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Effects of Grafting Time and Grafting Methods Used on Scion and Rootstock Compatibility of Physic Nut (*Jatropha curcas* L.)

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ABSTRACT

The activity was carried out to determine grafting technique supporting high compatibility by studying aspects of the time and method of grafting and morphological, histological and biochemical changes during early process of graft formation. The results showed that the best grafting technique was the combination of the 2 months old rootstock with the top cleft or V-shaped grafting. The high grafting compatibility was expressed by the high glucose content of plant leaves and little gap of total sugar content between above and below graft union. Assessment histologically demonstrated that tissue regenerated compatibility and rejoint of vessels from wound tissues on graft union grew and developed as a normal composite plant.

Key words: *Jatropha curcas*, old of rootstock, grafting method, compatibility

INTRODUCTION

Jatropha (*Jatropha curcas* L.) is a potential biodiesel feedstock crops (Fairless, 2007) because its seeds contain 30-35% of jatropha oil (Jongschaap *et al*., 2007). One effort to maintain the jatropha yield in dry land is by grafting the jatropha plants. Grafting is the process of combining rootstocks and scions from different plants in order to reach a unite compound and tissue at the junction of a graft union, in which ultimatelly grow as a new plant (Alnopri, 2005; Pina and Errea, 2005; Lee *et al*., 2010). The grafting combination usually used for jatropha planted in the dry area is a combination between the scion which has a high yield performance and the rootstock selected which is tolerant to the limited water availability.

Grafting compatibility is the ability of two different plants grafted to develop into one composite plant (Santamour, 1988). The unite plants will demonstrate through a high compatibility and productivity performance which is grown better than that of the original parent tree.

The finding results from the previous jatropha grafting studies showed that grafting combination between rootstocks and scions are not always successful which is expressed by the form of incompatibility. The unity-failure could be caused by unfavorable environmental conditions, pests-diseases attacked and histological incompatibility resulting in poor-joint-ability. The incompatibility is occured usually because: (1) The physiological responses are negative between graft partners and (2) Histological abnormalities of the vascular tissue in the callus bridge. This manner can be explained as an interruption in cambium and vascular continuity leading to a smooth break at the point of the graft union, resulting in grafting failure (Schmid and Feucht, 1981). The response to grafting incompatibility is a complex mechanism with a wide range of different morphological, physiological, biochemical and histological interaction. However, the biochemical and histological basis for grafting compatibility and the morphological change involved during early process of graft formation, were still less known. Grafting compatibility evaluation based only on morphological characters usually takes time and has the risk of huge losses. On the contrary, the evaluation based on morphological, histological and biochemical characters of composite plants that are observed simultaneously are expected saving time and costs. Furthermore, the assesment can be detected at the early stage of the grafted plants. This study was being done to examine the effect of grafting time and grafting method used by assessing the morphological, histological and biochemical responses on grafted plant.

MATERIALS AND METHODS

Study site and experimental design: The study was conducted on September 2012 to March 2013 at the experimental station of Indonesian Sweetener and Fiber Crops Research Institute, Malang, Indonesia. The experiment was arranged in factorial randomized block design (RBD) consisted of two factors with four replications. The first factor was 3 levels of rootstocks age (1, 2 and 3 months old). The second factor were 2 grafting methods (cleft grafting and whip grafting) and both grafting methods were illustrated in Fig. 1. Each experimental unit consisted of 5 grafted plants that were planted in 40×50 cm polythene bags.

Plant materials and grafting implementation: Plant materials were used in this experiment was drought tolerant rootstock (IP-3M) and high yield and oil content scion (IP-3A) based on the previous study. Rootstock cuttings were planted at different time among rootstock old treatments. Three months rootstock old treatment was planted two months earlier, followed treatment of two months rootstock old was planted one month earlier than treatment of one month rootstock old. Rootstock plant were grown in nursery to prepare uniform seedlings in small plastic bags (15×15 cm) using media of mixed compost and soil (1:1, v/v). The seedlings were prepared 4 weeks before transplanted to 40×50 cm polythene bag media for the treatments.

The seedlings were watered every two days and fertilized using NPK (15:15:15) compound fertilizer 1 g per polybag at the beginning of planting and 1.5 g per polybag 1 month after planting.

