HAYATI
Journal of Biosciences

Vol. 30 No. 3, May 2023 510-521
DOI:10.4308/hjb.30.3.510-521
ISSN: 1978-3019
EISSN: 2086-4094

Anatomical Studies and Evaluation of Genetic Stability in Plantlets Derived from Somatic Embryos of Arabica Coffee

Rina Arimarsetiowati[1,2], Budi Setiadi Daryono[3], Yohana Theresia Maria Astuti[4], Endang Semiarti[1,3*]

[1]*Biology Doctoral Study Program, Graduate Study Program, Faculty of Biology, Universitas Gadjah Mada, Yogyakarta 55281, Indonesia*
[2]*Indonesian Coffee and Cocoa Research Institute, Jember 68118, Indonesia*
[3]*Department of Tropical Biology, Faculty of Biology, Universitas Gadjah Mada, Yogyakarta 55281, Indonesia*
[4]*Faculty of Agriculture, Institut Pertanian Stiper, Yogyakarta 55283, Indonesia*

ARTICLE INFO

Article history:
Received August 24, 2022
Received in revised form November 4, 2022
Accepted January 4, 2023

KEYWORDS:
Anatomy,
Coffea arabica L.,
genetic variation,
Maragogipe,
Sigararutang,
*trn*L

ABSTRACT

Anatomical characteristics regenerant plantlet of Arabica coffee (*Coffea arabica* L.) were observed to determine the difference of plantlet performance between Sigararutang and Maragogige grown in shooting and rooting medium. Transverse sections of the fresh roots, stems and leaves of three-month-old plantlets from somatic embryos were collected and used for the study. Sigararutang and Maragogipe as the plantlet materials were chosen based on the bean size and the origin. Stomata were microscopically observed on the abaxial leaf paradermal section. A conformity test to compare between plantlet and the parent plant was observed to perform genetic stability. Assessment of genetic stability was measured by using the sequence of *trn*L (UAA) region. The result showed that all the anatomical roots, stems and leaves of the Maragogipe plantlet have a greater number than Sigararutang (root diameter, cortex thickness, distance of long stele, distance of short stele, endodermis thickness, stem diameter, cortex thickness, maximum stele diameter, minimum stele diameter, epidermis thickness, diameter of stomatal closing, length of stomatal closing, total stomatal density, adaxial epidermis density, midrib thickness, adaxial epidermis thickness, abaxial epidermis thickness, diameter of the vascular bundles, lamina thickness), except of epidermis thickness, diameter of the vascular bundles, diameter of stomatal aperture, diameter of stomatal opening, length of stomatal opening and abaxial epidermis density. Taxonomists may be able to use these anatomic traits as supplementary proof in the determination of Arabica coffee. Molecular analysis showed that there were genetically identical organisms between the plantlet and the parent plant. It was indicated there was no somaclonal variation during somatic embryogenesis in the micropropagation of Arabica coffee.

1. Introduction

Coffee is one of the most consumed beverages in the world and has high economic value (ICO 2018). Coffee has been developed over more than 11 million hectares (ha) of land in Africa, Asia, and the Americas. Around 70 percent of the global coffee supply is produced by small stakeholders with less than 5 ha, and over 80 million people rely on the harvest for livelihood (Simon-Gruita *et al.* 2019). Coffee is a perennial crop with morphologies, sizes, and agroecological conditions that vary greatly. It belongs to the Rubiaceae family and has more than 124 species, such as *Coffea arabica, Coffea canephora, Coffea liberica, Coffea excelsa* and *Coffea stenophyla* (Simon-Gruita *et al.* 2019). Development of industrial coffee is primarily based on two related species: *C. arabica* (Arabica coffee) and *C. canephora* (Canephora coffee), which represent 65 percent and 35 percent of global coffee production, respectively (https://coffee-genome.org/). Arabica coffee has been found in the shade of the tropical forest and has been used as a famous refreshment for centuries all over the world. It is a new allotetraploid (2n=4x=44) that originated from a natural crossover between *C. canephora* and *C. eugeniodes* (Cenci *et al.* 2012; http://coffee-genome.org/). Arabica beans have excellent coffee beans and

* Corresponding Author
E-mail Address: endsemi@ugm.ac.id

a more unique aroma. In its chemical composition, it contains more lipids, less caffeine and less chlorogenic acid (Barbosa *et al.* 2019).

Sigararutang is an Indonesian Arabica coffee specialty from North Tapanuli that has been officially released since the Ministry of Agriculture approved Regulation 205/Kpts/SR.120/4/2005 establishing Sigararutang to be an excellent local coffee specialty. It develops at elevations of over 1,000 meters above sea level in the highlands. Sigararutang coffee is a global specialty coffee with the most preferred qualities of coffee judges from around the world, according to cupping analysis (Hulupi 2016).

Maragogipe is a Typical variety mutant that once originated around Maragogipe, Bahia, Brasil. Maragogipe variety was first introduced to Indonesia in 1881 and planted in the Bogor Botanical Gardens. From the Botanical Gardens, the Maragogype variety spread to various coffee plantations in Indonesia (Nugroho *et al.* 2012). This phenotype includes tall plant height, long curved leaves that are large at the base, and large bean size, which are all greater than Typica. Margogipe is widely recognized is elephant bean in Arabica coffee variety that produces an extremely large bean. There are two types of fruit: yellow and red. Caffeine content is also lower in Maragogipe, at 0.6 percent vs 1.3 percent in Arabica (Wintgens 2007). It was not approved for major business cultivation in Brazil due to its lower profitability, but it is now more popular in Nicaragua, Guatemala, and Mexico. It has light cup quality (https://www.trespontas.com/pages/varietals).

The conversion of coffee to different light intensity, temperature and humidity from *in vitro* propagation by somatic embryogenesis has increased interest in several anatomical studies of this plant. It is recognized that plant that affects the conversion to different environmental conditions may be associated with various physical features (Larcher 2000). This structural modification aims to optimize the capture of water and radiation available as an energy source for photosynthesis in *in vitro* conditions. According to Rodrigues *et al.* (2014), anatomical characteristics are generally determined by the environmental factors in which plants grow and the result of a complex method that expresses the phenotypic variation of these species. Anatomy alterations are frequent in in vitro plants and the process of adapting to greenhouse cultivation leads to the alteration of the leaves, especially in the morphology and utilization of epidermal cells, as well as the thickness and differentiation of mesophyll tissues, and the quantity and arrangement of chloroplasts.

Traits involving the physiological processes and plant growth and development are significantly influenced by factors concerning the assimilation of resources such as carbon, water, and nutrients (Ackerly *et al.* 2000). Stomatal traits (density, frequency, and position) and epidermal traits (density, shape, and size of epidermal cells) determine the complexity of leaf surface morphology description (Jones 1998). Taxonomic knowledge is used to classify plants according to their similarities and distinctions. One of the taxonomic methods for organizing such data is anatomy, which is the actual representation of plant cells, tissues, and organs.

Tissue culture is an important technology in plant breeding programs to propagate superior cultivars which have valuable industrial advantages (Bhojwani and Dantu 2013). The advanced method of regeneration for woody plants is somatic embryogenesis (Guan *et al.* 2016). Somatic embryogenesis is recognized as advanced micropropagation because of its potential method to produce superior plants and maintain beneficial plant genetic resources. Somatic embryogenesis is an effective plant micropropagation method to produce transgenic plants, artificial seeds and germplasm conservation (Guan *et al.* 2016). Genetically identical production between the plantlet and the parent plant must be achieved. However, the application of this method on large scale carries the possibility of triggering genomic variation, also known as somaclonal differences because of the alteration during *in vitro* culture among plantlets in one parental line. Nucleotide alteration which was initiated in continuing callus during subculture, liquid culture and plantlet from micropropagation correlated to genomic variation. The subculture process in somatic embryogenesis propagation affects the development of true-to-type plantlets because of variation of explant tissue and cells, random mutation and stimulation of growth conditions of genomic material transposition (Bhatia *et al.* 2015). While certain alterations have no impact on agronomic characteristics or may result in significant improvement, creating variants with better qualities, several alterations could be harmful or even lethal, this directly impacts agricultural production (Hervé *et al.* 2016).

