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Morpho-anatomical structure and DNA barcode of *Sonchus arvensis* L.

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Abstract. *Wahyuni DK, Rahayu S, Purnama PR, Saputro TB, Suharyanto, Wijayanti N, Purnobasuki H. 2019. Morpho-anatomical structure and DNA barcode of* Sonchus arvensis *L. Biodiversitas 20: 2417-2426.* Tempuyung or show thistle (*Sonchus arvensis* L.) belongs to the Asteraceae. Morpho-anatomy and DNA (Deoxyribonucleic Acid) barcoding of the plant correlates with species identification and metabolite synthesis. This research aims to look at morpho-anatomical structures and analyze the DNA barcode of *Sonchus arvensis* L (tempuyung). Three samples used for morpho-anatomical analysis are leaves, stems, roots, fruit, and seeds. Anatomical samples are made using the embedding method. DNA barcode uses multiple locus from plastid genome: *rbcL* and *matK*. Morpho-anatomical structure of tempuyung showed a similar structure of *Sonchus* genus. The stem presents in the intercellular space, whereas the roots and leaves present in the vascular tissue and the seeds. Fruits present in each part of the body. Tissues that formed root are epidermis, cortex, endoderm, and stele. Tissues that formed stem are epidermis, cortex, and stele. Tissues that formed leaf are epidermis, cortex, phloem, and xylem. Tissues that formed the fruit and seed are paranormal and sclerenchyma tissues. *Sonchus arvensis* sequence for *rbcL* has a similar 100% maximum identity to *rbcL* gene of *S. arvensis*, *Sonchus asper*, and *Sonchus oleraceus*, whereas *Sonchus arvensis matK* sequence has a similar 99.31% maximum identity to other *S. arvensis matK* sequences in BLAST system. These findings provide morpho-anatomical features and DNA barcoding for identification of *S. arvensis.* from others species in the same genus. Thus also can be considered as pharmaceutical standard.

Keywords: Anatomy, DNA barcoding, *matK*, morphology, *Sonchus arvensis*, *rbcL*, tempuyung

INTRODUCTION

Indonesia has many potentials of medicinal plants that have not been studied, at least 9,600 species of plants have medicinal properties. One of them is *Sonchus arvensis* L., which is found throughout Indonesia and known as an invasive plant. The local name of *S. arvensis* in Indonesia is tempuyung. Tempuyung belongs to the Asteraceae family and is known to have many benefits for treating asthma, bronchitis, cough, and has antibacterial, anti-inflammatory, antioxidant, diuretic, sedative, and hypnotic activity (Delyan 2016). Most of *S. arvensis* metabolites are contained in the leaves. It has chemical compounds such as flavonoids (kaempferol, luteolin-7-glucoside, and apigenin-7-O-glucoside), coumarin, and taraxasterol (Sriningsih et al. 2012). Sulaksana et al. (2004) and Delyan (2016) also reported that tempuyung leaf contains high flavonoid and triterpenoids.

Species genus *Sonchus* is distinguished among themselves by a life form, lamina shape, stem character, number of flowers in inflorescences and color, number of edges on the achenes, achenes size and color, and so on (Svitlana et al. 2018). Mejias et al. (2012) reported that *Sonchus* could differ by the size of flowers, stamens size, and morphology of chromosomes. Qureshi et al. (2008) reported that *Sonchus* genus could differ by pollen analysis, whereas anatomical analysis of genus *Sonchus* has not been reported.

According to Sukandar and Safitri (2016), the use of tempuyung as medicine is safe even for pregnant women without side effects. Secondary metabolite compounds are located explicitly in particular part of the plant so that it may differ in cells, tissues, or organs of a plant. Secondary metabolite compounds in a plant can be detected by screening the plant extracts. Besides screening, an anatomical analysis is also crucial in determining the distribution of metabolite compounds in cells or tissues within each plant organ.

In addition to morpho-anatomical studies, it is also essential to do a molecular study for identification species. Previous research from Qureshi et al. (2008) also reported about pollen morpho-anatomical studies in *Sonchus* genus to find different and similar characters, but in vegetative organs not done. Species identification method living thing has developed from morphological identification to molecular identification based on short DNA pieces that are called "DNA barcode" (Hebert et al. 2013). DNA barcode has applicative functions for example for the ecological survey (Dick and Kress 2009), identification taxon-taxon cryptic (Lahaye et al. 2008), and confirmation of plant samples medicines (Xue and Li 2011). Consortium for Barcode of Life (CBOL) recommends the use of two

plastid genes, for examples *rbcL* and *matK* as barcodes standards (Hollongsworth et al. 2009).

Genes from the plastid genome are uniparentally inherited non-recombining, and an inherently stable genome (Kress et al. 2005). Current results designate that at least two plastid genes, better a multi-locus code, are required to specify a consistent plant DNA barcode, those are combination gene like *rbcL*, *matK* and internal spacer such as *trnH-psbA* (Lucas et al. 2012). *RbcL* is commonly used in phylogenetic analysis and can be simply to amplify; the sequence can also align in nearly all terrestrial plants. Thus *rbcL* is one of the right DNA barcoding regions for plants at the family and genus levels (Li et al. 2004).

