Each individual fibre is a plant cell. Its main characteristics are: (1) a very significant length (from a few hundred micrometers up for a number of centimetres) with the ratio between the length and diameter of cells ranging from 50 to 2000 or even more; (2) unusually thick cell wall, reaching up to 15 μm; and (3) mechanical function [4], [5], [9], [10],[11]. Phloem fibre plants, including marijuana (Cannabis sativa), has been used by humans since ancient times and now has a wide range of applications, both traditional and modern [12], [13],[14],[15]. Long and strong ramie fibres have a rich history of use for the production of ropes, paper, and textiles.

Ramie fibers are extracted from the stem of the herbaceous perennial plant *Boehmeria nivea* of the nettle family, native to eastern Asia. Bast fibres of ramie are soft extraxylary fibers, or aggregates [16]. The bundles consist of 10 to 25 elementary fibers, with a diameter of 10 - 50 μm.

Ramie has been used to produce an open weave fabric called mechera, which is suitable for warm climates. In the early 20th century the French painter Raoul Dufy designed patterns for prints on mechera that was used by the French shirtmaker Charvet [17]. Brazil began production in the late 1930s with production peaking in 1971. Since then, production has steadily declined as a result of competition with alternative crops, such as soybeans and the important synthetic fibres.

Nowadays, ramie fibres are successfully used in innovative technologies, such as reinforcement in composite materials, automotive industry, and biodiesel [14], [18], [19], [20], [21]. In addition to practical purposes, the fibre plant phloem interesting for fundamental studies of plant cell growth and cell wall formation, showing off (interrupt) a special type of elongation [5], [6], [10], [22], and in various types of fibre gelatin special cell walls tertiary which can function as a 'muscle plants' stems and pull the top and branches with shortening-cell fiber [10], [23], [24], [25].

According to Hearle and Peters, the chemical composition of ramie fibers is 91-93, 2.5, 0.63 and 0.65% of cellulose, hemicelluloses, pectin, and lignin respectively [5]. However, a more recent study suggests that the lignin content may be as high as 6-7% and hemicellulose as high as 13-16% [26]. Ramie fibers exhibit excellent mechanical properties, superior among bast fibre types (45-88 cN/tex) and, like most natural cellulose fibres, the strength increases by 25% when fibers are wet. Fibre diameter is generally 40-60 μm [27].

Fibre cells are durable and have good resistance to bacteria, mildew and insect attack. The main disadvantage of ramie is its low elasticity (elongation at break is 3-7%), which means that it is stiff and brittle. Fibres are oval to cylindrical in shape, translucent and high lustrous. The fibre surface is rough and characterized by small ridges, striations, and deep fissures. Ramie fibre can be easily identified by its coarse, thick cell wall, lack of twist, and surface characteristics [16].

Previous researchers [28], [29] proposed that besides plant morphology (stem diameter, leaf density, stem branching) and anatomy (cell diameter, cell wall thickness) several growth conditions (sowing date, climatological factors, plant density, nutrients, and harvest date) affect the quality of fibres. However, these effects have not been studied in detail in the *Boehmeria nivea* plant. Extensive data about the biological characteristics of local Indonesian clones of ramie and optimal age for harvest has yet to be obtained.

This study investigates the changes of anatomical characteristics over time of five clones of ramie. It was hypothesised that there would be a variation in the growth of different components of each clones' anatomy, especially the growth of the fibre and this would affect the optimum age of harvest.

II. Material and Method

A. Plant material

We obtained and cultivated five clones of ramie in adjacent rows in the Agricultural Faculty experimental field station of Andalas University in Padang *i.e.* clones of Bandung A, Indochina, Lembang A, Padang 3, and Ramindo. Samples were taken every week for 8 weeks to measure the length and fibre growth of an outermost layer of the stem.

Fiber samples were macerated with 10 ml of 67% HNO_3 then boiled in a water bath ($100^o \pm 2^0$C) for 10 min [30]. The resulting slivers were then washed in distilled water. They were then placed in small flasks with 100 ml of distilled water, and the fibre bundles were separated into individual fibers using a small mixer. The macerated fibre suspension was finally placed on a standard microscope slide by means of a medicine dropper. For fibre diameter, lumen diameter and cell wall thickness determination, cross-sections were obtained between nodes along the stem at points corresponding to the height of the plant at each week. Hence, after five weeks, five cross sections were taken from each individual stem sample. These were stained with fast green and safranin.

All fibre samples were viewed under a calibrated microscope; a total of 25 randomly chosen fibres were measured from each sample; a total of 75 fibere measurements from each clone of ramie. Measurements were taken of length, diameter and wall thickness of fibres of the five clones of ramie following the categories that have been reported by previous researchers on similar fibre crops [30].

