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▶	17102-1	sundaridu, Manuscript; Tolangara et al; Genetic Diversity Of Lemon (Citrus spp) Found In Ternate Island-12-12-2019.docx	January 22, 2020	Article Text
▶	17105-1	sundaridu, Covering Letter Tolangara et al 2020.pdf	January 22, 2020	Other
▶	17355-1	aseptiasari, Genetic Diversity of Lemon (Citrus spp) From .doc	January 27, 2020	Article Text
▶	17357-1	aseptiasari, !Biodiversitas-Guidance for Authors.pdf	January 27, 2020	Article Text

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8 dari 10

[biodiv] Submission Acknowledgement Eksternal Kotak Masuk x

Ahmad Dwi Setyawan <smujo.id@gmail.com> kepada saya
Kam, 23 Jan 2020 06:29

Inggris > Indonesia Terjemahkan pesan Nonaktifkan untuk: Inggris x

Abdulrasyid Tolangara:

Thank you for submitting the manuscript, "Genetic Diversity of Lemon Genetic Diversity of Local Lemon (Citrus spp) From Ternate Island Based on Morphological and Molecular Characters: Genetic Diversity of Lemon (Citrus spp) From Ternate Island" to Biodiversitas Journal of Biological Diversity. With the online journal management system that we are using, you will be able to track its progress through the editorial process by logging in to the journal web site:

Submission URL: <https://smujo.id/biodiv/authorDashboard/submission/5287>
Username: sundaridu

If you have any questions, please contact me. Thank you for considering this journal as a venue for your work.

Ahmad Dwi Setyawan

[Biodiversitas Journal of Biological Diversity](#)

Thank you for your response. Thanks a lot. Noted with thanks.

REVISION

The screenshot shows a Gmail interface. The search bar contains 'editors@smujo.id'. The email list on the left includes 'Kotak Masuk' (3,189), 'Berbintang', 'Ditunda', 'Ter kirim', 'Draf' (8), 'Ciutkan', 'Penting', 'Terjadwal', 'Chat', and 'Ruang'. The selected email is from 'Smujo Editors <smujo.id@gmail.com>' to 'saya' (me), dated '26 Mar 2020 19.52'. The subject is '[biodiv] Editor Decision'. The email content includes a decision to require revisions and a list of reviewer comments.

Reviewer A:

Dear author,

The manuscript 'Genetic diversity of lemon (*Citrus* spp.) from Ternate Island (Indonesia) based on morphological and molecular characters' has been reviewed. It has a significant contribution to biodiversity research, especially on species levels. However, the author should make major revisions to publish in Biodiversitas journal. The comments and suggestions of the manuscript are as follows:

1. Please rewrite all parts: abstract, introduction, materials and methods, results and discussion, and references.
2. Abstract: it should be contained research background, research aims, methods, results and discussion, research implementation.
3. Introduction: research problem has not presented in the introduction, add previous studies related to the current study, learn some terminology presented in this part.
4. Materials and Methods: please write some subtitles (see template how to write a manuscript in the Biodiversitas Journal), add a map that presented of research sites, add the scientific name of lemon cui, lemon nipis, lemon suaggi, and lemon.
5. Results and Discussion: please mention characters of vegetative and generative characters respectively, revise all tables and figures. On Figure 3, Please note A1, A2 C4, accessions of B3 using primer OPA2 is not amplified, How can you get the data? You should present successful visualization with all accessions using all primers amplified. Please learn how to decide on a cluster.
6. The last paragraph of Results and Discussion contained conclusions. Please refer to the research aims.
7. Reference: please follow guidance for author
8. All comments and suggestions are written in the manuscript. Please read carefully and make appropriate revisions.

Good luck

Recommendation: Revisions Required

Gmail interface showing an email from Rasyid Tolangara to editors@smujo.id. The email content includes:

Biodiversitas Journal of Biological Diversity

Rasyid Tolangara <rasyid_17@unkhair.ac.id>
kepada Smujo

Dear editor
I had sent revision of manuscript in the OJS System, thanks for good attention

Best regard
A.R Tolangara



Balas Teruskan

Gmail interface showing a notification email from Smujo Editors to editors@smujo.id. The email content includes:

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You have a new notification from Biodiversitas Journal of Biological Diversity:

You have been added to a discussion titled "Table of Response" regarding the submission "Genetic Diversity of Lemon Genetic Diversity of Local Lemon (Citrus spp) From Ternate Island Based on Morphological and Molecular Characters".

Link: <https://smujo.id/biodiv/authorDashboard/submission/5287>

Ahmad Dwi Setyawan

Biodiversitas Journal of Biological Diversity

Thank you! Thanks a lot. Congratulations!

Balas Teruskan

Short Communication:

Genetic diversity of lemon (*Citrus* spp.) from Ternate Island (Indonesia) based on morphological and molecular characters

Abstract. One effort to build the image of Lemon -Nusantara can be by utilizing local genetic resources of *Citrus* spp. One of the growth center lemon regions in North Maluku is Ternate Island. In Ternate island, cui lemon (*C. macrocarpa*) is dominantly cultivated besides that there are suaggi lemon (*C. hystrix*), nipis lemon (*C. aurantiifolia*), and lemon lemon (*C. sinensis*). The genetic diversity of these local lemons in North Maluku, especially in Ternate, has not been scientifically reported. Therefore, this study aimed to analyze the genetic diversity of local lemons found on the island of Ternate based on morphological character and RAPD molecular analyses. A total of 12 accessions of local lemons in Ternate island. How many accessions to studied for morphological and molecular characters? Observation of morphological and morphometric characters of the lemons on 117 sub-characters of vegetative and generative organs in lemons. Observation of molecular characters using three primer: OPA1, OPA 2 & OPA 3 in the RAPD technique. The morphological character and RAPD-molecular character analysis using UPGMA analysis showed similar results. Ternate Island has moderate diversity of lemon species (75% based on morphological characters and 73% based on RAPD markers). Cluster analysis indicates that cui lemon (*C. macrocarpa*) has the most distinctive autapomorphic character compared to the other local lemon varieties. Nipis lemon (*C. aurantiifolia*) and suaggi lemon (*C. hystrix*) shared 75% sinapomorphic character similarity. This genetic diversity information provides a novel insight into the lemon commodity breeding programs which can be encouraged through the selection of superior local lemons based on morphological and molecular markers.