So far, study about the molecular indicator of *Sonchus sp* has been widely conducted by using various marker such as ISSR (Psaroudaki et al. 2015; Subositi and Mujahid 2019), RAPD (Elkamali et al. 2010; Doğan et al. 2018), multiple-locus barcode *matK*-ITS (Kim et al. 2007; Mejías et al. 2018) moreover complete plastid genome (Cho et al. 2019; Kim et al. 2019). However, still, limited data provide molecular marker, primarily for *S. arvensis* using *rbcL* and *matK* locus.

Morpho-anatomy structures and DNA barcodes affect the biological systems of the plant, including the synthesizing process of secondary metabolites, so morpho-anatomical characters and DNA barcodes contribute the pharmaceutical standard. Because of *Sonchus* genus has several plant types so that it is vital to know the morpho-anatomical characters and DNA barcodes to prevent misusing plants. This study aims to describe morpho-anatomical characters and to analyze DNA barcodes (*rbcL+matK*) of *Sonchus arvensis* L.

MATERIALS AND METHODS

Plant materials

The material used is the tempuyung plant (*Sonchus arvensis* L.) which has been grown in Medicinal Plant Garden "Taman Husada Graha Family", Surabaya, Indonesia and determined in Purwodadi Botanic Gardens, Indonesian Institute of Sciences, Pasuruan, Indonesia.

Morpho-anatomical characterization

The component observed in the morphological study is a description of tempuyung's whole organs. The study was conducted on three different individual plants. The components observed in morphological studies are root, stem, leaves, flower, and fruit. The anatomical character studies are the cells contained in the tissue, secretion cells; tissue contains in the organs (root, stem, leaf, fruit, and seed). Sample preparation is using paraffin embedding with fixation, dehydration, dealcoholizing, infiltration, blocking in pure paraffin, slicing, gluing, staining, and closing (Sutikno 1989). The morpho-anatomical character was analyzed descriptively.

DNA extraction and quantification

Genomic DNA was isolated from 80 mg of fresh leaves of *S. arvensis*. The isolation was conducted using the Plant DNA Genomic Kit (Tiangen, China) according to the manufacturer's protocol. The quality and integrity of isolated DNA were checked by 1% gel electrophoresis (Promega, USA) and subjected to UV transilluminator.

PCR amplification and sequencing

Analysis of polymerase chain reaction (PCR) was carried out using two different primer pair. Both primers *RbcL* (Forward: 5'AAGTTCCTCCACCGAACTGTAG 3'; Reverse: 5'TACTGCGGGTACATGCGAAG 3') and *MatK* (Forward: 5' TGGTTCAGGCTCTTCGCTATTG 3'; Reverse: 5'CTGATAAATCGGCCCAAATCGC 3') were specific designed for Asteraceae family using Primer3 (Rozen and Skaletsky 2000). Selection conditions included: TM (57-60°C) and GC content (40-60%). A reaction (35µL) included 17.5 µL GoTaq®Green Master Mix, each 350 to 500 nM forward and reverse primers, 50 ng l^{-1} DNA template, and nuclease-free water until volume reached 35 µL. Thermocycling condition included: 5 min hot start at 94°C, 35 main cycles of 30 s at 94°C, 45 s at 56°C, and 45 s at 72°C. The reaction was completed with a final extension at 72°C for 5 min. PCR reactions were performed in an Eppendorf® master cycler personal. The PCR amplification efficiency was verified by 1% agarose (Promega, USA) gel electrophoresis using 0.5X TBE buffer. After verification of the success of PCR amplification, the reaction volumes were scaled up to 50 µL for sequencing at Macrogen Inc. (Korea).

Sequence and phylogenetic analysis

DNA sequences obtained from *rbcL* and *matK* were then analyzed using the rapid identification tool BLAST (Nucleotide BLAST: www.ncbi.nlm.nih.gov) to find regions of local similarity between sequences. The first five high similar sequences from each gene were aligned with ClustalW (Thompson et al. 1994) using BioEdit (Hall 1999). A cluster analysis was conducted using the distance method UPGMA (Unweighted Pair-Group Method with Arithmetic Mean). The output data were processed using MEGA 7 (Kumar et al. 2016) to build the phylogenetic trees.

RESULTS AND DISCUSSION

Morphological characters

Morpho-anatomical characters were analyzed descriptively. *S. arvensis* (Tempuyung) in Indonesia has a herbaceous habitus and erect, annual herb, but there is perrenial one in Pakistan (Qureshi et al. 2002). Tempuyung has a root rosette (Figure 1.A, B) and all the body has a white sap, this character can find in all member of the genus *Sonchus*. The sap can be found on the leaves, stems, and roots with various intensity. The root of the tempuyung is a taproot in yellowish-white color (Figure 1.C), long cone-shaped that grow downside and has a short root branch.

The stem is very short, herbaceous, round, and the nodus is clearly visible (Figure 1.D), green in color, has many trichomes and a monopodial bifocal. The trichomes

on the stem are a unique character because in another member of genus *Sonchus* have not many trichome (Mejias et al. 2012). Height of tempuyung reaches 64 cm tall. It is different from *S. oleraceus* that have 1.5 m tall (Chauhan et al. 2015). Another character in the stem that remarkably differs in genus *Sonchus* is branching, (Mejias et al. 2012).