Therefore it is very essential to understand genetic variability in regenerants for their profitable usage. There are many methods for identifying genetic diversity including phenotypic classification, cytological analysis and molecular technique. Molecular techniques have appeared recently as very powerful method for detecting genetic similarities or plantlet dissimilarities from somatic embryogenesis propagation. So strict quality checks to ensure the genetic stability of offspring become mandatory. Molecular techniques were used to evaluate and verify the genetic consistency of plantlets.

The *trn*L application has been popularized as one of the molecular markers commonly used to assess genetic diversity through sequence analysis. The *trn*L molecular marker is a chloroplastic DNA non-coding region that is capable of identifying the genetic diversity of plants. Because of the simple genome, the chloroplast *trn*L (UAA) has a benefit that is easily amplified in a large number of plant (highly successful PCR) (Rahadiantoro *et al.* 2013). The *trn*L region has been used to distinguish, identify species and analyze the phylogenetic relationships in *Lophophora* (Adrienne *et al.* 2015), *Atraphaxis* (Yurtseva *et al.* 2016), *Pandanaceae* (Buerki *et al.* 2012), ferns (de Groot *et al.* 2011), tea (Lee *et al.* 2016), *Myrtaceae* (Vasconcelos *et al.* 2017), *Cycas chenii* (Yang *et al.* 2016), wheatgrass *Elymus fibrosus* (Schrenk) Tzvelev (Wu *et al.* 2016). The primers are very effective in some species such as *Pandanaceae* (Callmander *et al.* 2012, 2013; Gallaher *et al.* 2015). The sufficiently small size of *trn*L allows the production of complete DNA sequences (Gielly and Taberlet 1994; Taberlet *et al.* 2007). *Trn*L has a medium genome length between 260-1,000 bp, a stable genetic structure and never or very rarely undergoes gene recombination, so it is easy to amplify and analyze (Dong *et al.* 2012; Hidayat *et al.* 2008). Alteration in the chloroplast *trn*L (UAA) sequence was identified to study the phylogenetic relationships between species of Coffee and Psilanthus (Maurin *et al.* 2007). Moreover, these regions display the highest mutation frequency (Baraket *et al.* 2010).

Plantlets usually have different shapes from each other, are not vigorous, unhealthy and decrease their regeneration. So it is necessary to evaluate their performance, such as anatomy and molecular genotyping. There have been several anatomical research on coffee such as leaf anatomy in C.arabica (Pompelli *et al.* 2012), anatomy and physiology of coffee (Castanheira *et al.* 2019), anatomy from micro-cuttings (Angelo 2019) and anatomy in Robusta coffee (Sakiroh and Ibrahim 2020). However, there are no reports of studies of the anatomical structure of Coffea arabica from *in vitro* plantlets. So that the study of anatomy becomes an important thing. The anatomy of the leaf and stem of plantlets is essential for micropropagation which is influenced by the color of light and culture medium (Smith *et al.* 2017; Su *et al.* 2013). Although the anatomy of leaves created intense plasticity in response to light conditions, there are several legacy impacts that cause light usage which can seriously affect the development and functioning plantlets during acclimatization in the field (Arena *et al.* 2016). If the demand for water rises and roots have not yet developed, the number and/or size of stomata will increase which can cause drought stress in plantlets (Batista *et al.* 2018; Jensen *et al.* 2018; Wang *et al.* 2016). Changes in the number of chloroplasts can influence assimilation after changing to a different light condition (Chen *et al.* 2020). The anatomy of the stem is important for *in vitro* and *ex vitro* plantlet development (Batista *et al.* 2018). The larger stem diameter is key for the simplicity of modifications of plantlets *in vitro* and ex vitro distribution (Zeps *et al.* 2022). When plantlets are delivered ex vitro, the thickness and anatomy of the xylem can play an important role in water supply absorptivity (Kwon *et al.* 2015), whereas phloem thickness provides the nutrient stock required for initial development (Batista *et al.* 2018). The leaf anatomy has a major function in adjusting plants to the ecological ambiance, and variation in the anatomy of leaves influences on photosynthesis of the plants (Terashima *et al.* 2011). Molecular genotyping is a rapid test to prove genetic stability. Genetic stability is an important factor for confirming the plantlet after subculture frequently in multiple stages of *in vitro* culture propagation. However, the genetic accuracy of the Sigararutang dan Maragogipe plantlet has not been evaluated. Type of explant source, genotype, type and concentration of plant hormones, culture period, and combination media are some factors that influence the occurrence of genetic and epigenetic diversity in regenerating plants (Kour *et al.* 2009). This research aimed to figure out the anatomy of roots, stems, leaves and stomata and genetic consistency evaluation according to the *trn*L (UAA) region of *C. arabica* in *in vitro* conditions as the result of somatic embryogenesis propagation to obtain supplementary aspects which could support

plant taxonomists in the classification of *C. arabica* to provide opportunities for further studies and to evaluate molecular characteristics of genotyping to ensure the stability of the genetic plantlets.

2. Materials and Methods

2.1. Plant Samples

Coffea arabica (L.) plantlets of Sigararutang and Margogipe were obtained from *in vitro* propagation by somatic embryogenesis from the second leaf explants from the tip of reproductive mother plant according to the protocol described by Arimarsetiowati (2011). Fresh leaves, stems and roots of three-month-old plantlets, developed in shooting and rooting medium were collected for anatomical study.

2.2. Slide Preparation and Anatomical Observation

Roots, stems and leaves anatomy slides were prepared using a semi-permanent method (Harijati *et al.* 2013). Stomata density was prepared based on the protocol of Khoiroh *et al.* (2014). The preparat was observed under an OLYMPUS CX31 light microscope. Images were recorded using a digital camera. It generates 25 characters which will be followed by the observation to measure the length, width, and thickness (Table 1). Analysis of variance was used to describe the experimental results. Duncan's test defines the dissimilarities between the treatments if there is a large difference.

2.3. Assessment of Genetic Stability

2.3.1. DNA Isolation

The CTAB technique was used to obtain total genomic DNA from the second leaf tissue from the tip of the *C. arabica* parent plant and plantlet (Doyle and Doyle 1990). The plantlet was from the 3-month-old from shooting and rooting medium. The healthy plantlet and complete performance with leave and roots were selected. The parent plant was 3 years old reproductive plant from the greenhouse. The 100 grams of leaves were cut for DNA extraction. The improvement technique by combining Phenol Chloroform-isoamyl alcohol and cold absolute ethanol was subsequently used to purify the nucleic acid. The qualitative of nucleic acid was performed by applying agarose electrophoresis gel method (1%) with Ethidium Bromide (EtBr) of 1 µl dissolved in 1x TBE solution. The isolated DNA samples were then visualized using a UV transilluminator with a DNA ladder of 1 Kb. The extraction of plant genomic DNA was stored at -2°C.

