Acceleration of productive age through candlenut [Reutealis trisperma (blanco) airy shaw] grafting method

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Abstract. The main constraints in toxic candlenut [Reutealis trisperma (Blanco) Airy Shaw] cultivation is late harvest time initiation, naturally begin to fruit at the age of 5-6 years after planting, so the crop management research is directed to more quickly harvest time (less than 5 years) through grafting. The study was carried out to determine grafting technique supporting high compatibility by studying aspects of scion sources and method of grafting, on grafting success and morphological performance during early process of graft formation and seedling stage, further plant growth. The treatment design used was Randomized Block Design (RBD) with four replications, consisting of five grafting treatments: 1) KS1/KS1 cleft grafting, 2) KS1/KS2 cleft grafting, 3) KS1/KS1 whip grafting, 4) KS1/KS2 whip grafting, and 5) KS1 plants from seed (ungrafted plant). The best grafting technique was treatment combination of scion of KS2 and top cleft or V-shaped grafting. Observation of 3 years old showed that the productive age of grafted plants faster than ungrafted plants. The highest percentage of flowering plants could be seen in the KS1/KS1 cleft grafting followed by KS1/KS1 whip grafting, KS1/KS2 cleft grafting and KS1/KS2 whip grafting. Whereas in plants that come from seeds had not entered the reproductive phase.

Keywords: compatibility, flowering, productive age, rootstock, scion.

1. Introduction
Toxic candlenut plant [Reutealis trisperma (Blanco) Airy Shaw] is an agricultural commodity that has been designated as an alternative energy source. In 2011 Indonesian Center for Estate Crops Research and Development (ICECRD) has released two superior varieties of Kemiri Sunan 1 and Kemiri Sunan 2 with seed production levels of 110.65 and 76.55 kg/tree/year respectively with oil yields of 38.1-42.0% and 47.2-56.0% (SK Mentan No 4000 dan 4044/ Kpts/SR.120/9/2011). Toxic candlenut (Kemiri sunan) is quite efficient as a raw material for biofuel because the production of toxic candlenut can reach 50-289 kg / tree / year or seeds as much as 4-6 tons of dry seeds per hectare per year is equivalent to 2-3 tons of crude oil per hectares per year. The yield of toxic candlenut seeds can reach 50 percent. The main obstacle in cultivating toxic candlenut plants is that their productive age is slow, naturally starting to bear fruit at the age of 5-6 years after planting so it is necessary to do research so that the harvest period is faster than 5 years through grafting techniques.

Grafting plants is the merging of two different parts of the plant, so that it is a unified whole and grows as a plant after tissue regeneration in the scars on the connection linkage [1,2,3]. The general grafting of plants aims to improve the quality and quantity of crop products, rehabilitate damaged or...
less productive plants, change cultivars from plants that have been produced, accelerate flowering and fruiting time, and modify the shape of plant growth.

Grafting techniques have been widely applied to horticulture plants, especially fruit plants. The purpose of grafting is to speed up the age of production, shorter habitus so that it will facilitate harvest operations, use of labor more efficiently, fewer chemical inputs per application, and higher quantity and quality of fruit per hectare [4,5]. The grafting plum is intended to reduce plant size and shorten the vegetative period [6].

Grafting compatibility is the ability of two different plants to combine to produce successful compounds and develop satisfactorily into one composite plant [7]. Plant material produced from joints that have high compatibility will be indicated by better growth and productivity than the parent tree from the rootstock and scion which is ungrafted.

Vascular continuity through the connection interface is very important and determines the compatibility between the rootstock and scion [8]. Compatibility of grafting was shown to regenerate and reunite vessel tissue in scar tissue in the joint surface to develop normally and grow as a composite plant. A good grafting technique will produce intimate contact between the upper cambium and rootstock area. Meristem tissue cells in the connection between the scion and rootstock must have complete contact, so that the linking process can continue.