Tempuyung's leaves are simple (single lamina). It is considered as incomplete because it has no petiole and vagina; there are two shapes of the leaves, which are spear and lanceolate (Figure 1.E). All member in genus *Sonchus* have two kinds of leaves with various shaped depends on species and location (Qureshi et al. 2002). Leaves on the root rosette part hugging rods, spear-shaped, pointed apex, flat base, pinnate vein, lobulated leaves margin, sleek adaxial (upper surface) colored in dark green (Figure 1.E1), while the abaxial (lower surface) colored in light green and has trichoma (Figure 1.E2). Edge of leaves *S. arvensis* in Pakistan is serrated, but in Indonesia, it is not serrated (Qureshi et al. 2002). *S. arvensis* has soft-thin leaves flesh like *S. oleraceus*; it differs from *S. asper* that has thick leaves (Mejias et al. 2012)

In the generative phase, leaves shaped in lanceolate, arranged alternately on the inflorescence, pointed apex, wavy base, pinnate vein, flat-sharp margin, thin leaves flesh with the rough surface (has trichome), the upper surface is greener (Figure 1.E1) than the lower part (Figure 1.E2). Subositi et al. (2018) reported that seat leaves and the edge of the seat could differ species in *Sonchus* genus.

The flower of genus *Sonchus* is resembled in all member but differ in size. The flower of *S. arvensis* is a terminal flower (Figure 1.F), compound inflorescence, monoecious, multi symmetry, cymose corymb, has flower ribbon, ovule covered by a cup-shaped part of the flower. There is small bract in the pedicle, shown by the white arrow in Figure F. The sepals are green and have soft brownish fur (Figure 1.H). The petals are colored in bright yellow (Figure 1.H). The ovule is located deep in the receptacle of the flower. Tempuyung's long central pedicle has generative leaves. According to Subositi et al. (2018), pedicle characteristic can differ species in *Sonchus* genus. When the flowers are fertilized, the petal will fall and form the fruit and seed (Figure 1.I).

The reproduction of tempuyung is relatively quick and accessible through the seeds. Tempuyung's fruit is considered as pure, dry indehiscent one-seeded fruit with a hard wall, brownish, and oval-shaped with 5-12 indentations along the sides (Figure 1.J). Pappus or white hairs present in one of the fruit edges, while the other edge is attached to the base of the flower. These accessories allow the fruit to be easily propagated by the wind. Tempuyung's fruit is so hard that it will not break when the seeds germinate. Seeds are protected in hard wall fruit, and there is only one seed in each fruit. Tempuyung's seed is tiny and colored in brownish-white. The seed of *Sonchus* has a high level of germination; it is about 90%. The seed can survive until one year in the soil surface. Light and salinity are not an absolute requirement in germination, because the germination more influenced by soil humidity. The size of the seed bank can be reduced by germination, insect predation, and decay (Chauhan et al. 2015).

Anatomical characters

Anatomical studies are critical to know the structure of the organ, the cells, and the tissues that are possible to synthesize secondary metabolite compounds (Sharma et al. 2017). Each organ has its characteristic, which indicates the location of secondary metabolites synthesis.

The cross-section structures of the basal side of tempuyung young root from the outside in are composed of epidermal, cortical, and pith tissue (xylem and phloem). Exodermis and endodermis are still not visible in the young root. The root epidermis is round, small, dense, and thickened walls (Figure 2.A1). The cortex is composed of several giant cells, and there are plenty of air spaces inside. The stele and cortex segments are separated by endodermis, radial transport system, and actynostele. The root base has a more differentiated transport system. In the root cap (Figure 2.A2), there is a small number of airspace; the cortical cells are composed of large, well-ordered cells. In the cortex, there are more dense-colored cells, indicating a secondary metabolite synthesis therein. Anatomical characters of the vegetative organ in genus *Sonchus* is similar so anatomical characteristic can be used to taxonomy studies (Kandemir et al. 2006).

Table 1. A morphological character among genus *Sonchus*

Character	*S. arvensis*	*S. oleraceus*	*S. asper*	*S. erzincanicus*
Habitus	herbaceous, 0,65-1,5 m	herbaceous, 0,3-1,25 m	herbaceous, 0,1-0,7 m	Herbaceous
Stem	erect, round, annual to perennial	erect, round, annual	erect, round, annual to biennial	erect, round, perennial
Leaf	spear to the lanceolate, margin of leaf serrated, soft-thin leaves flesh	Sagittarius, amount of serration on leaf margin, soft-thin leaves flesh	Auriculatus, the margin of leaf serrated and spiniest than other, fleshy and thick	oblong to the elliptic, margin of leaf serrated, spiny, fleshy and thick
Root	Taproot, root rosette	Taproot, root rosette	Taproot, root rosette	Taproot, root rosette
Flower	yellow, compound inflorescence, actinomorf	yellow, compound inflorescence, actinomorf	yellow, compound inflorescence, actinomorf	yellow, compound inflorescence, actinomorf
Pollen	Spheroidal	Spheroidal, tricolporate	Spheroidal, tetracolporate	anomocytic
Fruit and seed	Hard, brown, wrinkled	Hard, brown, narrow, wrinkled	Hard, brown, most wrinkled	Hard, brown, wrinkled
Branching	Near inflorescence	Near inflorescence	From stem	Near inflorescence

