C/C	Cervo (low land-medium land)	-
K/K	Karina (low land-high land)	-
T/T	Timoti (low land-medium land)	-
The rootstock of Gelatik		
C/G	Cervo	Gelatik
K/G	Karina	Gelatik
T/G	Timoty	Gelatik
The rootstock of EG 203		
C/E	Cervo	Line of EG 203
K/E	Karina	Line of EG 203
T/E	Timoty	Line of EG 203
The rootstock of S. *torvum*		
C/T	Cervo	*S.torvum*
K/T	Karina	*S.torvum*
T/T	Timoty	*S.torvum*

The grafting combinations included Cervo/Cervo, Karina/Karina, Timoty/Timoty, Cervo/Gelatik, Karina/Gelatik, Timoty/ Gelatik, Cervo/EG 203 line, Karina/EG 203 line, Timoty/EG 203 line, Cervo/S. *torvum*, Karina/S. *torvum*, and Timothy/S. *torvum*.

This study was repeated three times to obtain 12 x 3 = 36 units; each experimental unit consists of three plants so that the entire plant observed is 108 grafting plants. Observation parameters during the grafting unification period, by observing the occurrence of changes in plant tissues carried out using Olympus CX microscopes on 12 combinations consisting of the upper stem of tomatoes with eggplant stems connected top stems of tomatoes with the stems of the same-type tomatoes used as a control to compare the development of grafting unification. Observations were carried out on 5, 10, and 15 days after grafting (DAG) to study the development of the union of the upper and lower stems on the anatomy of the stem.

3. Results and Discussion

3.1. Observation for Five Days after Grafting

The anatomical structure of the scion consists of the epidermis, cortex, phloem, xylem, and pith (Figure 1). Some cambium formed on grafting union between the upper stem and the bottom stem whose observations began at five days after grafting (DAG) appears the connection between the top stem of the tomato and the tomato bottom stem as control and tomatoes connected with the bottom stem of the eggplant on average has not occurred a perfect union. A control stem over a tomato connected with a scion and bottom stem (Figure C/C, K/K, Ty/Ty) shows a better collaborative process than if the tomato crop is grafted to the eggplant bottom stem.

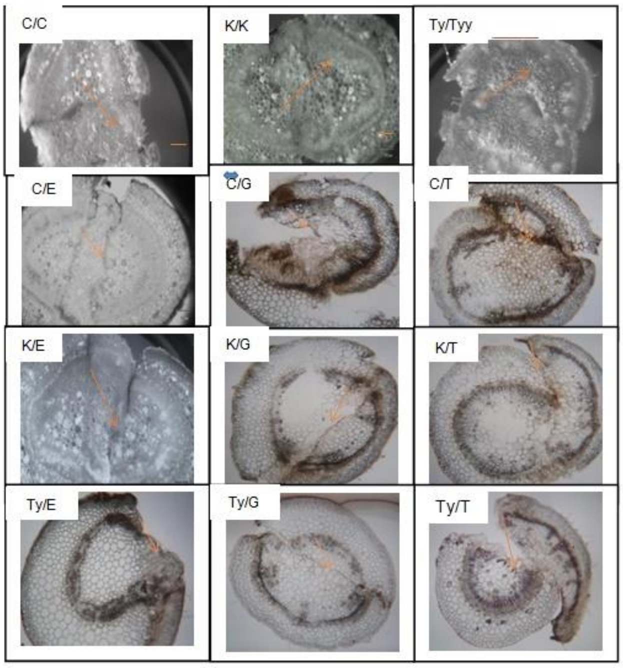

Fig. 1 Anatomical cross-section of tomato top stem and eggplant stem at the age of five days after grafting (DAG), C/C = Cervo Cervo 5 DAG, K/K= Karina Karina 5 DAG, Ty/Ty = Timoty Timoty 5 DAG C/E= Cervo EG 203 5 DAG, C/G = Cervo Gelatik 5 DAG, C/T= Cervo *S. torvum* 5 DAG, K/E = Karina EG 203 5 DAG, K/G = Karina Gelatik 5 DAG, K/T = Karina *S. torvum* 5 DAG, Ty/E = Timoty EG 203 5 DAG, Ty/G = Timoty Gelatik 5 DAG, Ty/T=Timoty *S. torvum* 5 DAG. The red arrows show the upper and lower bar linking lines. 1 scale = 50 μm

