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Anatomical and Physiological Characteristics of Reclamation Plant on the Post-Mining Land in Riding Panjang, Bangka

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ABSTRACT

Tin mining leaves disturbed land with sandy texture, damages natural drainage and habitats, and very low water holding capacity. The enrichment of species selection criteria for revegetating tin tailing with anatomical and physiological characters is needed to facilitate decision maker to select local species suitable for revegetation and to meet the economical need of the local people in post tin mining era. Besides exotic species, a handful of local tree species was planted recently. The need of more local species is especially needed to support biodiversity in mined land. Study of some leaf anatomical and root physiological characters of twelve months seedlings of leban (Vitex pinnata L-Verbenaceae) and ubak (Syzygium grande (Wight) Walp.–Myrtaceae) planted in unmined land and tin-mined land in Bangka Island was conducted using wholemount and paraffin methods. The findings showed that anatomical and physiological parameters of V. pinnata are more significant than those of S. grande. Therefore, V. pinnata is more adaptive grown on tin tailing than S. grande.

Keywords: *Stomatal density, leaf tissue ratio, root xylem conductivity, revegetation, tin tailing*

ABSTRAK

Penambangan timah meninggalkan lahan terganggu dengan tekstur pasir, merusak drainase dan habitat alami, dan daya ikat air yang sangat rendah. Upaya memperkaya kriteria seleksi tanaman untuk merevegetasi tailing timah dengan karakter anatomi dan fisiologi dibutuhkan untuk mempermudah pengambil kebijakan dalam memilih jenis lokal yang sesuai untuk revegetasi dan memenuhi kebutuhan masyarakat lokal di era pasca tambang timah. Di samping jenis eksotik, beberapa jenis lokal telah ditanam akhir-akhir ini. Kebutuhan lebih banyak jenis lokal terutama untuk mendukung keanekaragaman hayati di lahan pasca tambang. Penelitian beberapa karakter anatomi dan fisiologi dari semai berumur setahun dari leban (Vitex pinnata L-Verbenaceae) dan ubak (Syzygium grande (Wight) Walp.–Myrtaceae) yang ditanam di lahan tidak terganggu dan lahan pasca tambang timah di Bangka dilakukan dengan metode wholemount *dan* paraffin. *Hasil penelitian menunjukkan bahwa parameter anatomi dan fisiologi V. pinnata lebih signifikan dibandingkan parameter yang sama dari S. grande. Karenanya, V. pinnata lebih adaptif tumbuh di lahan pasca tambang timah dibandingkan S. grande.*

Kata kunci: *Kerapatan stomata, nisbah jaringan daun, konduktivitas xilem akar, revegetasi, tailing timah*

INTRODUCTION

Bangka is the largest tin producing island in Indonesia, contributing 40% of world demand of tin.[1] Tin mining leaves disturbed land with sandy texture, damages natural drainage and habitats, and very low water holding capacity. The total of mine-impacted lands, including other marginal lands, in the province totals 1,642 ha[2], or even more than 5,000 ha if those which have been already reclaimed and revegetated are included.[3] Sand tin tailings may have 95% sand, C-organics less than 2%, cation exchange capacity less than 1.0, its soil temperature may reach 45°C [4], and phosphate solubilizing bacteria and arbuscular mycorrhizal fungi readings were reported low.[5]

Reliance on natural succession to restore sand tin tailings without any human aid can be very slow.[5,6] From soil physical and chemical data simulation, heath forest restoration in Singkep Island was predicted takes 150 years.[7]

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A number of exotic species such as *Acacia mangium* has been widely used in rehabilitation programs since 1992 but ecological caution suggests it is unwise to continue to rely on such a limited species mix for all future rehabilitation efforts[8] as they may inhibit natural recolonisation. Therefore, the use of exotic species is gradually left. On the other hand, the use of local species has not been maximally developed to revegetate tin-mined lands in Bangka Island. Van Steenis mentioned *Ploiarium*, *Rhodamnia*, and *Rhodomyrtus* as plants to revegetate tin-mined land.[9]

Anatomical and physiological characters of three pioneer species i.e. *Trema orientalis*, *Commersonia bartramia*, and *Mallotus paniculatus* on tin-mined land was reported. From the study, *T. orientalis* was more adaptive on tin tailing than the other two with its root xylem conductivity was more significant on tin-mined land than the other two. To add more adaptive local tree species grown on tin-mined land, this study was conducted. The aim of this study is to find anatomical and physiological characteristics of reclamation plant on tin-mined land in Bangka. The result would enrich local plant selection criteria for revegetation disturbed land in general and to tin-mined land in particular.

