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Original Article

Morphological and Anatomical Evaluation of Grafted *Pinus merkusii*[1]

Arida Susilowati[2,3,†] · Apri Heri Iswanto[2,3] · Imam Wahyudi[4] · Supriyanto[5] · Iskandar Z Siregar[5]

ABSTRACT

Morphological and anatomical evaluation of grafted *P. merkusii* have been undertaken to obtain the information about compatible and incompatible symptoms of 18 years old grafts based on morphological observation and microscopic analysis. Samples of compatible and incompatible grafts were obtained from previous research conducted by the Silviculture Departement Team in 1994. Result showed that compatible grafts have normal stem form and secondary growth (diameter growth), but some abnormality symptoms like undulated pattern of annual growth rings, phloem thickening and abnormality resin ducts in inner and middle parts of the union area occurred. Incompatible ones showed abnormality of the stem form, cortex-bark necrosis and swelling in the union area. Microscopic observation showed abnormality of all parts of the union, undulated pattern of annual growth rings, phloem thickening, abnormal resin ducts, low numbers and discontinuity of vascular elements in the union area.

Keywords : grafting, compatible, incompatible, morphology, anatomy

1. INTRODUCTION

Grafting is a vegetative propagation which is commonly used to multiply plants with certain characters in line with the expectations (Darikova *et al*., 2011). Propagation by grafting is more desirable because it maintains genetic stability and certain superior properties compared to generative propagation (Leakey, 1985). This technique is also applicable to certain species that have proved to be technically difficult to root, such as *P. sibirica* (Kolegova, 1977); *P. merkusii* (Widowati, 1994); *P. sylvisteris* (Anderson & Hattemer, 1978). For *P. merkusii*, grafting methods also prospective way to solve the qualities and quantities seedlings problem of high resin yielder genotype (Susilowati *et al*., 2013).

The success of grafting technique depends on the role of several factors namely the combination of rootstock-scion, propagation technique, environments condition during and after graft-

1 Date Received June 27, 2016, Date Accepted November 13, 2016
2 Faculty of Forestry, University of Sumatera Utara, Medan
3 Affiliated Scientist at SEAMEO BIOTROP
4 Forest Product Departement, Faculty of Forestry IPB, Bogor
5 Silviculture Departement, Faculty of Forestry IPB, Bogor
† Corresponding author: Arida Susilowati (e-mail: arida_iswanto@yahoo.co.id)

ing, growth activity of union material, contamination by pests and diseases, and the role of plant growth regulators (Hartman *et al.*, 1990). In addition to these factors, genetic factors, physiology and anatomy of grafting material also determine the compatability and the forming of graft unions (Leakey, 1985). Furthermore, Mustard & Lynch (1977) proposed that the success of grafting technique was also very dependent on the presence of callus tissue proliferation between union area components and the existence of vascular tissue which was the origin of callus forming. Therefore, evaluation of graft compatibility is required as well as morphological and anatomical studies.

There is lack of information on anatomical and histological studies about the successful union in grafted *P. merkusii*. Some references only discussed graft incompatibility problems on other conifers (Mergen, 1954; Dormling, 1963). Specific studies about incompatibility and anatomical symptoms were proposed by Copes (1967; 1970) but on Douglas-fir. The results of those studies found that the union area during early growth of the grafted conifers occurred in several stages: the forming of the contact layer, cell enlargement, callus formation, phellogen layer development and finally cambium formation (Esau, 1965; Copes, 1969). However, the information about union compatibility on grafted mature trees is rare particularly in tropical wood species. Under these conditions, our research aimed to obtain information about the compability and incompability of 18 years old grafted *P. merkusii* both morphologically and anatomically through the histological study of formed wood.