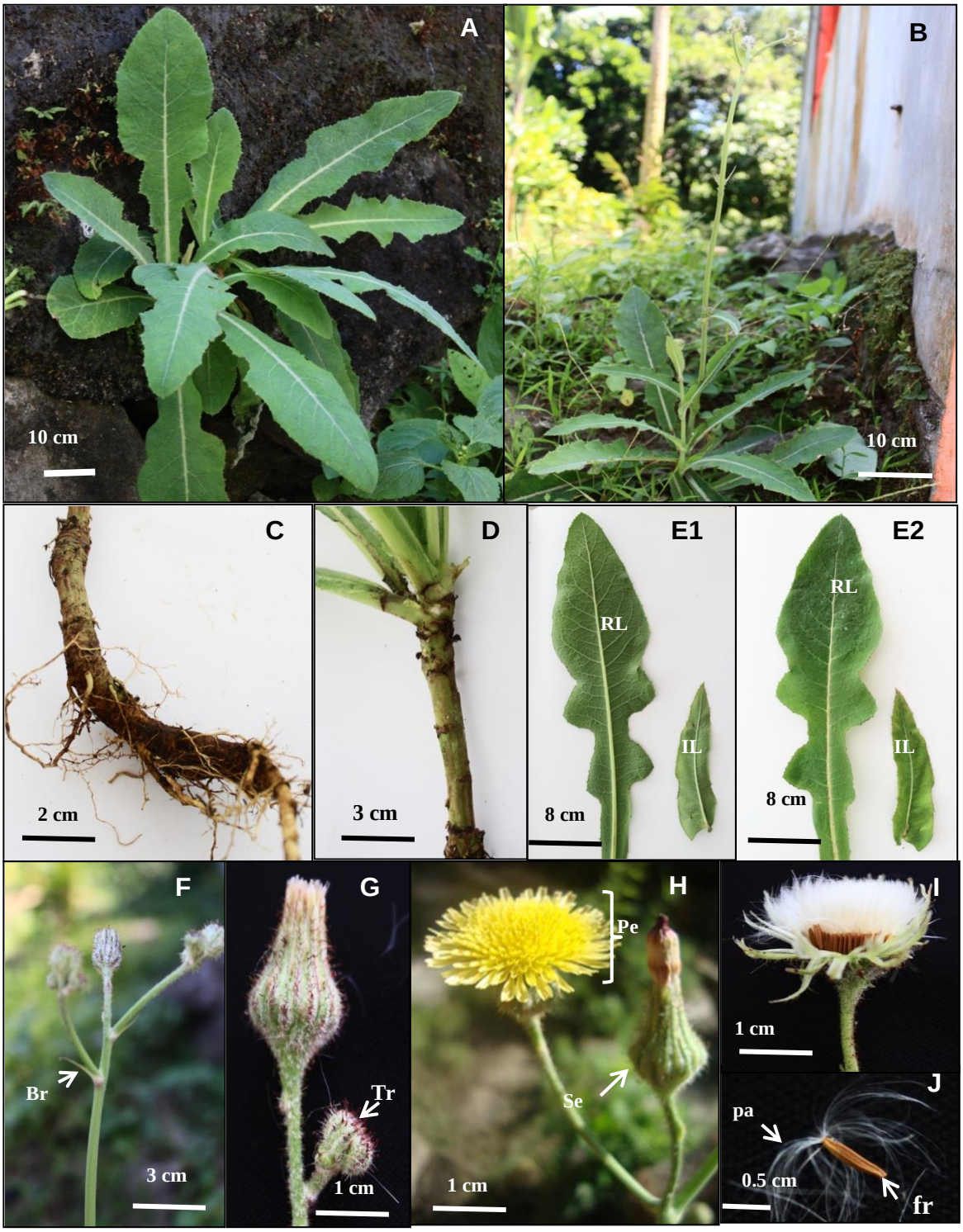

Figure 1. *Sonchus arvensis* L. morphology, A. Vegetative phase, B. Generative phase, C. Root, D. Shoot, E. Leaf; E1. Abaxial roset leaf (RL) and inflorescence leaf (IF), E2. Adaxial RL and IF, F. Bract (Br), G. Trichome on the flower (Tr), H. Petal (Pe) and Sepal (Se), I. Fruits arrangements in flower, J. Fruit (fr) and Pappus (pa)

Tempuyung's stem has a structure that is not much different from other Asteraceae plants too. The external anatomical arrangement of the stem is one layer of a flat-shaped epidermis, a single-layered of collenchyma, irregular cortical cell shape, and more abundant than epidermal cells (Figure 2.B). The cortex consists of 7-9 layers, usually oval-shaped or rectangular. The cortex containing latex are seen transparently. The transport system is open collateral (xylem and phloem are limited by cambium tissue) and arranged circularly around the pith. The xylem trachea cells are plentiful, and wall thickened, while the phloem is located outside the xylem and is

limited by the cambium tissue. Adult phloem cells do not have a cell nucleus, whereas, in young phloem, the cells are small and still have no cell lysis, thus resembling cambium and making it difficult to distinguish. Phloem sometime consists of amylum, its similar to other species in genus *Sonchus* like *S. erzincanicus* (Kandemir et al. 2006). Cambium cells are tiny and thin-walled, making it challenging to observe. The black arrows in Figure 2.B3 indicate the elongation of the transport system to the outer part of the cortex. This indicates the presence of leaf forming activity. In the deepest part, there is a pith that has a large-dense cell. There are many interstitial spaces in the cortex (Figure 2.B2) which have darker shades. Those may be the storages of secondary metabolite secretion.

