The Kinship Of Three Lactuca Species Based On Their Molecular Profiles On RAPD-PCR Markers

Alexander Jason[1], Pudji Widodo[2], Nurtjahjo Dwi Sasongko[3]

Fakultas Biologi, Universitas Jenderal Soedirman.
Jalan dr. Suparno 63 Purwokerto 53122.

Coresponding Author: Nurtjahjo Dwi Sasongko
Email: nurtjahyo.sasongko@unsoed.ac.id

ABSTRACT

Lactuca spp., Asteraceae, like: Green lettuce (L. sativa) is the most common type of lettuce grown, while the spiny lettuce (L. serriola) and wild lettuce (L. virosa) have not been being cultivated yet. The current study explores those three species in morphological and molecular characters. The data are then used to cluster the kinship of them which never been reported so far. Sampling was done in a composite sampling method, and analyzed by RAPD-PCR (Random Amplified Polymorphic DNA) applying 8 primers as follows: (OPF-1, OPF-2, OPF-3, OPF-4, OPC-11, OPAE-5, OPA-2, OPAM-7). Before PCR, the genomic DNA was extracted from each sample, following the DNA kit protocol and measured for their qualities and quantities. The total number of DNA bands were calculated for polymorphic or monomorphic patterns. The polymorphic bands were analyzed by the MEGA 6 software (Molecular Evolutionary Genetics Analysis) applying a maximum parsimony method to obtain the cladogram of the samples. The RAPD-PCR obtained a total of 91 amplicons where 84 of them are polymorphic (86,597%) and the rest were monomorphic. Primers whch produced the highest polymorphism are OPF-1, OPF-4, OPC-11, OPAE-5, dan OPA-2 (100%), and the OPAM-7 produced the ost amplicons 20 DNA bands. The kinship among these three species showed that L. serriola and L. virosa have the closest distance. The cladogram following the maximum parsimony analysis grouped them into two clad i.e; clad I and clad II. Clad I consists of L. serriola and L. virosa, clad II consists of L. sativa only.

Keywords: Asteraceae; Genetic Diversity; Lactuca; Lettuce; RAPD

INTRODUCTION

In Indonesia, horticulture, play significant roles because of their high economic value in both local market and so export. In the mean time, the demand of lettuce (*Lactuca spp.,*) in country, for example, is growing fast parallel with the growth of International/ chain restaurants in the country that serve this crop in their menus (Prasetyo & Lazuardi, 2019). On the hand, Indonesia, also exported this crop to some countries as as many as 47.920 tones in year 2018 and increased to 55.710 year after, and so classified as need to developed seriously for its high economic value (Samadi, 2019). Green lettuce, so far, has been cultivated quite massively (*L. sativa*), which is different from the (*L. serriola*) and (*L. virosa*) (Abdul-Jalil, 2020).

Most of the Indonesian farmers cultivate green lettuce to fulfill the market demands though this crop was originally come from west Asia and America (Prasetio, 2013).

In traditional medicament, some species of the genus Lactuca like the spiny lettuce (*L. serriola*) and wild lettuce (*Lactuca virosa*) are known to have medicinal compound and, therefore, were applied to cure some diseases (Mieslerova *et al*., 2013; Lebeda *et al*, 2021).

The genetic diversity of Lactuca genus might be detected through a *Polymerase Chain Reaction* (PCR) method, applying short primers called as *Random Amplified Polymorphic DNA* (RAPD) (Purnaningsih, 2013). This technique has proven to be a good molecular marker for analyses of genetic variability among the unknown

plants (Istiqomah *et al*., 2016). However, this method is known as low reproducible method thought it still be able to be solved by setting a consistent PCR condition (Wijaya *et al*., 2009).

Information on genetic diversity between the green lettuce and its wild kinship is important in developing this plant for any purposes like vegetable crop and medicament sources and further domestication of the wild lettuce ones (Yu *et al*., 2001; Khan *et al*., 2019); as well as ex situ conservation (El-Esawi *et al*., 2017; van Herwijnen & Manning 2017).