2. MATERIALS and METHODS

2.1. Materials

An 18-year-old top-cleft grafted *Pinus merkusii* which had been planted in a greenhouse at Silviculture Department, Faculty of Forestry, Bogor Agricultural University was provided as the experimental material. The tree was a result of research conducted by Silviculture Departement researchers in 1994. The rootstock originated from KPH Cianjur and the scion originated from the Tapanuli strain. Based on morphological observation for compatibilities, samples were then selected into 2 (two) categories that were either compatible or incompatible.

2.2. Methods

2.2.1. Sampling and data collecting

The sampling was conducted by piercing the stem at breast diameter height horizontally at union areas on four cardinal points using a 0.5 cm diameter and 30 cm length increment borer. The length of the samples were up to the pith limit. Furthermore, the union was separated into three parts; the outer, middle and inner wood so that a total of 24 samples was obtained. Morphological observation was conducted by observing the scion, union and rootstock conditions of each sample tree for the stem performance and the diameter increment.

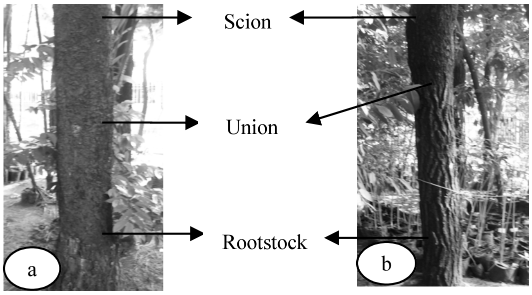

Fig. 1. The morphological performance of the stem, compatible graft (a) and incompatible graft (b).

2.2.2. Microscopical observation

The study of the graft anatomical structure was conducted by microscopical preparation according to the Sass Method (1960) with some modifications. Samples were soaked in a solution of PEG 2000 and ethanol in the ratio 1 : 5 to avoid wood cells damage when sliced. The observation of union condition was done by using *Axio Imager Almzeis* microscope with magnification set at 2.5-40 times to obtain clearer images. Following observation photographic documentation was made with the camera integrated into the microscope.

4. RESULTS and DISCUSSION

4.1. The morphology of the stem on compatible and incompatible grafts

The morphological observations of the compatible graft showed that the lower and upper parts of stem was fully joined as in generatively propagated individuals. The diameter increment was also relatively similar. Some specific morphological features compared to rootstock and scion were found in the union area. The bark was relatively smoother and also scarred at the border of the scion and rootstock (Fig. 1(a)). Generally, the stem was straight.

The observation on incompatible graft showed that the lower stem was smaller than the upper stem (abnormal). The diameter at breast height was also smaller compared to other normal pines of the same age (Fig. 1(b)). Some previous research proposed that the stem abnormality was a common symptom found on incompatible grafts. According to Hartman *et al.* (1997), the stem abnormality resulted in different growth dimensions between lower stem (root stock) and the upper stem (scion). The different growth dimensions were caused by time differences in vegetative growth between upper and lower stem. In this study it was concluded that scion had overgrown thus the stem was larger. Abnormalities of bark and the cortex area were also observed with swelling at union (Fig. 1(b)), but these are common symptoms of grafted trees (Copes, 1980; Ahlgren, 1971). Other than stem abnormalities, incompatibility symptoms on conifer were also shown by the short leaves that easily fall and disturbed cone form (Copes, 1980), but those features were not observed in this study.

The incompability symptoms did not always include graft necrosis at the early union process. However, some grafts were able to grow as one new individual until they had matured although stress and abnormal growth patterns were seen. Furthermore Hartmann *et al.* (2002) and Copes (1969) proposed other symptoms such as the decline in vegetative growth, early necrosis of trees, the growth difference or vigor between scion and rootstock, vegetative phase difference between scion and rootstock, dwarfed rootstock and clear faults between zones of union.

The diameter growth on compatible grafts showed a normal pattern as in 18-year-old generative propagated pine. Breast height diameter of a compatible graft and incompatible graft were 23.5 cm and 11 cm, respectively. To support the morphological evaluation, anatomical observation of union structure, both cross section and longitudinal section were conducted.