Keywords: lemon, morphological, molecular, diversity, Ternate

INTRODUCTION

Lemon (*Citrus* spp.) is one of the genera of the Rutaceae family that has the highest economic value. The potential of local fruits including lemons has the potential to be developed as a source of diversification of food and nutrition, medicines and components of environmental improvement according to local conditions (Karsinah et al., 2002). Indonesia is known to have a variety of local and national superior citrus species spread throughout the archipelago from Sabang to Merauke, and has the potential to be developed and utilized to support food security, especially to meet the needs of the community's vitamins and minerals as the population continues to increase from year to year (Budiyati et al., 2016).

Ternate Island is known for its high diversity of tropical fruits. One of the examples of tropical fruits that are commonly bred on this island is lemon. Local lemons species are widely spread on the island of Ternate. Lemon is a horticultural commodity of which demand has increased from year to year (Matakena 2017). Data obtained from the Department of Agriculture showed that national citrus production ranged between 17-25 tons/hectare from a potential of 25-40 tons/hectare (Agriculture Department 2007). Local lemon production should be improved to support food security, specifically to fulfil the requirements of society's mineral and vitamin daily intake. As the population grows, the need for fresh cleansing agents also increases from year to year. According to Rezkianti et al. (2016) and Adelina et al. (2017), several steps that can be carried out to preserve tropical fruit germplasm in Indonesia include exploration (inventory and identification), introduction to increased genetic diversity, collection of observations, and evaluation of fruit production and development. These efforts play a very crucial role in providing diverse plant genetic resources for plant breeding, environmental conservation, and plant species diversity that ultimately serve to support sustainable food security (Budiyati et al. 2016; Murtando et al. 2016).

The Agriculture in Indonesia targets the availability of new superior varieties that are productive and resistant to environmental stresses such as drought and pest attacks. To achieve this goal, having knowledge about genetic resource diversity is highly necessary. Access to genetic resources, both those obtained from exploration and from collection gardens can encourage the creation of new high-yielding varieties (Tobing, 2013). Ternate Island is rich in tropical fruit diversity (Sundari, et al., 2017). Various species of lemons such as cui lemon (*C. macrocarpa*), nipis lemon (*C. aurantiifolia*), suaggi lemon (*C. hystrix*) and lemon (*C. sinensis*) are widely cultivated in Ternate. These local lemons differ in terms of fruit and leaf morphology and habitat characteristics. Data on the genetic diversity of lemons in Ternate

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has not been scientifically reported. Thus, information about this, is a new thing brought to the plant breeding and conservation efforts. Efforts to preserve and develop local plants are very important in providing a variety of plant genetic resources for plant breeding, environmental preservation, and increasing diversity of plant species to support sustainable food security (Solikin, 2012). In addition, in Indonesia there are about 400 types of edible fruit plants (Verheij and Coroe, 1998) including the diversity of local lemon plants in North Maluku (Sundari et al., 2019). Several studies related to lemon breeding efforts have been any previous study of lemon using morphological and molecular characters, carried out in several countries including: Uzun, et al (2011) about the Investigation of genetic relationships among trifoliolate oranges and their hybrid relatives based on ISSR markers; Pal, et al. (2013) about Genetic Variability and Relationship Studies of Mandarin (*Citrus reticulata* Blanco) Using Morphological and Molecular Markers; Khiavi, et al. (2016) on Assessment of Lime Genetic Diversity in Three Regions of Iran, Using Morphological and ISSR Markers. This study aims to determine the genetic diversity of local lemons on Ternate Island based on morphology agronomie and molecular character of RAPD. This research is expected to contribute to the superior local lemon breeding model in North Maluku.

MATERIALS AND METHODS

Study area: This study was carried out from May to September 2019. Samples of this study were collected from seveeight villages in Ternate. A purposive sampling method was employed to collect the samples local lemon. The four local lemon speciesvarieties collected from Ternate Island are presented in Table 1.

Table 1. Sampling Locations

No	Location (villages)	Local Name	Number of Trees
1	Sango	Lemon Cui	7
		Lemon Nipis	6
		Lemon suanggi	6
		Lemon lemon	6
2	Kulaba	Lemon Cui	7
		Lemon Nipis	7
		Lemon suanggi	4
		Lemon lemon	7
3	Sulamadaha	Lemon Cui	8
		Lemon Nipis	7
		Lemon suanggi	6
		Lemon lemon	7
4	Loto	Lemon Cui	8
		Lemon Nipis	7
5	Rua	Lemon Cui	7
		Lemon Nipis	7
		Lemon suanggi	4
		Lemon lemon	7
6	Sasa	Lemon Cui	6
		Lemon suanggi	4
		Lemon lemon	8
7	Taduma	Lemon Nipis	7
		Lemon suanggi	3
		Lemon Cui	6
8	Kalumata	Lemon Cui	6
		Lemon lemon	5

Source: Survey Data (2019)

How many accessions do you use for morphological and molecular characters respectively? 4 accessions.
Procedure: Morphological characters of the lemons were observed based on the Descriptors for Citrus (Biodiversity International, IPGRI, 1999). Observations were made on 117 morphological and Morphometric characters of vegetative and generative organs in lemons. Observation of molecular characters using the RAPD technique consisted of DNA isolation was carried out using a Presto TM Mini KIT (Geneaid) DNA kit. The amplification process uses MyTag Red Mix (Bioline). Primary amplification of OPA 1, OPA 2, and OPA3 with 30 mL PCR reaction mixture composition (MyTag Red Mix, Primary, ddH₂O, and DNA templates). The PCR process was carried out under 94°C Pradenaturasi phases for 5 minutes, denaturation at 95 °C, annealing at 55 °C, and extension at 72°C and posting extensions at 72°C. DNA qualitative tests were performed using electrophoresis on 1.5% agarose gel with TBE 1X and photographed on GelDOC UV-transluminator, while the quantitative test was performed using a spectronanodrop. How many replication of each reaction did you make? Please mention
Data Analysis: Molecular data were obtained based on the presence and absent of DNA bands using primers OPA 1, OPA 2, and OPA 3. Score 1 shows the DNA bands that appear and score 0 for DNA bands that do not appear in each

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Please add scientific name of lemon cui, lemon nipis, lemon suanggi, and lemon lemon

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84 primary OPA (OPA 1-3). The data were analyzed using cluster analysis and the UPGMA (Unweight Pair Group Method
 85 with Arithmetic Mean) technique with the Multivariate Statistical Package (MVSP) program version 3.22 (Kovach,
 86 2007).