The arrangement of leaf tissue from the outside consists of a flat, one-layered epidermis which has thickened cuticle. There are many trichomes on the surface, but they are not visible (Figure 2.C). Midrib has a triangle-shaped and has 1 or 2 layers collenchyma under the epidermis. In the midrib of the leaf (Figure 2.C1), there is a thick irregularly shaped cortex, an open collateral transport system, and an unclear cambium wall. Therefore it is difficult to observe. In the mesophyll tissue section, there are more densely colored cells. In the phloem, there is a more concentrated interstitial space. This indicates the presence of secondary metabolite storage in that section. Sclerenchyma cells in bundles are dense.

The lamina (Figure 2.C2) is composed of a larger epidermis compared to the midrib, beneath the epidermis is a mesophyll tissue composed of the thick palisade, and spongy tissue are not distinct. The spongy tissue is spaced apart. On the sample presented, the sponge tissue was lysis during the preparation, so that it is difficult to observe. There is much chlorophyll in the mesophyll. The vegetative organ in genus *Sonchus* is similar so that it will work hard to distinct species by anatomical characteristic (Subositi et al. 2018).

The anatomical arrangement of fruits and seeds of tempuyung is very distinctive. The tempuyung fruit is grooved and hard, while the seeds are inside (Figure 3.D1). In younger fruits, the seeds have not formed so that the seed chamber is empty. Tempuyung's fruit wall is thickened. Most of the forming tissues of tempuyung's fruit are deep parenchymal tissues, in which there is a starch granule (shown by black arrows in Figure 3.D2) and contains bioactive compounds. Also, there is a thickening of sclerenchyme in the grooves of the fruit, small sclerenchyma cells, compacted, and has a thick cell wall. Besides strengthening, sclerenchyme tissue also serves as a transport system on the fruit and seeds.

The middle layer is thickened with a cell structure that extends over the seeds of the starch layer (Figure 3.D2). The structure of the seed endosperm is very distinctive, composed of densely tight, small meristematic cells and many starch granules which indicates secondary metabolite compounds therein. The endosperm of the tempuyung plant is in two pieces, so it is considered as dicotyledons.

DNA barcoding

Both *rbcL* and *matK* genes were successfully amplified in *S. arvensis*. These primers were specially designed to amplify both genes among the Asteraceae family.

Table 2. An anatomical character among *Sonchus* genus

Character	***S. arvensis***	***S. oleraceus***	***S. asper***
Root	• Epidermis small thick wall and round in shape • Cortex large, polygonal and dense color • There is air space in cortex • The transport system is radial • Stele type actynostele	• Epidermis small thick wall and round in shape • Cortex large, round • The transport system is radial • Stele type actynostele • Visible cambium	• Epidermis small thick wall and round in shape • Cortex polygonal • The transport system is radial • Stele type actynostele • Visible cambium
Stem	• One layer epidermis, flat in shape. • One layer collenchyma • 7-9 layers cortex, irregular in shape • The transport system is open collateral. • Phloem consist of starch	• One layer epidermis, flat in shape. • 2-3 layers collenchyma • 6-7 layers cortex consist of latex • The transport system is collateral. • Phloem consist of starch • Visible cambium	• One layer epidermis, rectangular. • 2-3 layers collenchyma • 6-7 layers cortex consist of oxalate crystals, polygonal in shape • The transport system is collateral. • Visible cambium
Leaf	• One layer epidermis with thick cuticula, and less trichoma, rectangular. • Palisade and sponge tissue are not distinct. • The transport system is open collateral	• One layer epidermis with thick cuticula, and many trichomas, rectangular. • Palisade and sponge tissue are not distinct. • The transport system is open collateral	• One layer epidermis with thick cuticula, and many trichomas, rectangular. • Palisade and sponge tissue are not distinct. • The transport system is open collateral. • Visible cambium.