4.2. Union forming on pine graft

The observation on radial board sortiment of matured graft (Fig. 2(a)) showed that the former scion pith is still attached on the rootstock (A), while the lower part of scion was immersed in the wood (C). These features indicated that even though the grafted tree was not able to heal graft scars (B) perfectly, it was still capable to grow and produce the new wood around the scars. The genetic difference between rootstock and scion was also seen at the newly wood formed (D) as represented by a clear different line between rootstock and scion. This line could be seen at the border between the upper part of rootstock and the lower part of the scion.

Similar conditions were observed on the high resin yielder grafted seedling (Fig. 2(b)) on a matured graft. The callus formed around union and immersed scion on the rootstock (B) was still present. The pith tissue on the border of scion and rootstock had also degenerated (brown and then becoming necrotic) because it did not contain meristematic cells and thus could not perform cell division.

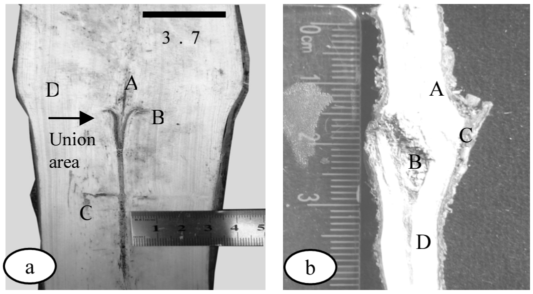

Fig. 2. Performance of radial cross-section of stem sortimen with 2x magnification, 18-year- old Aceh and Kerinci strain grafted pine (a), 1-year-old high resin yielder grafted seedling (b).

It was presumed that differences in the height of the initial union could affect the union forming pattern on the graft. Our results indicated that the lower height was able to produce an individual grafting with more perfect union, showed by straighter stem morphology. The observation on radial section at different heights of unions showed almost similar union forming patterns. Although the union was perfect, there were still immersed scion found on the rootstock and unification scars on different union height (Fig. 3).

Some anatomical studies on union zones had been undertaken by Barnett and Miller (1994); Barnett and Weatherhead (1988); Copes (1999) and Evans (1972). The results concluded that union graft was initiated through rapid division of scion and rootstock callus cells. It was com-

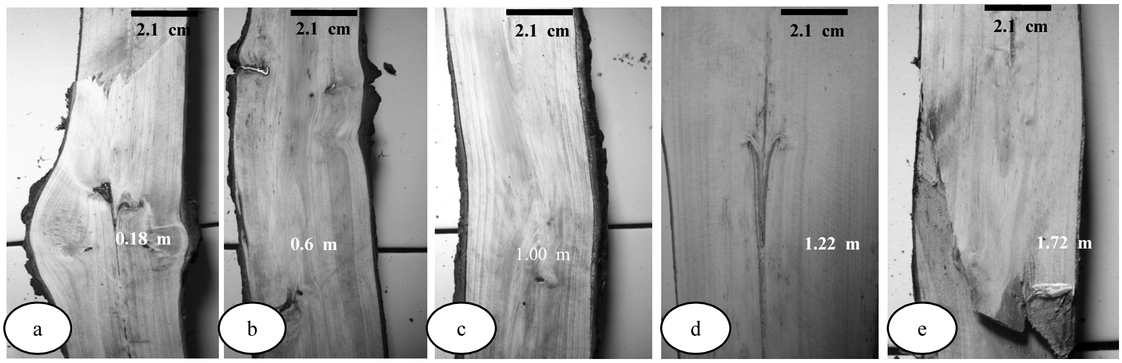

Fig. 3. Performance of the union (interface) of graft at different heights; interface area at 0.18 m of height (a) interface area at 0.60 m of height (b), interface area at 1.00 m of height (c), interface area at 1.22 m of height (d), interface area at 1.72 m of height (e).

pleted with differentiation process by the formation of vascular cambium (meristematic lateral) and then unification with the current vascular system. This happened because cambium cells had meristematic properties that can perform both functional and structural fusions. After the union growth fused the next 3 processes namely rootstock and scion adhesion, proliferation of callus cells at the union zone or callus bridge, and union zone differentiation were performed (Moe and Andersen, 1982). The success of the process of proliferation and differentiation would henceforth determine the tree growth.