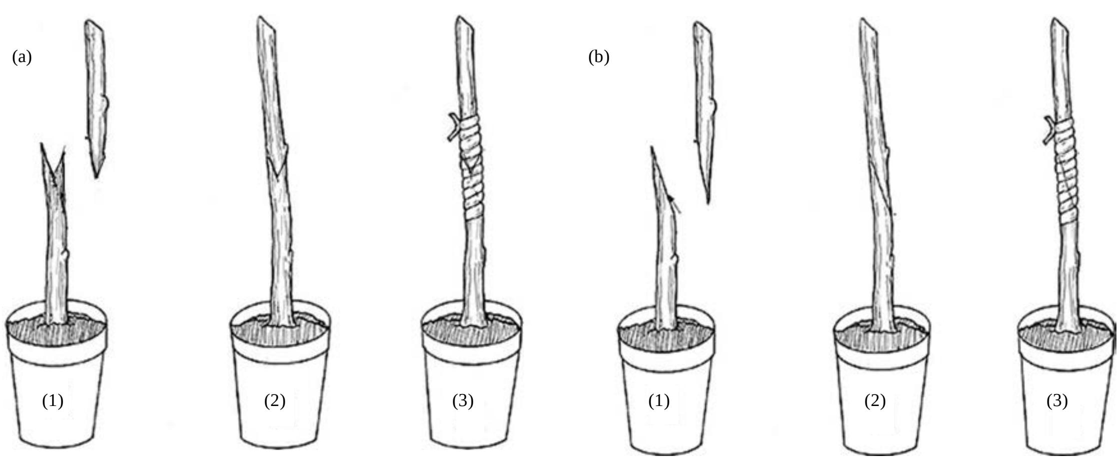

Fig. 1(a-b): Grafting methods (a) Top-cleft grafting and (b) Whip grafting

Scion materials were taken from mother plantations and yielded two month after pruning as shoot cuttings 12 cm in length. Grafting time was calculated simultaneously between rootstock old (1, 2 and 3 months old) and two kinds of grafting methods (cleft grafting and whip grafting). The grafted plant then maintained in the nursery under paranet which was shaded by 50% light intensity.

Plant growth measurement: The observations were started at 1 upto 4 month after grafting. The parameter observed including: the percentage of grafted plant success, plant growth (plant height, stem diameter: Below, above and at the graft union, number of branches and number of leaves). The root, stem, leaf and total plant dry weight were measured at the end of the experiment.

Biochemical and histological analysis: Proline contents were estimated by Bates *et al.* (1973) method while leaf glucose content and total sugar content were estimated by Yoshida *et al.* (1976) method. Histological observations were made using fresh material taken at 2 months after grafting. The probes consisted of 3-4 cm stem fragments of the grafting area which were fixed afterwards in ethylic alcohol 70%. In order to diagnose some aspects of grafting incompatibility, the samples were taken from three different parts of the grafted area: Above, below and at the joining area. The sections were made using SM 2000R microtome and performed transversal sections through the joining area. The sections thickness diameter were adjusted in between 30-40 μm. The surface which has been cut, then coloured by giving 1% solution of Safranine, put on preparate glass and then was given 50% glycerine solution. The probes obtained were analyzed using Olympus CH20 microscope with 100x size unit adjusted.

Statistical analysis: The differences between data in all tables were subjected to ANOVA and the comparison of quantitative traits means based on Duncan's Multiple Range Test (DMRT). Excel software 2010 was used for drawing figures. Grafting compatibility criteria was determined by plant growth and yield achieved, as well the biochemical properties at each grafting combination.

RESULTS

Effects of rootstock old and grafting method on plant growth

Success of grafting: The analysis of variance result showed that the success of the graft union was determined by the interaction between rootstocks and scions. The average of grafting success rate reached upto 78.57%, in which the higher grafting success rate was reached by grafting combination between 3 as well as 2 months rootstock old and top cleft grafting method (93.80 and 89.50%, respectively). The lowest grafting success rate was occured in grafted plant combination between 1 month rootstock old and whip grafting method (66.70%).

The result also showed that the older the rootstock, the higher the grafting success rate achieved (Fig. 2). 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.

Grafted plant growth: The high compatibility of grafted plant would give a better growth that were expressed by morphological characters performance. The statistical analysis result at 4 months after grafting showed that there was no significant interaction between older rootstock and grafting method on plant height, stem diameter, number of branches, number of leaves and root length.

