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Original Article

Tuber formation and endophyte dynamic in potato black nightshade grafting with application of goat manure liquid fertilizer

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Abstract

A research on the grafting of potato and black nightshade plants can be a new interesting report. This research was carried out in the greenhouse of Horticultural Seed Center Station in Pakem, Yogyakarta, Indonesia at an altitude of 780 m above sea level. In this research, a potato plant was used as the rootstock while a black nightshade plant was used as the scion. This research was conducted to examine the effect of grafting on tuber formation and endophyte dynamic of potato-black nightshade grafted with an application of liquid fertilizer made from goat manure. The plants consisted of three levels: potato plants, grafted plants, and black nightshade plants. Liquid fertilizer application consisted of two levels: without liquid fertilizer application, and with liquid fertilizer application. The liquid fertilizer was applied once a week in the range concentration of 1900-2000 μSCm^{-1} with total volume 0.5 liter per plants. Tuber formation was affected not only by the grafting of the plants but also by liquid fertilizer application. Potato with liquid fertilizer application produced the highest weight of tubers. There were about 66 % of grafted plants that produced tubers with sprouts, around 21.5% that produced irregular tubers and 12.5 % that produced regular tubers. Application of liquid fertilizer resulted a larger total bacteria in potato plants, in black nightshade plants, as well as in grafted plants. A population of bacteria in grafted plants was accumulated more in the connected stem, whereas the total of bacteria in the lower connected stem (rootstock) was larger than that in the upper connected stem (scion).

Keywords: Black nightshade scion, Grafted plants, Potato rootstock, Goat manure, Liquid fertilizer

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Introduction

Grafting has been used since several centuries ago to propagate horticultural plants to increase plant production, plant vigor, and plant resistance to disease (Petropoulos et al., 2014; Miles, 2012; Guan and Zhao, 2012; Davis et al., 2008; Davis and Veazie, 2006) as well as to increase plant tolerance of extreme temperatures (Johnson et al., 2011). This grafting technique is now broadly used to propagate muskmelon (Guan and Zhao, 2014), watermelon (Miles, 2012), and tomato (Johnson et al., 2011).

Grafting is generally known as a technique to connect two parts of a plant: the stem upper part is called "scion" and the stem lower part is called "rootstock or stock" (Goldschmidt, 2014). In order to have successful grafting, appropriate cutting of the stems both scion and stock are necessary (YouQun, 2011). To have a better fitting connection of the stems, the anatomical composition of stem tissues, especially stem cambium between scion and stock, must be properly connected (Johnson et al., 2011).

There have been no experimental reports on the grafting of potato (*Solanum tuberosum* L.) and black nightshade (*Solanum nigrum* L.) plants. In this research, a potato plant was used as the rootstock while a black nightshade plant was used as the scion. Both potato and black nightshade plants are in the same family "Solanaceae" so they have the possibility to be grafted (Kumar, 2011). The potato plant needs an optimum daily relatively low temperature of about 20^{o}C (Rykaczewska, 2013; Paul and Gogoi, 2013; Rykaczewska, 2015; Muthoni and Kabira, 2015), whereas the black nightshade plant has a good adaptation in low and high temperatures (Jain et al., 2011).

Many studies have been conducted to find out the effects of grafting on the growth and yield quality of plants. The rootstock of the cantaloupe plant can make better growth and yield of musk melon as the scion (Davis and Veazie, 2006; Petropoulos at al., 2014). The Rootstock of fruit vegetable plants having resistance toward soil-borne disease can increase yield quality on cucurbitaceous plants and tomatoes as the scion (Davis and Veazie 2008; Johnson et al., 2011; Guan and Zhao, 2012; Guan and Zhao, 2014).

This research was carried out to ascertain the effect of grafting on tuber formation and endophyte dynamic in grafted plants between the potato plant as the rootstock and black nightshade as the scion. Endophyte is microbes living in the plant tissues and several of them have symbiosis mutualism with host plant (Owen and Hundley, 2004) which can accelerate plant growth (Quambusch et al., 2014). Study on endophyte had been conducted on the potato plant (Sessitch et al., 2004; Andreote et al., 2009) and black nightshade (Nayak, 2015; Long et al., 2008).

Material and Methods

Research site and period

This research was carried out from March until July 2016 in the greenhouse of Horticultural Seed Center Station in Ngipiksari, Pakem, Yogyakarta, Indonesia at an altitude of 780 m above sea level. It has daily temperatures of 29^{o}C/22^{o}C.