87 **RESULTS AND DISCUSSION**

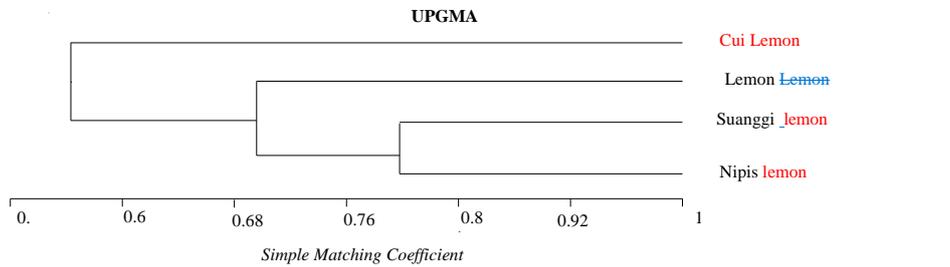
88 **Morphological Characters of Ternate Lemons**

89 There are 117 variations of morphological characters (phenotypes) found in the four local lemon species such as cui
 90 lemon (*C. macrocarpa*), nipis lemon (*C. aurantiifolia*), suanggi lemon (*C. hystrix*) and lemon (*C. sinensis*). These
 91 variations are identified in the vegetative organs (leaf and stem characters) and generative organs (characters of flowers,
 92 fruits, and seeds) of these lemons. Analysis on species similarity using the MVSP ver 3.22 software has produced a
 93 dendrogram consisting of two three lemon groups with a similarity value of 56%. The first main cluster with a similarity
 94 value of 56% consists of one type of lemon, namely Cui lime. The second group with a similarity value of 68% is
 95 comprised of one cluster only that is lemon lemon, while the third group with a similarity value of 75% is divided into two
 96 sub-clusters, consist Lemon and namely Nipis lemon and Suanggi lemon. (The similarity values for the three lemon
 97 groups are presented in Table 2 (genetic distance matrices) and Figure 2 (UPGMA Dendrogram UPGMA).

98 **Table 2. Genetic Distance Matrices of Ternate Lemon Morphometric Characters**

	Nipis lemon	Lemon	Suanggi lemon	Cui lemon
Nipis lemon	1			
Lemon	0,746	1		
Suanggi lemon	0,748	0,676	1	
Cui lemon	0,556	0,595	0,561	1

101 The results of the cluster analysis with dendrogram are presented below.



102 **Figure 1: Dendrogram of Ternate Lemon Morphometric Characters**

103 Based on the dendrogram above, it can be seen that the highest similarity is found in Nipis lime and Suanggi lime with
 104 a similarity value of 75%. Cui lime is the most distinctive type of lemon with the lowest similarity value, which is 56%.
 105 we know that Autapomorphic characters (unique characters in one taxon) owned by the first group (Cui lime) and the
 106 second group (lemon) are: two stem characters (stem height and stem surface), three leaf characters (leaf shape, length,
 107 and width), three fruit characters (skin shape, texture, and color), and two seed characters (seed shape and size).
 108 Synapomorphic characters (shared characters) that belong to the third group (Suanggilime and Nipis lime) consist of 10
 109 characters that include two stem characters, three leaf characters, three fruit characters, and two seed characters. The
 110 description of the autapomorphic and synapomorphic characters of the lemon species is shown in Table 3.

111 **Table 3. The Autapomorphic and synapomorphic characters of the four lemon species found in Ternate Island**

Cluster Group	Analysis	OTU	Character state
1	Autapomorphi c	Cui lemon	Medium stem height, barbed stem surface, orbicular-shaped leaf, small fruit size, smooth texture in dark green color fruit, ovoid-shaped seed

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- Commented [F63]: Please add scientific name of each species Which species is the most common in cultivation?
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- Commented [F66]: Please learn how to decide a cluster Suggestion: Dendrogram consists of 2 cluster: Cluster 1: cui lemon Cluster 2: lemon, suanggi lemon, nipis lemon Cluster 2 consists of 2 subclusters
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- Commented [N79R78]: character description of lemon
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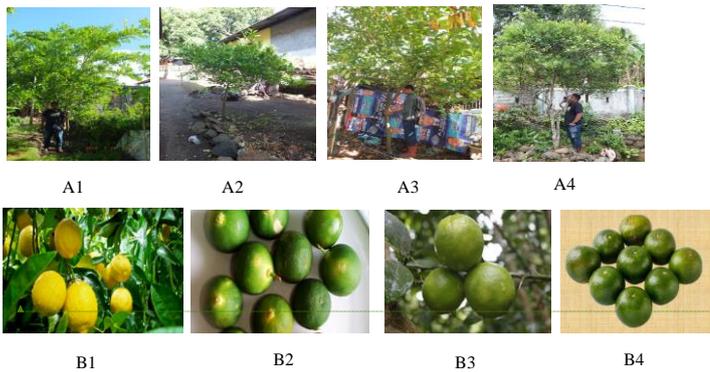
2	Autoapomorphic	Lemon Lemon	High stem height, barbed stem surface, abovate ovalic shaped leaf, medium-to-large fruit size, smooth texture in green color fruit, ovoid seed
3	Sinapomorphic	Suaggi lemon and Nipsis lemon	High stem height, smooth stem surface, elliptical-shaped leaf, medium fruit size, rough texture fruit, green color fruit, fusiform seed

The description of distinguished morphological characters of the lemons is presented in Figure 2

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RAPD molecular characters of Ternate lemons

Data on the DNA band appearance in the four local lemon species are presented in Table 4.