The current study was aimed to profilling the genetic diversity of the green lettuce (*L. sativa*), spiny lettuce (*L. serriola*), and wild lettuce (*L. virosa*) following the morphological and molecular approaches.

Material and Methods

The lettuce plants being studied were green lettuce (*L. sativa*), spiny lettuce (*L. serriola*) and wild lettuce (*L. virosa*).The genetic diversity among them then was compared with the out group plant *Chrysanthemum morifolium*. Initially these three different species were observed for their morphological characters which was done in the Laboratory of Plant Taxonomy, but, the molecular analysis was run in the Genetics and Molecular laboratory > Both laboratories belong t the Faculty of Biology University of Jenderal Soedirman from September to October 2022

The RAPD used 8 different primers namely: OPA-02, OPAM-07, OPC-11, OPF-1, OPF-2, OPF-3, OPF-4, and OPAM-5. For the PCR, the DNA os Lettuce were isolated using a GeneAid™ Plant DNA isolation mini kit. Following to this, the PCR was applied by mixing the solution of MyTaq PCR master mix, primer and nuclease-free water. The last step, visualization, was done in an agarose powder, mixed with TAE 1x buffer to make up a slab then added with lpading dye and a 1 Kb DNA ladder of Thermofischer.

Some tools like 1.5 mL microtubes , micro pipettes with their tips, micro centrifuge, autoclaved, analytical balance , a thermocycler machine, ultra-low -80˚C, Freezer -20˚C, UV Transiluminator, abd electrophoreses set.

The morphological characters of Lactuca were observed in the Plant Taxonomy Laboratory but eh molecular profilling was done in the Genetics and Molecular Biology Laboratory, bith are in the Faculty of Biology Unsoed from August to October 2022.

B. Design of the study

A random sampling technique was applied to obtain the samples of Lactuca spp., than taken to the laboratory ofor validation and identification. Following to this, the samples were subjected for molecular analysis using a PCR technique which based on the FNA concentration and so purity, then the appearance of DNA fragments on the agar gel.

a. Isolation of DNA genome

DNA genome of Lactuca spp., was done following the protocol of GeneAid™ plant DNA isolation mini kit as follows: ca. 1 cm leaf was cut from each plant species then cleanse with 70% alcohol and dried in an open air before being grind. Add 400 µL GPX1 buffer and transfer the sample into 1.5 mL microtube then 5 µL RNAse to hogenize in 30 seconds and incubated for 15 minutes at 60°C.and incubated for 3 minutes over the ice block. 100 µL GP2 buffer was also added to the tube containing the sample and homogenized. Sample was then transferred into 2 mL collection tube which completed with a filter column and centrifuged at 1000 x g for 8 minutes. Put the supernantant into a

new 1.5 mL microtube. Add the GP3 buffer as much as 1.5 times the supernantant volume then transferred into a GD column to be centrifuged at 15000 x g fort 2 minutes. Add 400 µL W1 buffer and centrifuged at 15000 x g for 30 seconds then throw the supernatant. Add 600 µL washing buffer into the GD column and centrifuged at 15000 x g for 30 seconds. After supernatant was thrown out, dry the GD column by centrifuging at 15000 x g for 3 minutes.

GD column was set to a 2 mL collection tube, then add. 100 µL warmed elution buffer and left for 5 minutes and centrifuged at 15000 x g for 30 seconds.

b. Quantitative and qualitative measurement of the DNA genome

For qualitative analysis, the DNA genome was visualized using a IV transilluminator over the agarose gel following the eletrophorese step. The DNA was measured at λ 260 nm, with a purity ratio of λ 260 - λ 280 nm. The measured DNA genome was then subjected for further study by dissolving it as much as it is neede for PCR and ampification using an RAPD primers.

c. RAPD-PCR

The total volume for PCR reaction was 12.5 µL, cosisting of 6.25 µL MyTaq PCR mix, 1 µL DNA template, 1 µL primer, and 4.25 µL *nuclease-freewater*. The DNA amplification was run in a thermal cycler peqlab primus 250, which set as the followimg: 1 cycle pre denaturation .at 95° for 2 minutes, After this step, the cycler runs for 35 cycles of denaturation at 95°C for 15 seconds and elongation for 15 seconds. The annealing temperature was set up at 38°C for 15 seconds and elongation at 72°C for 10 seconds plus final elongation 1 cycle at 72^0C for 5 minutes.

