January 2022

THE SECOND INTERNATIONAL CONFERENCE ON GENETIC RESOURCES AND BIOTECHNOLOGY: Harnessing Technology for Conservation and Sustainable Use of Genetic Resources for Food and Agriculture

Committees: The Second International Conference on Genetic Resources and Biotechnology

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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 2021virtually 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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Existing diversity profile for kernel characteristics of maize germplasm in IAARD-ICABIOGRAD gene bank

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Andari Risliawati, Sobir, Trikoesoemaningtyas, et al.

Existing Diversity Profile for Kernel Characteristics of Maize Germplasm in IAARD-ICABIOGRAD Gene Bank

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Abstract. Maize (*Zea mays* L.), a model genetic organism with immense genetic diversity, is one of the major commodities in Indonesia. The genetic diversity of this crop is an important resource for their genetic improvement through breeding activities, thus properly conserving and managing the germplasm is necessary. This valuable maize *ex situ* germplasm is being prioritized to be conserved in the Indonesian Agency for Agricultural Research and Development-Indonesian Center for Agricultural and Biotechnology and Genetic Resources Research and Development Gene Bank (IAARD-ICABIOGRAD Gene Bank) for decades. However, the diversity information of the whole germplasm has been not reported due to the large collection which is challenged for the management. The research aimed at investigating the genetic diversity from whole maize germplasm conserved in IAARD-ICABIOGRAD Gene Bank, specifically on kernel characteristics as a major trait contributes to maize diversity. A total of 1,279 accessions were characterized for their kernel characteristics using a modified maize descriptor and followed by a series of diversity analyses. The results showed a moderate-high genetic diversity existed among the accessions. Two out of nine kernel morphological characters revealed the major contribution to genetic diversity among the accessions. These characters successfully differentiated all accessions into ten clusters, of which specific traits were observed as cluster identity. This preliminary investigation of kernel diversity within maize germplasm may become valuable information for gene bank managers to determine the appropriate decision for germplasm management and inform users about the existing diversity profile of the germplasm.

INTRODUCTION

Maize (*Zea mays* L.) is a cereal crop that can be multi-use as food, feed or biofuel [1]. As a food source, maize is one of the cereal crops that provide calories for more than half of the world's population [2]. To meet the demand, a larger planting area and high-yielding maize varieties are needed. These varieties can be developed through a plant breeding approach which not only targeted on high-yielding varieties but also varieties well-adapted to climate change and having better nutritional content to combat malnutrition [3].

Many factors involve in a successful of plant breeding program, of which a broad genetic diversity might be developed through crossing and mutation or those that come from natural resources [4]. All these genetic resources usually prone to extinction, particularly when their genetic potential have been not discovered yet. Therefore, an intervention must be carried out to ensure the availability of plant genetic resources, specifically through *ex situ* conservation in the form of gene bank [5]. Conservation of maize germplasm in the form of seeds within a gene bank, might be the most convenient method and may prolong the longevity of the seed [6]. In Indonesia, maize

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germplasm has been prioritized to be conserved by the Indonesian Agency for Agricultural Research and Development-Indonesian Center for Agricultural and Biotechnology and Genetic Resources Research and Development Gene Bank (IAARD-ICABIOGRAD Gene Bank) under the Ministry of Agriculture for decades. This gene bank has been conserved around 12,000 accessions from 40 species, including maize with a total number of 1,279 accessions [7].

To exploit more the potency of this huge collection, such as base materials for breeding, main component for particular food or feed industry, and as gene sources for biotic stress resistance or abiotic stress tolerance, further germplasm characterization and evaluation activities have to be carried out. However, this becomes challenging for gene bank manager to simultaneously conserving and managing the germplasm for optimal use. Therefore, for the initial step, there is an urge for understanding the global diversity extent and pattern of genetic diversity within collections. This information will not only important for the development of conservation strategies, but also the efficient use of germplasm [8].

Among the part of the maize plant, kernel or seed is known to have a high variation among varieties [9]. The kind of product utilization of maize also determines by the kernel characteristic, as we can see on the different kernel characteristic which is shown among sweet corn, waxy corn, and flint/dent corn. Moreover, the use of seed as genetic material to assess the global diversity of the maize collection is considered as the most convenient way. It is non-destructive and can be carried out simply by observing the phenotype based on standardizing descriptor.