Table 4 Primer sequences with the number of scorable amplified and polymorphic RAPD Markers, DNA Sequence, and Polymorphism (%) of Four Lemon Species Found in Ternate Analyzed Using the UPGMA Technique

Primer	Seq 5 to 3	Seq 5 to 3 band number of amplified	Number of Polymorphic bands	Number of Monomorphic bands	Polymorphic bands (%)
OPA 1	AGT CAG CCA C	11	11	00	100
OPA 2	AAT CGG GCT G	07	04	03	57.14
OPA 3	CAG CAC CCA C	08	05	03	62.5
Total		26	20	6	73.21

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The total number of DNA bands found was 26 bands with 20 polymorphic bands and 6 monomorphic bands. The average percentage of polymorphism was 73.21% (Table 4). Matrix similarity analysis is based on the appearance of DNA bands (DNA profile), score 1 shows the DNA bands that appear and score 0 for DNA bands that do not appear in each primary OPA (OPA 1-3) (Figure 3).

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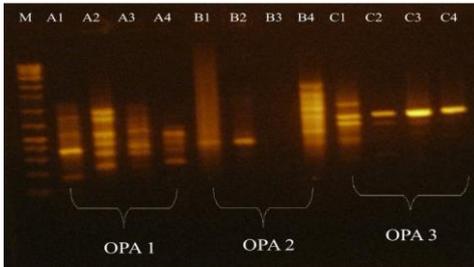


Figure 3. Visualization of DNA Bands based on OPA 1-3 RAPD primers

Diversity analysis of Ternate lemons was analyzed based on DNA band visualization (Figure 3). The Similarity Matrix of Lemon DNA is shown in Table 5.

Table 5. Similarity Matrices of the Ternate Lemon Species

	Nipis lemon	Lemon Lemon	Suanggi lemon	Cui lemon
Nipis lemon	1			
Lemon Lemon	0.706	1		
Suanggi lemon	0.783	0.696	1	
Cui lemon	0.554	0.595	0.545	1

The highest similarity matrix value (0.783) is found in Suanggi lemon and Nipis lemon (Table 5), suggesting that the two species share many identical features. Dendrogram generated based on the average value of similarity matrices of the Ternate lemon DNA is shown in Figure 4.

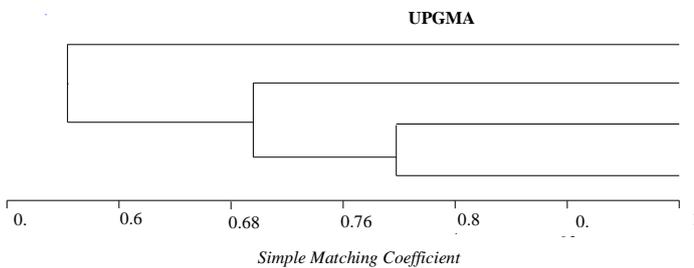


Figure 5. Dendrogram of RAPD Analysis

UPGMA cluster analysis based RAPD molecular characters shows two main cluster with a similarity value of 54%. The first main cluster with a similarity value of 54% consists of Cui lemon, the second group with a similarity value of 70% is comprised of lemon, and sub cluster divided into Nipis lemon and Suanggi lemon.

Discussion

Ternate Island has moderate diversity of lemon species (75% based on morphological characters and 73% based on RAPD markers according our results. Lower similarity values indicate higher diversity this is in line with Wijayanto et al. (2013) and Rachma et al. (2017) stated that the smaller the coefficient of similarity (close to 0), the more distant the relationship; conversely, the greater the coefficient of similarity (close to 1), the closed the relationship. From the results of this study and referring to Nei (1987), the value of local lemon genetic diversity on Ternate Island was classified as moderate. This result can be used as a reference in local Lemon breeding activitie. Based on the results of this study, it can be understood that the genetic diversity of lemons in Ternate is moderate than the intraspecies level of Durio spp from

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Cluster 1: cui lemon
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214 Ternate island (Sundari et al., 2017). According to (Calinski and Harabasz 1974) and Sturn et al. (2002), grouping or
 215 cluster analysis can be used to classify individuals or objects into groups with different characteristics. Individuals or
 216 objects that belong to the same group will have relatively homogeneous properties this is in line with opinion of (Daryono
 217 et al. 2019). The data of this study have revealed that local lemon Ternate are enough diverse. The close relationship
 218 between Suanggi lemon and Nipis lemon is presented in a dendrogram. Cluster analysis performed in this study used
 219 euclidean distance that varies from 0.0 to 0.1 to express the similarity level of traits among lemon species. Genetic
 220 distance was used in this study because each of the species characters has a comparable scale (Figure 3 and Figure 5).
 221 UPGMA cluster analysis based on RAPD molecular characters shows three main groups with a similarity value of
 222 54%. The first main group with a similarity value of 54% consists of Cui lemon, the second group with a similarity value
 223 of 70% is comprised of lemon, and the third group with a similarity value of 78% is divided into Nipis lemon and Suanggi
 224 lemon.
 225 Ternate Island has moderate diversity of lemon species (75% based on morphological characters and 73% based on
 226 RAPD markers according our results. Lower similarity values indicate higher diversity this is in line with Wijayanto et al.
 227 (2013) and Rachma et al., (2017) stated that the smaller the coefficient of similarity (close to 0), the more distant the
 228 relationship; conversely, the greater the coefficient of similarity (close to 1), the closer the relationship. From the results of
 229 this study and referring to Nei (1987), the value of local lemon genetic diversity on Ternate Island was classified as
 230 moderate. This result can be used as a reference in local Lemon breeding activities. Based on the results of this study, it can
 231 be understood that the genetic diversity of lemons in Ternate is moderate than the intraspecies level (Sundari, et al., 2017).
 232 According to (Calinski, T., and Harabasz, 1974) and Sturn, et al., (2002), grouping or cluster analysis can be used to
 233 classify individuals or objects into groups with different characteristics. Individuals or objects that belong to the same
 234 group will have relatively homogeneous properties this is in line with opinion of (Daryono et al. 2019). The data of this
 235 study have revealed that local lemon Ternate are enough diverse. The close relationship between Suanggi lemon and
 236 Nipis lemon is presented in a dendrogram. Cluster analysis performed in this study used euclidean distance that varies
 237 from 0.0 to 0.1 to express the similarity level of traits among lemon species. Euclidean distance was used in this study
 238 because each of the species characters has a comparable scale (Figure 3 and Figure 5).
 239 According to Rezkianti, et al., (2016); Adelina, et al., (2017), environment is a determinant factor in the diversity of a
 240 plant population in an area. The environment factor consist: altitude, rainfall, temperature and humidity are supporting
 241 factors of plant growth. This means that differences in one of the environmental factors will affect the characters (both
 242 morphological and physiological) of similar plant populations (Ismail et al. 2019). For an example of the morphology of
 243 the color of the leaves and the structure of the rind of a Nipis lemon.
 244
 245 In conclusion, this study has unveiled the similar result between the morphological analysis and RAPD molecular
 246 analysis conducted to four local lemon species found on Ternate Island, has moderate diversity of lemon species (75%
 247 based on morphological characters and 73% based on RAPD markers). Cluster analysis shows that Cui lemon has the most
 248 distinctive autopolymorphic characters compared to the other local lemons. Nipis lemon and Suanggi lemon have the
 249 moderate similarity value (75%) and shared similar sinapomorphic characters.