d. Electrophorese

Visaulaization of the DNA genome and the PCR products were done in an agarose gel and UV Transiluminator. The gel agarose for those two matters were different on their thickness, a 1.0% thick was used to cek the qualitative of the DNA genome, and 2% for all PCR products. The visualization pattern was captured by the camera and calculated for their polymorphics bands and analyzed by the MEGA-6 software (Molecular Evolutionary Genetics Analysis) with a *maximum parsimony* method.

Data analyses

The appeared DNA fragments on the agar gel of a particular primer were used to termne the polymorphisms pattern. The fragments are called as polymoprhic when they have more than 95% fragments at the same distance. For analysis of the plant's kinship, the presence or absence of the fragment was then scored using a binary score (0/1). Score 1 is used when the DNA fragment present on the agar gel while score 0 is oppositely, and further the data are used to determine genetic diversity among those three different samples of *L. sativa*, *L. serriola*, dan *L. virosa* by a pplying a *maximum parsimony* method in the MEGA 6 (*Molecular Evolutionary Genetics Analysis*) software. Plants grouping which based on the kinship can be seen on the cladogram.

RESULTS AND DISCUSSION

A. Morphologocal Character of the Lactuca spp.

Observation on the morphological characteristic of the Lettuce plants beame a prerequisite before identification processes, analyzes of genetic diversity, and scoring the accession numbers (Bermawie, 2005). Table 1. shows the

results of the morphological diversity among the three lettuce plants.

Table 1. Morphological differences of *Lactuca sativa., L. serriola*, and *L. virosa.*

Spesies	*Lactuca sativa*	*L. serriola*	*L. virosa*
Bud number on stem base	Many	Many	Many
Leaf petiole	*sessile*	*sessile*	*sessile*
Leaf shape	*orbicular*	*oblonceolate*	*spathulate*
Leaf shape	White	White	Purple
Costa lower surface	Rough	Smooth	Rough
Leaf surface	*rugose*	*rugose*	*rugose*
Leaf apex	*rounded*	*acute*	*acuminate*
Leaf margin	Smooth-Dentate	Lobed	Dentate
Leaf hairs	None	Long	Short
Leaf upper surface	Light green	Yellowish-light green	Greeinsh-Blue
Leaf under surface	Light green	Light green	White

Table 1 shows some similarities and dissimilarities of the three lettuce (*Lactuca spp.*)/ Of these morphological characters we could drawn the kinship of them, however, these morphological characters much depend on their geographical distribution which are different from molecular characters. The current study, therefore, applied RAPD markers in dividing the cluster of the Lettuce plants instead

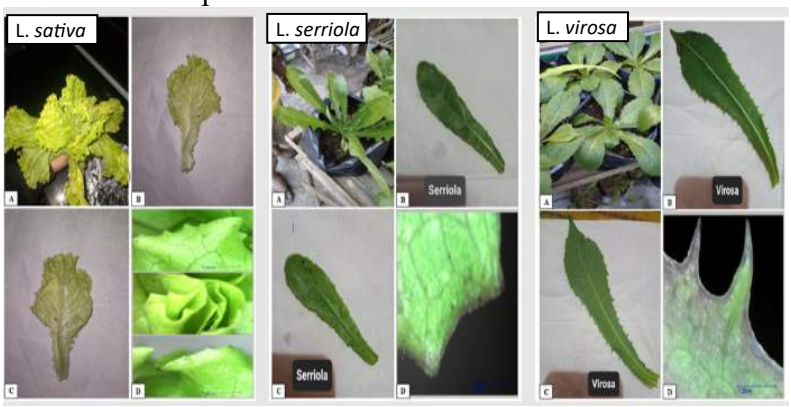


Figure 1. Morphological characters of three different species of Lettuce (*Lctuca spp*.,)

Remark: (A) Lettuce Plant, (B) The Upper Surface Leaf, (C) The Lower Surface Leaf, and (D) Leaf Margin.