The success of grafting is shown by the good union between the scion and rootstock so that the plants from the joints grow optimally. Several factors such as cellular suitability, formation of plasmodesmata, connection of vessel tissue affect the success of the grafting [9]. By observing the development of vascular tissue it can be concluded whether the combination is appropriate or not, and the combination process takes place in good condition [12]. The development of toxic candlenut plants as an alternative energy source requires optimal cultivation technology support as a standard for proper farming and precision. The proper application of toxic candlenut cultivation technology is needed to increase productivity in an effort to meet the provision of alternative energy from biofuels sustainably. The main obstacle in cultivating sunan toxic candlenut plants is that their productive age is slow, naturally starting to bear fruit at the age of 5-6 years after planting, so research needs to be done so that the reproductive phase is faster than 5 years through grafted plants and management techniques. The objectives of the research was to accelerate the reproductive phase of toxic candlenut plants.

2. Material and methods

The study was conducted from 2014 to 2017, preceded by nursery activities, nurseries and connections were carried out at Karangploso Research Station in 2014, then planting in the field was carried out in the Kalipare Research Station on January 2015.

The material used is the KS-1 variety toxic candlenut seeds as rootstock which has been seeded for 4 months, while the scion used is KS-1 and KS-2 varieties which have already been produced. Other materials in the form of fertilizer, bamboo, plastic and paranet for nurseries, as well as several research aids such as: ruler, scales, handcounters, and knives, and laboratory equipment for histological observation.

The treatment design used was Randomized Block Design (RBD) with four replications, consisting of five joint plant treatments, namely 1) KS1/KS1 cleft grafting, 2) KS1/KS2 cleft grafting, 3) KS1/KS1 whip grafting, 4) KS1/KS2 whip grafting, and 5) KS1 plants from seed (ungrafted plant).
Plant maintenance consists of fertilization, pest and disease control, weed control, and irrigation. The dosage of fertilizer used is 10 tons/ha of manure (4 kg/plant), 50 kg N + 30 kg P₂O₅ + 30 kg K₂O/ha, or equivalent to 50 kg Urea + 200 kg Phonska/ha. In one year twice, fertilizing I with ½ dose is given at the beginning of the rainy season (December), second fertilization with ½ dose is given before the dry season (March-April). Fertilizing by immersing 50 cm on the side of the plant as deep as 15-20 cm. Weeding was done 3-4 times a year using hoes or livestock.

Observations of plant growth were including: grafting success rate, plant height, stem diameter below, above the connection joint, and histological character. Observations were made on 6 sample plants per plot. The data obtained were analyzed statistically using Analisys of variance (Anova). If there is a significant difference between treatments, proceed with Duncan's at the 5% level.

3. Results and discussion

3.1. Plant growth
The general grafting of plants aims to improve the quality and quantity of crop productions, rehabilitate damaged or less productive plants, change cultivars from plants that have been produced, accelerate flowering and fruiting time, and modify the shape of plant growth. The material of grafting plant which has high compatibility is indicated by better growth and productivity than the parent tree from the rootstock and scion which is ungrafted. Toxic candle nut of KS1 has the advantage of stronger canopy and root performance, but its oil quality is lower than that of KS2. Through grafting techniques by utilizing the superiority of KS1 which is sturdy as rootstock and KS2 which has productivity, oil content and oil quality height as scion, so that two superior characters converge into one composite plant.
Figure 2. Plants resulting from grafting a vigorous thick root rootstock and scion which has high productivity and oil content.

Grafting activities is carried out when the weather (air temperature and soil conditions) are not too dry. It is necessary to pay attention about the polarity of scion and rootstock, the base of the scion must be connected to the top of the rootstock. Then after the connection was done, it was covered with a transparent plastic bag as a hood to maintain temperature stability.

Figure 3. Implementation of grafting in the nursery: branches to be used as scion are removed from the leaves; the cleft grafting to tap down into a V shape.