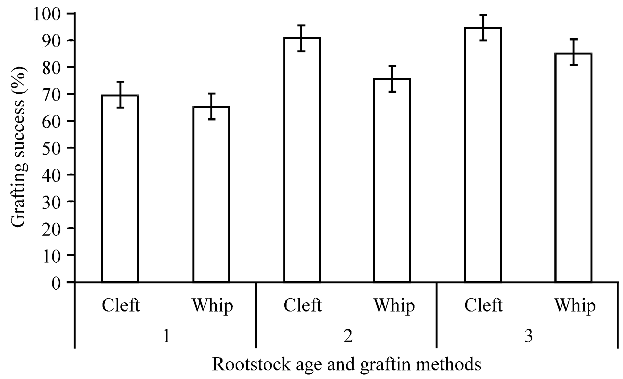

Fig. 2: Grafting success rate at 3 levels of rootstock age (1, 2, 3 months old) and 2 grafting methods used (cleft grafting and whip grafting) on grafted plants

Table 1: Effects of rootstock age and grafting method used on plant height, stem diameter, number of branches, number of leaves and root length of grafted plants

Treatments	Plant height (cm)	Stem diameter: Below (cm)	Stem diameter: At graft union (cm)	Stem diameter: Above (cm)	No. of branches (cm)	No. of leaves	Root length
Rootstock age							
1 month	64.50[b]	2.97[b]	2.82[b]	2.41[b]	2.50[c]	25.50[a]	66.38[a]
2 months	72.75[a]	3.10[ab]	2.81[b]	2.61[ab]	3.13[b]	19.50[a]	70.38[a]
3 months	78.13[a]	3.25[a]	3.10[a]	2.70[a]	3.75[a]	22.00[a]	68.00[a]
Grafting methods							
Cleft grafting	74.42[a]	3.27[a]	3.12[a]	2.74[a]	3.50[a]	26.33[a]	71.09[a]
Whip grafting	69.17[a]	2.95[b]	2.70[b]	2.40[b]	2.76[b]	18.33[b]	65.42[a]

Values in the same row and the same column followed by same letters were not significantly different at 5% level based on the DMRT test

However, a rootstock old as a single factor treatment gave a significantly effect on plant height, stem diameter and number of branches. While the grafting method independently influenced on almost all growth parameters observed except root length (Table 1).

Effects of rootstock age and grafting method on plant dry weight: The observation on cleft grafting method showed that the root, stem, leaf and total plant dry weight were higher than that of whip grafting method. Biomass accumulation was in agreement with the old of rootstock used. The rootstock that was grafted at 3 months old gave the highest plant dry weight but was not significantly different from 2 months old rootstock. One month old rootstock gave the lowest plant dry weight (Table 2).

Effects of rootstock age and grafting method used on biochemical characters

Leaf glucose content: The increasing of rootstock age was being followed by the increasing of leaf glucose content. The highest leaf glucose content was achieved by grafted plant that used 3 months old rootstock, followed by 2 months and 1 month old rootstock, respetively. Leaf glucose content that was yielded by top cleft grafting was higher than that of whip grafting (Fig. 3).

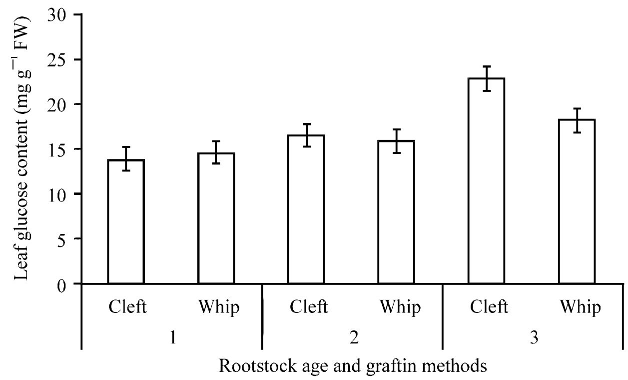

Fig. 3: Leaf glucose content yielded by grafting plant combination between 3 levels of rootstock old (1, 2, 3 months old) and 2 types of grafting methods (cleft and whip grafting)

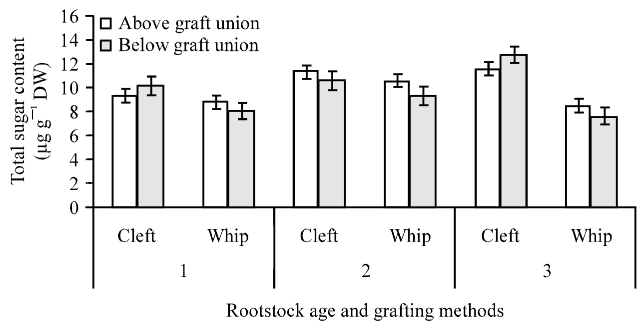

Fig. 4: Bark total sugar content yielded by above and below graft union derived from a combination between 3 levels of rootstockold (1, 2, 3 months old) and 2 types of grafting method (top cleft grafting and whip grafting)