2.3.2. PCR Amplification

The primer used is *trn*L forward (5'CGAAATCGGTAGACGCTACG-3') and reverse (5'GGGGATAGAGGGACTTGAAC-3') (Taberlet *et al.* 2007). PCR was performed in a reaction mixture of 30 µl volume. 6 µl ddH_2O, 15 µl PCR mix 2x solution, 3 µl DNA (100-350 ng/µl), 3 µl primer forward dan 3 µl primer reverse (30 pmol/µl) were used in the reaction. The *trn*L (UAA) region thermocycling phase was 95°C for 5 minutes, 35 cycles of 95°C for 45 seconds, 61.3°C for 45 seconds, and 72°C for 45 seconds, with a final extension of 72°C for 10 minutes. On a 1 percent agarose gel stained with Ethidium Bromide, the amplicons were interpreted. DNA PCR products were sequenced at 1st Base Sequencing INT Singapore applying the Sanger technique on an ABI PRISM 3730xl (Genetic Analyzer developed by Applied Biosystem, USA). To compare the sample DNA sequences resembling the Gene Bank report, it applied The Basic Local Alignment Search Tool (BLAST) (www.blast.ncbi.nlm.nih.gov/) (Altschul *et al.* 1990). Furthermore, gene sequence analysis was performed using MEGA5 software to determine the percentage of similarities.

3. Results

The structural patterns of the arrangement of roots, stems, leaves and stomata showed the same in Sigararutang (Figure 1A-E) and Maragogipe varieties (Figure 1F-J). The main variations in the physical properties of both varieties of roots, stems, leaves and stomata are presented in Table 1. Anatomical root traits consisted of root diameter, cortex thickness, distance of long stele, distance of short stele, epidermis thickness and endodermis thickness. Anatomical stem traits included stem diameter, cortex thickness, diameter of the vascular bundles, maximum stele diameter, minimum stele diameter and epidermis thickness. Anatomical leaf traits were comprised of diameter of stomatal aperture, diameter of stomatal opening, diameter of stomatal closing, length of stomatal opening, length of stomatal closing, total stomatal density, adaxial epidermis density, abaxial epidermis density, midrib thickness, adaxial epidermis thickness, abaxial

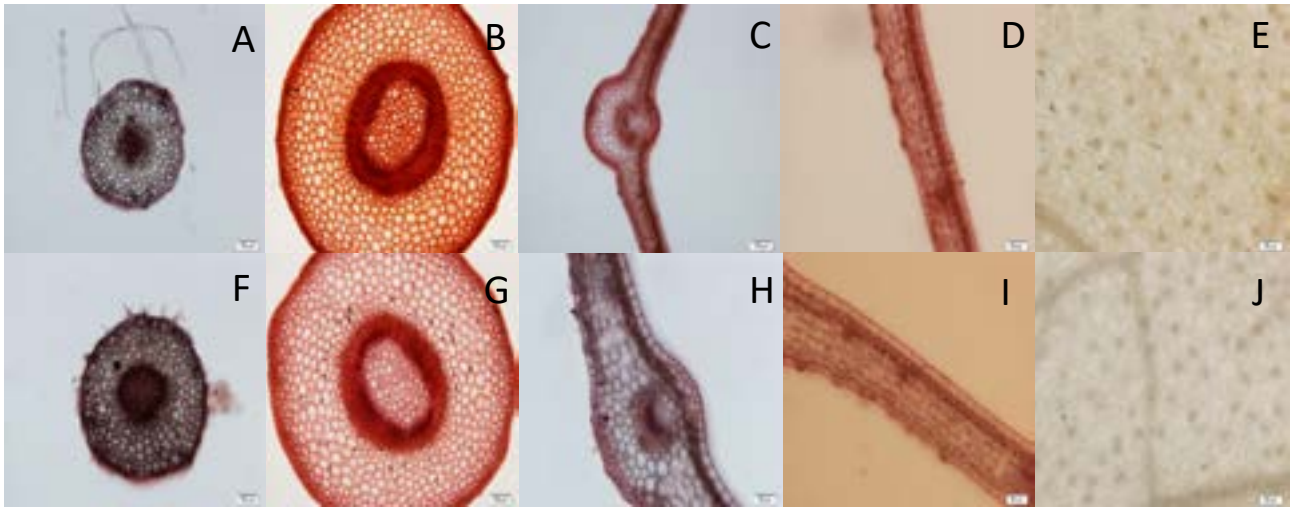

Figure 1. Cross section of roots, stems, leaves and abaxial surface of leaf formed *in vitro* of somatic embryo derived-plantlet of *Coffea arabica*. Cross section of roots (A), stems (B), leaves (C and D) and stomatas (E) of Sigararutang plantlets. Cross section of roots (F), stems (G), leaves (H and I) and stomatas (J) of Maragogipe plantlets. Bars = 100 µm (A, B, C, F, G) and 50 µm (D, E, H, I, J)

Table 1. The comparison of the thickness of the anatomical properties of the roots, stems and leaves of Sigararutang and Maragogipe Arabica coffee grown *in vitro* by somatic embryogenesis

Anatomical traits (µm)	Sigararutang	Maragogipe
Anatomical root traits:		
Root diameter	537.25±40.39[b]	679.14±3.59[a]
Cortex thickness	178.69±8.24[b]	227.89±8.68[a]
Distance of long stele	179.08±8.48[b]	227.06±22.17[a]
Distance of short stele	183.71±16.70[b]	246.90±10.29[a]
Epidermis thickness	24.08±0.21[a]	16.27±1.79[b]
Endodermis thickness	38.54±1.85[a]	39.76±3.40a
Anatomical stem traits:		
Stem diameter	1169.04±68.54[a]	1258.03±46.25[a]
Cortex thickness	350.07±8.08[a]	383.55±22.29[a]
Diameter of the vascular bundles	133.88±30.45[a]	120.82±11.43[a]
Maximum stele diameter	291.78±96.57[a]	376.94±40.49[a]
Minimum stele diameter	232.39±51.73[a]	251.10±25.39[a]
Epidermis thickness	23.53±3.81[a]	46.20±43.97[a]
Anatomical leaf traits :		
Diameter of stomatal aperture	11.17±0.23[a]	7.92±1.36[b]
Diameter of stomatal opening	41.56±2.90[a]	22.68±2.09[b]
Diameter of stomatal closing	20.49±3.57[a]	26.12±1.19[a]
Length of stomatal opening	31.67±1.65[a]	28.49±3.44[a]
Length of stomatal closing	32.50±2.22[a]	32.90±2.10[a]
Total stomatal density	293.00±23.00[a]	296.00±84.00[a]
Adaxial epidermis density	155.80±9.7[a]	160.49±5,91[a]
Abaxial epidermis density	222.35±25.87[a]	187.12±7.55[a]
Midrib thickness	286.69±64.54[a]	333.63±9.40[a]
Adaxial epidermis thickness	21.97±1.42[b]	26.51±1.42[a]
Abaxial epidermis thickness	18.80±2.32[a]	20.22±0.22[a]
Diameter of the vascular bundles	136.45±40.47[a]	154.10±7.07[a]
Lamina thickness	115.32±9.23[b]	151.05±5.66[a]

The values of similar characters in the same line are not significantly different by Duncan test ($p<0.05$). The anatomical properties was measured at different locations with means ± SD are shown

epidermis thickness, diameter of the vascular bundles and lamina thickness.

Statistical analysis shows a significant difference ($P<0.05$) of anatomical root traits and anatomical leaf traits between Sigararutang and Marogogipe varieties. Otherwise, the anatomical stem traits are not significantly different between Sigararutang and Marogogipe varieties. Root traits of Sigararutang and Maragogipe were measured from 24.08-537.25 µm thickness and 16.27-679.14 µm thickness, respectively. Stem traits of Sigararutang and Maragogipe were calculated from 23.53-1169.04 µm thickness and 46.20-1258.03 µm thickness, respectively. Leaf traits of Sigararutang and Maragogipe were computed from 11.17-286.69 µm thickness and 7.92-333.63 µm thickness, respectively. Maragogipe's thickness of anatomical traits was found to be greater than those of Sigararutang, except the epidermis thickness (16.27 µm), diameter of the vascular bundles (120.82 µm), diameter of stomatal aperture (7.92 µm), diameter of stomatal opening (22.68 µm), length of stomatal opening (28.49 µm) and abaxial epidermis density (187.12 µm).