The anatomical structure of the stem consists of the tissues of the epidermis, cortex, phloem, xylem, cambium, and pith (Figure 2). Anatomical evaluation in the cross-section of the connection can provide the main information about the grafting compatibility [12]. The tissue anatomy is observed by controlling the development of callus tissue, cambium continuity, and vascular tissue development on the grafting surface [13].

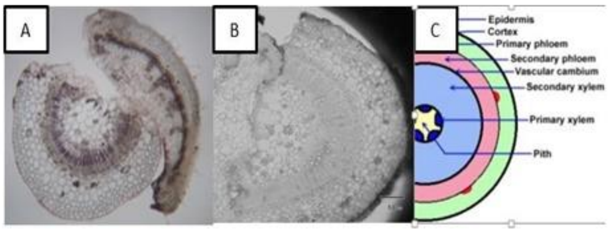

Fig. 2 Timoty connected Timoty (A) and Timoty *connected S. torvum* (B) at the age 5 DAG compared to the anatomical structure of the tomato stem

According to [14], the success of grafting pays attention to the skills and ability to graft, the survival of the upper and lower stems, and environmental conditions. However, it is still rare to see the attention to the anatomy of the upper and lower stems used, so failure is often due to the incompatibility of these anatomical factors. The incompatibility of joints is also due to anatomical and biochemical discrepancies. Anatomical incompatibility is shown in the decrease in the number of vascular vessel beams differentiated in the grafting union, thus inhibiting the transport of nutrients from the lower stem to the upper stem [8]. Signs to predict incompatible plant connections according to [15] are seen from external symptoms such as swelling on the surface of the union, death or decrease in vegetative growth conditions, upper stem resistance, and differences in upper and lower stem growth.

Several combinations of upper and lower stems showed that the Timoty and S. *torvum* varieties on 5 DAG (Figure 1. Ty/Ty) had not shown any union at all and still separate the connections of Cervo and Gelatik variety (Figure 1. C/G). According to [16], this connecting age begins with the death of the cell layer on the grafting surface wound by cutting. According to [6], this age begins with the death of the layer of cells on the surface grafting wounds due to cutting. Then Karina grafted with Gelatik at 5 DAG (Figure 1. K/G), Karina with S. *torvum* on 5 DAG (Figure 1. K/T), and Timoty grafted with Gelatik on 5 DAG (Figure 1. T/G) were still separate despite showing a closer unification position and still faint connection. Also as illustrated by Figure 1. C/E, Cervo grafted with EG 203 on 5 DAG began to show the union of the upper and lower stems and merge to differentiate, as well as what happened in the K/E, which is the connection between Karina's upper scion and the lower stem of EG 203 5 DAG. In Timoty grafted with EG 203 on 5 DAG (Figure 1. Ty/E) and Cervo grafted with *S. torvum* at the age of 5 DAG (Figure 1. C/T), it is enough to show the unification process characterized by the formation of callus and the differentiation of callus in the parenchymal network shown by thickening the linking line between the upper and lower stems. The type of species and plant tissues affect the time it takes for grafting unification processes [10].

According to [17], the first connection between the scion and rootstocks stems generally occurs at 4 DAG. As stated in [6], there is an attachment between the upper and lower stems in the first one or two days after grafting at the intersection. Then, the cell wall's constituent material, such as pectin, is removed. Next, the necrosis layer of the cell form will die. Furthermore, cell division begins from the cambium, cortex, and cells around the phloem and xylem in both the upper and lower stems, from which the callus begins to form. In this phase, there is an increase in the unification power of grafting that runs very slowly.