Table 2: Effects of rootstock age and grafting method used on root, stem, leaf and plant dry weight of grafted plants

	Dry weight			
	Root	Stem	Leaf	Plant
Treatments	(g plant^{-1})			
Rootstock age				
1 months	12.70^{b}	48.85^{a}	7.46^{b}	69.01^{b}
2 months	17.63^{a}	52.09^{a}	11.71^{a}	81.43^{a}
3 months	18.95^{a}	55.33^{a}	13.63^{a}	84.27^{a}
Grafting methods				
Cleft grafting	20.42^{a}	57.83^{a}	13.53^{a}	91.78^{a}
Whip grafting	12.43^{b}	46.36^{b}	6.82^{b}	64.69^{b}

Values in the same row and the same column followed by same letters were not significantly different at 5% level based on the DMRT test

Total sugar content of bark: The increasing of rootstock old was being followed by the increasing of bark glucose content. The highest bark glucose content was achieved by grafted plant that used 3 months old rootstock, followed by 2 months and 1 month old rootstock, respectively. Bark glucose content that was yielded by top cleft grafting was higher than that of whip grafting (Fig. 4).

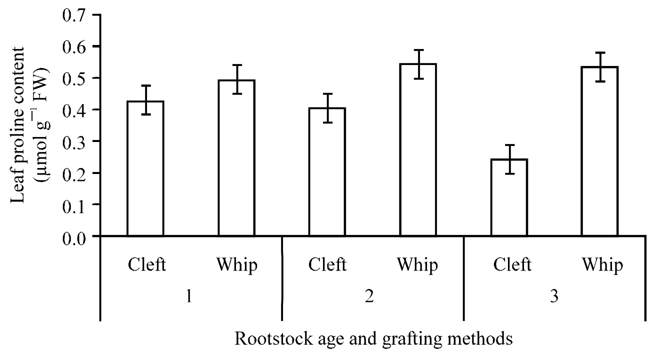

Fig. 5: Leaf proline content of grafted plant derived from a combination between 3 level rootstock (1, 2, 3 months old) and 2 grafting method (cleft grafting and whip grafting)

Leaf proline content: The observations on the plant biochemical properties at three months after grafting showed that the highest leaf proline content was yielded by 1 month rootstock old, followed by 2 and 3 months rootstock old. The observation also showed that whip grafting yielded leaf proline content higher than that of top cleft grafting (Fig. 5).

DISCUSSION

Effects of rootstock old and grafting method on plant growth

Success of grafting: The grafting succeed is determined by the ability of scions and rootstocks to develope a new union then grows into one composite plant. This process is affected by some factors, i.e., close relationship of species used as a scion or rootstock, rootstocks old, grafting method and weather conditions at and after grafting. The result showed that the older the rootstock, the higher the successull rate of grafting was achieved (Fig. 2). It could be due to both 3 and 2 month old grafting rootstocks has stem diameter wider than that of 1 month old grafting rootstocks, thus would be able to expand the graft union area, support to regenerate its vessel tissue. Composite plant that derived from a younger rootstock provided a slower-growth which was probably due to the reduction in vessels diameter. The decrease in vessel diameter would followed by decreasing hydraulic conductivity that cause a decrease in stem water potential and stomatal conductance.

The assessment on cleft grafting treatment showed that this method gave a higher grafting success percentage than that of whip grafting. The higher grafting success percentage on cleft grafting would be due to the stable linkage position provided and the graft union surface between rootstocks and scions provided was wider than that of the other grafting method. This result is in agreement with the findings of Islam *et al.* (2004), who indentified the highest percent survival of grafted mango plant produced by modified cleft grafting might be due to the long cambial layer connection and the easiness of wrapping of graft union hindering the entrance of the rain water. One of incompatibility symptoms of grafted plant was very low percentage of successful union. Nevertheless, successfull grafted plant was indicated by fast grafted plant growth and suitable graft union.

Combining two different plant genotypes to be one unite by grafting the upper part onto the below plant part would produce growing pattern that are different from those when each part is grown separately. Martinez-Ballesta *et al.* (2010) stated that 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.

