

The Second International Conference on Genetic Resources and Biotechnology

**Harnessing Technology for Conservation and Sustainable
Use of Genetic Resources for Food and Agriculture**

Bogor, Indonesia • 24–25 May 2021

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Preface: The Second International Conference on Genetic Resources and Biotechnology

The Second International Conference on Genetic Resources and Biotechnology, which is the continuation of the first event held in 2018, focuses on topics related to advances in biotechnology to create more opportunities for effective conservation and sustainable utilization of genetic resources for food and agriculture. This year conference's theme is Harnessing Technology for Conservation and Sustainable Use of Genetic Resources for Food and Agriculture. The conference was organized by Indonesian Agency for Agricultural Research and Development (IAARD), Ministry of Agriculture, Indonesia, in collaboration with Indonesian Biotechnology Consortium and held on 24th-25th of May 2021 virtually due to the pandemic of COVID-19.

The conference aims to share and exchange current scientific information and technological developments on biotechnology and their applications for conservation and sustainable use of genetic, to encourage and promote quality, efficiency, and modernization of management and utilization of genetic resources, and to facilitate national and international collaboration among participants. There are five scopes discussed in this conference. They are effective management of conservation and sustainable use of genetic resources for food and agriculture, application of genomics and molecular markers for genetic resource conservation and crop adaptation to climate change, application of innovative crop improvement techniques for conservation and sustainable use of plant genetic resources for food and agriculture, plant cell and tissue culture for conservation and effective utilization of genetic resources, and the use of microbial genetic resources as biological control agents of agricultural pests and diseases, and for soil bioremediation.

Five speakers from the United States of America, Japan, India and Indonesia were invited to discuss about their expertise and knowledge on relevant subjects in the plenary sessions. This conference was attended by more than 100 participants including 75 presenters and 44 listeners worldwide. They came from diverse governmental, private, or academic institutions and also scientific communities. The presented materials have undergone peer review processes and only qualified papers were selected. Furthermore, all papers were subjected to double blind peer-review and expected to meet the scientific criteria of significance and academic excellence to be published in a conference proceedings indexed in a well-known, reputable service.

We would like to express our sincere gratitude to our speakers, presenters and all participants for their contributions in this conference. We would also like to express our appreciation for the generosity of our sponsors that support this conference: PT CropLife, PT ITS Science Indonesia, PT Fajar Mas Murni and PT Prima Instrument Analitika. Lastly, special thanks to all committee members for their exceptional work and contributions in the conference and publication.

Chair of Organizing Committee

Dr. Toto Hadiarto

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Abstract. Clove is a native spice from Indonesia, originating from Maluku Islands. Indonesia is the largest producer and consumer of cloves, but productivity is less than 800 kg of dried clove buds. To improve national clove productivity, it is necessary to select high-yielding mother trees (HYMTs) and used them as seed sources in clove planting. The objective of present study was to characterize morphological characteristics of the progenies and calculate the relationships of the selected clove HYMTs. The research was conducted at the Plant Breeding Division, Indonesian Spice and Medicinal Crops Research Institute, Bogor from December 2018 to December 2020. Seeds of 129 HYMTs collected from thirteen regions in Indonesia were sown at the greenhouse, and the germinated seedlings were planted in polybags. The observation was made on seedlings at 4 and 8 months after planting. The result showed that clove progenies of different HYMTs varied morphologically. However, this variation was relatively low indicated by the similarity value of 72% observed among the clove progenies. Based on dendrogram generated from morphological characters of the clove progenies derived HYMTs were divided into three groups. The group I consisted of HYMTs from Banggai Kepulauan, Semarang, Karo, Ende, Gorontalo, and Solok; the group II consisted of HYMTs from Cianjur, Buru Selatan, Karanganyar, and Bogor; the group III consisted of HYMTs from Trenggalek, East Manggarai, and Alor. HYMTs from Ende and Trenggalek had good seedling performance based on morphological characters and the farthest distance, indicating their different genetic backgrounds and beneficial for selecting parental for breeding programs.