3.2. Observations 10 Days after Grafting

After ten days of grafting development, it appears that the joints between tomatoes and eggplant have fused but not yet perfected. At some joints, it appears once thickening the necrotic layer in the grafting link between the upper stem and the bottom stem (Figure 3). Thickening of necrotic layers is more visible in Cervo grafted with Cervo (Figure 3. C/C 2), Karina with Karina (Figure 3. K/K 2), Cervo with EG 203 (Figure 3. C/E 2), Cervo with Gelatik (Figure 3. C/G 2), Cervo with S. *torvum* (Figure 3. C/T 2), Timoty with S. *torvum* (Figure 3. Ty/T) and Timoty with Gelatik (Figure 3. Ty/G 2), where the seven combinations also showed the improvement of the process of connecting the upper and lower stems compared to the combination of Karina and Gelatik (K/G 2) and Timoty with EG 203.

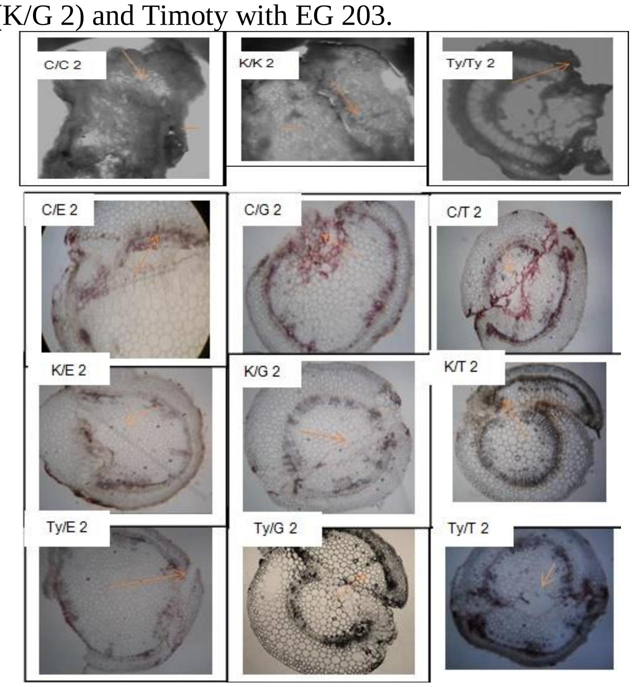

Fig. 3 Anatomical cross-section of tomato top stem and eggplant bottom stem ten days after grafting (DAG). C/C 2 = Cervo Cervo 10 DAG, K/K 2 = Karina Karina 10 DAG, Ty/Ty 2 = Timoty Timoty 10 DAG, C\/E 2 = Cervo EG 203 10 DAG, C\/G 2 = Cervo Gelatik 10 DAG, C/T 2 = Cervo *S. torvum* 10 DAG, K/E 2 = Karina EG 203 10 DAG, K/G 2 = Karina Gelatik 10 DAG, K/T 2 = Karina *S. torvum* 10 DAG, Ty/EG 2 = Timoty EG 203 10 DAG, Ty/G 2= Timoty Gelatik 10 DAG, Ty/T 2 = Timoty *S. torvum* 10 DAG. The red arrow shows the upper and lower bar link lines, one scale = 50 μm

The combination of grafted between Karina upper scion and Gelatik bottom stem (Figure 3, K/G 2) has not shown the consolidation process; the upper and lower stems are still separated. Timoty grafted to Timoty on 10 DAG (Figure 3, Ty/Ty 2) has also not yet reflected the proper unification. The connection between the Karina variety and S. *torvum* bottom stem (Figure 3, K/T 2) shows the availability of unification but not yet perfect because there is still a thickening of the connection, similar pictures are also displayed by Timoty connections with the Gelatik bottom stem (Figure 3, Ty/G 2). Timoty's top stem grafted with the EG 203 bottom stem at 10 DAG (Figure 3, Ty/E 2) shows almost integration with the formation of a transparent furry seemingly ambiguously necrotic layer on the connection.