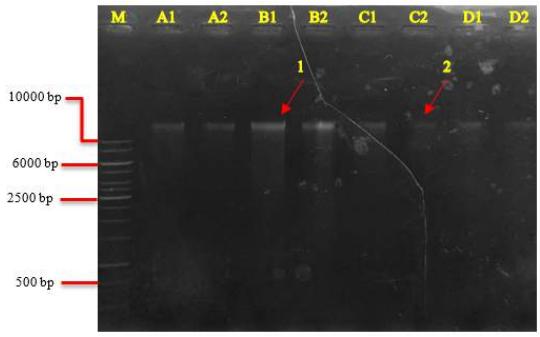
Figure 1. visualize the morphological characters as noted in the Table 1, mainly on the leaf shape, apex, margins, hairs, and both surfaces upper and under .

A. Molecular Characteristic

Molecular analyzes was done by applying an RAPD-PCR method, the total numbers of DNA fragments were then used to be applied as binary numbers to develop a cladogram of the three lettuce plants kinship according to the following steps.

1. Isolation of the genome DNA of Lactuca spp..

To ensure the quality of the isolated DNA genome, they were treated for an electrophorese and visualized on the agar gel. This step revealed some clear and thick DNA fragments except for the *L. sativa*



(Figure 2) because of its low quality (Millah *et al*., 2012). Nugroho *et al*. (2016) reminded there area some factors nightr affect the isolated DNA genome quality like how to keep the fresh material before being isolated, the types of the organism tissue, the solution compositions, and the homogenization technique along the isolation processes.

Following to the checking step for the DNA genome quality, the isolated DNA genome were measured for their quantity and purity by using a nanodropspectrometer at λ 260/ λ 280 nm wavelengths for their ratio (Prayogo *et al*., 2020). Table 2. Shows the ratio of the isolated DNA genome vary between 1.755 and 1.796 which according to Tiwari *et al*. (2017), these ratio are lowerr= than ideal for PCR. Furthermofre, Prayogo *et al*. (2020), stated that the pure DNA genome supposed to have ratio between 1.8 and 2.0. The ratio lower than 1.8 indicating that the DNA genome is contaminated by either, carbohydrate, protein, opr phenolic compounds; but when the ratio is over 2.0 the DNA genome is contaminated by RNA

Gambar 2. Visualisasi DNA Genom *Lactuca* spp. dan *Chrysanthemum morifolium*.
Remarks: (M = 1 kb DNA ladder, 1= Hasil Visualisasi Pita DNA Yang Cukup Jelas, 2= Hasil Visualisasi Pita DNA Yang Kurang Jelas, A1 = *L. serriola*, A2 = *L. serriola* Duplo, B1 = *L. virosa*, B2 = *L. virosa* Duplo, C1 = *L. sativa*, C2 = *L. sativa* Duplo, D1 = C. morifolium, D2 = C. morifolium Duplo).

These contamination might due to practical errors as stated by Sari *et al*. (2014), the treatment of the samples during isolation processes will strongly affect the resulting of isolated DNA genome. This study nioted that the DNA genome of those three samples *L. seriola*, *L. virosa*, and *L. sativa* vary between 33,4 and 103 ng/µL. Hutami *et al*. (2017), the concentration of DNA genome lower than 50 ng/µL is classified as poor that might be affected by some factors like temperature and time during the incubation period Triani (2020). Table 2 shows a variation of the DNA genome concentrations of the samples

Table2. Quality and quantity of the DNA genome of *Lactuca serriola., L. virosa,* and *L. sativa.*

Sample	Duplo	λ 260/ λ 280 Absorbance ratio	Concentration (ng/µL)

Lactuca serriola	1	1.766	41.5
	2	1.755	43
Average	=	1.760	42.25
Lactuca virosa	1	1.796	102
	2	1.793	104
Average	=	1.794	103
Lactuca sativa	1	1.763	33.5
	2	1.795	35
Average	=	1.779	34.25