INTRODUCTION

Clove (*Syzygium aromaticum* [L.]) originating from Maluku Islands is a valuable spice that can stimulate economic development, especially in Indonesia. Cloves have numerous potential and benefits among the other spices. Cloves have antimicrobial, antibacterial, antifungal, antiviral, and anticarcinogenic compounds [1]. The main volatile compound of clove is eugenol. Rana *et al.* [2] reported that the essential oil of clove could inhibit all fungal pathogens through agar well diffusion test. It is also used as a food flavoring agent [3], cosmetic products, and has antioxidant activities [4]. Clove is often used in traditional medicine to treat respiratory ailments so that its compounds may also be potential in the fight against COVID-19 disease [5].

The national demand for cloves increases every year, while its national production decreases due to many factors, such as low-yielding ability, yield fluctuation/biennial bearing, pest and disease attacks, and changing climatic conditions. Recently the development of the clove industry has begun to restore the glory of Indonesian spices. The total area of cloves in 2018 has reached 569,052 hectares, with 66.84% was cultivated by farmers [6].

Various obstacles are faced in developing the national clove industry, including the narrowing of the clove plantation area. Besides, the national clove productivity is still low, accounted for 260–333 kg/ha. This condition causes national clove production to be very low. In contrast, the potential productivity of cloves can reach 2,000 kg/ha. In general, farmers have not planted superior varieties and are still heterogeneous. Breeding of new clove varieties with high yield trait will contribute to increasing national clove productivity. The high Indonesia cloves

genetic resources have an essential role in the assembly of cloves superior varieties, especially in selecting their parental lines for the breeding program.

Hartung and Schiemann [7] stated that plant breeding can be done through various techniques ranging from only selecting plants (selection) with the desired characteristics to be propagated, to methods that utilize genetic and chromosomal knowledge, to more complex molecular techniques. To the present date, breeding on clove plants has only been done through selection from the existing onfarm population. Five superior clove varieties, namely Zanzibar Karo [8], Zanzibar Gorontalo [9], Tunj Bursel [10], AFO [11], and Siantan Agribun [12], were selected from existing clove population belonged to farmers. Selection was based on yield and clove quality. Further clove improvement will only be achieved through the implementation of an effective breeding strategy.

The success of a plant breeding program through selection is essentially depends on the presence of genetic diversity and the value of heritability estimates [13]. Knight [14] stated that the estimation of the value of genetic diversity and the estimated value of heritability varies depending on environmental factors. If the level of genetic diversity is narrow, this indicates that the individuals in the population are relatively uniform. Thus the selection for trait improvement becomes less effective [15]. On the other hand, the wider the genetic diversity, the greater the chance for successful selection in increasing the frequency of the desired gene. In other words, the opportunity to get a better genotype through selection is getting bigger [16].

To further optimize in obtaining superior varieties of cloves, selection of high-yielding mother trees (HYMTs) have been done in several clove production centers in Sumatra, Java, Sulawesi, Nusa Tenggara, and Maluku Islands. Progenies from these mother trees have been collected and planted. Understanding the growth of the progenies of the selected HYMTs are needed to determine the inheritance of the desired characteristics from the parents to the progenies. It is important that the desired traits are passed from the superior plants to their offspring. Clove has been known as open pollinated crops [17], progenies derived from generative seeds are generally heterogenous and morphologically vary. The objective of the present study was to characterize morphological characteristics of the progenies and calculate the relationships of the selected clove HYMTs. These informations are usefull for the next clove breeding program.

MATERIALS AND METHODS

Plant Materials

This study was conducted at the Plant Breeding Division, Indonesian Spice and Medicinal Research Institute, Bogor from December 2018 to December 2020. About 129 clove HYMTs derived from thirteen regions in Indonesia were used in this study (Table 1). Seeds of the selected HYMTs from various regions were collected and sown in the greenhouse. The good growth seedlings were planted in poly bags. The number of seedlings varies from 29–193 plants in each HYMTs.

Morphological Observation

Parameters of morphological clove characters consisted of the percentage of live seeds, the percentage of less vigor seeds, the percentage of red shoots, the percentage of seedlings with reddish shoots, the percentage of green shoots, plant height, number of leaves, and the number of leaves stem diameter, and number of branches. Twelve sample plants derived from each HYMTs were selected randomly and observed twice, at 4 and 8 months after planting (MAP).