Grafted plant growth: The older the rootstock, the better the appearance of its morphological characters. This was expressed by yielding a higher plant, a wider stem diameter at the position of below, above and at graft union, as well as the number of branches formed that were more accomplished on rootstocks grafted at 3 months, followed by 2 and 1 month rootstock old, respectively (Table 1). While the number of leaves formed and root length were not influenced by rootstock old.

The longest root length character was reached by scion that was grafted at the age of 2 months (Table 1 and Fig. 6). Observation at graft union found that grafting between rootstocks and scions that had the same stem diameter will make the surface of graft union easy to be attached by each other, so the meristem tissue in between the contacted area would be attached perfectly. The

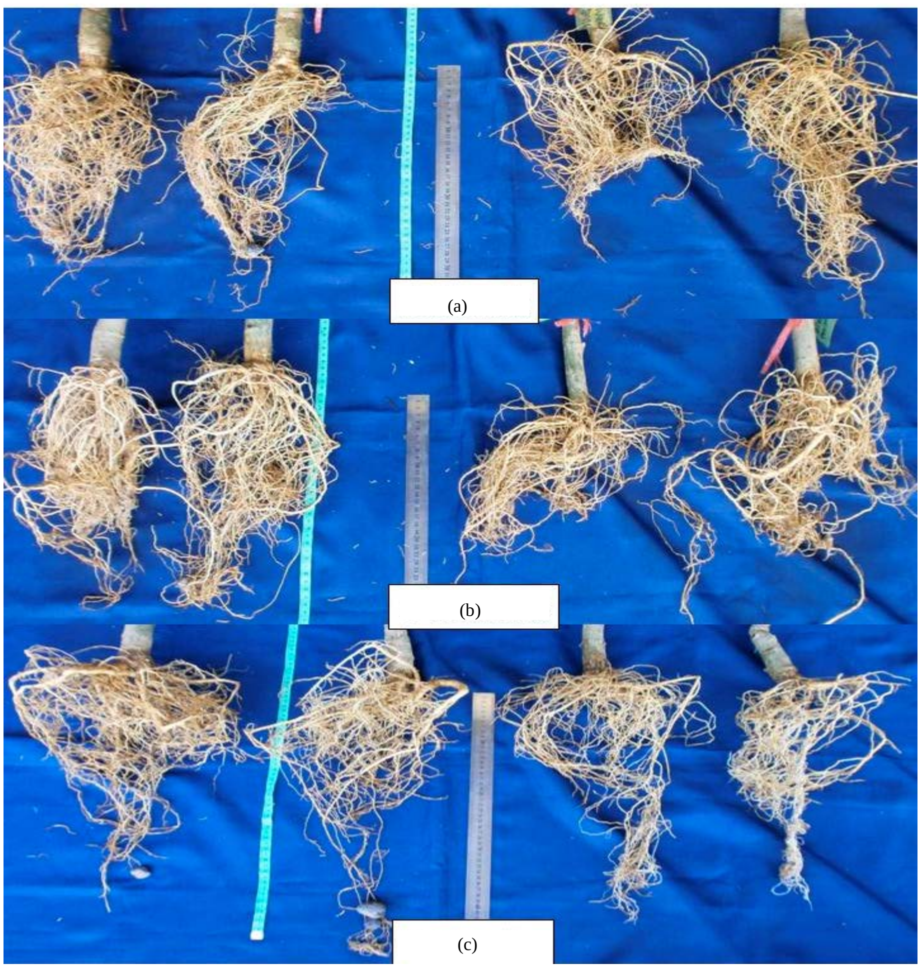

Fig. 6(a-c): Roots performance of jatropha provenances derived from grafting combination between rootstock age and grafting method (a) 1, (b) 2 and (c) 3 months old continuity cambium and vascular tissue then will be formed quickly resulting in a normal cycle of plant metabolism. Furthermore, the grafting treatment used the oldest (3 months old) or the youngest (1 month old) rootstock would cause the meristem tissue in between cutting contacted area attached not perfectly. As the vessel tissues were only coincide on one side so regeneration and continuity of vascular tissue would be inhibited that ultimately would inhibit water, nutrients and assimilates of transported photosynthesis product.

The unsuccessful and unsuitable grafting treatment between rootstocks and scions are shown by the form of incompatibility. Grafted plant observation showed that the stem diameter increased at graft union was faster than that at above and below graft union. This result is agreed with the finding results of Tshokoeva and Tsonev (1995), who reported that there was a marginal difference between scion and rootstock diameter in grafted plant but the stem diameter increased significantly was occurred at the graft union area.