Potato and black nightshade seedlings preparation

Forty-eight seedlings of black nightshade were grown in plastic pots with a diameter of 14 cm and a height of 10 cm. At the age of 14 days, 24 seedlings were transplanted to the plastic pots with a diameter of 38 cm and a height of 30 cm, whereas the shoots of another 24 seedlings were taken to be used as scions. Forty-eight seedlings of potato "granola" variety with an average tuber weight of 25 g were planted in plastic pots with a diameter of 38 cm and a height of 30 cm. At the age of 14 days, 24 seedlings were used as rootstock grafted with the scion of black nightshade, whereas another 24 seedlings were planted without grafting. All seedlings were planted in the potting media with the composition of air-dried regosol soil and rice husk charcoal in the ratio 2:1 (v/v).

Grafting method of the plants

Potato seedlings as rootstock and shoots of black nightshade seedlings as scions were grafted using the cleft grafting method (Johnson et al., 2011). On 14 days after grafting, the rootstock of potato seedlings and scion of black nightshade shoots were connected well.

Liquid fertilizer preparation and application to the plants

The liquid fertilizer was made from materials consisting of air-dried goat manure, sugar, Ammonium Sulfate (ZA), and Effective Microorganisms (EM) formulated by (Higa and Parr, 1994). All materials were fermented through the steps conducted by (Sunaryo et al., 2018[b]) as follow: (1) goat manure, sugar, and ZA were mixed in ratio to 20 L of water. Sugar + ZA were diluted by 20 L of water in the plastic bucket, then it was added by 20 ml EM and 2 kg goat manure. (2) The mixed materials were stirred manually around 5 minutes using wood stick. (3) All of mixed combination materials were fermented in the plastic bucket of 25 liter volume. All of the fermented plastic buckets were capped properly and they were put inside the room in condition of air room temperature. (4) Every day, in the morning and afternoon, all of the mixed fermented materials were stirred manually around 2 minutes using wood stick. (5) During 3 weeks while fermentation, the pH and EC of the solution were observed. The pH and EC were

observed using portable pH/EC/TDS/ Temperature meter Hanna Instruments H19811-5. In order to get the real value of EC and TDS, and due to the fertilizer solution which was very high concentrated, it was diluted by adding sterile water in ratio of 1:20 (v/v).
The plants were arranged in three levels: potato plants (P), grafted plants (G), and black nightshade plants (B). Liquid fertilizer application consisted of two levels: without liquid fertilizer application (F_0), and with liquid fertilizer application (F_1). The experiment was arranged in a completely randomized design. Plant to plant distance was 80 cm. Each experimental unit was replicated four times, and each replication used three plants. The plants with application of liquid fertilizer (PF_1, GF_1, and BF_1) were given the liquid fertilizer every a week in the range concentration of 1900-2000 μSCm^{-1}, whereas the pH was in the range of 5.5-6.5, with total volume 0.5 liter per plants. All plants were watered with well water by spraying the water on the surface of the potting media.

Tuber observation
Both tubers of potato plants and tubers of grafted plants were observed 45 days and 70 days after planting. Tuber observation was finished at the plant age of 70 days because the plant growth of both potato plants and grafted plants was terminated. Tuber observation was done on the total number of tubers per plant, tuber fresh weight per plant, tuber diameter, tuber length, and the shape of the tubers.

Endophytes observation
Endophytes were observed on the stem of plants at the age of 45 days after planting. On grafted plants, the observation was conducted on the three parts of the stem: the lower connected stem (Ls), on the connected stem (Cs), and the upper connected stem (Us) of grafting. Endophytes observation used plate agar technique (Nayak, 2015).

Results and Discussion

Grafting plants could result in different tuber shapes and a greater number of tubers per plant (pot) compared to those of potato plants both on the application of liquid fertilizer and without liquid fertilizer application. Grafting plants could result in unusual or irregular tuber shapes of potato, tubers with sprouts (Figure 1. left), tubers with irregular shape (Figure 1. middle), and tubers with regular shape (Figure 1. right) and a greater number of tubers per plant (Table 1). There were about 66 % of grafted plants that produced tubers with sprouts, around 21.5% that produced irregular tubers and 12.5 % that produced regular tubers.

Figure 1. Representative tubers of grafted plants have sprouts (left), tubers of plants have irregular shape (middle), and tubers of plants have regular shape (right).