The objective of this study was to investigate the diversity of whole maize germplasm within the IAARD-ICABIOGRAD Gene Bank based on the kernel characteristic assuming that these have a high contribution to the diversity. The preliminary investigation of kernel diversity along with the existing available information in the database may become valuable information for gene bank managers to determine the appropriate decision for germplasm management and inform users about the existing diversity profile of the germplasm.

MATERIALS AND METHODS

The study was carried out in the Seed Quality Testing Laboratory of IAARD-ICABIOGRAD Gene Bank from August 2020 to March 2021. Genetic materials used in this study comprised 1,279 accessions of maize which are acquired since 1980s and conserved in short-term facilities of IAARD-ICABIOGRAD Gene Bank located in ICABIOGRAD, Bogor, West Java, Indonesia. The production dates of the seed vary ranging from the year 2006 to 2020. The sample was randomly selected from the seed container in the short-term storage, weighed 250–300 g per accession, and immediately returned to the container after observation. A total of nine kernel characters were observed based on maize plant descriptor from the International Maize and Wheat Improvement Center (CIMMYT) with slight modification [10] as shown in Table 1.

TABLE 1. Observation method and scoring for characterization of the kernel in maize [10].

No.	Character	Observation method and scoring
1	Kernel type	1 = floury, 2 = semi floury, 3 = dent, 4 = semi dent, 5 = semi flint, 6 = flint, 7 = pop, 8 = sweet, 9 = opaque-2/QPM, 10 = tunicate, 11 = waxy
2	Kernel color	1 = white, 2 = yellow, 3 = purple, 4 = dark purple, 5 = brown, 6 = orange, 7 = mottled, 8 = yellow-orange, 9 = red
3	White cap	0 = absent, 1 = present
4	Mottled type	1 = yellow group (yellow, yellow-orange, and orange), 2 = white-purple, 3 = white-yellow group, 4 = red-yellow group, 5 = red-brown, 6 = white-red-yellow group, 7 = purple-red-yellow group, 8 = white-purple-red-yellow group
5	1,000-kernel weight (g)	Based on the ISTA method, 100 seeds were randomly selected and weighing, replicated 8 times. One thousand kernel weight is means of 8 times sampling multiply by 10
6	Kernel length (mm)	Average of 10 kernels, measured with a caliper
7	Kernel width (mm)	Average of 10 kernels, measured with a caliper
8	Kernel thickness (mm)	Average of 10 kernels, measured with a caliper
9	Kernel upper surface shape	1 = shrunken, 2 = indented, 3 = level, 4 = rounded, 5 = pointed, 6 = strongly pointed

ISTA = International Seed Testing Association, QPM = quality protein maize.

Descriptive analysis was carried out to reflect the structure of the collection based on the kernel data as well as the information of passport data, followed by calculation of genetic diversity from each character based on the Shannon-Weaver diversity index [11] using the formula:

$$I = \sum_{i=0}^{n} p_i \log_e p_i$$

where p_i = frequency of phenotypic class data, n = number of class data.

To keep the value of the Shannon-Weaver index (H') between 0 and 1, each I value above was divided by its maximum value ($\log_e n$) and normalized. To support the profile of descriptive analysis, a heatmap clustering was performed using 'gplots' package under R software v.3.6.1 for particular traits. Meanwhile, cluster analysis was also generated using web-based software, PBSTAT-CL (www.pbstat.com). The cluster analysis was carried out in agglomerative-average linkage method with Gower genetic dissimilarity [12] since variables with different data types were used in the study.

RESULTS AND DISCUSSIONS

Structure of the Maize Collection

The total number of maize germplasm in IAARD-ICABIOGRAD Gene Bank was 1,279 accessions which comprised of 819 local varieties/landraces (64.03%), 17 improved varieties (1.33%), 197 introduction varieties (15.40%), 239 inbred lines (18.69%), and 15 accessions with no information in their passport data (1.17%) (Fig. 1). Local variety was accounted majorly to the collection and these come from various regions in Indonesia. Most of these local varieties originated from eastern region of Indonesia (35%), i.e. Bali, West Nusa Tenggara (NTB), East Nusa Tenggara (NTT), Maluku, and North Maluku Provinces. In addition, the other region next to the eastern region that having a major contribution in local germplasm origin was Java Island (16.81%). Local variety or landrace has a common characteristic such as historical origin, high genetic diversity, well known by the community, lack of genetic improvement, and adaptive to the local environment [13]. This characteristic made them valuable genetic resources because of carrying hidden potency that might be useful in the future, especially to deal with climate change. Therefore, conserving landrace is a priority to secure the diversity.