B. Cross-section

Samples were prepared by cutting a very thin slice from each fresh stem of ramie sample using a scalpel and placing the sample on a glass microscope slide, covered with a drop of water and a cover glass. A total of ten randomly chosen bast fibres bundles were measured from each sample; a total of 30 bast fibre bundles measurements from each clone of ramie.

III. Results and Discussion

A. Results

1) Fiber type

Fibres are classified according to their shape at the point of the cross-section; type I being rounded, type II flattened on the tangential cell wall and type III flattened on the radial cell walls (Figure 1). The number of fibres of each type was counted.

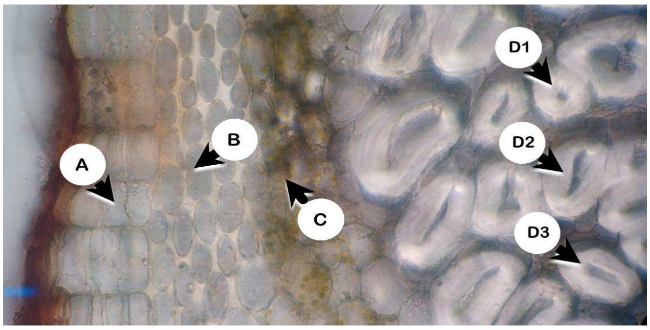

Fig. 1. Transverse section of an outer layer of *Boehmeria nivea* stem showing bast fibres. A. Multiple epidermises, B. Collenchyma (Angular type), C. Chlorenchyma, D1. Round fiber (type I), D2. Tangential flattened fibre (type II), D3. Radial flattened fibre (type III).

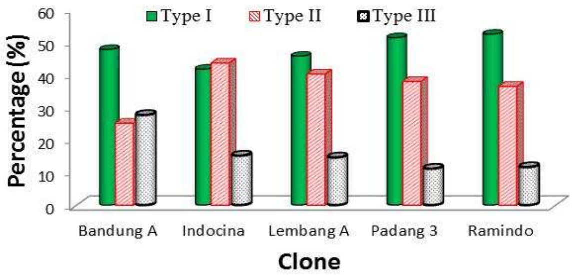

Fig. 2. Average fiber number in a fiber bundle for five ramie clones

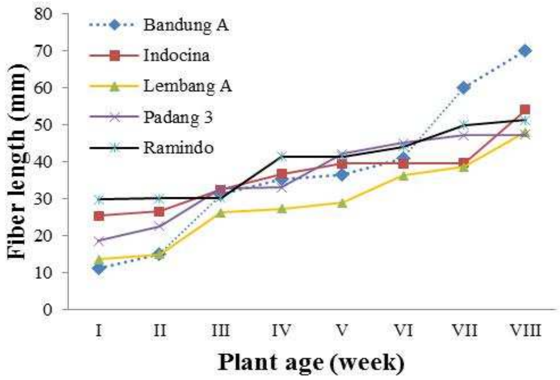

Fig. 3. Comparative growth rate of five ramie clones from 1-8 weeks age.

The growth rate of fibre length from highest to lowest is from Bandung A (132 µm/day), Lembang A (77 µm/day), Indochina and the Padang 3 (64 µm/day), and Ramindo (48 µm/day) (Fig 3 and Table II).

Table II shows that the range of the fibre length is from 11 mm at 1 week for Bandung A clone to 70 mm at 7 weeks for the same clone. The lengths of fibres in the other four clones fell between these two values.

Fibre diameter ranged from 24-69 µm (Lembang-Ramindo) (Table III). Figure 3 and Table III show that the growth of the fibre diameter from one week until the age of maturity varies among the ramie clones. The diameter Ramindo clone fibres are larger at 69 µm than that of other clones that range from 50-52 µm.

TABLE I
AVERAGE PERCENTAGE OF EACH TYPE OF FIBERS IN FIBRE BUNDLE OF FIVE RAMIE CLONES

Clone name	Fiber Type (%)		
	Type I	Type II	Type III
Bandung A	48	24	28
Indochina	42	43	15
Lembang A	45	40	15
Padang 3	51	38	11
Ramindo	52	36	12
Average (species level)	48	36	16