On grafting, scions would not grow normally if the vascular tissue at the union zone was not developing properly. On the other hand, if the phloem on union zone was disturbed, carbohydrate and other metabolites from scion to root system would also be disturbed causing rootstock degeneration. This probably was the reason why compatible and incompatible grafts had different growth pattern. Basically, incompatibility occurred as a result of disharmonious growth between scion and rootstock cambiums. If the rootstock grew faster than the scion, the lower part of the union would be larger (Fig. 3(a)). In the contrary if the scion grew faster than the rootstock, the upper part of the union would be larger (Fig. 3(d)). The union was not formed perfectly on the part where scion and rootstock made contact; thus it became necrotic (Fig. 3(b)). According to Jayawickrama *et. al.* (1997), the scion is a very important part of a grafted tree. The scion would determine the properties of the grafted tree, included its growth and reproduction.

Cellular studies on the effect of grafting on tree growth have been conducted by Pina *et al.* (2009) who concluded that the compatibility of rootstock-scion cells and intercellular communication were the deciding factors for the success of grafting. One of the important features in successful graft intercellular communication was the existence of plasmodesmata. The plasmodesmata is a channel on cells wall, acting as a

Table 1. Macroscopical characteristics of compatible and incompatible grafts

Observation variable	Part	Compatible (∅: 23.5 cm)		Incompatible (∅: 11 cm)	
		Length (cm)	Percentage (%)	Length (cm)	Percentage (%)
New wood cells	union 2	12.2	51.9	8.5	77.3
	union	6.6	28.09	3	27
	rootstock	0.3	2.55	3	27
Damaged wood cells	union 2	None	0	8.5	77.3
	union	None	0	3	27
	rootstock	None	0	3	27
Seperated rootstock pith	union 2	None	0	0.7	6.3
	union	None	0	0.7	6.3
	rootstock	None	0	None	0
Necrotic cells	union 2	None	0	1.6	14.5
	union	None	0	0.4	3.63
	rootstock	None	0	0.1	0.9
Unpatched union	union 2	None	0	1.6	14.5
	union	None	0	1.6	14.5
	rootstock	None	0	1.6	14.5

pathway for solutions and macromolecules among the cytoplasm of surrounding cells. The incompability of grafts is related to low intercelluler transport capacity and the low number of functional plasmodesmata resulting in growth being disturbed.

4.3. Macroscopical Anatomy of Rootstock and Union Areas

General macroscopical observations in this study was performed at the union scar and scion-rootstock border. On compatible grafts the new wood was formed around the union and bark area indicating that the tissues of scion and rootstock had united. No significant damage was found but the scion scar was still found immersed in the rootstock, buried and united with surrounding cells. The border between scion and rootstock in the union area was indicated by the color difference of wood and the newly formed.

Incompatible grafts bark was found tucked in the edge of the union, indicating rootstock and scion barks had not united. One side of former rootstock pith was found separated from the scion pith (scions pith was sticking out towards the bark on the right side), causing asymmetrical growth increments. There was a clear wood border between scion and rootstock on compatible grafts. The wood properties on a compatible grafting union area followed the scions character which was seen to be the same wood color. Meanwhile on incompatible grafts most wood which had been formed followed that of the scion although some still followed