Black nightshade as scion had a strong effect on not only the quantity of tubers but also tuber shapes of potato as rootstock of potato-black nightshade grafted plants. In general, observation on grafted plants was conducted on the effect of rootstock on the scion, i.e. rootstock effect is clearly identifiable in the development of fruit firmness and fruit weight of some sweet cherry grafted plants (Hajagos et al., 2012). Furthermore, grafting of woody plants have a positive effect on the morphological change on leaves and fruits, the number of branches, thorns and stem on the scion of plants (Eltayb et al., 2014).
Grafted plants could produce a greater number of tubers compare that of potato plants (Table 1). The total number of tubers per plant (pot) both on potato plants and grafted plants was not affected by the application of liquid fertilizer (Table 1).
Application of liquid fertilizer could result in greater tuber weight of both potato plants and grafted plants (Table 1). This result was in a line with the research conducted by (Sunaryo et al., 2018[a]) stating that the application of goat manure liquid fertilizer had a positive significant effect on the growth of foliage vegetable in hydroponics. Liquid fertilizer made from goat manure have high organic acid content such as lactic acid, acetic acid, and citric acid (Sunaryo et al., 2018[b]), which can promote plant health and growth (Seddik et al, 2016). The application of a liquid organic humate fertilizer and growth stimulator "humustim" could promote growth and development of plants and result in high quality crop yields (Vasileva et al., 2017).

Table 1. Effect of grafting and liquid fertilizer application on number of tubers per plant, tuber weight per plant, and tuber diameter

Treatments	Tuber parameter		
	Number of tubers plant^{-1}	Tuber weight plant^{-1} (g)	Tuber diameter (cm)
PF_0	8.7±1.20a	108.7±4.91a	3.7±0.26b
PF_1	9.0±1.15a	189.7±10.39c	4.6±0.15c
GF_0	16.3±1.20b	110.0±8.14a	2.8±0.11a
GF_1	17.0±1.00b	153.3±10.97b	3.3±0.15ab

Numbers in column followed by same letter are not significantly different by DMRT 5%
PF_0: Potato without liquid fertilizer application
PF_1: Potato with liquid fertilizer application
GF_0: Grafted plant without liquid fertilizer application
GF_1: Grafted plant with liquid fertilizer application

Grafted plants produced a lower weight of tuber per plant than the potato plants with application of liquid fertilizer (Table 1). Tuber weight per plant (pot) of both potato plants and grafted plants was low, less than 200 g per plant (pot). This experiment was conducted in a lower area having an elevation of 780 m above sea level with daily temperatures of 29^{o}C/22^{o}C. This higher temperature condition is not appropriate for potato growth. The potato crop is characterized by specific temperature requirements and it needs cooler temperatures of around 18^{o}C to 20^{o}C for its growth (Paul and Gogoi, 2013; Rykaczewska, 2013), so the crop requires a higher altitude area. In appropriate conditions, potato "granola" could produce tubers of 298 g per hill (Hossain et al., 2015).

Tuber diameter of either potato plants or grafted plants was affected by liquid fertilizer application. The plants treated by liquid fertilizer could produce bigger tubers, whereas grafted plants produced smaller tubers (Table 1). Grafted plants could produce not only tubers that looked like potato tubers but also fruits that looked like black nightshade fruits. Nevertheless, the plant figure of grafted plants was less vigorous than black nightshade plants (Figure 2), and they produced a fewer number of fruits. Grafted plants had tubers and fruits so they had two important sinks which needed more assimilate for their growth. This condition resulted in the slower growth of the vegetative parts so the grafted plants had either less vigorous canopy or fewer tubers weight. Potato tuber yield is mainly affected by source capacity via the net photosynthetic rate, total leaf area and leaf lifespan (Li et al., 2016).

Figure 2. Representative potato-black nightshade grafted plants (left) were less vigorous than black nightshade plants (right) at the age of 45 days after planting

The application of liquid fertilizer could result in the higher population of endophyte in potato plants (Figure 3. left), black nightshade plants (Figure 3. right) as well as in connected stem of grafting plants (Figure 4). There was no significant effect of liquid fertilizer on endophyte population both in lower stem and upper stem of grafting plants (Figure 4).