TABLE II
COMPARATIVE OF LENGTH AND DAILY GROWTH OF FIBRE OF FIVE RAMIE CLONES FROM 1-8 WEEKS OLD

Age (weeks)	Clone				
	A	B	C	D	E
1	11	25	14	19	30
2	15	27	15	22	30
3	31	32	26	33	30
4	35	37	27	33	41
5	36	39	29	42	41
6	41	39	36	45	44
7	60	40	39	47	50
8	70	54	48	47	51
Average (mm)	7	4	4	4	3
Daily Growth (µm)	132	64	77	64	48
A, Bandung A; B, Indochina; C, Lembang A; D, Padang 3; E, Ramindo					

TABLE III
THE GROWTH OF FIBRE DIAMETER (µM) OF FIVE RAMIE CLONES FROM 1-8 WEEKS OLD

Clone	Age (week)							
	I	II	III	IV	V	VI	VII	VIII
Bandung A	33	33	37	42	44	46	50	50
Indochina	25	31	44	45	48	49	50	51
Lembang	24	29	35	37	38	42	43	50
Padang 3	37	43	45	46	47	49	50	52
Ramindo	35	43	50	50	52	52	68	69

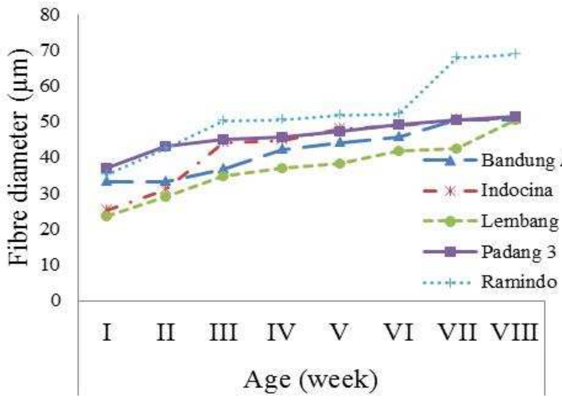

Fig. 4. Comparison of growth of fibre diameter for the five ramie clones from 1-8 weeks

Figure 4 shows that Ramindo clones not only have larger fiber diameter but also significantly thicker fibre cell walls (22 µm) compared to other clones (10-14 µm) (Figure 5 and Table IV). The wall thickness of the fibre appears to grow twice as fast in the first week compared to the growth in week 8 for all clones. This could also contribute to the higher rigidity Ramindo clone fibres which makes them less suitable for clothing textile manufacture.

TABLE IV
AVERAGE CELL WALL THICKNESS OF FIVE RAMIE CLONES FROM 1-8 WEEKS OLD (MM)

Clone	Age (week)							
	1	2	3	4	5	6	7	8
Bandung A	7	8	10	10	10	10	10	11
Indochina	6	6	8	8	9	10	10	10
Lembang	5	6	7	8	9	9	10	11
Padang 3	8	10	10	12	12	13	14	14
Ramindo	9	9	11	12	13	13	16	22

Figure 5 shows how the fibre growth pattern varied between clones. All clones grew rapidly between 2-3 weeks. This may be because each clone has similar genetic traits controlling early growth or that favourable environmental factors induced all fibres to grow rapidly. More research is needed to determine which of these factors is dominant.

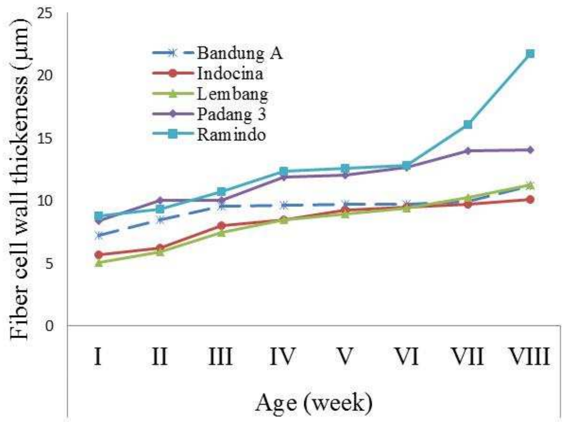

Fig. 5. Comparison of growth of cell walls of fibre for the five ramie clones from 1-8 weeks

Figure 6 shows that after five weeks Padang 3 clones cease to have any more significant growth. For Padang, 3 clones harvesting is best done at week 6 because if they are left to grow longer, the fibre this will result in unnecessary time and expense in terms of land use, labour and delayed delivery to the manufacturer and subsequently to the market. For similar reasons, based on fibre length alone, Bandung A clones should be harvested on the sixth week, Indochina, Lembang A, and Ramindo clones harvested after the seventh week. This is sooner than the usual *Boehmeria nivea* harvest period based on morphological characteristics which are generally at 68 -75 days or 10 -11 weeks.

However, in the case of Ramindo, harvesting after only 6 weeks will result in a softer more flexible fiber as the fibre cell walls grow rapidly after that time resulting in a more rigid fiber.