Data Analysis

The difference in morphological trait of HYMTs were compared using analysis of variance (ANOVA). Data were analyzed using Tukey's test if there was a significant difference. All statistical analyses were performed using the Statistical Tool for Agricultural Research (STAR) software. A dendrogram of genetic relationship among HYMTs were generated using Pearson correlation coefficient (Agglomeration method: Unweighted pair-group average).

TABLE 1. Location and number of high-yielding mother trees (HYMTs) selected from various region.

No.	Province	Region	Number of HYMTs	Number of plants	Label of HYMTs
1	North Sumatra	Karo	10	1,059	KR1, KR5, KR6, KR8, KR11, KR12, KR16, KR17, KR18, KR20
2	West Sumatra	Solok	7	613	BRS1, BRS4, BRS5, BRS6, BRS10, BRS13, BRS15
3	West Java	Cianjur	8	718	CJR2, CJR3, CJR5, CJR7, CJR11, CJR12, CJR14, CJR15
		Bogor	8	497	CM15, CM16, CM17, CM27, CM28, CM38, CM39, CM41
4	Cetral Java	Semarang	5	483	SMR2B, SMR46, SMR47, SMR68, SMR86
		Karanganyar	12	845	KRA1, KRA2, KRA3, KRA4, KRA5, KRA6, KRA7, KRA8, KRA9, KRA10, KRA11, KRA12
5	East Java	Trenggalek	11	552	L1, L2, L3, L4, L5, L7, L8, S2, S4, S5, PAR5
6	East Nusa Tenggara	Alor	10	1,239	ALOA4, AL2OA4, AL11OA1, AL13OA1, AL17OA1, AL22OA1, AL23OA1, AL29OA1, AL30OA1, AL31OA1
		East Manggarai	6	503	MT28, MT41, MT50, MT52, MT53, MT58
		Ende	4	334	ENE1, ENE2, ENE3, ENE4
7	Central Sulawesi	Banggai	31	2,736	BP1, BP2, BP3, BP4, BP5, BP6, BP7, BP8, BP9, BP10, BP11, BP12, BP13, BP14, BP15, BP16, BA1, BA2, BA3, BA4, BA5, BA6, BA7, BA8, BA9, BA10, BA11, BA12, BA13, BA14, BA15
		Kepulauan			
8	Gorontalo	Gorontalo	17	2,461	GTB1, GTB2, GTB3, GTB4, GTM5, GTM6, GTM7, GTM8, GTM9, GTL10, GTL11, GTL12, GTL13, GTL14, GTL15, GTL16, GTL17
9	Maluku	Buru Selatan	2	169	PB1, PB2

RESULTS AND DISCUSSIONS

Cloves are found in various regions in Indonesia, the production centers in Sumatra, Java, East Nusa Tenggara, Sulawesi, and Maluku Islands. In general, it is known that there are four superior clove varieties, such as Zanzibar, AFO, Turni Bursel, and Siantan Agribun. This variety was obtained from the selection of HYMTs in high-yielding blocks at farmer's field [18].

Tidbury [19] stated that the pollination system in cloves was not known definitively. Pool and Bermawie [17] reported that the most of Zanzibar flowers anthesis in the afternoon and were immediately followed by the breaking of pollen tube. Pollen viability and stigma receptivity were obtained simultaneously after 48 h. In general, HYMTs in high-yielding blocks were heterozygous and have high genetic diversity morphologically. This indicated that cloves are cross-pollinated. Bermawie [20] reported that offspring originating from the same HYMTs still varied in morphology, production and fluctuation so that cross-pollination was suspected.

The genetic variation between HYMTs were observed morphologically including qualitative and quantitative characters. The quantitative morphological characterization revealed variability among the thirteen HYMTs at 4 MAP (Table 2). Results showed that there was six HYMTs which showed tall seedling, lots of leaves, and large stem diameter, i.e. HYMTs from Semarang, Karanganyar, Trenggalek, Ende, Banggai Kepulauan, and Gorontalo, based on Tukey's test with 95% confidence level. Of these, the Ende's HYMTs showed good performance with tallest seedling compared to the other HYMTs. Meanwhile, the Semarang's HYMTs had the largest number of leaves compared to the other HYMTs. Most of the HYMTs had a large branch diameter at 4 MAP. Meanwhile, the East Manggarai's HYMTs had short seedling, less number of leaves, and small stem diameter.