2. The RAPD profiles of *Lactuca* spp.

8 different RAPD primers (Table 3) used in this study generated 91 amplicons sized of 90 to 1150 bp, and polymorphism pattern between 66.6 and 100% indicating that the genome DNA samples have complementary sequences to these primers (Sulistyawati & Widyatmoko, 2017). The OPAM-7 primer, however, generated the most DNA fragments with a total of 20 bands where 16 bands of them were polymorphic and the rest 4 were monomorphic followed by the OPC-11 which produced 28 bands and the least band was showed by the OPF-2 primer (3 bands). In the case of the OPF-2 primer, Herman *et al*. (2018) reminded that some factors like annealing temperature, the PCR mix composition, the purity of the DNA genome and the total cycles during the PCR might affect the appearance of the DNA bands on the agar gel.

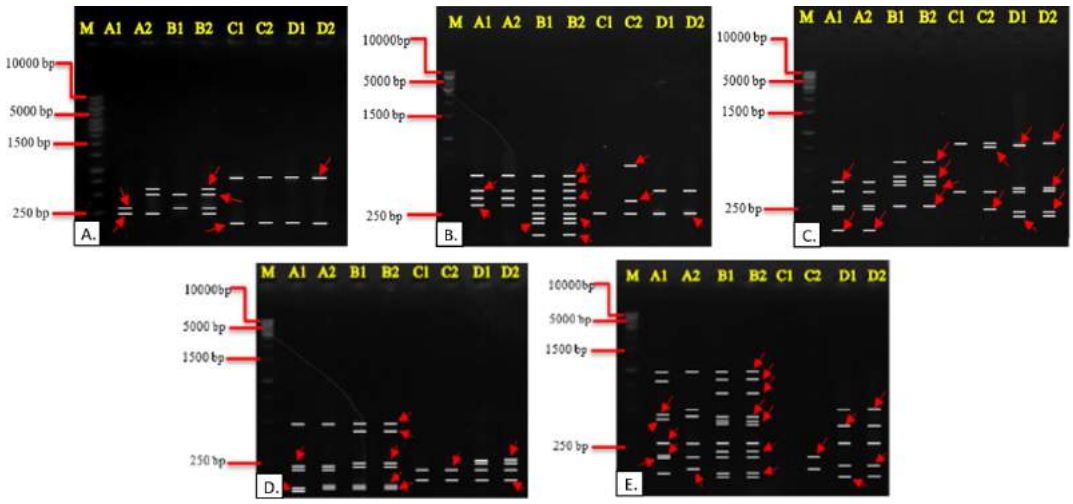


Figure 3. Amplification of the RAPD-PCR of the *Lactuca* spp. and *Chrysanthemum morifolium* with their polymorphism pattern values 100% shown by (A) OPF-1, (B) OPA-2, (C) OPF-4, (D) OPAE-5, (E) OPC-11.

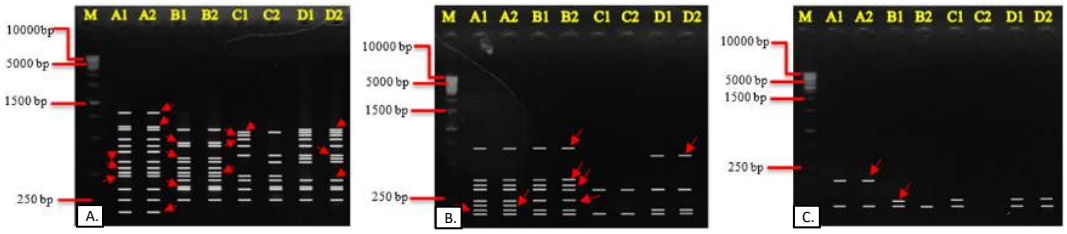


Figure 4. Amplification of the RAPD-PCR of the *Lactuca* spp. and *Chrysanthemum morifolium* with their polymorphism pattern values vary between 66.6 and 80% (A) OPAM-7 (B) OPF-3, (C) OPF-2.