Four days after grafting, irrigation is not carried out to avoid the accumulation of water on the incision in grafting union which can trigger infection, and to accelerate the process of adaptation and
formation of new callus. Furthermore, watering is carried out routinely according to field conditions, to prevent drought and to accelerate the process of unification between the two trees.

Figure 4. Maintenance of composite plants resulting from grafting in the nursery

The success of a grafting is determined by the compatibility between the rootstock and scion, the quality of the rootstock and scion, the time of grafting, supporting equipment, skills and accuracy in carrying out the grafting, as well as maintaining the grafted plant. In an effort to increase the productivity of the grafted plants, the success of the grafting must be followed by the application of good cultivation practices. The average of grafting success rate at 2 months after grafting reached up to 80%. Grafting using KS-2 scion gave higher percentage of successful composite plants than KS-1 scion. This shows that the KS-2 rootstock source had high compatibility and was capable of compounding with KS-1 rootstock into one composite plant.

The cleft grafting method showed grafting success rate compared to whip grafting method. In the cleft grafting method the surface of grafting union between the rootstock and scion was wider, and the position of the linkage is more stable or not shifting so that the success rate of grafting was higher. When connecting scion to the rootstock, it must be observed that the scion cambium and rootstock cambium were attached to each other. In general, the diameter of the rootstock is used for grafting according to scion (equal or slightly bigger). In the condition of the rootstock is not as large as the upper stem, then one side is tried to coincide so that the cambium can unite even if only one side. In order for the linking process to continue, the cell or meristem network between the cutting regions must have contact attached/perfect interlocking.

The result also showed that the older the rootstock, the higher the grafting success rate achieved (Fig. 5). The 3 months rootstock old used as a grafting material provided the highest grafting success rate, followed by 2 months and 1 month old rootstock, respectively.
Figure 5. Grafting success rate at 3 levels of rootstock age (1, 2, 3 months old) and 2 grafting methods used (cleft grafting/V and whip grafting/M) on grafted plants

Combining two different plant genotypes to be one unite by grafting the upper part onto the below part would produce growing pattern that are different from those when each part is grown separately. The nature of the rootstock-scion relationship is very complex and differs among genotypes used as a source for grafting. The interaction between rootstock and scion include: (1) histological factor, (2) nutritional and carbohydrate levels and (3) absorption and translocation of water-nutrients [10].

Grafting between the rootstock and scion are not always successful, and unsuitable grafting union between rootstock and scion are shown in the form of incompatibility. Grafting incompatibility is a disturbance in the continuity of cambium and vascular tissue which leads to the cessation of growth in grafting union. Normal vascular tissue does not develop in grafting union. Incompatibility between the types of plants that are grafted is shown by low success rate of grafted plant, and there are differences in the rate of growth between the rootstock and scion.

The results of the observation of 2-year-old toxic candlenut plants showed that the tallest crop was reached in the treatment ungrafting (from seed), followed by the plants resulting from K1/K2 by cleft grafting method, and K1/K2 by whip grafting method. The shortest plants are found in plants with K1/K1 whip grafting method (Figure 6). The KS-2 scion source has high compatibility and is capable of compounding with KS-1 rootstock into one composite plant unit. It is assumed that the KS-1 and KS-2 varieties still have close relationship, because the closer the level of relationship of plant genotypes, the higher the level of compatibility when grafted into one composite plant.
Figure 6. Performance of the toxic candlenut plant height in scion source and grafting method

Cleft grafting had a higher compatibility than whip grafting, which had been shown in the performance of stem diameter from cleft grafting as composite plant combination of K1/K1 and K1/K2 were greater than whip grafting.

Figure 7. Performance of stem diameter below and above grafting union in scion source and grafting method.