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Best regard
A.R Tolangara



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Short Communication: Genetic diversity of lemon (*Citrus* spp.) from Ternate Island (Indonesia) based on morphological and molecular characters

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Abstract. Tolangara A, Corebima AD, Mas'ud A, Sundari. 2020. Short Communication: Genetic diversity of lemon (*Citrus* spp.) from Ternate Island (Indonesia) based on morphological and molecular characters. *Biodiversitas* 21: xxxx. One effort to build the image of Lemon Nusantara can be by utilizing local genetic resources of *Citrus* spp. One of the growth centra lemon regions in North Maluku is Ternate Island. In Ternate island, cui (*C. macrocarpa*) is dominantly cultivated besides that there are Suanggi (*C. hystrix*), nipsis (*C. aurantiifolia*), and lemon (*C. sinensis*). The genetic diversity of these local lemons in North Maluku, especially in Ternate, has not been scientifically reported. Therefore, this study aimed to analyze the genetic diversity of local lemons found on the island of Ternate based on morphological character and RAPD molecular analyses. A total of 12 accessions of local lemons in Ternate island to studied for morphological and molecular characters. Observation of morphological and morphometric characters of the lemons on 117 characters of vegetative and generative organs in lemons. Observation of molecular characters using three primer: OPA1, OPA 2 & OPA 3 in the RAPD technique. The morphological character and molecular character using UPGMA analysis showed similar results. Ternate Island has moderate diversity of lemon species (75% based on morphological characters and 73% based on RAPD markers). Cluster analysis indicates that cui (*C. macrocarpa*) has the most distinctive autapomorphic character compared to the other local lemon varieties. Nipsis (*C. aurantiifolia*) and Suanggi (*C. hystrix*) shared 75% sinapomorphic character similarity. This genetic diversity information provides a novel insight into the lemon commodity breeding programs which can be encouraged through the selection of superior local lemons based on morphological and molecular markers.

Keywords: Lemon, morphological, molecular, diversity, Ternate

INTRODUCTION

Lemon (*Citrus* spp.) is one of the genera of the Rutaceae family that has the highest economic value. The potential of local fruits including lemons has the potential to be developed as a source of diversification of food and nutrition, medicines and components of environmental improvement according to local conditions (Karsinah et al. 2002). Indonesia is known to have a variety of local and national superior citrus species spread throughout the archipelago from Sabang to Merauke, and has the potential to be developed and utilized to support food security, especially to meet the needs of the community's vitamins and minerals as the population continues to increase from year to year (Budiyati et al. 2016).

Ternate Island is known for its high diversity of tropical fruits. One of the examples of tropical fruits that are commonly bred on this island is lemon. Local lemons are widely spread on the island of Ternate. Lemon is a horticultural commodity of which demand has increased from year to year (Matakana 2017). Data obtained from the Department of Agriculture showed that national citrus production ranged between 17-25 tons/hectare from a potential of 25-40 tons/hectare (Agriculture Department

2007). As the population grows, the need for fresh cleansing agents also increases from year to year. According to Rezkianti et al. (2016) and Adelina et al. (2017), several steps that can be carried out to preserve tropical fruit germplasm in Indonesia include exploration (inventory and identification), introduction to increased genetic diversity, collection of observations, and evaluation of fruit production and development. These efforts play a very crucial role in providing diverse plant genetic resources for plant breeding, environmental conservation, and plant species diversity that ultimately serve to support sustainable food security (Budiyati et al. 2016; Murtando et al. 2016).

The Agriculture in Indonesia targets the availability of new superior varieties that are productive and resistant to environmental stresses such as drought and pest attacks. To achieve this goal, having knowledge about genetic resource diversity is highly necessary. Access to genetic resources, both those obtained from exploration and from collection gardens can encourage the creation of new high-yielding varieties (Tobing 2013). Ternate Island is rich in tropical fruit diversity (Sundari et al. 2017). Various species of lemons such as cui (*C. macrocarpa*), nipsis (*C. aurantiifolia*), Suanggi (*C. hystrix*) and lemon (*C. sinensis*)

are widely cultivated in Ternate. These local lemons differ in terms of fruit and leaf morphology and habitat characteristics. Data on the genetic diversity of lemons in Ternate has not been scientifically reported. Thus, information about this, is a new thing brought to the plant breeding and conservation efforts. Efforts to preserve and develop local plants are very important in providing a variety of plant genetic resources for plant breeding, environmental preservation, and increasing diversity of plant species to support sustainable food security (Solikin 2012). In addition, in Indonesia there are about 400 types of edible fruit plants (Verheij and Coroe 1998) including the diversity of local lemon plants in North Maluku (Sundari et al. 2019). Several studies related to lemon breeding efforts have been any previous study of lemon using morphological and molecular characters, carried out in several countries including: Uzun. et al (2011) about the Investigation of genetic relationships among trifoliolate oranges and their hybrid relatives based on ISSR markers; Pal et al. (2013) about Genetic Variability and Relationship Studies of Mandarin (*Citrus reticulata* Blanco) Using Morphological and Molecular Markers; Khiavi et al. (2016) on Assessment of Lime Genetic Diversity in Three Regions of Iran, Using Morphological and ISSR Markers. This study aims to determine the genetic diversity of local lemons on Ternate Island based on morphology and molecular character of RAPD. This research is expected to contribute to the superior local lemon breeding model in North Maluku.