Tabel 4.3. Total numbers of DNA bands produced by the 8 Primers.

Primer	Jumlah Total Pita	Pita Monomorpik	Pita Polimorpik	Persentase Polimorpik (%)
OPA-2	11	0	11	100
OPAM-7	20	4	16	80
OPC-11	18	0	18	100
OPF-1	6	0	6	100
OPF-2	3	1	2	66,6
OPF-3	9	2	7	77,7
OPF-4	14	0	14	100
OPAE-5	10	0	10	100
Total	91	7	84	86,597

3. Phylogenetic relationship between *Lactuca* spp. and *Chrysanthemum morifolium.*

The obtained molecular markers were then set as binary numbers before being analyzed by the MEGA 6 (*Molecular Evolutionary Genetics Analysis*) software to see the kinship of the samples and the out group species.

The *maximum parsimony* showed that the *Consistency Index* (CI) of thew samples and outgroup was 0.83; and *Retention Index* (RI) of 0.8; and *Retention Consistency* (RC) of 0.73 at the *Bootstrap* 1000X. Hidayanti (2021), stated that the value which is closed to 1 was quite high to determine the resolution and consistency of the phylogenetic tree. Furthermore, Sari *et al*. (2014), aid that the *Consistency Index* (CI) of ca. 1 shows a character changes in the DNA bases pair with a parsimony character, meanwhile the *Retention Index* (RI) around 1 shows a complete and consistant sharacter with the phylogeny. Thus the high *CI* and *RI* values determines the high parsimony (Hidayanti, 2021).

The strength of the phylogenetic tree nodes were checked with the *Bootstrap* 1000X, where the higher interval confidence of the *bootsrap* means the higher confidence of the phylogenetic tree (Bousquet *et al*., 1992). The *Bootstrap* value between 70 and 100 means the cladogram might have a change on its node but whren the score is < 70 means the possibility of nodes change is extremely high, and the nodes position could change quite often (Rosdiani *et al*., 2013).

The *maximum parsimony* analyzes done in the current study produced two different main clads tclled as clad I consisting of *L. serriola* and *L. virosa,* with the *Bootstrap* score 100%, and clad II has a monophyletic of *L. sativa* (Figure 3). Hidayanti, (2021) stated that the *Bootstrap* score of 100% determines that the possibility of node changes is very low. It might then concluded that the *L. serriola* and *L. virosa* are sister plants. Rosidiani *et al*. (2013), the monophyletic clad has a very close relationship and came from the common ancestor. Furthermore, Hidayat *et al*. (2008), stated the formation monophyletic clad noted in this study suggested taht the three samples of Lactuca spp., have similar material genetic and so biochemical compounds from the ancestor. Grouping the *L. serriola* and *L. virosa* in pone clad was due to those two species have very close relationship as revealed also in the RAPD-PCR results (Figure 4).

CONCLUSIONS

The RAPD-PCR profile of three *Lactuca* spp. Consisting of *L. serriola*, *L. virosa*, and *L. sativa,* and *Chrysanthemum morifolium* an out group plant applying 8 different primers was able to generated 91 amplicons and 84 of them are polymorphic wbile the rest 7 bands are monomorphic.

He genetic diversity among the green lettuce (*L. sativa*), spiny lettuce (*L. serriola*), and wild letgtuce (*L. virosa*) show that they have different morphological characteristics on their leaves, leaf's edges, leaf's hair, and the color of leaf's upper surface. However, the *maximum parsimony* analyzes with the *Bootstrap* score of 1000X of the three species show that they are having a close relationship.