The stem diameter increased at the grafted union could probably related to metabolites (could be phenols and carbohydrates) accumulated as a result of partial cambium continuity in grafted union. Errea (1998) reported that the gap of assimilates translocation between scion and rootstock parts of grafted plant leads to accumulating some compounds at grafted union. In addition, high levels of callus formed into undifferentiated parenchyma cells resulted in swollen grafted union. Physical assay from the previous study also showed that rootstock had stem bark that is significantly thicker than that of scion ones which caused the differences in growth rate and callus formation between the two parts.

Effects of rootstock old and grafting methods on plant dry weight: Grafted plants that had a high compatibility resulted in a successful synchronization of growth and development of rootstocks-scions as composite plants. Regeneration and reunification of vessel tissues in wounded area of graft union was developed normally, so the vessel tissues would give a proper function. By continuity of cambium formation and vascular tissue, both water and nutrient were transported smoothly to support scion while the assimilate produced by the canopy would be distributed properly to the rootstock to support the root growth. The accumulation of net photosynthesis manifested in the form of biomass accumulation or plant dry weight (Table 2). The older the rootstock being attached, the greater the plant diameter and the more extensive root distribution formed. These features will increase the plant ability to exploit water and nutrients to support the biomass accumulation as an end product of photosynthesis.

Effects of rootstock old and grafting methods on biochemical characters

Leaf glucose content: Physiological and biochemical assay are needed to understand the growth and sucrose storage mechanisms, as well as the relationship between the leaves activity as a source of photosynthesis and the sucrose accumulation in the sink in jatropha. Source is a metabolic source referred to an organ or a tissue that produce and/or transported out photosyntate, i.e., leaves which is responsible to the process of photosynthesis. Sink is metabolic source referred to an organ and/or tissue that utilize and/or storing photosyntate, such as bud, young leave, root, fruit and seed. In general, the source is a part of plant that produces photosyntate and the sink is a the other part of plant utilized or stored photosyntate.

In plant metabolism, the homeostatis between photosynthesis and respiration must be maintained in order to achive a positive net photosynthesis that will be used to support plant growth and development and ultimately is being expressed by increasing plant dry weight.

Communication between the plant source and sink plays an important role in photosynthate partitioning patterns which are not only determined by the sink size available but are also influenced by sink strength. Plant will allocate its photosynthate directed to root growth to improve the ability of exploiting water and nutrients in soil depth layers in order to overcome drought condition. The older rootstock was grafted, the wider of plant diameter produced and the more extensive root distribution (Fig. 4) thereby increasing its ability to exploit water and nutrients that give a better support to the photosynthesis process and biomass accumulation as an end product of photosynthesis. Result finding was in agreement with Usenik and Stampar (2001) result, who reported that the different rootstocks used gave the different effects on plant performance connections, as well as the phenolic compounds accumulated in the upper stem tissue. While, the grafting study has been carried out by Goncalves *et al.* (2006) using three sweet cherry cultivars in combination with five rootstocks showed that the genotype treatment affected the rootstocks performance physiologically (water relations, leaf gas exchange, chlorophyll fluorescence, light transmittance canopy, leaf photosynthetic pigments and metabolites).

Total sugar content of bark: The high concentration of carbohydrate accumulated probably associated with overgrowth at graft union. The histological differences between plant scions and rootstocks would limit the soluble sugar transported or starch mobilization, resulting in a local accumulation of sugar or starch above graft union. The grafting plant often shows a cross-sectional area at the graft union slightly wider compared to that of the ungrafting plant. Furthermore, histological differences between the two plants usually found out in that area (Olmstead *et al.*, 2006; Goncalves *et al.*, 2007) which has a potency to increase the hydraulic resistance (Cohen *et al.*, 2007). Soumelidou *et al.* (1994) reported that reducing polar auxin transport in composite plants will reduce the cambium activity, particularly the xylem formation at the graft union area.