MATERIALS AND METHODS

Study area

This study was carried out from May to September 2019. Samples of this study were collected from eight villages in Ternate, North Maluku, Indonesia, includes 4 accession namely cui, nipis, suanggi, and lemon. A purposive sampling method was employed to collect the samples local lemon. The four local lemon varieties collected from Ternate Island are presented in Table 1.

Procedure

Morphological characters of the lemons were observed based on the Descriptors for Citrus (Bioersity International, 1999). Observations were made on 117 morphological and Morphometric characters of vegetative and generative organs in lemons. Observation of molecular characters using the RAPD technique consisted of DNA isolation was carried out using a Presto TM Mini KIT (Geneaid) DNA kit. The amplification process uses MyTag Red Mix (Bioline). Primary amplification of OPA 1, OPA 2, and OPA3 with 30 mL PCR reaction mixture composition (MyTag Red Mix, Primary, ddH₂O, and DNA templates). The PCR process was carried out under 94°C Pradenaturasi phases for 5 minutes at 94°C, denaturation at 95 °C for 5 min, 45 cycles for 30 sec annealing at 55 °C, and extension at 72°C for 90 seconds and posting extensions at 72°C for 7 minutes. DNA qualitative tests were performed using electrophoresis on 1.5% agarose gel with TBE 1X

and photographed on GelDOC UV-transluminator, while the quantitative test was performed using a spectronanodrop. How many replication of each reaction did you make? Please mention.

Table 1. Sampling locations in Ternate, North Maluku, Indonesia

Location (Villages)	Local name	Number of trees
Sango	Cui	7
	Nipis	6
	Suanggi	6
Kulaba	Lemon	6
	Cui	7
	Nipis	7
Sulamadhaha	Suanggi	4
	Lemon	7
	Cui	8
	Nipis	7
Loto	Suanggi	6
	Lemon	7
	Cui	8
Rua	Nipis	7
	Cui	7
	Nipis	7
Sasa	Suanggi	4
	Lemon	7
	Cui	6
Taduma	Suanggi	4
	Lemon	8
Kalumata	Nipis	7
	Suanggi	3
	Cui	6
	Lemon	5

Data analysis

Molecular data were obtained based on the presence and absent of DNA bands using primers. Score 1 shows the DNA bands that appear and score 0 for DNA bands that do not appear in each primary OPA (OPA 1-3). The data were analyzed using cluster analysis and the UPGMA (Unweight Pair Group Method with Arithmatic Mean) technique with the Multivariate Statistical Package (MVSP) program version 3.22 (Kovach 2007).

RESULTS AND DISCUSSION

Morphological characters

There are 117 variations of morphological characters (phenotypes) found in the four local lemon such as cui (*C. macrocarpa*), nipis (*C. aurantiifolia*), Suanggi (*C. hystrix*) and lemon (*C. sinensis*). These variations are identified in the vegetative organs (leaf and stem characters) and generative organs (characters of flowers, fruits, and seeds) of these lemons. Analysis on species similarity using the MVSP ver 3.22 software has produced a dendrogram consisting of two lemon groups with a similarity value of 56%. The first main cluster with a similarity value of 56% consists one of lemon, namely Cui lime. The second group with a similarity value of 68% is comprised of one cluster

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group with a similarity value of 75% is divided into two sub-clusters, consist Lemon and namely Nipis and Suanggi (The similarity values for the three lemon groups are presented in Table 2 (genetic distance matrices) and Figure 2 (Dendrogram UPGMA). The results of the cluster analysis with dendrogram are presented in Figure 1. The description of distinguished morphological characters of the lemons is presented in Figure 2. Diversity analysis of Ternate lemons was analyzed based on DNA band visualization (Figure 3). The similarity matrix of lemon DNA is shown in Table 5.

Based on the dendrogram above, we know that autapomorphic characters (unique characters in one taxon) owned by the first group (Cui lime) and the second group (lemon) are: two stem characters (stem height and stem surface), three leaf characters (leaf shape, length, and width), three fruit characters (skin shape, texture, and color), and two seed characters (seed shape and size). Synapomorphic characters (shared characters) that belong to the third group (Suanggilime and Nipis lime) consist of 10 characters that include two stem characters, three leaf characters, three fruit characters, and two seed characters.

The description of the autapomorphic and synapomorphic characters of the lemon species is shown in Table 3.

Molecular characters

Data on the DNA band appearance in the four local lemon species are presented in Table 4.

The total number of DNA bands found was 26 bands with 20 polymorphic bands). The average percentage of polymorphism was 73.21% (Table 4). Matrix similarity analysis is based on the appearance of DNA bands (DNA profile), (Figure 3).

Table 2. Genetic distance matrices of Ternate lemon morphometric characters

	Nipis	Lemon	Suanggi	Cui
Nipis	1			
Lemon	0,746	1		
Suanggi	0,748	0,676	1	
Cui	0,556	0,595	0,561	1

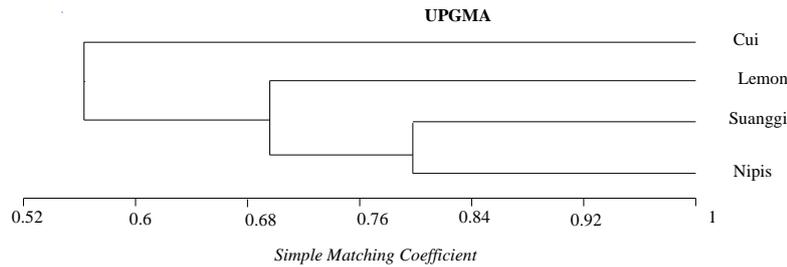


Figure 1. Dendrogram of Ternate lemon morphometric character

Table 3. The Autopomorphic and synapomorphic characters of the four lemon species found in Ternate Island