Quantitative morphological variability was also showed among the thirteen HYMTs at 8 MAP, especially stem diameter character (Table 3). Present study showed that there was seven HYMTs showing tall seedling, lots of leaves, and large stem diameter, i.e. HYMTs from Karo, Cianjur, Semarang, Karanganyar, Ende, Banggai Kepulauan, and Gorontalo, based on Tukey's test with 95% confidence level. The Semarang's HYMTs showed

good performance with tallest seedling compared to the other HYMTs. The Ende's HYMTs had the largest number of leaves and stem diameter compared to the other HYMTs.

TABLE 2. High-yielding mother trees morphology at 4 months after planting.

Region	Plant height (cm)	Number of leaves	Stem diameter (cm)
Karo	13.59±3.11 efg	11.50±2.35 abc	1.96±0.34 ab
Solok	10.28±3.28 g	7.92±2.53 e	2.05±0.39 a
Cianjur	13.85±4.13 defg	9.62±2.10 cde	2.28±0.34 a
Bogor	13.91±2.65 cdefg	10.00±1.81 bcde	2.00±0.28 a
Semarang	19.41±4.73 ab	12.47±2.80 a	2.09±0.23 a
Karanganyar	18.06±3.51 abcd	10.44±1.58 abcd	1.96±0.28 ab
Trenggalek	18.24±3.83 abc	10.53±1.96 abcd	2.06±0.32 a
Alor	16.90±3.72 bcdef	11.40±2.75 abc	2.09±0.77 a
East Manggarai	12.83±3.56 fg	8.88±2.01 de	1.61±0.30 b
Ende	21.32±4.35 a	12.04±2.04 ab	2.10±0.22 a
Banggai Kepulauan	17.49±3.35 abcde	12.36±1.98 a	1.97±0.29 ab
Gorontalo	19.14±3.36 ab	11.73±2.16 abc	2.01±0.31 a
Buru Selatan	16.55±1.73 bcdef	11.88±1.68 ab	2.06±0.13 a
CV (%)	20.84	15.10	11.51

Different letters in the same column indicated significantly different according to the test of Tukey's 5%.

All the HYMTs had the same number of branches at 8 MAP based on Tukey's test with 95% confidence level. There was three HYMTs which showed short seedling, less of leaves, and small stem diameter, i.e. HYMTs derived from Solok, Alor, and East Manggarai. Of these, the East Manggarai's HYMTs had shortest seedling size and smallest in stem diameter in comparison with other HYMTs. The East Manggarai's HYMTs had slowest growth from the beginning of seedling. The Solok's HYMTs had the least of leaves compared to other HYMTs.

TABLE 3. High-yielding mother trees morphology at 8 months after planting.

Region	Plant height (cm)	Number of leaves	Stem diameter (cm)	Number of branches
Karo	46.46±5.84 a	28.89±7.47 abc	3.31±0.46 ab	2.34±1.62 a
Solok	27.80±8.92 c	19.30±5.09 d	2.61±0.36 de	0.72±1.07 a
Cianjur	43.51±10.86 ab	26.82±8.37 abcd	3.23±0.48 abc	1.67±1.70 a
Bogor	45.82±8.84 ab	22.77±4.85 abcd	3.04±0.35 bcd	1.82±1.41 a
Semarang	47.61±9.21 a	28.18±7.92 abcd	3.24±0.29 abc	1.78±1.56 a
Karanganyar	41.23±10.20 abc	24.04±6.04 abcd	3.11±0.41 abcd	1.58±1.60 a
Trenggalek	36.44±10.65 abc	24.51±7.88 abcd	3.04±0.47 bcd	2.15±1.34 a
Alor	31.35±11.16 bc	19.68±7.34 bcd	2.68±0.45 cde	1.40±1.43 a
East Manggarai	28.66±11.98 c	19.47±5.11 cd	2.30±0.53 e	1.26±1.36 a
Ende	43.43±8.45 ab	30.66±8.36 a	3.66±0.63 a	2.35±2.06 a
Banggai Kepulauan	40.05±9.56 abc	28.92±8.66 ab	3.26±0.54 ab	1.99±2.72 a
Gorontalo	39.88±13.98 abc	25.74±10.19 abcd	3.39±0.52 ab	1.85±1.70 a
Buru Selatan	41.88±7.14 abc	21.04±6.57 bcd	3.54±0.33 ab	1.75±1.54 a
CV (%)	23.40	23.47	13.54	51.63