Figure 2 showed that the confirmation of the *trn*L primer DNA of all Maragogipe and Sigararutang samples were amplified successfully. A unique band of approximately 530 bp was observed in all samples using *trn*L primers for amplification.

Nucleotide sequences of the *trn*L coding region between Sigararutang and Maragogipe samples were compared to further characterize and distinguish between parent plant and plantlet (Figure 3). Parent plant and plantlet both of Maragogipe and Sigararutang have 100 percent identity (Table 2).

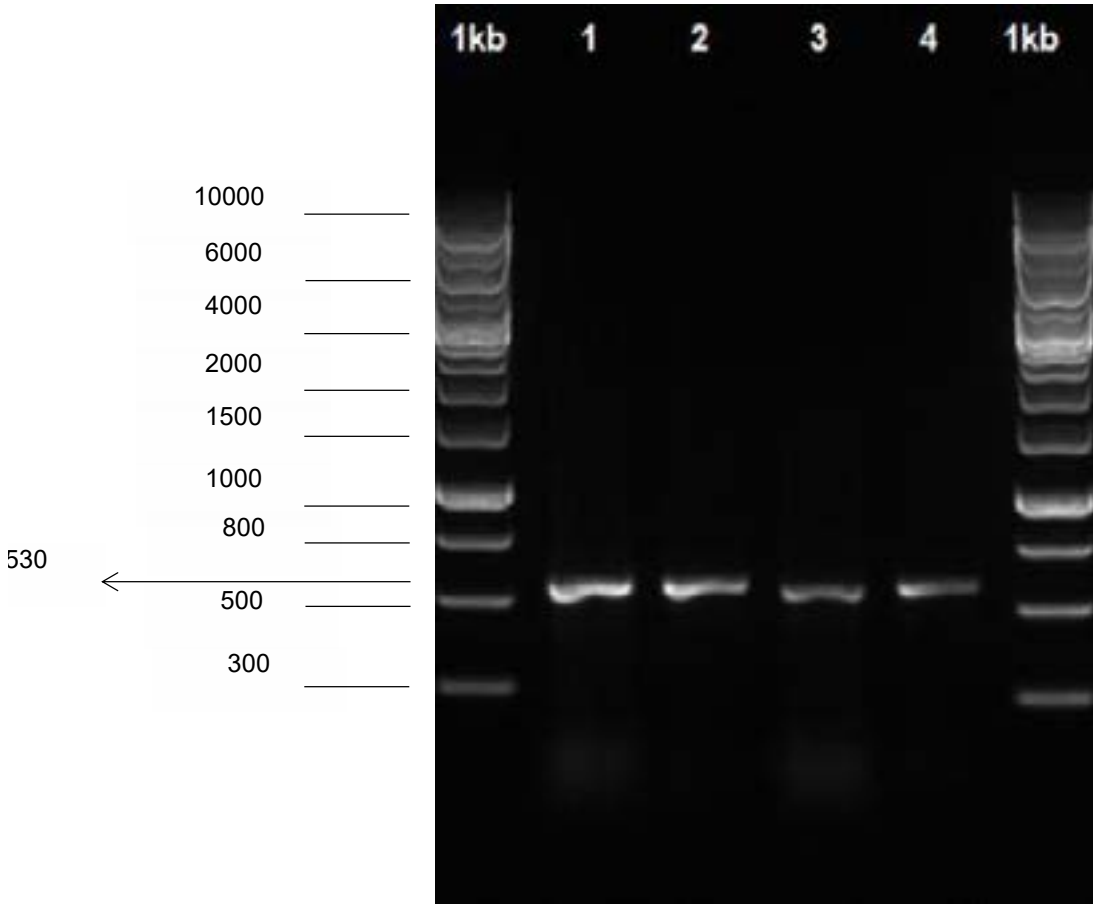

Figure 2. PCR product from trnL primers: 1. Maragogipe's parent plant, 2. Maragogipe's planlet, 3. Sigagarutang's parent plant, 4. Sigararutang's planlet

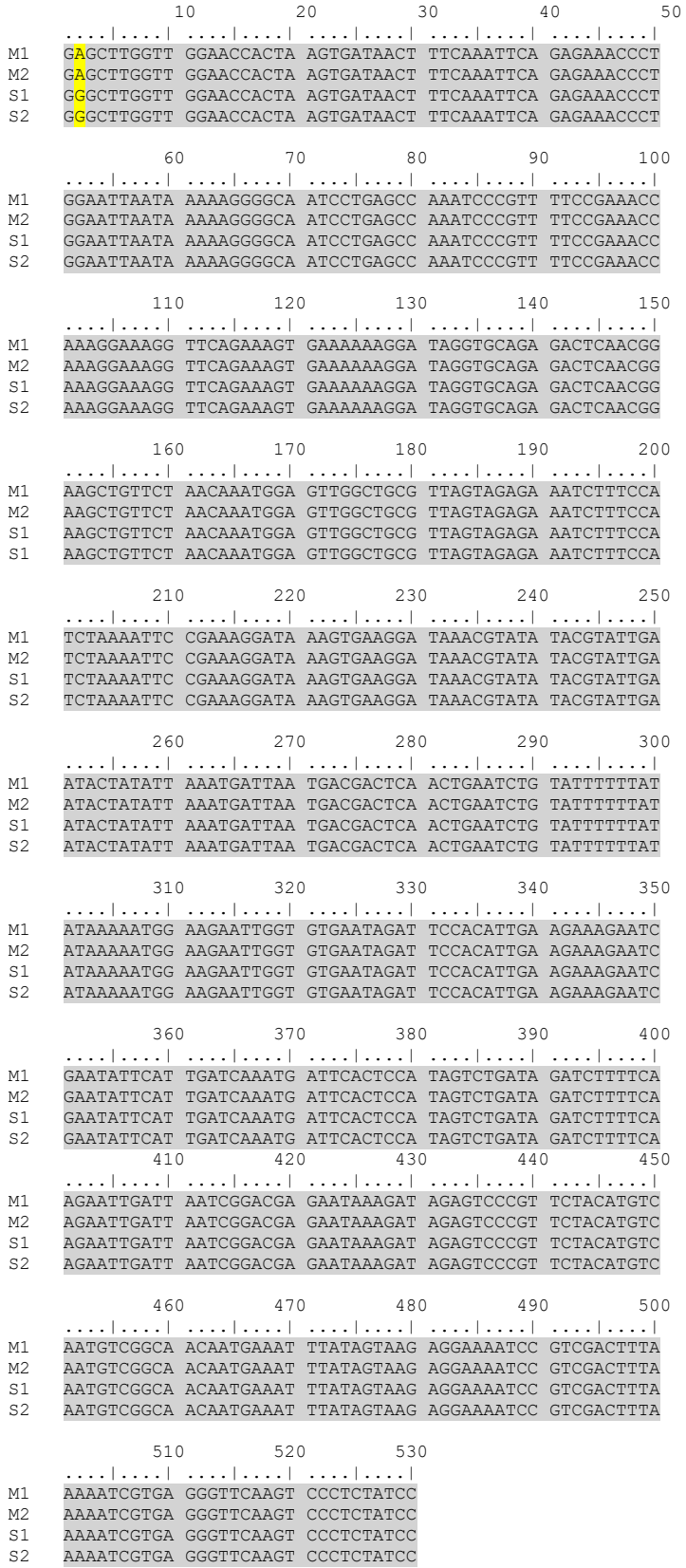

Figure 3. Alignment of the *trn*L DNA sequences of *Coffea arabica* (L.). M1 and M2 are sampled populations of Maragogipe as parent plant and plantlet, respectively. S1 and S2 are sampled populations of Sigararutang as parent plant and plantlet. The grey block indicates that the character states are the same. The yellow block indicates that the character states are different

Table 2. Nucleotide sequence similarity of *trn*L sequence for Maragogipe and Sigararutang. M1 and M2 are sampled population of Maragogipe as parent plant and plantlet, respectively. S1 and S2 are sampled population of Sigararutang as parent plant and plantlet. The values were calculated using MEGA5

Samples	M1	M2	S1	S2
M1	100.0			
M2	100.0	100.0		
S1	99.8	99.8	100	
S2	99.9	99.8	100	100

4. Discussion

Anatomical characters were observed between the two genotypes based on different origins and bean sizes from *in vitro* propagation through somatic embryogenesis. Not only the anatomical study but also the genetic stability suitability test between parent plants and plantlets were mostly caused by differences in genotype and phenotypic plasticity (Majada *et al.* 2000).