Cluster	Analysis	OTU	Character state
1	Autopomorphic	Cui	Medium stem height, barbed stem surface, orbicular-shaped leaf, small fruit size, smooth texture in dark green color fruit, ovoid-shaped seed
2	Autopomorphic	Lemon	High stem height, barbed stem surface, abovate ovalic shaped leaf, medium-to-large fruit size, smooth texture in green color fruit, ovoid seed
3	Sinapomorphic	Suanggi and Nipis	High stem height, smooth stem surface, elliptical-shaped leaf, medium fruit size, rough texture fruit, green color fruit, fusiform seed

Table 4. Primer sequences with the number of scorable amplified and polymorphic

Primer	Seq 5 to 3	Number of amplified	Number of polymorphic bands	Number of Monomorphic bands	Polymorphic bands (%)
OPA 1	AGT CAG CCA C	11	11	00	100
OPA 2	AAT CGG GCT G	07	04	03	57.14
OPA 3	CAG CAC CCA C	08	05	03	62.5
Total		26	20	6	73.21

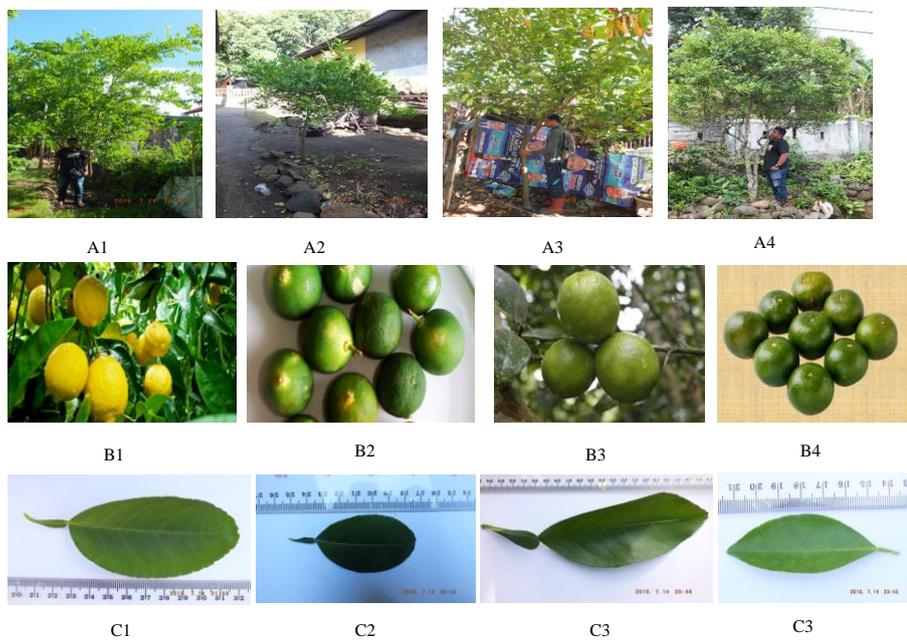


Figure 2. The distinguished characters of ternate lemon morphology. (A1: Cui of tree; A2: Suanggi of tree; A3: Nipis lemon of tree; A4: Lemon of trees; B1: Nipis ; B2: Suanggi; B3: Cui; B4: Lemon; C1: Nipis; C2: Cui; C3: Suanggi; C4: Lemon).

The highest similarity matrix value (0.783) is found in Suanggi and Nipis (Table 5), suggesting that the two species share many identical features. Dendrogram generated based on the average value of similarity matrices of the Ternate lemon DNA is shown in Figure 4.

UPGMA cluster analysis based RAPD molecular characters shows two main cluster with a similarity value of 54%. The first main cluster with a similarity value of 54% consists of Cui, the second group with a similarity value of 70% is comprised of lemon, and sub cluster divided into Nipis and Suanggi.

Table 5. Similarity matrices of the ternate lemon species

	Nipis	Lemon	Suanggi	Cui
Nipis	1			
Lemon	0.706	1		
Suanggi	0.783	0.696	1	
Cui	0.554	0.595	0.545	1

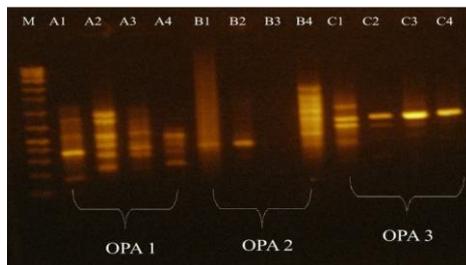


Figure 3. Visualization of DNA bands based on RAPD primers

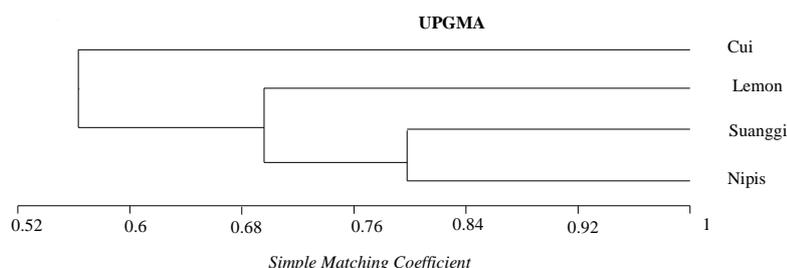


Figure 5. Dendrogram of RAPD analysis

Discussion

Ternate Island has moderate diversity of lemon species (75% based on morphological characters and 73% based on RAPD markers according our results. Lower similarity values indicate higher diversity this is in line with Wijayanto et al. (2013) and Rachma et al. (2017) stated that the smaller the coefficient of similarity (close to 0), the more distant the relationship; conversely, the greater the coefficient of similarity (close to 1), the closer the relationship. From the results of this study and referring to Nei (1987), the value of local lemon genetic diversity on Ternate Island was classified as moderate. This result can be used as a reference in local Lemon breeding activities. Based on the results of this study, it can be understood that the genetic diversity of lemons in Ternate is moderate than the intraspecies level of *Durio* spp from Ternate island (Sundari et al., 2017). According to (Calinski and Harabasz 1974) and Sturn et al. (2002), grouping or cluster analysis can be used to classify individuals or objects into groups with different characteristics. Individuals or objects that belong to the same group will have relatively homogeneous properties this is in line with opinion of (Daryono et al. 2019). The data of this study have revealed that local lemon Ternate are enough diverse. The close relationship between Suanggi and Nipis is presented in a dendrogram. Cluster analysis performed in this study used euclidean distance that varies from 0.0 to 0.1 to express the similarity level of traits among lemon species. Genetic distance was used in this study because each of the species characters has a comparable scale (Figure 3 and Figure 5).