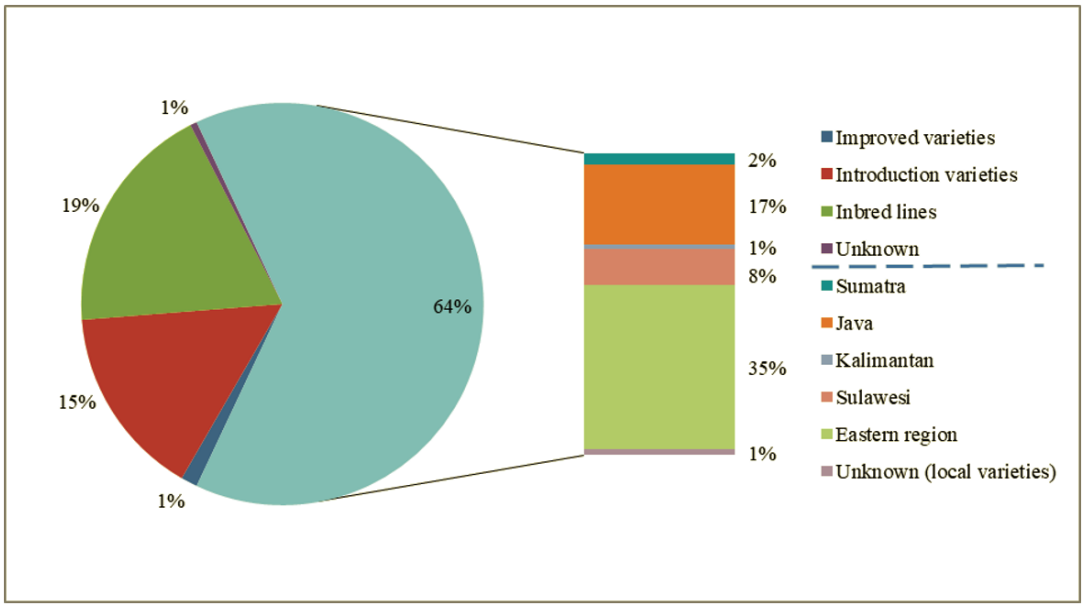

FIGURE 1. Collection structure of maize germplasm in IAARD-ICABIOGRAD Gene Bank based on passport data in 2021. Eastern region = Bali, West Nusa Tenggara (NTB), East Nusa Tenggara (NTT), Maluku, and North Maluku.

A wide range of maize utilization is determined by their kernel characteristic. Yellow and orange flint or dent corn is commonly utilized in feed and industrial factories, while white waxy corn, sweet corn, and popcorn are consumed by the people due to their starch and sugar content [14]. In this study, we observed the kernel characteristic visually and identified 7 out of a total of 11 kernel types. Since we only observed the kernel visually, other methods were needed to identify the unidentified kernel types. The majority of kernel type that has been conserved in the IAARD-ICABIOGRAD Gene Bank is flint corn (1,015 accessions) which is accounted for 79.36% of the whole collection. The remains were waxy corn, semi flint corn, semi dent corn, sweet corn, and popcorn, i.e. 116 (9.07%), 80 (6.26%), 26 (2.03%), 23 (1.80%), 14 (1.09%), and 5 (0.39%) accessions, respectively. Notably, all variations of kernel color were identified in this gene bank. Kernel with the yellow group color (yellow, yellow-orange, and orange) and white color dominated the entire collection, i.e. 501 accessions (39.17%) and 674 accessions (52.7%), respectively (Table 2). Moreover, if these two characters were combined, the type of maize that dominated the collection was the flint corn with yellow-orange and white color of the kernel (Fig. 2).

TABLE 2. The number of maize accessions in *ex situ* conservation of the IAARD-ICABIOGRAD Gene Bank for each kernel type and kernel color.