4.1. Anatomy of Roots, Stems and Leaves

Anatomical structure studies are very important for plant identification. The existence of similar characteristics indicates the existence of kinship between species in a family in the same habitat (Nabilah *et al.* 2011). The roots, stems, leaves and stomata of both species (Figure 1) revealed similar structural patterns of arrangement. However, the main differences in the anatomical features in the roots, stems, leaves and stomata of both species are outlined in Table 1. During *in vitro* somatic embryogenesis propagation, the rate of roots, stems, leaves and stomata differentiation and development differs between genotypes. A morphological analysis of *C. arabica* L. showed that the size of the root, stem and leaves of Maragogipe were larger than Sigararutang but most of the traits did not differ among samples (Table 1). Some of the features of Maragogipe were shorter in size than Sigarautang. The thickness of the root epidermis, the diameter of the vessel stem, the diameter of stomatal aperture, the diameter of stomatal opening, the length of stomatal opening and the density of abaxial Sigararutang epidermis were larger than that of Maragogipe. It could be the identity of the variety. However, no differences were seen between the samples. The anatomy of the stem is almost similar to that of the root. These can be considered a clear indications of anatomical characteristics from *C. arabica*.

Sigagarutang with shorter roots, stems and leaves are generally a species that grows on steep slopes, is exposed to the drying effects of sun and wind, and experiences low water uptake during rainy periods due to steepness. Maragogipe has a larger size than Sigagarutang most likely because it grows in the highlands under trees close to water sources so there is more water so that the roots, stems and leaves are bigger than Sigararutang. Phenotypic variability is influenced by both genotype and environment and is a key factor for most evolutionary and ecological mechanisms (Hahn *et al.* 2019; Zirbel and Brudvig 2020). Genetic variation is defined posses major impacts on trait expression (Agrawal and Hastings 2019a), creating variation for selection to respond to throughout evolution (Potts and Hunter 2021). Besides contributing to phenotypic variation (Couture *et al.* 2015; Decker *et al.* 2019), environmental variation is a source of powerful selection on specific phenotypes, influencing which genetic traits may succeed (Beemelmanns and Roth 2017; Jay *et al.* 2012). When environment is changing, population numbers may become developmentally stunted (Jay *et al.* 2012; Patankar *et al.* 2013; Sorte *et al.* 2013), and if the genetic variation is inadequate, the threat of extinction increases. Considering the concept of cellular totipotency, somatic embryogenesis aims to regenerate identical plants from the parent plant (Henao-Ramírez and Urrea-Trujillo 2020).

4.2. Evaluation of Genetic Consistency

Based on the DNA PCR product it can be seen that all Maragogipe and Sigararutang samples were successfully amplified (Figure 2). Then the band is analyzed for the sequencing stage. Somaclonal variation is defined as genetic or epigenetic changes that arise *in vitro* between clonal regenerants and their corresponding donor plants. The genetic changes are cytogenetic abnormalities and alterations to specific sequences of DNA; epigenetic changes are alterations of gene expression without changes to DNA sequences. Somaclonal variation, independent from the mechanisms involved, has been reported for several plant species. The occurrence of somaclonal variation in tissue culture has a negative effect on the rapid production of clonal plants of elite cultivars but may promote the production of novel horticultural crop genotypes (Leva and Rinaldi 2017). Somaclonal differences are the most common issues observed while the somatic embryogenesis system (Bairu *et*

al. 2011; Bhojwani and Dantu 2013) due to long-term treatment of culture and irregular development stag es between plantlets, the high concentration of plant growth regulator, the frequent of subculture and genotype dependence, pre-existing variation of the explants, activation of transposable elements and hypo or hypermethylation of DNA (Roostika *et al,* 2015). Somaclonal variation may lead to loss of genetic fidelity. Thus, evaluating genetic stability is crucial in the propagation strategy for analyzing genomic consistency between plantlet and parent plants (de Oliveira *et al.* 2019). According to Aydin *et al.* (2016), somaclonal variation should be detected during the early stage of plant tissue culture.

This study confirmed that the Margogipe and Sigararutang coffee plantlets resulting from somatic embryogenesis propagation had no genetic variation compared to the parent plant. This is shown by polymorphism analysis of 530 nucleotides on the aligned *trn*L primers of Maragogipe and Sigararutang coffee (Figure 3). The alignment of the *trn*L (UAA) DNA marker results shows that the parent plants and plantlets have the same character and conservation area, as well as there are no deletions or insertion of nucleotides between the four samples. Protected areas are areas that have the position of the nucleotide bases that do not change, so they are primitive (plesiomorph) (Hidayat and Pancoro 2008). In contrast, Maragogipe and Sigararutang varieties differ from 1 nucleotide base. The bases in position 2 show differentiation of adenine (A) to guanine (G). There is no gap or missing nucleotide bases. The *trn*L (UAA) of four Coffea arabica samples obtained in this research was similar in length matched to the GenBank sequence (https://www.ncbi.nlm.nih.gov/nuccore/AF543029.1). Moreover, they have a greater standard of homology (similarity rate 99 percent for *C. arabica*). Those varieties appear to be identical with limited genomic variation from the perspective of phytogeography. The differences between Maragogipe and Sigararutang have been investigated by the low level of genetic replacement in sequences, indicating that *trn*L (UAA) sequences are suitable for genetic stability assessment. Parent plant and plantlet both of Maragogipe and Sigararutang have 100 percent identity. Sequences are expected to be very similar (Table 2). Otherwise, Maragogipe towards Sigagarutang varieties has 99.8 percent identity because of the one nucleotide base difference (Figure 3). According to Landey *et al.* (2013), the molecular analyses indicated that the occurrence of somaclonal variation was very low, and possible genetic and epigenetic alterations occurred during somatic embryogenesis of elite F1 hybrids of *C. arabica*.

In conclusion, the anatomy of the roots, stems, leaves and stomata of the two species shows a similar structural pattern or arrangement. Most of the sizes of root, stem and leaves of Maragogipe are larger than Sigararutang and most of the properties did not differ between samples. The anatomic traits revealed in this research may be used as supplementary evidence in describing *C. arabica*. Sequence analysis showed that genetic stability between the parent plant and plantlet from somatic embryogenesis was achieved. Both of the samples are identical, there is no somaclonal variation. The *trn*L (UAA) sequence can detect genetic variation and is successful as a marker for conformity testing.

Acknowledgements

This research was supported by the Ministry of Research, Technology and Higher Education of the Republic of Indonesia on the scheme of PDD research grant 2021, contract number: 2232/UN1/DITLIT/DIT-LIT/PT/2021) with ES as PI.