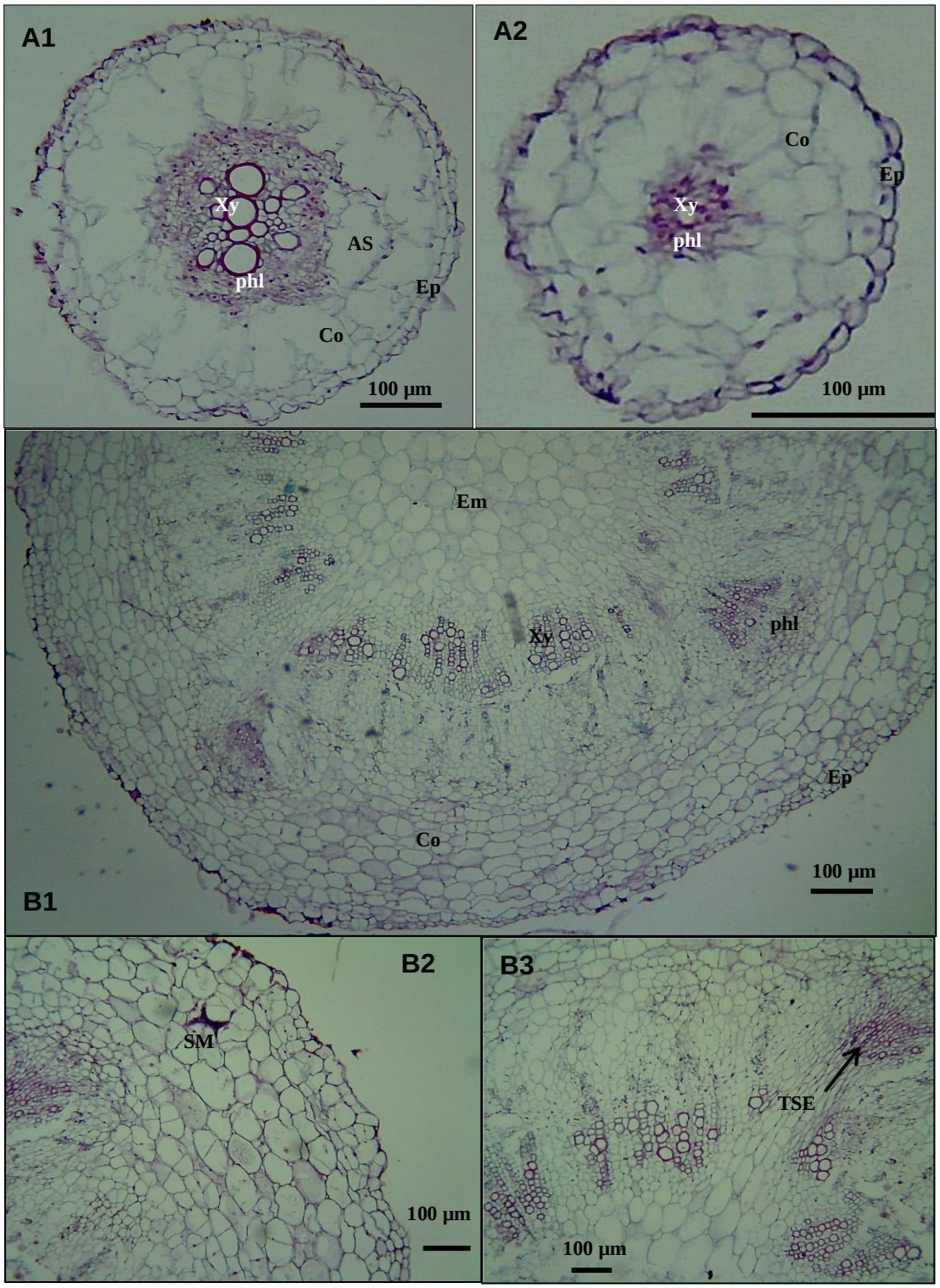

Figure 2. A Cross-section of roots, A1. Root base [Epidermis (Ep), Cortex (Co), Air space (AS), Phloem (Phl), Xylem (Xy)]. A2. Root cap [Epidermis (Ep), Cortex (Co), Phloem (Phl), Xylem (Xy)]. B. Cross-section of shoots, B1. Shoot layers [Epidermis (Ep), Cortex (Co), Air space (AS), Phloem (Phl), Xylem (Xy)], B2. Secondary metabolite accumulation (SM), B3. Transport system elongation (TSE)

The sequence length from *rbcL* was 433 bp, and *matK* was 288 bp, whereas GC contents were 42.7% and 35.4%, respectively. The BLAST result (Table 3) for *rbcL* barcode displayed that *S. arvensis* MN206020 (this study) for *rbcL* has a similar 100% maximum identity to *rbcL* gene of *S. arvensis* (JX848427.1), *Sonchus oleraceus* (KM360989.1), *S. oleraceus* (EU385018.1), *Sonchus asper* (MF135322.1), *S. asper* (HM850372.1). Furthermore, close relationship to *S. arvensis* MN218598 (this study) for *matK* sequence was shown in Table 4 which has a similar 99.31% maximum identity to other *S. arvensis matK* sequences (MH265200.1; MF770209.1; MG225099.1; MG225031.1; MG 225020.1).

Nucleotide base differences are found in nucleotides number 32 and 33 from *S. arvensis* MN218598 sequence against other comparative sequences (Table 5). The *rbcL* amplicon of *S. arvensis* MN206020 seems in the conserved region of *Sonchus* genera. It can be seen in BLAST result which indicated only one sequence intraspecies (*S. arvensis* JX848427.1) and others were from inter-species (*S. oleraceus* KM360989.1, *S. oleraceus* EU385018.1, *S. asper* MF135322.1, *S. asper* HM850372.1). This result can be caused the *rbcL* sequences to evolve slowly, and this locus has the faintest divergence of plastid genes in Angiospermae (Kress et al. 2005) considerably. Hence, it is not suitable at the species level due to its scanty discriminatory ability (Fazekas et al. 2008; Lahaye et al. 2008). Even though *rbcL* by itself can not make the favored feature of a barcoding locus. It is possible that *rbcL* in combination with diverse plastid or nuclear loci can make precise identification (Chase et al. 2007; Kress and Erickson 2007).