Kernel type	Kernel color								
	White	Yellow	Dark purple	Brown	Orange	Mottled	Yellow-orange	Red	Total
Dent	16	2				1	4		23
Semi dent	18	2			3	2	1		26
Semi flint	27	5			16	11	19	2	80
Flint	329	75	1	8	213	77	312		1,015
Popcorn	2				1		2		5
Sweet		7			5		2		14
Waxy	109	2			1	2	2		116
Total	501	93	1	8	239	93	342	2	1,279

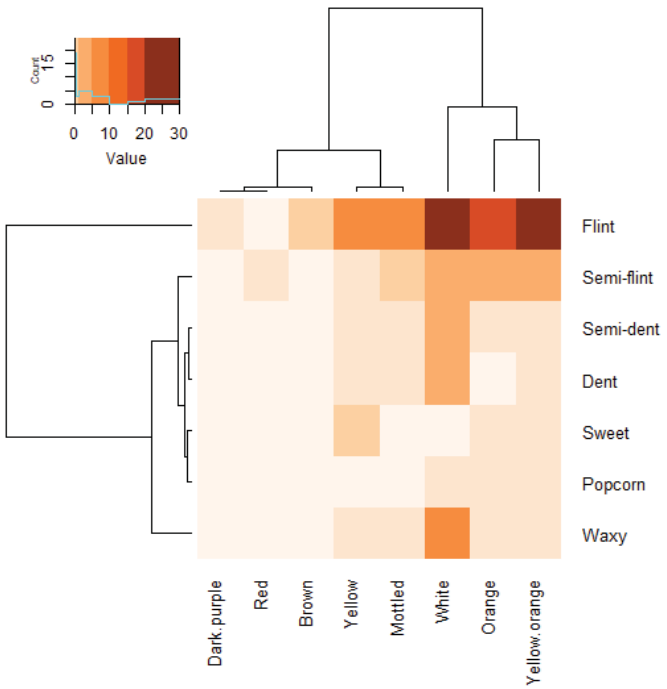

FIGURE 2. Heatmap of the structure collection of maize germplasm at IAARD-ICABIOGRAD Gene Bank based on kernel type and color. The value represented the percentage of the characters in the entire collection.

Only 7 kernel types were identified from a total of 11 variations that existed in maize. The unidentified kernel types were floury, semi floury, quality protein maize (QPM), and tunicate type. These kernel types should be observed by other methods which utilizing advanced technologies that have been reported by other researcher. The floury and semi floury kernel types can be assessed by iodine solution in a fluorescence microscope [15], while QPM kernel type can be estimated by molecular markers [16]. The need for a further testing method is necessary since the kernel morphology had no relationship with vitreous and floury texture in maize endosperm [17]. It is expected that among dent-flint kernels with yellow color, there were floury or QPM kernels existed. From the heatmap graphic, we can figure out that a few accessions revealed unique kernel colors, i.e. purple, red, and brown (Fig. 3). In maize, orange kernels might contain high carotenoids, and purple kernels might contain high anthocyanin which both are beneficial for human health [18, 19]. Therefore, to increase the genetic diversity of maize collection in the gene bank, the acquisition of such maize as well as sweet corn and popcorn that also presented a few number in the existing collection, are highly considerably collected in the future mission.

Maize Diversity Based on Kernel Characteristic

The Shannon-Weaver diversity index (*H'*) was used to assess the genetic diversity of kernel characters. Quantitative traits (0.68) had higher means of Shannon-Weaver diversity compared to the qualitative traits (0.64). Among the qualitative traits observed, a white cap in the kernel upper surface showed the highest *H'* value because in this character, all variations existed and comparable, in contrast to the kernel type with the lowest diversity index which only 7 out of total 11 variations that existed (Table 3). Moreover, only one character in kernel type, i.e. flint type, dominated and accounted for 79.36% of the entire collection. However, the value of the Shannon-Weaver index for kernel type of maize from southern Brazil was higher (0.66) than that of our study results although this value was categorized as medium-high [20]. This was understandable since these Southern Brazil are the micro center of maize diversity in the world. Moreover, it is well known that the majority of maize utilization in Indonesia was for the feed industry purposes, allowing flint corn to become the main target of maize breeding program in Indonesia and predominantly has being conserved in the IAARD-ICABIOGRAD Gene Bank.

TABLE 3. Shannon-Weaver diversity index (*H'*) for qualitative traits in maize kernel characters.