Different letters in the same column indicate significant difference according to the test of Tukey's 5%.

The Trenggalek's HYMTs had the largest value for both length and width of leaf compared to other HYMTs. The Trenggalek's HYMTs also had long leaf petiole after Bogor's HYMTs (Table 4). In general, all the HYMTs showed the same value of the clove leaf shape (Table 5). Leaf shape of all HYMTs was elliptic but ratio between length and width of the leaves at each HYMTs differed. Diversity of leaf qualitative characters was sown on leaf

color. Mature leaf color was divided into two groups, namely green and yellowish green groups. Young leaf color was divided into two groups, namely yellowish green and greenish yellow groups. The highest leaf diversity was observed in the tip of young leaf color. While its color was divided into three groups namely yellowish green, greenish orange, and greyish red groups.

TABLE 4. Quantitative morphological character of high-yielding mother trees leaf.

Region	Leaf length (cm)	Leaf width (cm)	Petiole pength (cm)
Karo	11.60±1.14	3.84±0.35	1.76±0.23
Solok	11.54±0.72	3.56±0.21	1.72±0.18
Cianjur	10.83±0.72	3.98±0.28	1.73±0.21
Bogor	10.62±0.87	3.90±0.49	1.98±0.04
Semarang	11.28±1.17	3.54±0.34	1.64±0.19
Karanganyar	12.14±0.61	3.92±0.18	1.86±0.22
Trenggalek	13.06±1.09	4.78±0.76	1.96±0.15
Alor	11.67±0.58	4.03±0.40	1.80±0.17
East Manggarai	12.42±2.11	4.22±0.63	1.90±0.33
Ende	11.62±0.66	3.58±0.26	1.75±0.47
Banggai Kepulauan	11.87±0.51	3.51±0.27	1.74±0.42
Gorontalo	12.26±1.21	4.08±0.40	1.56±0.09
Buru Selatan	12.04±1.40	4.26±0.59	1.86±0.13
CV (%)	9.78	13.35	14.54

TABLE 5. Qualitative morphological character of high-yielding mother trees leaf.

Region	Leaf shape			Leaf color		
	Whole	Base	Tip	Mature	Young	Tip of young leaf
Karo	Eliptic	Cuneate	Acuminate	GG137D	YGGN144C	GOG175A
Solok	Eliptic	Cuneate	Acuminate	GG137C	YGGN144C	GOG166C
Cianjur	Eliptic	Cuneate	Acuminate	GG137B	YGGN144C	GOG175C
Bogor	Eliptic	Cuneate	Acuminate	YGG147A	YGG144A	GOG176A
Semarang	Eliptic	Cuneate	Acuminate	YGG147A	YGGN144C	GOG176C-YGG152D
Karanganyar	Eliptic	Cuneate	Acuminate	GG137A	YGGN144C	GOG175B-166B
Trenggalek	Eliptic	Cuneate	Acuminate	GG137B	YGG144C	GRG178B-GOG166B
Alor	Eliptic	Cuneate	Acuminate	GG137B	YGGN144C	GOG175D
East Manggarai	Eliptic	Cuneate	Acuminate	GG137B-GG147A	YGG144B	GOG175C
Ende	Eliptic	Cuneate	Acuminate	GG137A	YGGN144B	GOG175B
Banggai Kepulauan	Eliptic	Cuneate	Acuminate	GG137B	YGG144B	GOG175A-YGG146C
Gorontalo	Eliptic	Cuneate	Acuminate	GG137B	YGG144C	GOG175D-YGG152C
Buru Selatan	Eliptic	Cuneate	Acuminate	GG137B	YGGN144C	GOG166C

GG = Green Group, YGG = Yellowish Green Group, YGGN = Greenish Yellow Group, GOG = Greenish Orange Group, GRG = Greyish Red Group.