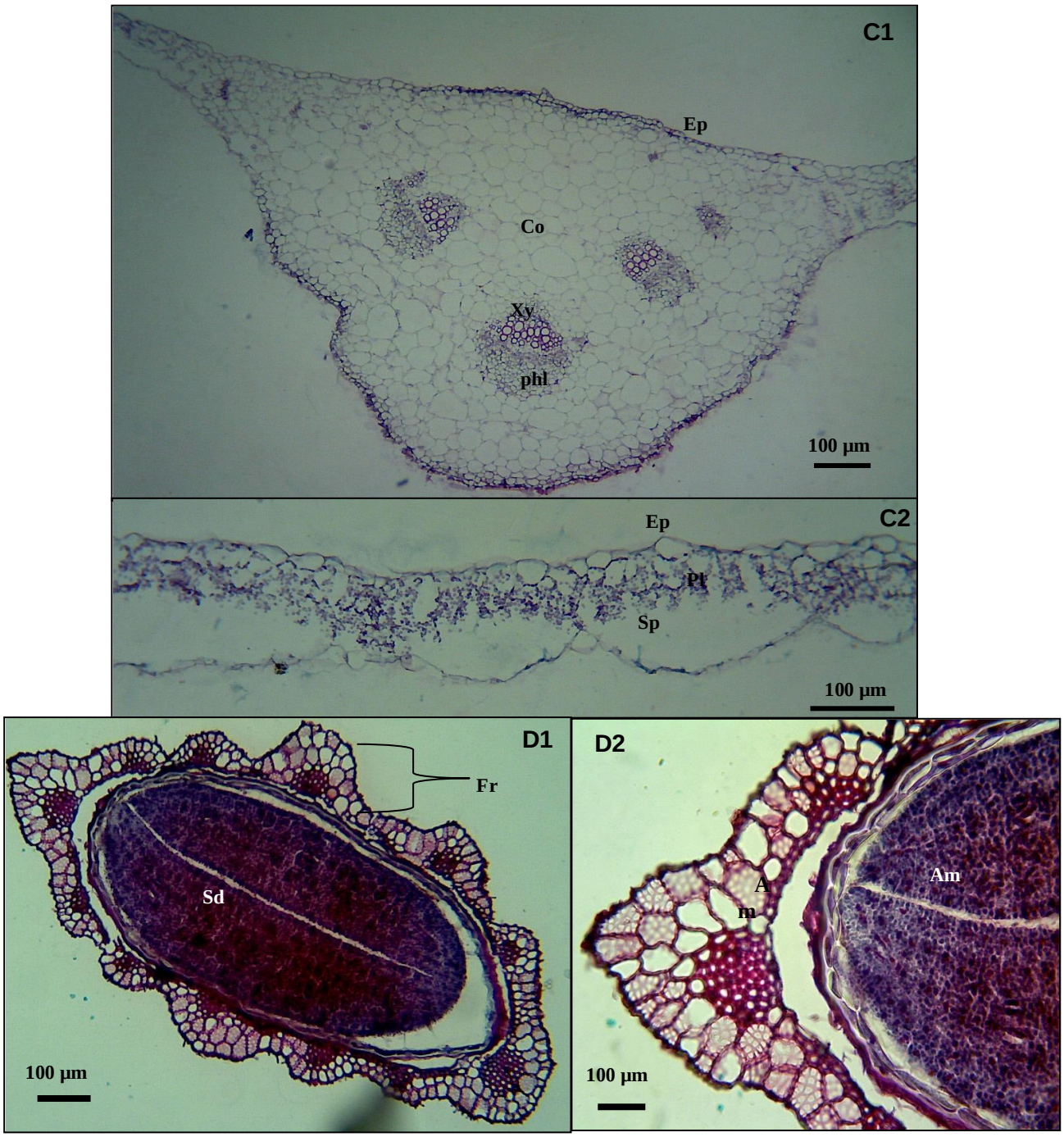

Figure 3. C. Cross-section of a leaf, C1. The midrib of the leaf; Ep: epidermis, Co: Cortex, phl: phloem, Xy: xylem, C2. Lamina of leaf; Ep: epidermis, Pl: palisade, Sp: Sponge. D1. Cross-section of fruits (Fr) and seed (Sd), D2. Amylum (Am) in fruits and seed

Table 3. Local alignment results obtained from BLAST of *Sonchus arvensis* MN206020

Species	Max score	Total score	Query cover	E-value	Per. ident.
Sonchus arvensis JX848427.1	800	800	100%	0.0	100.00%
Sonchus oleraceus KM360989.1	800	800	100%	0.0	100.00%
Sonchus oleraceus EU385018.1	800	800	100%	0.0	100.00%
Sonchus asper MF135322.1	800	800	100%	0.0	100.00%
Sonchus asper HM850372.1	800	800	100%	0.0	100.00%

Table 4. Local alignment results obtained from BLAST of *Sonchus arvensis* MN218598

Species	Max score	Total score	Query cover	E-value	Per. ident.
Sonchus arvensis MH265200.1	521	521	100%	5E-144	99.31%
Sonchus arvensis MF770209.1	521	521	100%	5E-144	99.31%
Sonchus arvensis MG225099.1	521	521	100%	5E-144	99.31%
Sonchus arvensis MG225031.1	521	521	100%	5E-144	99.31%
Sonchus arvensis MG225020.1	521	521	100%	5E-144	99.31%

Table 5. Genetic variation in 288 bp of partial maturase K (*matK*) gene of *Sonchus arvensis* species. Position based on the sequence of the first sequence obtained from *Sonchus arvensis* MN218598

Species	Identity (%)*	Nucleotide differences at position	
		32	33
Sonchus arvensis MN218598	100%	A	C
Sonchus arvensis MH265200.1	99.31%	T	T
Sonchus arvensis MF770209.1	99.31%	T	T
Sonchus arvensis MG225099.1	99.31%	T	T
Sonchus arvensis MG225031.1	99.31%	T	T
Sonchus arvensis MG225020.1	99.31%	T	T