No.	Kernel characters	Proportion (%)	*H'*	No.	Kernel characters	Proportion (%)	*H'*
1	**Kernel type**		0.33	4	**Kernel upper surface shape**		0.50
	Dent	1.80			Shrunken	1.17	
	Semi dent	2.03			Indented	3.99	
	Semi flint	6.26			Level	54.26	
	Flint	79.36			Rounded	40.27	
	Popcorn	0.39			Pointed	0.31	
	Sweet	1.09					
2	**Kernel color**		0.68	5	**Mottled type**		0.71
	White	39.01			Yellow group	23.66	
	Yellow	7.43			White-purple	6.45	
	Dark purple	0.08			White-yellow group	26.88	
	Brown	0.63			Red-yellow group	37.63	
	Orange	18.69			Red-brown	1.08	
	Mottled	7.27			White-red-yellow group	1.08	
	Yellow-orange	26.27			Purple-red-yellow group	2.15	
	Red	0.63			White-purple-red-yellow group	1.08	
3	**White cap**		0.99				
	Presence	50.20					
	Absent	49.80			**Average**		**0.64**

The size and weight of the maize kernel among accessions in the gene bank were varied which indicated by high range value of each character. Diverse in such morphological character also reflected the variation in the kernel type because they are correlated each other. For example, popcorn commonly had a smaller kernel size in comparison to flint-dent corn. Similarly, the sweet corn had a lighter weight than that in flint-dent corn (Fig. 3). The highest Shannon-Weaver diversity index observed among quantitative traits was the 1,000-kernel weight and the lowest ones was observed in the kernel length (Table 4). The kernel length showed the lowest index because the majority of accessions had kernel length of 50–130 mm, with only a few accessions showing kernel length of 200–250 mm, indicating their potential donor for a breeding program. Interestingly, these accessions were local varieties/landraces originated from East Java Province. Thus, in relevance to the IAARD-ICABIOGRAD Gene Bank management, conserving landrace is important since this genetic resource prone to extinction due to land use conversion or monoculture practices [21, 22], and could undergo genetic erosion due to its genetic systems [23].

TABLE 4. Shannon-Weaver diversity index (*H'*) for quantitative traits in maize kernel characters.

No.	Kernel characters	Means±SE	Range	*H'*
1	Kernel length (mm)	84.81±0.31	52–250	0.39
2	Kernel width (mm)	79.81±0.21	52–100	0.75
3	Kernel thickness (mm)	48.55±0.19	30–80	0.72
4	1,000-kernel weight (g)	220.23±1.32	88–365	0.84
	Average			**0.68**

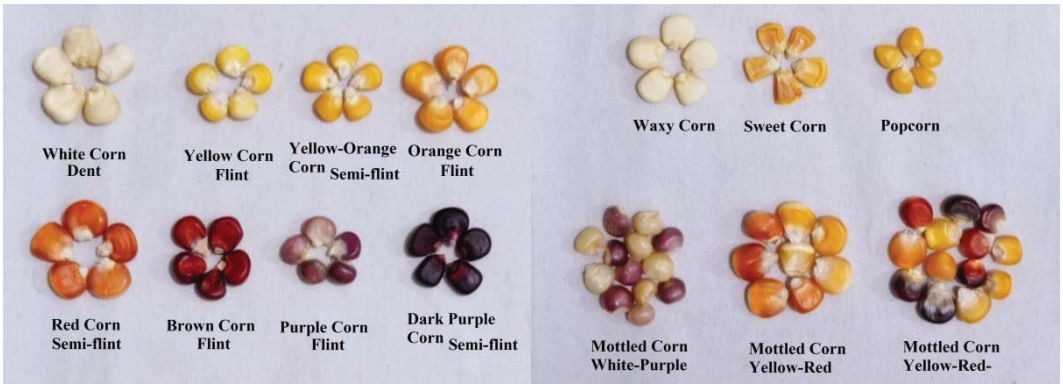

FIGURE 3. Genetic variation based on kernel morphological characters of the maize germplasm collection at IAARD-ICABIOGRAD Gene Bank.