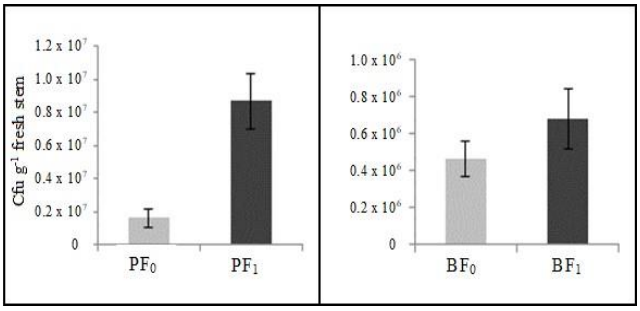

Figure 3. Total of bacteria of potato plants (left) without application of liquid fertilizer (PF_0) and with application of liquid fertilizer (PF_1). Total of bacteria of black nightshade plants (right) without application of liquid fertilizer (BF0) and with application of liquid fertilizer (BF_1). Bar line indicates the standard error of mean (n=8)

The result of this experiment was congruent with the result of an experiment conducted by Seghers et al. (2004) stating that the composition of root endophytic community was clearly affected by different fertilizer treatments. The result of the experiment indicated also that distribution of bacteria in the grafted stem was

accumulated more in the connected stem both without application of liquid fertilizer and with the application of liquid fertilizer. The total number of bacteria in the lower connected stem (rootstock) was higher than that in the upper connected stem (scion) (Figure 4). The result of the research conducted by (Liu et al., 2018) indicated that the more vigorous rootstock was observed to have a greater quantity of growth-promoting bacterial taxa. Nevertheless, the mechanism by which an apple genotype, either rootstock or scion, had a determinant effect on the composition of a microbial community was not known.

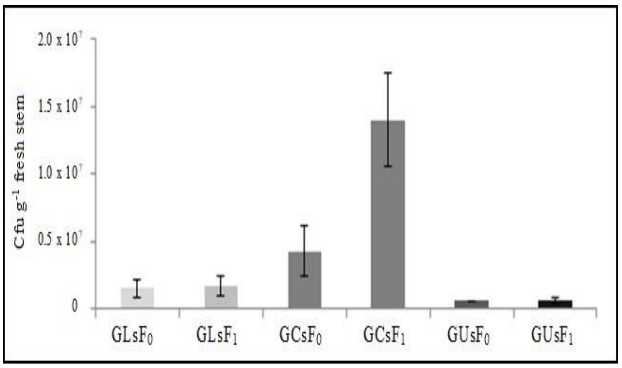

Figure 4. Distribution of bacteria population in the grafted stem.

Total of bacteria in the lower connected stem without application of liquid fertilizer ($GLsF_0$) and with application of liquid fertilizer ($GLsF_1$). Total of bacteria in the connected stem without application of liquid fertilizer ($GCsF_0$) and with application of liquid fertilizer ($GCsF_1$). Total of bacteria in the upper connected stem without application of liquid fertilizer ($GUsF_0$) and with application of liquid fertilizer ($GUsF_1$). Bar line indicates the standard error of mean (**n=8**)

The result of the experiment indicated that tuber formation was affected not only by grafting of the plants but also by liquid fertilizer application. The grafting produced a greater quantity of tubers and it could result in irregular shapes of tubers. There were about 66 % of grafted plants that produced tubers with sprouts, around 21.5% that produced irregular tubers and 12.5 % that produced regular tubers. The application of liquid fertilizer resulted in greater tuber weight, bigger tuber diameter, a larger total of endophyte bacteria of both potato plants and grafted plants, and a higher total of endophyte bacteria of black nightshade plants. The Population of endophyte bacteria in grafted plants was accumulated more in the connected stem, whereas the total of endophyte bacteria in the lower connected stem is greater than that in the upper connected stem.

Conclusion

Tuber formation was affected by both grafting and liquid fertilizer application. The Grafting could increase quantity number of tubers with different shape i.e. tubers with sprouts, irregular tubers, and regular tubers. The liquid fertilizer application could increase the size and weight of tubers, as well as the population of endophyte bacteria of both potato plants and grafted plants.

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Contribution of Authors

Sunaryo Y: Conceived Idea, Literature Search, Data Collection, Literature Review, Manuscript Writing.
Purnomo D: Design Research Methodology, Data interpretation, Statistical Analysis, Manuscript final reading and approval.
Darini MT: Literature Search, Data Collection.
Cahyani VR: Conceived Idea, Literature Review, Manuscript Writing, Manuscript final reading and approval.

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