According to [6], the process that occurs a few days to a week after grafting forms a necrotic layer begins to spread, then callus makes a connection between the upper stem and the lower stem. Then a cell wall is formed between the upper and lower stems of the new cell wall material stored like pectin. The cell wall thins and forms a plasmodesma between the upper and lower stems. Then there is an increase in the strength of the substance for the unification of grafting. According to [18], when the production of callus increases, the necrotic layer is absorbed to form a callus bridge between the upper and lower stems. The callus cells are intertwined to form a callus bridge [19], in which symplast and apoplast connect the flow of water between the upper and lower stems and mechanically increase the union.

Furthermore, there is the proliferation of callus bridges on grafting surfaces, differentiation of new vascular cambium, and restoring new vascular tissue [6]. In herbaceous plants, the process of grafting unification takes a faster time than grafting on woody plants. This type of Arabidopsis requires 7-12 days of unification process [6]. That vascular vessels in Arabidopsis can function again in three days after grafting [20].

3.3. Observations 15 Days after Grafting

The observations 15 days after the average grafting on the control treatment (Figure 4. C/C 3, K/K 3 and Ty/Ty 3) already showed the perfect grafting union followed by the loss of necrotic layers along the connecting surface, where callus cells have been linked. The stronger connecting of the callus bridge causes the connecting network of vascular vessels to form perfectly (Figure 4).

The tomato stem connected using the *S. torvum* bottom stem of its consolidation has not been perfect, still seems to have the thickening of the necrotic layer along with the rotation and shows the unfolding process, given there is still a detached space of Cervo, Karina or Timoty grafted with *S. torvum* bottom stem (Figure 4. C/T 3, K/Ty). This is the possibility of compatible but the process runs slower or occurs incompatible, where the meaning of incompatibility does not always mean failure in the connectivity of crops. Some combinations of connection crops can indicate symptoms of incompatibility but remain growing, produce, and profitable [21]. Incompatibility is defined in [22] as a different character between the upper and lower stem accommodating failure to become a strong union, failure to grow healthily, and death after connectivity.

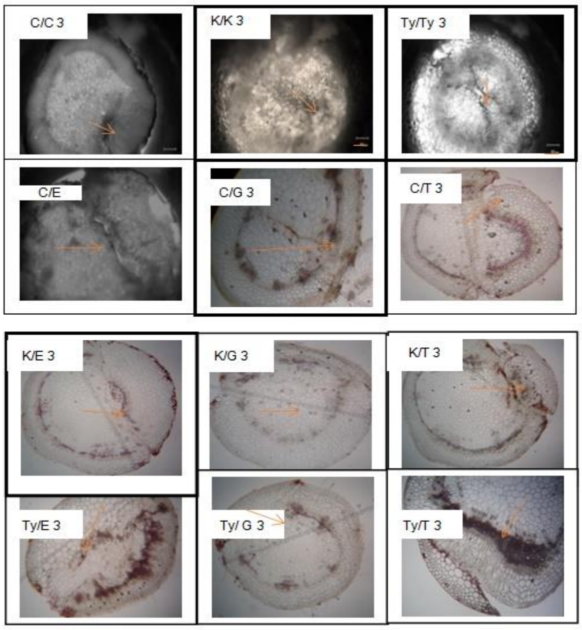

Fig. 4 Anatomical cross-section of tomato top stem and eggplant stem at 15 days after grafting (DAG), C/C 3= Cervo Cervo 15 DAG, K/K 3= Karina Karina 15 DAG, Ty/Ty 3= Timoty Timoty 15 DAG, C/E 3= Cervo EG 203 15 DAG, C/G 3= Cervo Gelatik 15 DAG, C/T 3= Cervo *S. torvum* 15 DAG, K/E 3= Karina EG 203 15 DAG, K/G 3 = Karina Gelatik 15 DAG, K/T 3 =Karina *S. torvum* 15 DAG, Ty/E 3 = Timoty EG 203 15 DAG, Ty/G = Timoty Gelatik 15 DAG, Ty/T = Timoty *S. torvum* 15 DAG. Red arrows indicate upper and lower bar linking lines, one scale = 50 µm

Until now, the causes of incompatible connections in plants are very diverse. Among them are environmental conditions that do not support up to the skills or expertise of the connecting personnel. According to [22] that the symptoms of incompatibility vary greatly depending on the species. The earliest method for detecting incompatibility is by knowing the visual appearance of external (anatomical) symptoms after the connection is made. Internal symptoms usually precede external symptoms, where internally the phloem tissue is more often played a role than the xylem tissue, the combination of several symptoms gives a strong indication of the occurrence of incompatibility; if only one symptom is seen, then it can be suspected as a result of climate, environment or poor connection techniques [23].