Cluster Analysis among Accessions

The relationship among accessions was investigated using a cluster analysis based on Gower genetic dissimilarity to combine all different types of variables in a single analysis [24]. In present study, all maize accessions grouped into 10 clusters at a cophenetic distance of 0.25 (Fig. 4). Of this, cluster 7 and 10 were the clusters with the highest number of accession member among the other eight clusters, i.e. 537 accessions and 509 accessions, respectively. Four clusters were the minority group in the maize germplasm collection, i.e. cluster 1, cluster 2, cluster 5, and cluster 9 which consisted of 14, 7, 2, and 6 accessions, respectively. Whereas the remaining clusters i.e. cluster 3, 4, 6, and 8 consisted of 59, 34, 44, and 67 accessions, respectively.

Each cluster had its specific characters as cluster identity foremost based on the kernel type and color. The most distinct clusters were cluster 1 and 2 comprising of sweet corn and popcorn groups, respectively. Both types had specific kernel character. Sweet corn had a shrunken shape of the upper surface of the kernel and popcorn possessed a pointed shape, while the remaining accessions had an indented or level shape. The shrunken shape in sweet corn is caused by the high levels of total sugars and higher moisture content than other corn types when they are dry [25].

The remaining clusters were divided into five main nodes. The first node of the cluster grouped mottled corn in cluster 3 and 4. Generally, the mottled corns were landraces, thus a mixture of kernel color is prevalent. In the non-cultivated field, it is common that the farmer preferred to cultivate a mixture of maize variety for decades and conducting less selection, as represented by mottled corn. The next node, i.e. the second node of the cluster differentiated accessions into dent and flint type of kernel. It is noted that there were only a few accessions that had dent kernel. Similar to the sweet corn and popcorn, the dent corn also had a specific shape of the upper surface of the kernel, i.e. indented shape, which were not possessed by other types. As a result, they are specifically grouped in cluster 5 and 6 (Fig. 4).