References

Ackerly, D.D., Dudley, S.A., Sultan, S.E., Schmitt, J., Coleman, J.S., Linder, C.R., Sandquist, D.R., Geber, M.A., Evans, A.S., Dawson, T.E., Lachowicz, M.J., 2000. The evolution of plant ecophysiological traits: Recent advances and future directions. *Bioscience*. 50, 979-995. https://doi.org/10.1641/0006-3568(2000)050[0979:TEOPET]2.0.CO;2

Adrienne, E.Ng., Sandoval, E., Murphy, T.M., 2015. Identification and individualization of Lophophora using DNA analysis of the *trn*L/*trn*F region and *rbc*L gene. *Journal of forensic sciences*. 61, 226-229. https://doi.org/10.1111/1556-4029.12936

Agrawal, A.A., Hastings, A.P., 2019a. Plant defense by latex: Ecological genetics of inducibility in the milkweeds and a general review of mechanisms, evolution, and implications for agriculture. *Journal of Chemical Ecology*. 45, 1004-1018. https://doi.org/10.1007/s10886-019-01119-8

Altschul, S.F., Gish, W., Miller, W., Myers, E.W., Lipman, D.J., 1990. Basic local alignment search tool. *Journal of Molecular Biology*. 215, 403-410. https://doi.org/10.1016/S0022-2836(05)80360-2

Angelo, P.C.S., 2019. Study on the anatomy of young coffee plants from micro-cuttings and induced to sprout. *Plant Tissue Culture and Biotechnology*. 29, 185-194. https://doi.org/10.3329/ptcb.v29i2.44507

Arena, C., Tsonev, T., Doneva, D., De Micco, V., Michelozzi, M., Brunetti, C., Centritto, M., Fineschi, S., Velikova, V., Loreto, F., 2016. The effect of light quality on growth, photosynthesis, leaf anatomy and volatile isoprenoids of a monoterpene-emitting herbaceous species (*Solanum lycopersicum* L.) and an isoprene-emitting tree (*Platanus orientalis* L.). *Env. Exp. Bot.* 130, 122-132. https://doi.org/10.1016/j.envexpbot.2016.05.014

Arimarsetiowati, R., 2011. Pengaruh auksin 2,4-D dan sitokinin 2-ip terhadap pembentukan embriogenesis somatik langsung pada eksplan daun *Coffea arabica* L. *Jurnal Pelita Perkebunan.* 27, 68-76.

Aydin, M., Arslan, E., Taspinar, M.S., Karadayi, G., Agar, G., 2016. Analyses of somaclonal variation in endosperm-supported mature embryo culture of rye (*Secalecereale* L.). *Biotechnology and Biotechnological Equipment*. 30, 1082-1089, https://doi.org/10.1080/13102818.2016.1224980

Bairu, M.W., Aremu, A.O. , Van Staden, J., 2011. Somaclonal variation in plants: causes and detection methods. *Plant Growth Regul.* 63, 147-173. https://doi.org/10.1007/s10725-010-9554-x

Baraket, G., Abdelkrim, A.B., Saddoud, O., Chatti, K., Mars, M., Trifi, M., Salhi-Hannachi, A., 2010. Molecular polymorphism of cytoplasmic DNA in *Ficus carica* L. insights from non-coding regions of chloroplast DNA. *Scientia Horticulturae*. 125, 512-517. https://doi.org/10.1016/j.scienta.2010.04.043

Barbosa, M.S.G., Scholz, M.B.S., Kitzberger, C.S.G., Benassi, M.T., 2019. Correlation between the composition of green Arabica coffee beans and the sensory quality of coffee brews. *Food Chemistry*. 292, 275-280. https://doi.org/10.1016/j.foodchem.2019.04.072

Batista, D.S., Felipe, S.H.S., Silva, T.D., Motta de Castro, K., Mamedes-Rodrigues, T.C., Miranda, N.A., Ríos-Ríos, A.M., Faria, D.V., Fortini, E.A., Chagas, K., Torres-Silva, G., Xavier, A., Arencibia, A.D., Otoni, W.C., 2018. Light quality in plant tissue culture: does it matter? *In Vitro Cell Dev. Biol. Plant*. 54, 195-215. https://doi.org/10.1007/s11627-018-9902-5

Beemelmanns, A., Roth, O., 2017. Grandparental immune priming in the pipefish Syngnathus typhle. *BMC Evolutionary Biology*. 17, 1-15. https://doi.org/10.1186/s12862-017-0885-3

Bhatia, S., Sharma, K., Dahiya, R., Bera, T., 2015. *Modern Applications of Plant Biotechnology in Pharmaceutical Sciences*, first ed. Academic Press. https://doi.org/10.1016/B978-012-802221-4.00005-4

Bhojwani, S.S., Dantu, P.K., 2013. *Plant Tissue Culture: An Introductory Text*. Springer, London. https://doi.org/10.1007/978-81-322-1026-9

Buerki, S., Callmander, M.W., Devey, D.S., Chappell, L., Gallaher, T., Munzinger, J., Haevermans, T., Forest, F., 2012. Straightening out the screw-pines: a first step in understanding phylogenetic relationships within *Pandanaceae. TAXON*. 61, 1010-1020. https://doi.org/10.1002/tax.615008

Callmander, M.W., Booth, T.J., Beentje, H., Buerki, S., 2013. Update on the systematics of Benstonea (*Pandanaceae*): when a visionary taxonomist foresees phylogenetic relationships. *Phytotaxa*. 112, 57-60. https://doi.org/10.11646/phytotaxa.112.2.4

Callmander, M.W., Lowry, P.P., Forest, F., Devey, D.S., Beentje, H., Buerki, S., 2012. Benstonea Callm. and Buerki (*Pandanaceae*): characterization, circumscription, and distribution of a new genus of screw-pines, with a synopsis of accepted species. *Candollea*. 67, 323-345. https://doi.org/10.15553/c2012v672a12

Castanheira, D., Voltolini, G., Rezende, T.T., Netto, P.M., 2019. Growth, anatomy and physiology of coffee plants intoxicated by the herbicide glyphosate. *Coffee Science*. 14, 76. https://doi.org/10.25186/cs.v14i1.1530

Cenci, A., Combes, M.C., Lashermes, P., 2012. Genome evolution in diploid and tetraploid Coffea species as revealed by comparative analysis of orthologous genome segments. *Plant Mol Biol.* 78, 135-145. https://doi.org/10.1007/s11103-011-9852-3

Chen, L., Zhang, K., Gong, X., Wang, H., Gao, Y., Wang, X., Zheng, Z., Hu, Y., 2020. Effects of different LEDs light spectrum on the growth, leaf anatomy, and chloroplast ultrastructure of potato plantlets *in vitro* and minituber production after transplanting in the greenhouse. *J. Integr. Agric.* 19, 108-119. https://doi.org/10.1016/S2095-3119(19)62633-X

Coffea arabica trnL gene, partial sequence, chloroplast gene for chloroplast product 2020. Available at: https://www.ncbi.nlm.nih.gov/nuccore/AF543029.1. [Date accessed: 15 November 2020]

Coffee Genome Hub 2020. Available at: https://coffee-genome.org/. [Date accessed: 14 November 2020]

Couture, J.J., Serbin, S.P., Townsend, P.A., 2015. Elevated temperature and periodic water stress alter growth and quality of common milkweed (*Asclepias syriaca*) and monarch (*Danaus plexippus*) larval performance. *Arthropod-Plant Interactions*. 9, 149-161. https://doi.org/10.1007/s11829-015-9367-y

de Groot, G.A., During, H.J., Maas, J.W., Schneider, H., Vogel, J.C., Erkens, R.H.J., 2011. Use of *rbc*L and *trn*L-F as a Two-Locus DNA Barcode for Identification of NWEuropean Ferns: an ecological perspective. *PLoS ONE*. 6, e16371. https://doi.org/10.1371/journal.pone.0016371

de Oliveira, K.C., de Souza-Guimarães, P., Bazioli, J. M., Martinati, J.C., dos Santos, M.M., Padilha, L., Guerreiro-Filho, O., Maluf, M.P., 2019. Effects of somatic embryogenesis on gene expression of cloned coffee heterozygous hybrids. *Acta Physiologiae Plantarum*. 41, 118. https://doi.org/10.1007/s11738-019-2917-7

Decker, L.E., Soule, A.J., de Roode, J.C., Hunter, M.D., 2019. Phytochemical changes in milkweed induced by elevated CO_2 alter wing morphology but not toxin sequestration in monarch butterflies. *Functional Ecology*. 33, 411-421. https://doi.org/10.1111/1365-2435.13270

Dong, W., Liu, J., Yu, J., Wang, L., Zhou, S., 2012. Highly variable chloroplast markers for evaluating plant phylogeny at low taxonomic levels and for DNA barcoding. *PLoS ONE*. 7, e35071. https://doi.org/10.1371/journal.pone.0035071

Doyle, J.J., Doyle, J.L., 1990. A rapid total DNA preparation procedure for fresh plant tissue. *Focus*. 12, 13-15.