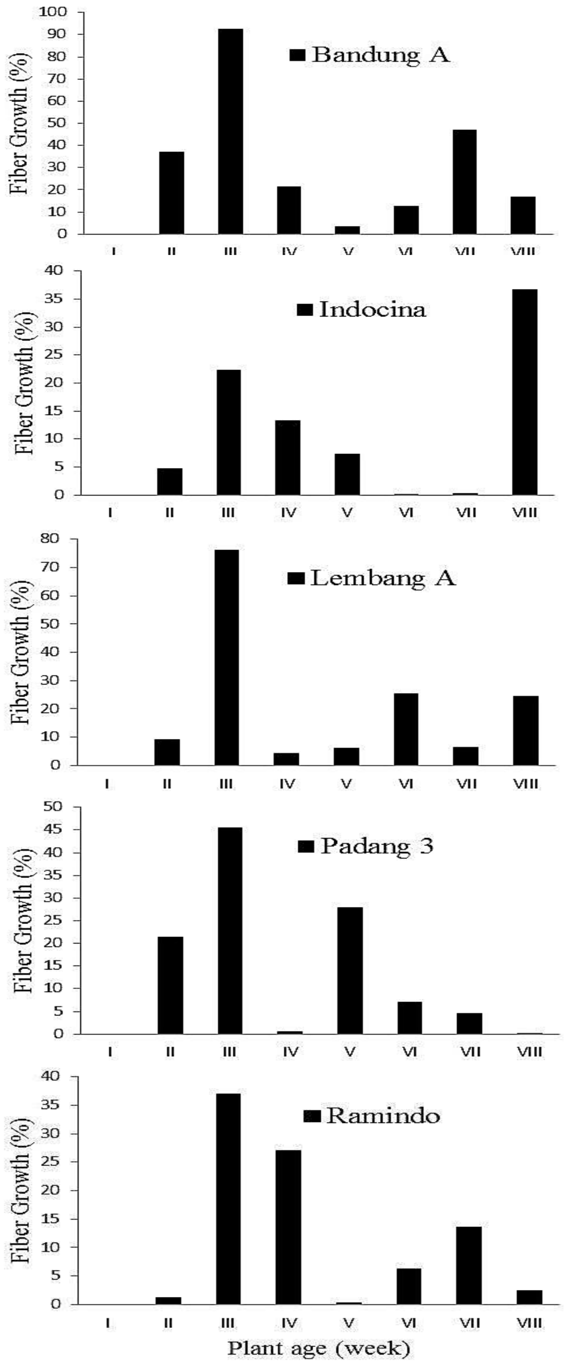

Fig. 6. Comparison of growth of ramie fibre from 1-8 weeks.

B. Discussion

Rami bast fibre clearly shows extra xilary fibre that is found in the cortex around floem. Fibres like this developed from the basic network. Fibre elongation process is extremely slow and can take months. From current research, fibres for all 8 weeks old ramie clones have reached their maximum length. Floem primer fibre elongation process that is carried out in phases involved development which is complicated by the secondary walls. Meanwhile, the walls remain thin for fibre growth through symplastic. Later, when the ends start to grow intrusively, only the cell walls at the ends will remain thin. The development of the secondary walls will start from the centre of the fibre that has stopped lengthening. In the ramie process, this occurs in phases until a new lamella from the secondary wall is added via the centripetal method in a cylindrical form whereby the two ends split opened. Simultaneously, lamella that was formed initially continues to elongate towards the ends of the fibre until it stops lengthening [4], [5].

The top end of the fibre continues to grow longer than the bottom end. Sometimes not all layers are able to reach the ends of the actual fibres, and on several fibres, there are chambers made at the ends by inward growth towards lumen cells on the lamellas [6], lamella on the phloem primer fibre or juvenile fibre usually is not adhered to strongly. This can be seen in Photo 1, whereby there are chambers between one fibre cell and another (cell wall does not stick to the other).

There is a growth difference between fibre on primer body and fibre in the secondary body. Initially, primer fibres appear first before organ in the fibres elongated. Hence, fibres can grow elongated through symplastic together with neighbouring cells that continue to divide themselves. Symplastic growth will increase with growths at the ends of the fibres intrusively which will pierce through other surrounding cells. Initially, surrounding cells develop on organs that have stopped growing length-wise. Therefore, secondary fibre can only grow intrusively. This is the reason primer fibres are longer compared with secondary fibres of similar growth. For this reason, it is better to harvest before secondary growth occurs if long fibres are desired to be achieved [4], [5].