A dendrogram which generated based on morphological characters of clove samples demonstrated a high genetic similarity between Ende and Gorontalo cultivars. The group obtained in clove cultivars was very useful in future plant breeding due to the genetic relationship among HYMTs on the basis of morphological characters could be clearly differentiated. The seed with many similarities in both characters (quantitative and qualitative) means having a close kinship relationship. Meanwhile, genotypes that have little or no similarity in many characters means having a distant kinship relationship [21]. A dendrogram showed that at the similarity of about 72%, HYMTs were divided into three groups. The group I consisted of HYMTs derived from Banggai Kepulauan, Semarang, Karo, Ende,

Gorontalo, and Solok. In details, the group I revealed the HYMTs were group of clove cultivars from provinces which located on different islands, except for North Sumatra and West Sumatra. The group II consisted of HYMTs collected from Cianjur, Buru Selatan, Karanganyar, and Bogor that dominated by HYMTs derived from Java Island. Meanwhile, the group III consisted of HYMTs from Trenggalek, East Manggarai, and Alor which dominated by HYMTs from East Nusa Tenggara. Overall, the HYMTs collected from Trenggalek, East Manggarai, and Alor were among clove samples showing the farthest genetic distance from the other HYMTs.

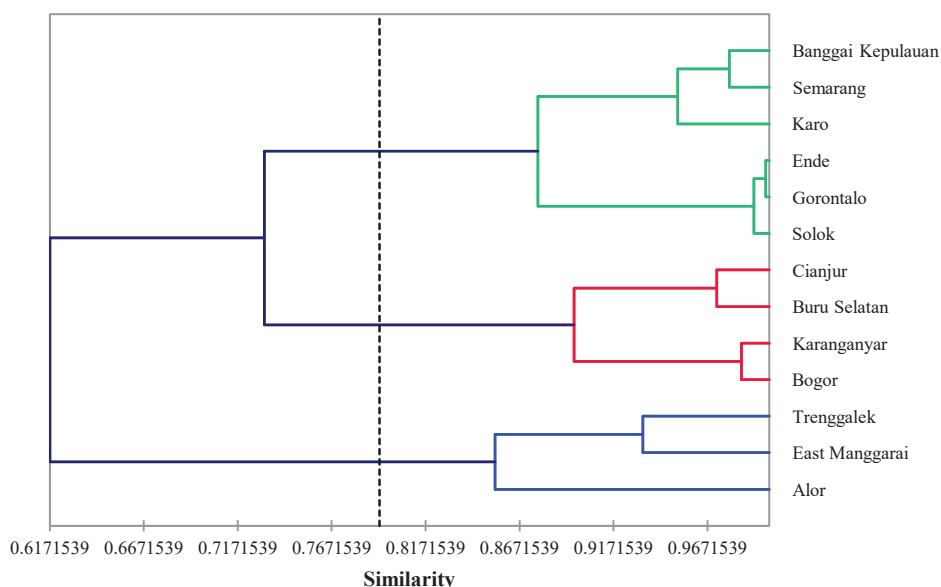


FIGURE 1. Dendrogram of high-yielding mother trees from various region based on morphological characters.

CONCLUSION

A dendrogram generated from morphological characters showed that at the similarity of about 72%, HYMTs were divided into three groups. The group I consisted of HYMTs from Banggai Kepulauan, Semarang, Karo, Ende, Gorontalo, and Solok; the group II consisted of HYMTs from Cianjur, Buru Selatan, Karanganyar, and Bogor; the group III consisted of HYMTs from Trenggalek, East Manggarai, and Alor. HYMTs from Ende and Trenggalek had good seedling performance based on morphological characters and the farthest distance, indicating their differences in genetic backgrounds and beneficial for selecting parental for breeding programs.

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