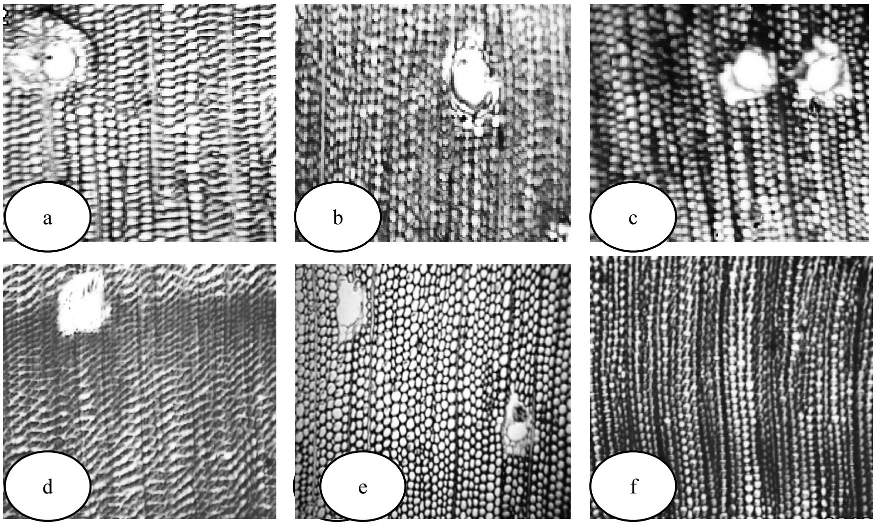

Fig. 4. Anatomical appearance of cross section; outer wood of compatible graft (a), middle wood (b), inner wood (c) and outer wood of incompatible graft (d), middle wood (e), inner wood (f).

that of the rootstock. The comparison of macroscopical characteristics of compatible and incompatible grafts is presented below (Table 1).

Incompatible grafts lacked the formation of new cells and overall the new cells that formed were damaged. This finding is consistent with those of Mckeand & Raley (2000) who stated that new cells formed were derived from callus in the union scar area and was a positive response and one of the macroscopical indicators of union success, while in incompatible grafts this phenomenon was very rare.

4.3.1. Cross-section

The appearance of union cross-sections from both graft types showed different results. On compatible grafts there were some abnormalities such as phloem thickening which differed with those in the surrounding areas (Fig. 4(a)), an undulated pattern of annual growth rings on the inner union (Fig. 4(c)) and abnormal resin duct structure (Fig. 4(b)) still occurred. Most abnormalities in compatible grafts were found in the inner and middle parts which, the contact areas of the scion and rootstock.

Similar symptoms were also recognized in the incompatible union. Undulated annual growth rings in the inner union (Fig. 4(f)), phloem thickening (Fig. 4(e)) and abnormal resin duct structure (Fig. 4(d), 4(e)) on almost all of outer, inner and middle areas of wood. This symptom was presumed to be one of the reasons to cause the slow growth. Similar symptoms have been reported by grafting result evaluation on other cloned pine seed orchards on its 4 and 8-year-old grafts (Copes, 1980).

4.3.2. Tangential section

Tangential sections of the union on both grafts also showed the different results.