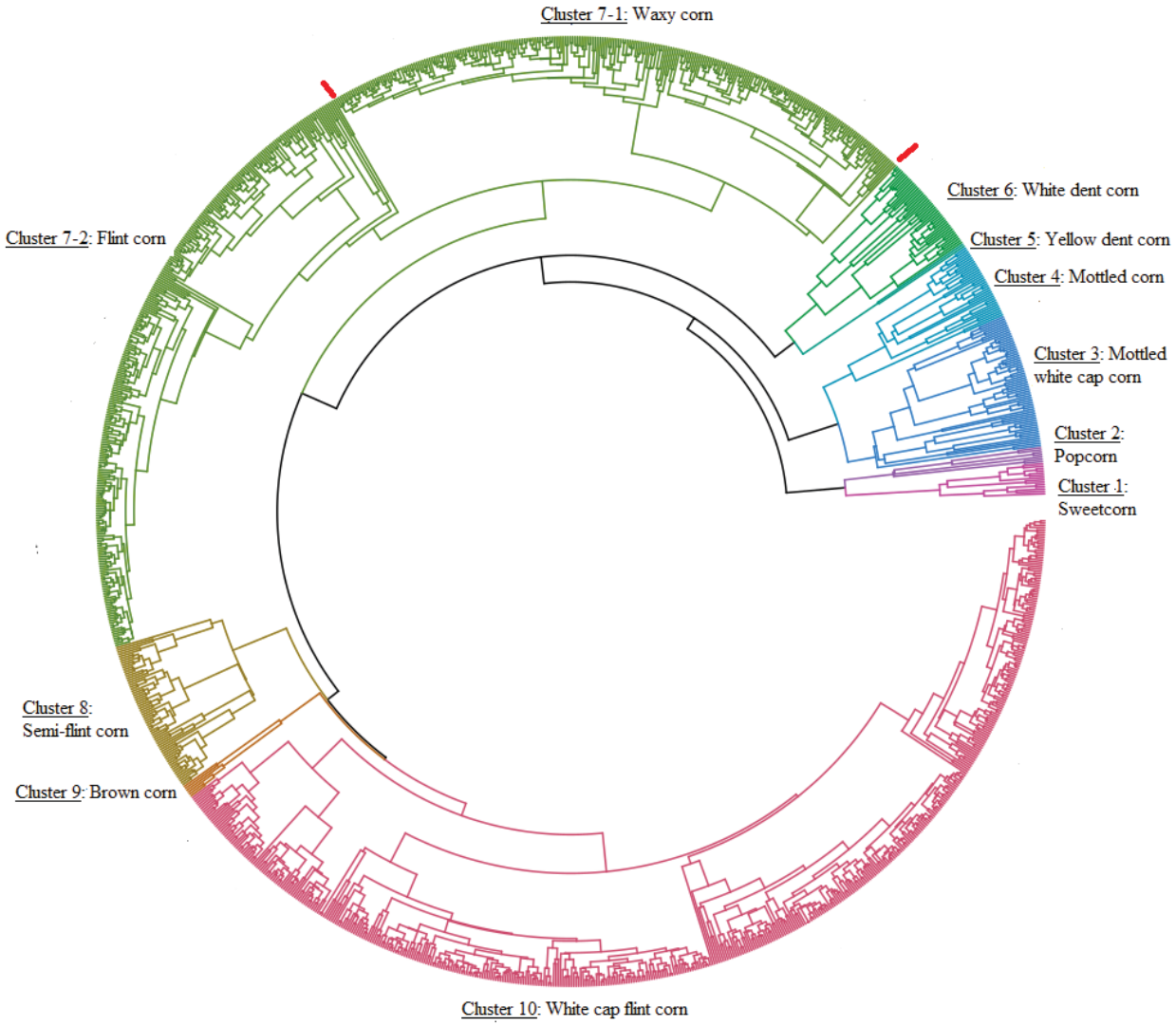

FIGURE 4. Dendrogram of 1,279 accessions of maize germplasm in IAARD-ICABIOGRAD Gene Bank based on kernel characters.

The other nodes, i.e. the third node of the cluster differentiated all remaining accessions based on the presence of the white cap which means the presence of a dense white color in the upper surface of the kernel. It may prevalent both on the white or yellow kernel. This phenotype was controlled by dominant alleles, *Wc,* which has negative regulation of carotenoid accumulation in the developing maize kernel [26]. For remaining accessions of which those with no white cap grouped in cluster 7. In this cluster, there were two types of maize existed, i.e. waxy and flint corn. The waxy corn grouped in this cluster representing the dense white color in the whole surface of the kernel. This character made the waxy corn can be distinguished from the white flint corn showing a white cap in their upper

surface of the kernel (Fig. 3). Waxy corn contains less amylose than other types of maize because of exclusive amylopectin (95–100%) controlled by a recessive allele, *wx1* [27]. Waxy corn is being popular in Asia and is usually consumed fresh by the people due to the better flavor [14]. In Indonesia, this corn has widely been planted and popular in Eastern Indonesia, such as in Sulawesi, West Nusa Tenggara, East Nusa Tenggara, and Maluku [28]. Hundreds accessions of the local variety of this corn have been also conserved at IAARD-ICABIOGRAD Gene Bank and has been supported the national breeding program of maize. Up to date, there were two improved varieties of waxy corn that released nationally which was developed from local maize variety [29].

The remaining two nodes, the fourth and fifth nodes could differentiated the rest of maize the accessions into semi flint corn in cluster 8 and flint corn in cluster 9 and 10. Cluster 9 consisted of specialty maize with brown flint. The brown color, as well as the reddish color in the maize kernel, were controlled by *midrib2* (*bm2*) gene located in chromosome 1, encoding a methylenetetrahydrofolate reductase that contributes to lignin accumulation [30]. The brown maize exhibited the flint type phenotype which is affected by lignin known as the contributor to the strength of the tissue. The kernel strength of brown maize is beneficial for the plant as protection, but it has a negative effect on forage quality and cellulosic biofuel production, thus this kind of corn is not recommended to be utilized in feed or bio-industrial sectors [31]. The last cluster name as cluster 10 was consisted of flint corn that exhibits white cap phenotype in the upper surface of the kernel. The flint corn in this cluster differed with the flint corn in cluster 7, suggesting that the white cap character had a high contribution to the diversity of the germplasm collection which was also supported by the high value of the genetic diversity index as stated previously (Table 3).

CONCLUSION

Moderate-high diversity was observed among maize accessions on the basis of the kernel character. Kernel type and kernel color were the main characters contributed to the diversity of the whole maize collection and differentiated all the maize accessions into ten clusters. This preliminary investigation of kernel diversity within maize germplasm may become valuable information for gene bank managers to determine the appropriate decision for germplasm management as basic consideration for core collection development. The information of the existing diversity profile of the germplasm in the IAARD-ICABIOGRAD Gene Bank could be beneficial for a wide range of users in different fields to assist further needs in breeding and other relevant researches.

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