The observation of 3-year-old toxic candlenut plants shows that the plants produced by grafting of productive age are faster than ungrafted plant from seeds. The highest percentage of flowering plants can be seen in the K1/K1 V-grafting method followed by K1/K1 whip grafting, K1/K2 V-grafting and K1/K2 whip gartting. Whereas in plants that come from seeds (ungrafted plants) have not entered the reproductive phase (flowering). The acceleration of flowering is due to scion that was used to come from plants that have already produced, so that the composite plant combination of two individual plants can accelerate the productive phase of the plant.
Figure 8. Percentage of flowering plants in scion source and grafting method of toxic candlenut plant

In addition, habitus of composit grafted plants was shorter and horizontal canopy growth make it easier to operate the harvest. Dwarf tree will facilitate cultivation practices, such as spraying, pruning, harvesting, and so on.

Figure 9. Performance of 4 years old toxic candlenut ungrafted plants from seeds and grafted plants

3.2. Histological character

Observations on the histological grafting combination of plant tissue samples taken at short time after grafting, will provide a quick information about the grafting compatibility [11]. The success of grafting is characterized by the callus tissue formed by rootstocks and scions. By observing the development of vascular tissue can be inferred if the combination is appropriate or not and the combination takes place in well condition [12]. Histological observations showed that in the V-shaped grafted plants, the necrotic patch was less than whip grafted plants, where the presence of the necrotic layer can inhibit continuity of vascular tissue. Histology observations of grafted plant with V-shape and whip grafting method at 2 months seedlings at above, at the point of the graft union and below of grafting union were shown in Figure 10.

Vascular continuity through the connection interface is very important and determines the compatibility between the rootstock and scion [8, 13]. Compatibility of grafting was shown to
regenerate and reunite vessel tissue in scar tissue in grafting union to develop normally and grow as a composite plant. A good grafting technique will produce intimate contact between scion and rootstock cambium area. Meristem tissue cells in grafting union between scion and rootstock must have complete contact, so that the linking process can continue.

![Histology of plant stems connecting slits and sloping at the age of 2 months of seedling in the connection joint section. BA: scion, BB: rootstock, Lap.Neck: necrotic layer.](image)

**Figure 10.** Histology of plant stems connecting slits and sloping at the age of 2 months of seedling in the connection joint section. BA: scion, BB: rootstock, Lap.Neck: necrotic layer.

The success of grafting was shown by the good union between scion and rootstock so that the grafted plants can grow optimally. Several factors such as cellular suitability, formation of plasmodesmata, connection of vessel tissue affect the success of grafting [9]. By observing the development of vascular tissue it can be concluded whether the combination is appropriate or not, and the combination process takes place in good condition [12]. The general sequence of events in grafting regeneration has the following steps [14]: the formation of intimate contact between rootstock and scion cambium region and the production of callus tissue parenchymatic cells by the two joint components in the cambium region; differentiation of certain parenchymatic cells from callus into new cambium cells connected to the original cambium of rootstock and scion; and the production of new xylem and phloem vessel networks by the new cambium, forming the vascular relationship needed for successful grafting union.

The major causes for graft incompatibility include: (1) physiological and biochemical factors, (2) modification of cells and tissues at the graft union, and (3) cell recognition between grafting partners. The success of grafting depends primarily on the identification of stress and pathogen-resistant rootstocks and on the compatibility of the graft union in terms of fast formation of the vascular connections between the rootstock and the scion and fast renewal of root and canopy growth [15]. This variation among plant species and cultivars in their grafting ability is probably related to their ability to produce callus parenchyma and differentiate a vascular system across the callus bridge. Thus better graft ability was indicated by less necrotic layer due to better tissue regeneration between scion and rootstock at graft union.

### 4. Conclusion

Kemiri Sunan 2 (K2) variety had a higher success rate of grafting than Kemiri Sunan 1 (K1) variety as a source of scion. The top cleft or V-shaped grafting had higher compatibility than whip grafting,
wiched indicated by higher success rate of grafting. The observation of 3 years old of Kemiri Sunan plants showed the most suitable combination of V-connecting K1/K2 which was indicated by faster growth in plant height and stem diameter than other joint combinations. In an effort to increase the productivity of the intercropping plants, the success of the connection must be followed by the application of good cultivation practices.

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