METHODS

Samples were collected from the two hectares revegetated tin-mined land located at Riding Panjang, Bangka Island (lat. 01°59'53.46"S; long. 106°06'45.32"E; 30 m asl.) and 0.02 hectare unmined land (lat. 01°51'49.0"S; long. 106°07'09.5"E; 30 m asl.). Mean annual rainfall (1996–2005) was 2,408 mm, and temperature ranges from 23.8–31.5°C with an average of 26.8°C.[10] Laboratory preparation and observation were conducted at Laboratory Plant Anatomy and Physiology, Departement of Biology, Institut Pertanian Bogor, Bogor.

Plant tissue sampling

Leaf and root samples of 12 month old after planting leban (*Vitex pinnata* L.–Verbenaceae) and ubak (*Syzygium grande* (Wight) Walp.–Myrtaceae) from mined and unmined sites were collected. Leaf samples were collected from three branches each individu with three individu per species as repetition. For paradermal cut preparation, one leaf from the fourth position from apex was taken and fixed with 70% alcohol. For transversal cut preparation, one leaf from the third position from apex was taken and cut 1 x 1 cm and fixed in FAA solution (5:5:90 of formaldehid, asetate acid glacial, and 70% alcohol) in plastic bottle for two days. Primary root tip samples were transversally cut ± 0.5 cm. Root samples were collected from three individuals each species as repetition. Those samples were put in plastic bottles, and fixed in FAA solution for four days.

Plant tissue preparation

Paradermal cut was prepared in a semi permanent slide and colored with 1% safranine according to wholemount[11] method by Sass through steps: fixation in 70% alcohol, washed and soaked in aquadest, softened with 30% HNO_3 solution for 24 hours, washed with aquadest, sliced with knife, chlorophyll extraction with chlorine solution in few minutes and then washed with aquadest, colored with 1% safranine for 3-5 minutes, and covered with cover glass with 10% glicerine. Transversal cut was prepared according paraffin [12] method by Johansen using a series of Johansen TBA solution as dehidrant.

Data collection and analysis

T-test was run to compare every variable measured from plants grown in mined and unmined sites. Leaf anatomy variables i.e. stomata density, stomata length and width, leaf thickness, palisade thickness, sponge thickness, and epidermal thickness, and leaf tissue ratio (palisade thickness divided by leaf thickness). Root anatomy variables i.e. root conductivity (total area of xylem bundles), and root conductivity ratio (root conductivity divided by cross section of root area).

RESULTS AND DISCUSSIONS

Leaf anatomical structure

Both stomata of leban leaf (*V. pinnata*) and ubak leaf (*S. grande*) has anisositic type and hypostomatic type or abaxial.

Stomata density of *V. pinnata* grown on mined site was smaller (431 mm^{-2}) and significantly different from those on unmined site (586 mm^{-2}), while those of *S. grande* grown on mined site was bigger (610 mm^{-2}) significantly different from those on unmined site (539 mm^{-2}) (Table 1) (Fig. 1 and 2).

The increase of stomatal density and significantly different on mined site compared to those on mined was shown at *Trema orientalis* and *Commersonia bartramia*.[13] These findings similar to Willmer's[14] that plants grown on dry area which has abundant sunlight has bigger stomatal density compared to those on humid and sheltered condition.[14-16]

The increase of stomatal density is to compensate the decrease of leaf are of those which suffer water stress to reduce transpiration.[16] Besides, water stress reduces stomata development as the condition hampers differentiation of

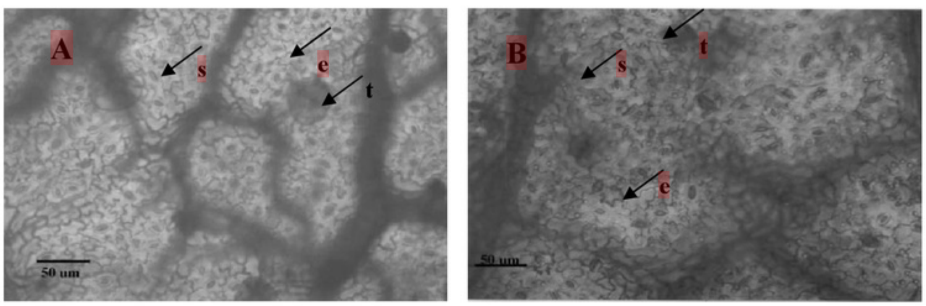

Figure 1. Lower paradermal of *V. pinnata*, A = unmined; B = mined site; e = epidermal cell; s = stoma; t = trichome