Gallaher, T., Callmander, M.W., Buerki, S., Keeley, S.C., 2015. A long distance dispersal hypothesis for the Pandanaceae and the origins of the *Pandanus tectorius* complex. *Molecular Phylogenetics and Evolution*. 83, 20-32. https://doi.org/10.1016/j.ympev.2014.11.002

Gielly, L., Taberlet, P., 1994. The use of chloroplast DNA to resolve plant phylogenies: noncoding versus *rbc*L sequences. *Molecular Biology and Evolution*. 11, 769-777.

Guan Y., Li S., Fan X., Su Z., 2016. Application of somatic embryogenesis in woody plants. *Frontiers in. Plant Scicience*. 7, 908. https://doi.org/10.3389/fpls.2016.00938

Hahn, P.G., Agrawal, A.A., Sussman, K.I., Maron, J.L., 2019. Population variation, environmental gradients, and the evolutionary ecology of plant defense against herbivory. *American Naturalist*. 193, 20-34. https://doi.org/10.1086/700838

Harijati, N., Azrianingsih, R., Prawaningtyas, E.A., 2013. The study of anatomy and fiber banana leaf as a potensial wrapping. *American Journal of Plant Sciences*. 4, 1461-1465. https://doi.org/10.4236/ajps.2013.47179

Henao-Ramírez, A.M., Urrea-Trujillo, A.I., 2020. Somatic embryogenesis for clonal propagation and associated molecular studies in cacao (*Theobroma cacao* L.), in: Chong, P., Newman, D., Steinmacher, D. (Eds.). *Agricultural, Forestry and Bioindustry Biotechnology and Biodiscovery*. Cham: Springer. https://doi.org/10.1007/978-3-030-51358-0_5

Hervé, E., Guyot, R., Beulé, T., Breitler, J.C., Jaligot, E., 2016. Plant fidelity in somatic embryogenesis-regenerated plants, in: Loyola-Vargas, V.M., Ochoa-Alejo, N. (Eds.), *Somatic Embryogenesis Fundamental: Aspects and Applications*. Springer International, Switzerland, pp. 121-150. https://doi.org/10.1007/978-3-319-33705-0_8

Hidayat, T., Kusumawaty, D., Yati, D.D., Muchtar, A.A., Mariana, D., 2008. Molecular phylogenetic analysis of *Phyllanthus niruri* L. (Euphorbiaceae) using Internal Transcribed Spacer (ITS). *Jurnal Matematika Dan Sains.* 13, 16-21.

Hidayat, T., Pancoro, A., 2008. Molecular phylogenetic studies provide a basic knowledge of improving genetic resources. *Agrobiogen.* 4, 35-40. https://doi.org/10.21082/jbio.v4n1.2008.p35-40

Hulupi, R., 2016. *Panduan Determinasi Varietas dan Klon Kopi Indonesia Berdasarkan Sifat Morfologi*. Pusat Penelitian Kopi dan Kakao Indonesia, ...

[ICO] International Coffee Organization, 2018. Trade Statistics. Available at: https://www.ico.org/trade_statistics.asp. [Date accesessed: 15 November 2020]

Jay, F., Manel, S., Alvarez, N., Durand, E.Y. , Thuiller, W., Holderegger, R., Taberlet, P., François, O., 2012. Forecasting changes in population genetic structure of alpine plants in response to global warming. *Molecular Ecology*. 21, 2354-2368. https://doi.org/10.1111/j.1365-294X.2012.05541.x

Jensen, N.B., Clausen, M.R., Kjaer, K.H., 2018. Spectral quality of supplemental LED grow light permanently alters stomatal functioning and chilling tolerance in basil (*Ocimum basilicum* L.). *Sci. Hortic.* 227, 38-47. https://doi.org/10.1016/j.scienta.2017.09.011

Jones, H.G., 1998. Stomatal control of photosynthesis and transpiration. *Journal of Experimental Botany*. 49, 387-398. https://doi.org/10.1093/jxb/49.Special_Issue.387

Khoiroh, Y., Harijati, N., Mastuti, R., 2014. Pertumbuhan serta hubungan kerapatan stomata dan berat umbi pada *Amorphophallus muelleri* Blume dan *Amorphophallus variabilis* Blume. *Biotropika: Journal of Tropical Biology*. 2, 249-253.

Kour, G., Kour, B., Kaul, S., Dhar, M.K., 2009. Genetic and epigenetic instability of amplification-prone sequences of a novel B chromosome induced by tissue culture in *Plantago lagopus* L. *Plant Cell Reports*. 28, 1857-1867. https://doi.org/10.1007/s00299-009-0789-9

Kwon, A.R., Cui, H.Y., Lee, H., Shin, H., 2015. Light quality affects shoot regeneration, cell division, and wood formation in elite clones of *Populus euramericana. Acta Physiol. Plant.* 37, 65. https://doi.org/10.1007/s11738-015-1812-0

Landey, B.R., Cenci, A., Georget, F., Bertrand, B., Camayo, G., Dechamp, E., Herrera, J.C., Santoni, S., Lashermes,P., Simpson, J., Etienne, H., 2013. High genetic and epigenetic stability in *Coffea arabica* plants derived from embryogenic suspensions and secondary embryogenesis as revealed by AFLP, MSAP and the phenotypic variation rate. *PLoS One*. 8, e56372. https://doi.org/10.1371/journal.pone.0056372

Larcher, W., 2000. *Ecofisiologia Vegetal*. Rima. São Carlos, São Paulo.

Lee, S.C., Wang, C.H., Yen, C.E., Chang, C., 2016. DNA barcode and identification of the varieties and provenances of Taiwan's domestic and imported made teas using ribosomal internal transcribed spacer 2 sequences. *Journal of Food and Drug Analysis*. 25, 260-274. https://doi.org/10.1016/j.jfda.2016.06.008

Leva, L.M.R., Rinaldi, 2017. *Breeding Genetics and Biotechnology in Encyclopedia of Applied Plant Sciences,* second ed. Academic Press, Cambridge.

Majada, J., Tadeo, F.R.., Fal, M.A., Sánchez-Tamés, R., 2000. Impact of culture vessel ventilation on the anatomy and morphology of micropropagated carnation. *Plant Cell, Tissue and Organ Culture.* 63, 207-214. https://doi.org/10.1023/A:1010650131732

Maurin, O., Davis, A. P., Chester, M., Mvungi, E. F., Jaufeerally-Fakim, Y., Fay, M.F., 2007. Towards a phylogeny for coffea (*Rubiaceae*): identifying well-supported lineages based on nuclear and plastid DNA sequences. *Annals of botany*. 100, 1565-1583. https://doi.org/10.1093/aob/mcm257

Nabilah, M., Nurnida M.K., Noraini T., Ruzi A.R., Amalia, Nurshahidah, M.R., Mohd-Arrabe', A.B., 2011. Leaf anatomical adaptation of some mangrove species (*Rhizophoraceae*). *In Proceeding: of 10th International Annual Symposium of University Malaysia Terengganu (UMTAS2011), Empowering Science, Technology and Innovation towards a Better Tomorrow, University Malaysia Trengganu*. Terengganu, Malaysia: University Malaysia Terengganu Publisher. pp. 445-449.