The increasing of rootstock old was being followed by the increasing of bark total sugar content. Bark total sugar content that was yielded by top cleft grafting was higher than that of whip grafting (Fig. 4). Scion overgrowth caused by blocking of assimilates translocating from the scion to the rootstock, causing a weak root system. Size differences between crown and root usually associated with partition or allocation differences of carbohydrates to the scions-canopy and rootstocks-roots. The higher the gap will give a negative affect to the plant growth and production (Whiting and Lang, 2004). Graft union can be a barrier to plant nutrients and water transported as a result of the negative histological development (Simons and Chu, 1983; Cohen *et al.*, 2007), incompatibility of scion-rootstock grafted (Schoning and Kollman, 1997) and the differences in transporting carbohydrates into rootstocks (Salvatierra *et al.*, 1998).

Leaf proline content: Osmotic adjustment is an important physiological mechanism by which plants synthesize and accumulate compounds act as osmolit in cells in response to water deficit (Seki *et al.*, 2007). This adjustment causes the accumulation of sugars, amino acids, sugar alcohols and quaternary ammonium to lower the osmotic potential (Morgan, 1984; Gomes *et al.*, 2010) and to increase the cell osmotic pressure, taking water into the cells and tissues thereby contributing to turgor maintenance.

The observations on the plant biochemical properties at two months after grafting showed that the highest leaf proline content was yielded by 1 month rootstock old, followed by 2 and 3 months rootstock old (Fig. 5). The younger rootstock old used, the higher leaf proline content yielded. It could be due to the ability of water and nutrient exploitation at that stage is still worse, thus more sensitive to environmental stress conditions (water, temperature and humidity). The increasing of leaf proline content at young rootstock was expected to keep maintaining the plant turgor pressure.

The observation done also showed that whip grafting yielded leaf proline content higher than that of top cleft grafting (Fig. 5). The analysis of leaf proline content on whip grafting showed that the increasing its leaves proline content is a mechanism to respond to environment stress conditions. Proline and quaternary ammonium compounds such as glycinebetaine, choline, prolinebetaine is key osmolit, contribute to plant osmotic adjustment (Huang *et al*., 2000; Kavi Kishore *et al*., 2005). Proline accumulation is a plant response to environmental stress (Sairam *et al*., 2000). High levels of proline allows plants to maintain low water potential, thus allowing water to be taken from the environment. This condition will maintain water homeostatic in plant (Kumar *et al*., 2003).

Effects of grafting method on histological characters: 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 (Tekintas *et al*., 2008). 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 (Moore, 1984).

Some theories suggested that the phenomenon of cellular response might be involved in the development of vascular functional linkage since first callus formed. However, callus formation could be a passive response to the wound without implications for compatibility. Different reasons which may have an influence on the success of grafting are: Inherent system of cellular incompatibility, plasmodesmata formation, vascular tissue connection and the presence of growth regulating hormones and peroxidase. Phloem mobile proteins found at graft union which are convergence and steady connection to well metabolism functions.

Early detection of graft incompatibility in fruit trees is greatly facilitated since this process can be detected histologically within weeks after grafting (Errea *et al*., 1994). Histological observations were expected to describe the grafting incompatibility and swelling at graft union area. The study conducted by Goncalves *et al*. (2007) showed that there were a xylem histological structure variations between dwarf and vigorous rootstocks.

Histological observation found that grafting between 2 month rootstock old and 2 month old scions that used in this study showed that both had the same stem diameter will make the surface of graft union easy to be attached by each other, so the meristem tissue in between the contacted area would be attached perfectly. Furthermore, the grafting treatment used the oldest (3 months old) or the youngest (1 month old) rootstock will cause the meristem tissue in between cutting contacted area attached not perfect.

The new vascular elements formed in the grafting area of grafting combination between 2 months old rootstock and cleft grafting that had good compatibility were presented in Fig. 7 can be noticed a higher number of vessels with less necrotic layer at graft union. While in less incompatible of grafting combination between 2 months old rootstock and whip grafting could be noticed a lower number of vessels with big number of necrotic layer at graft union. These negative differences may explain vessels discontinuity between scion and rootstock and the blockage in water and nutrients transport.

Histological assay on plant stem of top cleft grafting and whip grafting using the 2 months old rootstock were presented in Fig. 7.