The difference in the shape and size of the fibres in the fibre bundles found in the ramie clone currently studied can be due to the position and age of different fibre cells in a fibre bundle. In general, each fibre cell can increase in length because there are two stages of growth which are first, symplastic growth stage and secondly, intrusive growth [1], [3],[29],[30]. The length of the fiber that can reach several millimeters is commonly found in various plant species [1],[3],[12],[16]. Furthermore, that in the *Boehmeria nivea* can reach more than 55 cm (550,000 μm). Initially, the fiber length is only 20 microns, and within a few months, it can grow to 27,500 times the original length [7]. During its growth, the tip of the ramie fibre, as well as other fibre species, must slip in the middle of tens of thousands lamella of other fibre cells [4],[5],[6].

The five ramie clones examined in the present study have more fibres in each bundle than those reported from earlier studies on various types of bast fibre. This suggests that these clones have a greater potential than other crops that produce similar fibres.

The amount and size of extra xilary fibres of each plant vary greatly. In the ramie clone currently examined, ~~now~~ there is no anatomical character and the number of fibres that can be used to distinguish between ramie clone.

Understanding of the pattern of arrangement and functions of cells in plant phyto is still limited [6]. In some plants, the dicotyledons secondary axial phloem system consists of filter elements, companion cells, parenchyma, and fibre or sclereid bundles (e.g., [1],[4]). The network pattern in the secondary phloem is mostly studied from a descriptive point of view (e.g., [6],[8],[9]) and not much from the problem of the arrangement pattern.

The results of the previous research show great variation in data regarding the size and length of ramie fibre [31]. The longest fibre recorded in the present study was 70 mm in Ramindo clones considerably shorter than the 125 mm reported by previous researchers. It is possible that the clones examined have different characteristics than those previously reported due to different genetic traits and environmental conditions such as soil temperature and fertility and rainfall.

The results of the study suggest a harvest time based on determining the point at which growth rate slows at 6 – 8 weeks depending on clone rather than waiting unit the plant morphology shows characteristics of maturity at about 3 months would be advantageous from an economic point of view without sacrificing fibre length. Padang 3 fibres stop growing sooner than the other clones.

Although stem anatomy can be utilised to distinguish between various species of the same genus, distinguishing between various clones of the same species is not so straightforward. The anatomical characteristics of ramie fibre investigated are insufficient as distinguishing markers to differentiate between the ramie clones used in the present study. Of all the characteristics of the fibers analyzed in the present study, namely; the composition of fibres in the fibre bundle, the daily growth of fibre, the number of fibres in a fiber bundle, fiber diameter and growth rate and growth of fiber wall for the first 8 weeks there are few markers that distinguish between these clones.

Distinguishing characteristics that could possibly be used to identify a particular clone from the others was the percentage of Type II and III cells which were higher and cell diameter that became significantly larger after 6-weeks in Bandung A clone and the continuing thickening of cells walls that occurred in Ramindo clone after 6 weeks. However, further research under different growing conditions is necessary to determine the robustness of this method of clone identification.

IV. CONCLUSION

Of the clones studied, the Ramindo clones had the highest number of fibers in each bundle of fiber at 69 fiber cells per bundle, while the smallest number was found in Bandung A clones; that is 40 cells per fiber bundle. Overall fiber bundles consisted of an average of 48% type I, 36% type II and 16% type III fiber cells. The fibers in each fiber bundle increased in diameter from the cambium to the outside and are dominantly round shaped (type I) in the center , flattened on the tangential wall (type II) in the intermediate region and flattened on the radial wall (type III) in the outside region. The length of fiber found the present study of 11-70 mm was shorter than in previous reports. Generally, ramie fiber cells growth in length, diameter, and thickness of cell wall occur in step with each other. Padang 3 had the fastest growth rate at 132 μm/day, while at 48 μm/day Ramindo had the lowest one. The fastest growth rate for all clones occurred at 2-3 weeks. Based on fiber growth, Padang clones should be harvested when they are 6 weeks old, Indochina, Lembang A, and Ramindo harvested 8 weeks and Lembang A clone after the 8 weeks. This harvesting period is sooner than when harvesting is carried out based on morphological appearance which is generally between the age of 10-11 weeks.

Padang 3 has the advantage of being ready for harvest; the fastest but has fewer fibres per bundle than Ramando. However, the cell walls of Ramindo fibres are thicker making them rigid and less suitable for textiles for clothing. Bandung A takes significantly longer to mature than Padang 3 but produces the longest fibers which may provide some mechanical advantages for textile manufacture.

More research is needed to see if the apparently unique characteristics of Ramindo and Bandung A are sufficiently robust to provide a guide for clone identification.

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