Very high genetic diversity of citrus can be demonstrated by the high number of species and hybrid taxonomic units (Cottin, 2002). Polyembryonies, hybridization, mutations, and phenotypic diversity are one of the causes of the difficulty of identification and classification of citrus. There is a large collection of citrus germplasm originating from local varieties with names according to their home regions, or the same cultivars with different names (Machado et al., 1996). Exploration results

from the species of citrus and their wild relatives indicate that Indonesia is rich in sources of germplasm. Citrus plants spread almost all over Indonesia. In some areas, there are many types of citrus with unknown origins, as if they were native to the area and known as local cultivars (Sugiyarto and Supriyanto 1992).

Lemon leaves vary greatly so that they can provide information about characters that are systematically manifest (John 1965; Stuessy 1990; Williams 2010; Aydin et al. 2013). Leaf morphological characters are normally used to determine diversity of plant species (Murtando et al. 2016). Leaf color can vary depending on the condition of the plant's habitat, water and food supplies, and irradiation. In general, upper and lower surfaces of a leaf have different colors. The upper leaf surface usually looks greener, shinier, and more slippery than the lower leaf surface (Tjitrosoepomo 2009; Murtando et al. 2016). Ternate citrus leaf shape is mostly elliptical or orbicular with acute (pointed) leaf tip, serrated leaf edges, smooth leaf surface, oblique leaf shape, and green color. The color of the leaf flush petiole also varies from light green to green.

According to Rezkianti et al. (2016); Adelina et al. (2017), environment is a determinant factor in the diversity of a plant population in an area. The environment factor consist: altitude, rainfall, temperature and humidity are supporting factors of plant growth. This means that differences in one of the environmental factors will affect the characters (both morphological and physiological) of similar plant populations (Ismail et al. 2019). For an example of the morphology of the color of the leaves and the structure of the rind of a Nipis.

The results of molecular analysis in this study are in line with morphological analysis, this shows that the morphological characters used in identification are quite representative to study genetic diversity of local lemon in Ternate island. According to Sunaryono (1982) several steps that must be carried out in the context of efforts to preserve germplasm fruit plants in Indonesia, including citrus plants, among others; exploration (identification and

identification), introduction to increase genetic diversity, and collection of observations and evaluations in order to increase production and development. This research is an exploration of genetic diversity through an inventory and identification of local lemons on the island of Ternate. **This genetic diversity data will be used as a basis for local lemon crossing techniques in order to improve quality. Lemon cui is the most distant lemon, it can be used as a crossed parent.**Please add implementation of this current study

In conclusion, this study has unveiled the similar result between the morphological analysis and RAPD molecular analysis conducted to four local lemon species found on Ternate Island has moderate diversity of lemon species (75% based on morphological characters and 73% based on RAPD markers). Cluster analysis shows that Cui has the most distinctive autapomorphic characters compared to the other local lemons. Nipis and Suanggi have the moderate similarity value (75%) and shared similar sinapomorphic characters.

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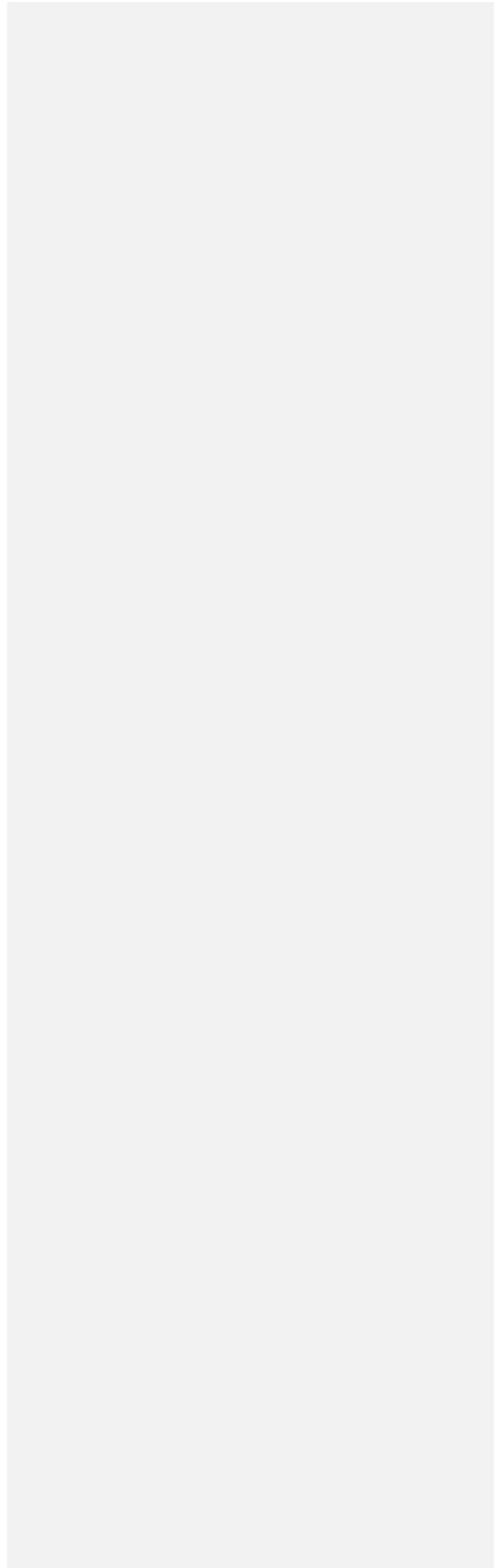
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