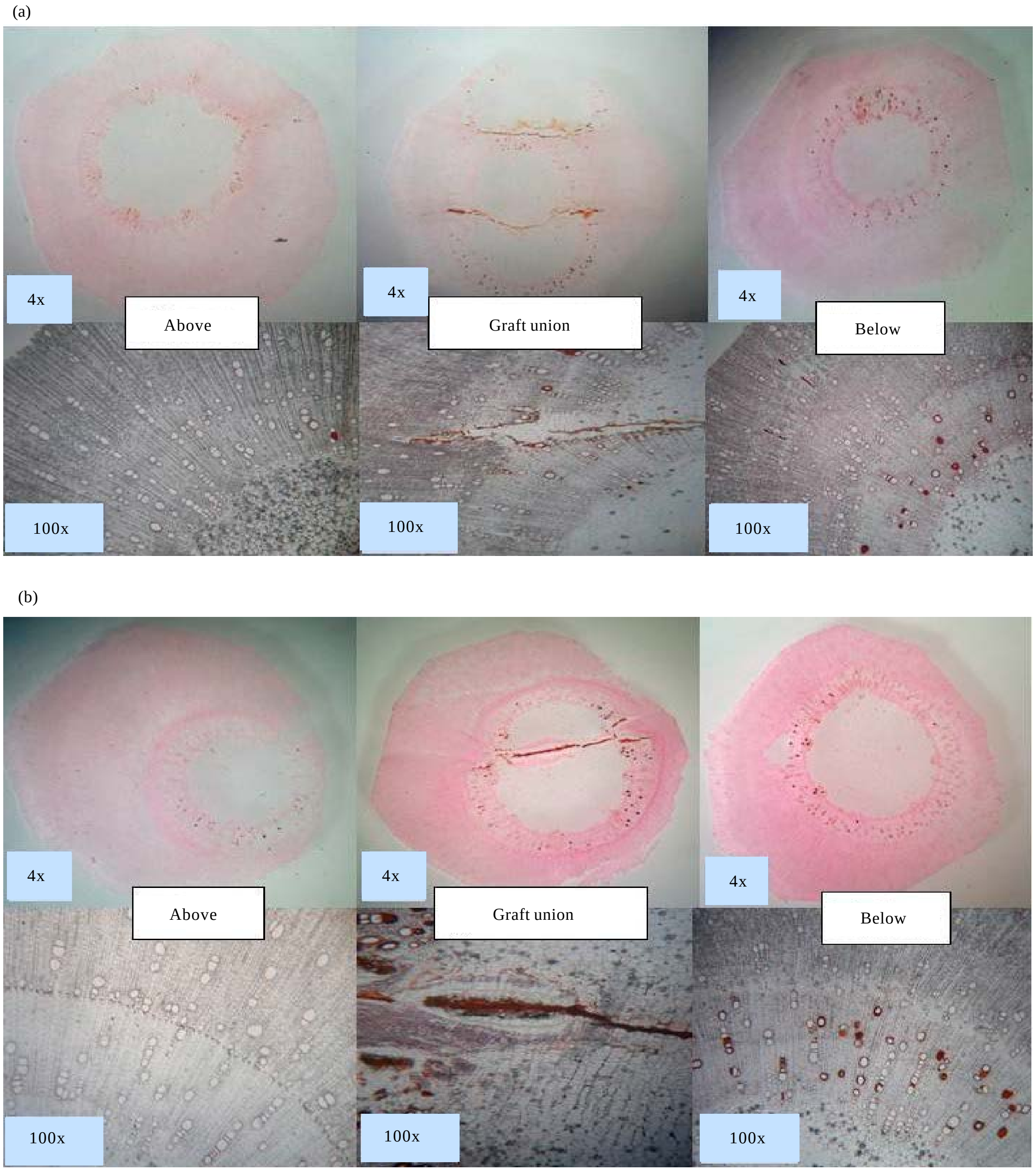

Fig. 7(a-b): Stem histology of cleft grafting and whip grafting of 2 months old seedlings in above, below and graft union. (a) Cleft and (b) Whip grafting

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 (Cohen *et al.*, 2007). 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 tesssue regeneration between scion and rootstock at graft union.

Based on the observation of morphological, biochemical and histological characters showed that treatment of three and two month old rootstock had better performance compared to one month old rootstock. Thus the two-month-old rootstock was selected as the best grafting time as it saved time and costs. Cleft grafting method gave better performance in all parameters were observed compared with whip grafting method.

CONCLUSION

The best grafting compatibility was provided by grafting combination between two months old rootstock and cleft grafting. The high grafting compatibility was expressed by the high leaves glucose content and litle gap of total sugar content between above and below graft union. Histologically assay demonstrated that compatibility was shown by the ability of tissue to regenerate and vessels of wound tissues to rejoint togetherthen grow and develope as a vigorous composite plant. Attemp to increase the productivity of grafted plant should be followed by the application of good agricultural practices.

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