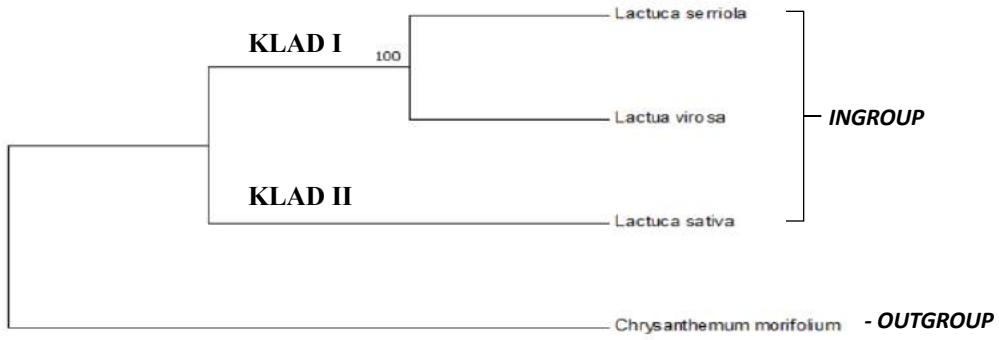


Figure 4. The phylogenetic relationship among the *Lactuca* spp. Nd *Chrysanthemum morifolium* using a *Maximum parsimony.*

ACKNOWLEDGEMENT

The author would like to pass his gratitude to any parties who helped the author to complete this report.

REFERENCES

Abdul-Jalil, T.Z., 2020. *Lactuca serriola*: Short Review of its Phytochemical and Pharmacological Profiles.

Bermawie, N. 2005. Karakterisasi plasma nutfah tanaman, hal. 38-52. dalam Buku Pedoman Pengelolaan Plasma Nutfah Perkebunan. *Pusat Penelitian dan Pengembangan Perkebunan.* Bogor.

Bousquet, J., Strauss, S. H., Doerksen, A. H., & Price, R. A., 1992. Extensive Variation In Evolutionary Rate of

rbcL gene SequencesAmong Seed Plants. *Proc. Natl. Acad. Sci*. USA. 89: 7844-7848.

El-Esawi, M.A., Elkelish, A., Elansary, H.O., Ali, H.M., Elshikh, M., Witczak, J. & Ahmad, M., 2017. Genetic transformation and hairy root induction enhance the antioxidant potential of Lactuca *serriola* L. *Oxidative Medicine and Cellular Longevity*, 2017.

Herman, H., Nainggolan, M. & Roslim, D.I., 2018. Optimasi Suhu Annealing untuk Empat Primer RAPD pada Kacang Hijau (Vigna radiata L.). *Dinamika Pertanian*, 34(1), pp.41-46.

Hidayat, T., Kusumawaty, D., Kusdianti, Yati, D. D., Muchtar, A. A., Mariana, D., 2008. Analisis Filogenetik Molekuler pada Phyllanthus niruri L. (Euphorbiaceae) Menggunakan Urutan Basa DNA Daerah Internal Transcribed Spacer (ITS*). Jurnal Matematika dan Sains*. 13(1): 16-21.

Hidayanti, R. E., 2021. Analisis Filogenetik Nibung (Oncosperma Tigillarium (Jack) Ridl.) "Flora Maskot" Riau Berdasarkan Sekuen Gen rbcL Dengan Metode *Maximum parsimony* (MP) [Skripsi]. Riau: Universitas Riau.

Hutami, R., Idzni, N., Ranasasmita, R., & Suprayatmi, M., 2017. DNA Extraction Method For Molecular Detection. *Jurnal Pertanian*, 8(2): 106-122.

Istiqomah, C.R.P., Pancasakti, H. & Kusdiyantini, E., 2016. Keragaman Genetik Jahe (Zingiber officinale Roscoe) Menggunakan Teknik Penanda Molekuler Random Amplified Polymorphic DNA. *Jurnal Biologi*, 5(2), pp.87-97.

Khan, M. K. U., Muhammad, N., Uddin, N., Ali, N., Khan, I., Ullah, R., 2019. Genetic polymorphism within the wild population of Rhynchosiahi malensis Benth. ex Baker. *International Journal of Biosciences*14:54-64.

Lebeda, A., Křístková, E., Khoury, C. K., Carver, D., & Sosa, C. C., 2021. Distribution and ecology of wild lettuces Lactuca *serriola* L. and Lactuca *virosa* L. in central Chile. *Hacquetia*. 21(1), pp. 1-14.