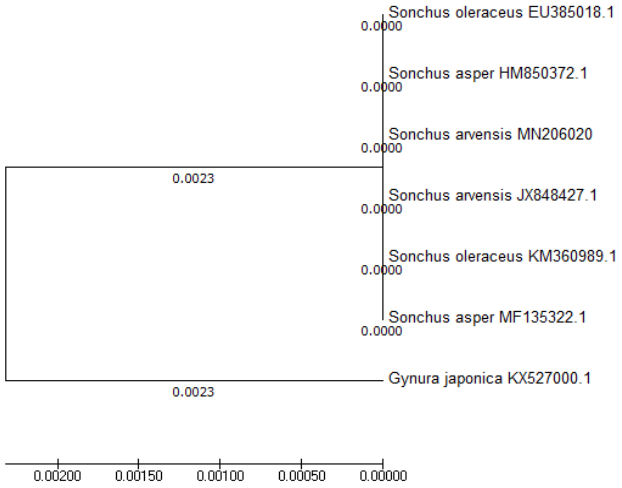

Figure 4. Phylogenetic tree of partial ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (*rbcL*) sequence 433bp of 5 species of *Sonchus*. *Gynura japonica* (KX527000.1) as outgroup

Presenting the different view, *matK* has a high evolutionary rate, compatible length, and noticeable interspecific divergence as well as a low transition/ transversion rate (Min and Hickey 2007). As seen in Table 5 that transversions take place at position nucleotide 32 and transitioning at position nucleotide 33. Thus, it can affect the circumstances of amino acid from Phenylalanine to Tyrosine.

Phylogenetic analysis reveals a little deviation in the *rbcL* tree could be as a result of the symmetry in *rbcL* among a sequence of *Sonchus* genera (Figure 4). In other words, no difference between the intraspecies and inter-species alteration was noticed in the present study. Dissimilar results shown by *MatK* tree which *S. arvensis* MN218598 was disjoined from cluster *S. arvensis* acquired from database NCBI with branch length 0.0035 (Figure 5). *Gynura japonica* (KX527000.1) is placed to outgroup in *rbcL* tree as those are out of *Sonchus* genera but still within an Asteraceae family. Whereas, *S. oleraceus* (EU385397.1) is positioned as outgroup in *matK* tree since the following members are intraspecific of *S. arvensis*.

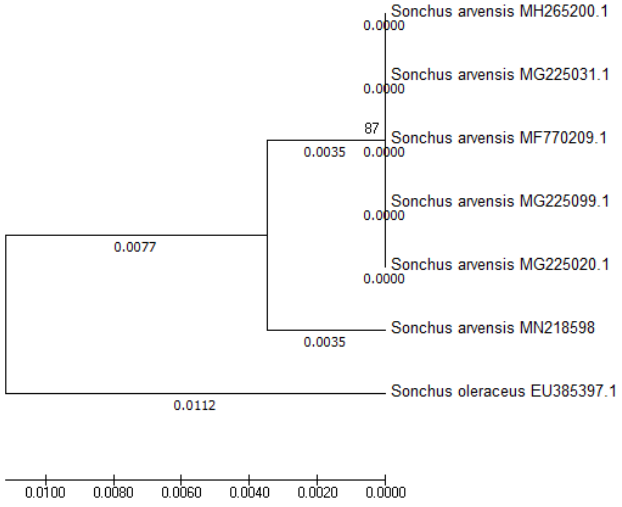

Figure 5. Phylogenetic tree of partial maturase K (*matK*) sequence 288bp of 7 species of *Sonchus*. *Sonchus oleraceus* (EU385397.1) as outgroup

Total cpDNA size of *Sonchus* sp has range 152.071 to 152.194 bp (Cho et al. 2019) with 1428 bp of *rbcL* gene (Kim et al. 2019) and 1530 bp of *matK* gene (Kim et al. 2007). Refer to Kim et al. (2007) and Mejías et al. (2018) that *Sonchus brachyurous* and *S. arvensis* are closely related base on *matK* region and put them together into a single clade. This study presents a new phylogenetic analysis of *S. arvensis* using *rbcL* barcode since numerous deposited sequence in Genbank are unpublished.

Another barcode that employed for DNA barcoding *Sonchus* sp is ITS (Mejías et al. 2018; (Kim et al. 2007). The ITS spacer is a robust phylogenetic marker at the species level indicating high levels of interspecific separation (Alvarez and Wendel 2003). The higher inequitable power of ITS over plastid regions at low taxonomic levels has been extensively considered leading to it also being recommended as a plant barcode (Stoeckle 2003; Kress et al. 2005; Sass et al. 2007).

Study of morpho-anatomy and DNA barcode have contributed to expanding the information about *S. arvensis*. Morpho-anatomy of *S. arvensis* has been describing. It was shown that the root, leaves, stem, flower, fruit and seed present morpho-anatomical characteristics that are useful in the identification and differentiation from other species of this genus and are also essential parameters for the quality control of vegetable raw material. For supporting the morpho-anatomical study, the DNA barcode, *rbcL* and *matK*, are also potential to rely on two barcode loci for identification *S. arvensis*.

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