NCBI, 2020. Available at: https://www.blast.ncbi.nlm.nih.gov/. [Date accessed: 13 November 2020]

Nugroho, D., Mawardi, S., Yusianto, Arimersetiowati, R., 2012. Karakterisasi mutu fisik dan cita rasa biji kopi Arabika varietas Maragogip (*Coffea arabica* L. var. Maragogype Hort. ex Froehner) dan seleksi pohon induk di Jawa Timur. *Pelita Perkebunan*. 28, 1-13. https://doi.org/10.22302/iccri.jur.pelitaperkebunan.v28i1.159

Patankar, R., Quinton, W. L., Baltzer, J.L., 2013. Permafrost-driven differences in habitat quality determine plant response to gall-inducing mite herbivory. *Journal of Ecology*. 101, 1042-1052. https://doi.org/10.1111/1365-2745.12101

Pompelli, M., Pompelli, G.M., Cabrini, E.C., Alves, M.C.J., Ventrella, M., 2012. Leaf anatomy, ultrastructure and plasticity of *Coffea arabica* L. in response to light and nitrogen. *Revista Biotemas*. 25, 13-28. https://doi.org/10.5007/2175-7925.2012v25n4p13

Potts, A.S., Hunter, M.D., 2021. Unraveling the roles of genotype and environment in the expression of plant defense phenotypes. *Ecol Evol.* 11, 8542-8561. https://doi.org/10.1002/ece3.7639

Rahadiantoro, A., Hakim, L., Arumingtyas, E. L., 2013. Genetic variation of *Dacrycarpus imbricatusin* Bromo Tengger Semeru National Park(BTS-NP), East Java based on *trn*L (UAA) intron region. *The Journal of Tropical Life Science*. 3, 127-131. https://doi.org/10.11594/jtls.03.02.10

Rodrigues, S.P., Picoli, E.A.D.T., Oliveira, D.C., Carneiro, R.G.S., Isaias, R.M.S., 2014. The effects of *in vitro* culture on the leaf anatomy of *Jatropha curcas* L. (Euphorbiaceae). *Biosci. J.* 30, 1933-1941.

Roostika, I., Khumaida, N., Ardie, S.W., 2015. RAPD Analysis To De Te Ct somaclonal variation of pine apple culture S during *in vitro* micropropagation. *BIOTROPIA.* 22, 109-119. https://doi.org/10.11598/btb.2015.22.2.422

Sakiroh., Ibrahim, M.S.D., 2020. Morphological, anatomical, and physiological characterization of seven superior clones of robusta coffee. *Jurnal Tanaman Industri dan Penyegar*. 7, 73-82. https://doi.org/10.21082/jtidp.v7n2.2020.p73-82

Simon-Gruita, A., Pojoga, M.D., Constantin, N., Duta-Cornescu, G., 2019. *Caffeinated and Cocoa Based Beverages, Genetic Engineering in Coffee.* Woodhead Publishing, Sawston . https://doi.org/10.1016/B978-0-12-815864-7.00014-3

Smith, H.L., McAusland, L., Murchie, E.H., 2017. Don't ignore the green light: exploring diverse roles in plant processes. *J. Exp. Bot.* 68, 2099-2110. https://doi.org/10.1093/jxb/erx098

Sorte, C.J.B., Ibáñez, I., Blumenthal, D.M., Molinari, N.A., Miller, L.P., Grosholz, E.D., Diez, J.M., D'Antonio, C.M., Olden, J.D., Jones, S.J., Dukes, J.S., 2013. Poised to prosper? a cross-system comparison of climate change effects on native and non-native species performance. *Ecology Letter.* 16, 261-270. https://doi.org/10.1111/ele.12017

Su, N., Wu, Q., Shen, Z., Xia, K., Cui, J., 2013. Effects of light quality on the chloroplastic ultrastructure and photosynthetic characteristics of cucumber seedlings. *Plant Growth Regul.* 73, 227-235. https://doi.org/10.1007/s10725-013-9883-7

Taberlet, P., Coissac, E., Gielly, L., Miquel, C., Brochmann, C., Valentini, A., Vermat, T., Willerslev, E., 2007. Power and limitations of the chloroplast *trn*L (UAA) intron for plant DNA barcoding. *Journal of Natural History*. 35, 1-8. https://doi.org/10.1093/nar/gkl938

Terashima, I., Hanba, Y.T., Tholen, D., Niinemets, U., 2011. Leaf functional anatomy in relation to photosynthesis. *Jornal of Plant Physiology*. 155, 108-116. https://doi.org/10.1104/pp.110.165472

Trespontas, 2021. Available at: https://www.trespontas.com/pages/varietals. [Date accessed: 22 Maret 2021].

Vasconcelos, T.N.C., Proença, C.E.B., Ahmad, B., Aguilar, D.S., Aguilar, R., Amorim, B. S., Campbell, K., Costa, I.R., De-Carvalho, P.S., Faria, J.E.Q., Giaretta, A., Kooij, P.W., Lima, D.F., Mazine, F.F., Peguero, B., Prenner, G., Santos, M.F., Soewarto, J., Wingler, A., Lucas, E.J., 2017. Myrteae phylogeny, calibration, biogeography and diversification patterns: increased understanding in the most species rich tribe of *Myrtaceae. Molecular Phylogenetics and Evolution* 109, 113-137. https://doi.org/10.1016/j.ympev.2017.01.002

Wang, J., Lu, W., Tong, Y.X., Yang, Q.C., 2016. Leaf morphology, photosynthetic performance, chlorophyll fluorescence, stomatal development of lettuce (*Lactuca sativa* L.) exposed to different ratios of red light to blue light. *Front. Plant Sci.* 7, 250. https://doi.org/10.3389/fpls.2016.00250

Wintgens, J.N., 2009. *Coffee: Growing, Processing, Sunstainable Production,* second ed. Wiley-VCH, Weinheim.

Wu, D.C., He, D.M., Gu, H.L., Wu, P.P., Yi, X., Wang, W.J., Shi, H.F., Wu, D.X., Sun, G., 2016. Origin and evolution of allopolyploid wheatgrass *Elymus fibrosus* (Schrenk) Tzvelev (Poaceae: Triticeae) reveals the effect of its origination on genetic diversity. *PLoS ONE.* 11, e0167795. https://doi.org/10.1371/journal.pone.0167795

Yang, R., Feng, X., Gong, X., 2016. Genetic structure and demographic history of *Cycas chenii* (Cycadaceae), an endangered species with extremely small populations. *Plant Diversity.* 39, 44-51. https://doi.org/10.1016/j.pld.2016.11.003

Yurtseva, O.V., Kuznetsova, O.I., Mavrodiev, E.V., 2016. A broadly sampled 3-loci plastid phylogeny of *Atraphaxis* (Polygoneae, Polygonoideae, Polygonaceae) reveals new taxa: I. *Atraphaxis kamelinii* spec. nov. from Mongolia. *Phytotaxa.* 268, 1-24. https://doi.org/10.11646/phytotaxa.268.1.1

Zeps, M., Kondratovičs, T., Grigžde, E., Jansons, Ā., Zeltiņš, P., Samsone, I., Matisons, R., 2022. Plantlet anatomy of silver birch (*Betula pendula* Roth.) and hybrid aspen (*Populus tremuloides* Michx. × *Populus tremula* L.) shows intraspecific reactions to illumination *in vitro*. *Plants (Basel)*. 11, 1097. https://doi.org/10.3390/plants11081097

Zirbel, C.R., Brudvig, L.A., 2020. Trait-environment interactions affect plant establishment success during restoration. *Ecology*. 101, 1-7. https://doi.org/10.1002/ecy.2971