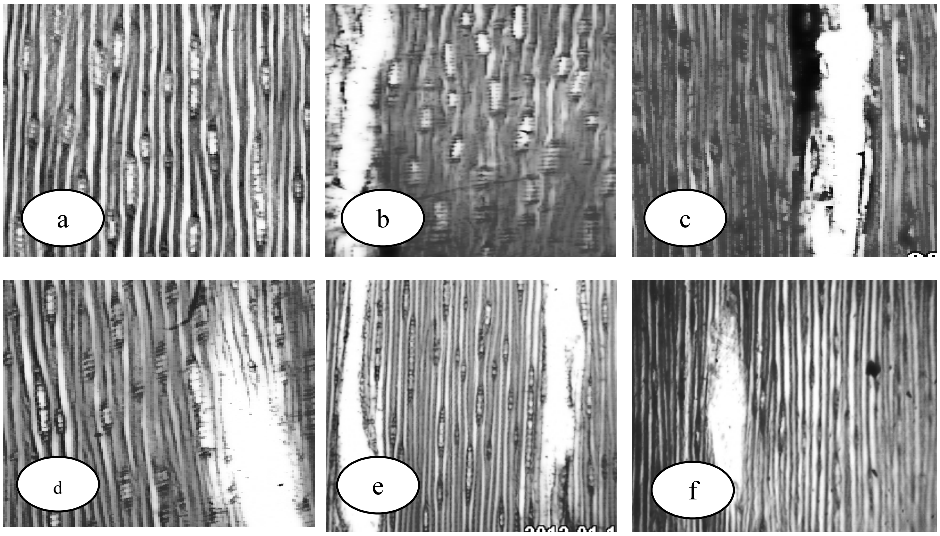

Fig. 5. Anatomical appearance of longitudinal section; outer part of compatible graft (a), middle wood (b), inner wood (c) and outer part of incompatible graft (d), middle wood (e), inner wood (f).

Tangential section of compatible grafts still showed abnormalities of radius cells, in particular at middle part of union, although the outer part of the union looked normal. On this location very thin new cells, and soft wood tissue which damaged when cut were observed (Fig. 5(b), 5(c)). These thin cells were presumably a result of callus tissue proliferation at the union which would build a cambial bridge between the scion and rootstock. Furthermore, it would form secondary vascular tissues in the stem. New soft cells indicated that the recovery of the union scar was still progressing. Anatomical performance of new wood at the union area (interface) was following the pattern of the scion.

The radius cells abnormalities in incompatible grafts were found on all parts of the union (outer, middle and inner). Abnormal cells were also found and these are presumed to originate from former scion material of the ungrown union (hard tissue) and with no soft tissue cells around it. This is in agreement with Pina *et al.* (2009) who stated that graft incompatibility could be recognized by the lack of vascular elements, discontinuity of vascular tissue, and phloem degeneration in the union area. They also stated that the lack of vascular tissue, discontinuity of vascular tissue and phloem degeneration caused disturbance of nutrition and water transfer through the xylem and photosynthate transfer through phloem. Furthermore the low number of plasmodesmatal formed during the initial growth was one of the causes of incompatibility because it was a pathway to the cell wall for macromolecules and solutions from the surrounding cells (Pina *et al.*, 2009; Darikova *et al.*, 2011).

On grafting technique, the probability occurrence of incompatible union was very large due to various causal factors. For this reason early detection of the graft type to minimalize incompatibility in the grafted trees is of prime importance. Some recent studies have been successful in detecting the presence of phenol and other compounds in the union area as indicator of successful union (Errea *et al*., 2001; Mng'omba *et al*., 2008; Zarrouk *et al*., 2010). The utilization of biochemical markers such as phenol, starch, polypeptide and peroxidase activities are being researched with the prospect of detecting union success at an early stage of growth.

5. CONCLUSION

The morphological and anatomical evaluation of compatible grafts exhibited a relatively straight stem and normal diameter growth and were similar to the pine growth by generative propagation. However anatomical examination revealed abnormalities such as undulated annual growth rings, phloem thickening, abnormal resin ducts in the inner and middle parts of the union area although the outer part was relatively normal. New soft tissue in the union was also found, presumably being a result of callus tissue proliferation in the graft union which would form a cambial bridge between the scion and rootstock and furthermore would form secondary vascular tissue in the stem.

Incompatible grafts showed abnormality of stem form (asymmetric, curved and unproportioned) as a result of different growth dimensions between the scion and rootstock with overgrowth symptoms on one of the unions, cortex and bark necrosis and swelling in the union area. Anatomical examination demonstrated abnormality from all parts of the union (outer, middle and inner), undulated pattern of annual growth rings, phloem thickening, abnormal resin ducts, and lack of numbers of vascular elements. These are considered to be causal factors of the slow growth occurred in incompatible grafts. The detection of biochemical markers such as phenol, starch, polypeptide and peroxidase is considered to be potentially promising for the early recognition of incompatible grafts and improving the success rate of grafting in pine trees.

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