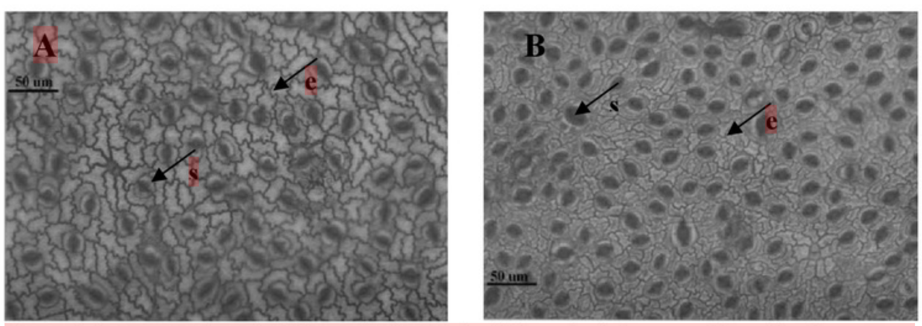

Figure 2. Lower paradermal of *S. grande*, A = unmined; B = mined site; e = epidermal cell; s = stoma

Table 1. Stomatal density, leaf thickness, palisade thickness, sponge thickness, epidermal thickness, and leaf tissue ratio of *V. pinnata* and *S. grande* grown on unmined and mined sites.

Parameters	Unit	V. pinnata		S. grande	
		unmined	mined	unmined	mined
Stomatal density	mm^{-2}	585.88	430.7 *	539.11	609.78 *
Leaf thickness	µm	168.63	176.83 *	363.5	373.3
Palisade thickness	µm	74.36	72.46 *	107.76	104.56 *
Sponge thickness	µm	65.4	68.06 *	218.63	225.73
Upper epidermal thickness	µm	16.36	18.86 *	18.03	18.63
Lower epidermal thickness	µm	11.26	15.76 *	13.36	13.46
Leaf tissue ratio		0.08	0.1 *	0.04	0.04

Asterix (*) indicates that means was significantly different between treatments (0.05) guard cells.[17] Similar findings are also reported in some soybean (*Glycine max*) genotips[18], *Vinca rosea*[19], and *Lotus creticus* [20]. On the other hand, stomatal density of plants exposed to water stress which is lower or similar to those on undisturbed site shows adaptation to water stress.[18,21]

Leaf thickness, palisade thickness, sponge thickness, and epidermal cells thickness of *V. pinnata* leaf grown on mined site were bigger than those on unmined site. In *S. grande* leaf only palisade cells was bigger than those on unmined site (Fig. 3 and 4). Thicker palisade cells of plants grown on water stress environment might shown their tolerant to water stress.[18] Palisade cells enlarged their cells on water stress environment[18] and chloroplast increase of those on water stress environment might take place in enlarged palisade cells.

Leaf thickness changes on some tissues cause leaf tissue ratio. At *V. pinnata* the leaf tissue ratio increases but unsignificantly different at *S. grande*. Tolerant genotip has low leaf tissue ratio.[22,18] This finding supports plant growth in the field that the growth and survival of *S. grande* were higher than those of *V. pinnata*.

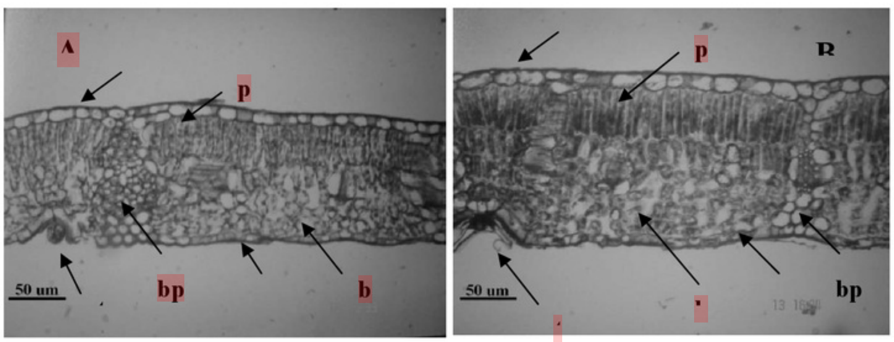

Figure 3. Leaf transversal of *V. pinnata*, A = unmined; B = mined site; e = epidermal cell; p = palisade tissues; b = sponge tissues; t = trichome; bp = bundles

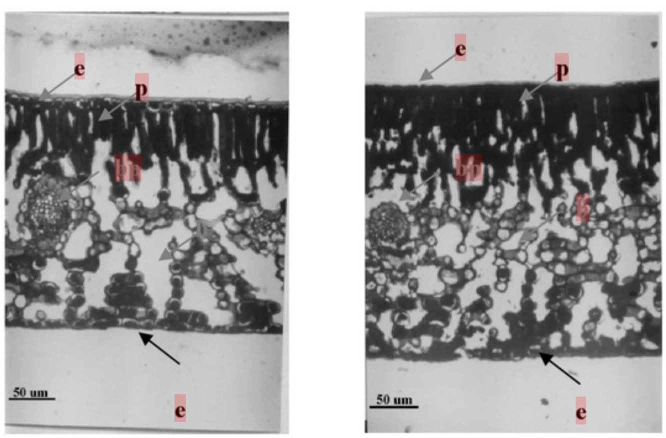

Figure 4. Leaf transversal of *S. grande*, A = unmined; B = mined site; e = epidermal cell; p = palisade tissues; b = sponge tissues; t = trichome; bp = bundles

ROOT CONDUCTIVITY

Root conductivity and root conductivity ratio of *V. pinnata* grown on mined site were bigger than those on unmined site. At *S. grande* root conductivity on mined site was smaller and significantly different than those on unmined site, but, root conductivity ratio grown on mined site was bigger and significantly different than those on unmined site (Table 2) (Fig. 5 and 6).