In Figure 4, it appears that the diameter of the upper scion and the diameter of the *S. torvum* bottom stem is quite different. The connection of tomato varieties of Cervo with the bottom stem of Gelatik (Figure 4. C/G 3) is already attached. However, the linkage of callus is not yet perfect. In contrast, Timoty's connection with the lower stem of the strain EG 203 (Figure 4. Ty/E 3), then Cervo that grafted with the lower stem of EG 203 (Figure 4. C/E 3) still shows the thickening of the necrotic layer in the connecting area. However, the upper and lower stems show a better union than the tomato graft with *S. torvum* bottom stem.

In Figure 4, it appears that Karina with EG 203 (Figure 4. K/E 3) and Karina with Gelatik (Figure 4. K/G 3), and Timoty with Gelatik (Figure 4. Ty/G 3) show a strengthened connection with the thinning of the necrotic layer, callus cells intertwine, proliferation and differentiation from a new vascular network so that the transport of nutrients and water is re-connected. It resembles a cross-sectional linkage of tomato control joints (Figure 4. C/C 3, K/K 3 and Ty/Ty 3).

The results of [6] showed that despite the good growth of plant connections in the field indicated by the production of new bud, but anatomically seen, the linking results are not completely perfect because there is still necrotic tissue between the two connection areas. Incompatibility based on anatomy is observed from the absence of vascular tissue in connection linkages due to the presence of lignin layers that interfere with the continuity of the cambium or the presence of premature aging as a result of physiological and biochemical processes. According to [23], failure to form strong and perfect linkages is caused by physiological processes that cannot adapt at the cellular level due to metabolic, developmental, or anatomical differences. According to [14], the incompatibility process involves anatomical, biochemical, physiological, and molecular interactions between the lower and upper stems. This process can occur in a short time or a little longer after grafting.

The results of [24] study, that different upper and lower stem diameter sizes affect the ability of success to graft. According to [19], the cambium area inequality in the upper and lower stems causes tissue death in the injured area from the lower stem to the upper stem to the next upper scion. The results of the study [6] reported that the difference in the diameter of the upper and lower stems during grafting union caused the accumulation of carbohydrates in the wound area due to the continuity of the partial vascular vessels, the high formed callus, which develops into indifference parenchymal cells that cause swelling in the union. Also, according to [25], cell division that occurs above and below the necrotic layer causes the grafting unification area in the swollen cambium area.

4. Conclusion

Compatibility or incompatibility between the scion and rootstocks depends on plant genetics, unfavorable environmental conditions, and the skill or expertise of the connective force.

1. At the age of 15 DAG, the self-grafting scions (Cervo-Cervo, Karina-Karina, and Timoty-Timoty) had shown complete union in the grafting chamber with a temperature of 15-320, while the combination of tomato as scions (Cervo, Karina, and Timoty) with eggplant as a rootstock (EG 203, Gelatik and *S. torvum*), and anatomically up to 15 DAG were not fully fused. However, it had shown a strengthened connection with the thinning of the necrotic layer, intertwined callus cells, proliferation, and differentiation of new vascular tissue so that the transport of nutrients and water is re-connected.
2. The combination of tomato as a scion (Cervo, Karina, and Timoty) with eggplant as a rootstocks EG 203 line and Gelatik resulted in a more compatible anatomical connection than *S. torvum* rootstock. Inappropriate stem size between tomato and *S. torvum* as a rootstock caused slower grafting.
3. The EG 203 line resulted from genetic manipulation, prepared as rootstocks to construct a more compatible connection. Meanwhile, Gelatik is a local eggplant with a similar description to the EG 203 line, so it is more suitable than *S. torvum*.

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