Mieslerova, B., Lebeda, A., Petrželová, I. R. E. N. A., & Korbelova, P., 2013. Incidence of lettuce downy mildew (Bremia Lactucae) and powdery mildew (Golovinomyces cichoracearum) in natural populations of prickly lettuce (Lactuca *serriola*). *Plant Protection Science*, 49(Special Issue), pp.S24-S32.

Millah, M. I., Habibah, N. A., Suwarni, E., & Rertoningsih, A., 2012. Analisis Keanekaragaman Genetika dan Diferensiasi Jati Jawa dan Madura Berdasarkan Marka Mikrosatelit untuk Mendukung Fingerprinting Jati. Biosaintifika: *Journal of Biology & Biology Education*, 4(2).

Nugroho, K., Terriyana, R. T., Rijzaani, H. & Lestari, P., 2016. Metode Ekstraksi DNA pada Jatropha spp. Tanpa Menggunakan Nitrogen Cair. *Jurnal Littri*, 22(4), pp.159-66.

Prasetio, Bambang., 2013. *Budi daya Sayuran Organik di Pot*. Yogyakarta: Lily Publisher.

Prasetyo, J. & Lazuardi, I. B., 2019. Pemaparan Teknologi Sonic Bloom Dengan Pemanfaatan Jenis MusikTerhadap Pertumbuhan

Vegetatif Tanaman Selada Krop (Lactuca *sativa* L). *Jurnal Keteknikan Pertanian Tropis dan Biosistem*, 5(2), pp.178-188.

Prayogo, F. A., Budiharjo, A., Kusumaningrum, H. P., Wijanarka, W., Supriyadi, A. & Nurhayati, N., 2020. Metagenomic Applications in Exploration and Development of Novel Enzymes From Nature: A Review. *Journal of Genetic Engineering and Biotechnology*, 18(39), pp. 1-10.

Samadi, Budi, 2019. *Rahasia Budi daya Selada Teknik Budi daya Pertanian Organik dan Anorganik*. Jakarta: Pustaka Mina.

Sari, S. K., Listyorini, D., Mazieda, M. N., & Sulasmi, E. S. (2014). Optimasi Teknik Isolasi dan Purifikasi DNA pada Daun Cabai Rawit (Capsicum frutescens cv. Cakra Hijau) Menggunakan Genomic DNA Mini Kit (Plant) GENEAID. *In Proceeding Biology Education Conference: Biology, Science, Enviromental, and Learning* (Vol. 11, No. 1, pp. 65-70).

Sulistyawati, P. & Widyatmoko., 2017. Keragaman genetik populasi kayu merah (Pterocarpus indicus Willd) menggunakan penanda Random Amplified Polimorphism DNA. *Jurnal Pemuliaan Tanaman Hutan*, 11(1), pp. 67-76.

Tiwari, S., Tomar, R. S., Tripathi, M. K. & Ahuja, A., 2017. Modified Protocol for Plant Genomic DNA Isolation. *Indian Society of Genetics, Biotechnology Research and Development,* 9(4), pp. 478-485.

Triani, N., 2020. Isolasi DNA tanaman jeruk dengan menggunakan metode CTAB (cetyl trimethyl ammonium bromide). G-Tech: *Jurnal Teknologi Terapan*, 3(2), 221-226.

van Herwijnen, Z. O. & Manning, J. C., 2017. A review of the history and taxonomy of the enigmatic southern African endemic wild lettuce Lactuca dregeana DC. (Asteraceae: Lactuceae: Lactucinae). *South African Journal of Botany* 108:352-358.

Wijaya, A., Lakitan, B., & Surahman, M., 2009. Identifikasi Beberapa Aksesi Jarak Pagar (*Jatropha curcas* L.) Melalui Analisis RAPD dan Morfologi. *Jurnal Agronomi Indonesia (Indonesian Journal of Agronomy)*, 37(2).

Yu, J., Mosjidis, J., Klingler, K., Woods, F., 2001. Isozyme Diversity in North American Cultivated Red Clover. *Crop Sci.* 41:1625-1628.