The average number of xylem bundles of *V. pinnata* grown on mined site and unmined site were 99 and 69 respectively, while those of *S. grande* grown on mined site and unmined site were 72 and 82 respectively. This means that there is an increase of root xylem density on mined site but decrease its diameter of *V. pinnata*, while the opposite shown at *S. grande*. Both species showed the increase of leaf tissue ratio on mined sites. Water stress on *L. creticus* causes the increase of root and shoot xylem density.[20] The increase of root xylem density and diameter might be a kind of response to water stress. The less water availability might be followed with the more number and diameter of root xylem bundles.

Table 2. Root conductivity and root conductivity ratio of *V. pinnata* and *S. grande* grown on unmined and mined sites

Parameters	Unit	V. pinnata		S. grande	
		unmined	mined	unmined	mined
Root conductivity	μm^2	124,796	500,602 *	374,537	191,446 *
Root conductivity ratio		0.06	0.11 *	0.10	0.11 *

Asterix (*) indicates that means was significantly different between treatments (0.05)

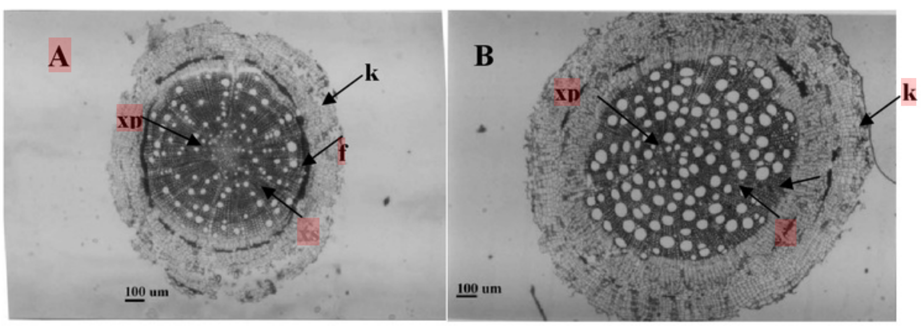

Figure 5. Root cross section of *V. pinnata*, A = unmined; B = mined site; k = cortex; xs = secondary xylem; xp = primary xylem; f = phloem

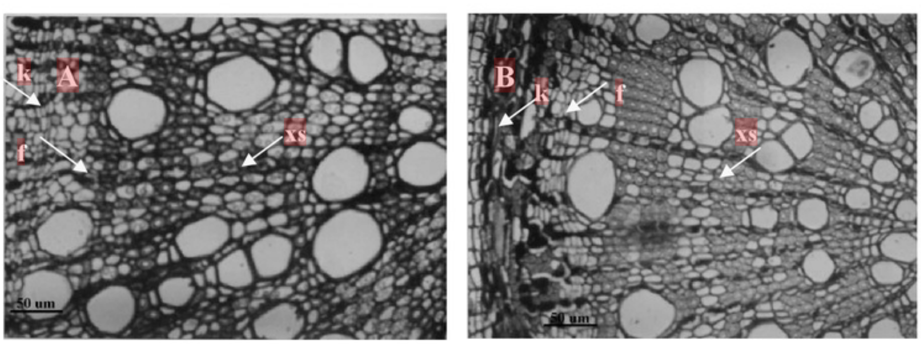

Figure 6. Root cross section of *S. grande*, A = unmined; B = mined site; k = cortex; xs = secondary xylem; f = phloem

The twelve month after planting monitoring data showed that *S. grande* has better canopy cover (0.25 m^2) and survival rate (90.2%) compared to canopy cover (0.07 m^2) and survival rate of *V. pinnata* (68.8%).[4] This data suggests that more morphological and physiological variables need to be added to draw a more accurate conclusion. To identify whether a species is sensitive or tolerant to water stress, it is valuable to study the free proline concentration.[19]

CONCLUSIONS

There is response combination of stomatal density, leaf tissue ratio, and root conductivity ratio of plants grown on mined site. Those might enrich plant selection criteria for revegetating tin tailing. Based on the findings, *V. pinnata* is more adaptive grown on tin tailing than *S. grande*. Further study is required to validate this result by measuring more morphological and physiological parameters, as well as free proline concentration.

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