



## Morphological Diversity of Local Sorghum Cultivar (*Sorghum bicolor*) of East Nusa Tenggara, Indonesia

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### Abstract

Sorghum has great potential to be developed in marginal lands of Indonesia including East Nusa Tenggara. However, the information about sorghum diversity in this area was very limited. This research aimed to describe morphological variation, and assessing the value of genetic diversity based on morphological characters of local sorghum cultivar of East Nusa Tenggara. The exploration and sample collection were conducted in 3 islands, i.e.: Timor, Sumba, and Flores. The observation of morphological characters was based on sorghum descriptor. Morphological character similarities were analyzed using Simple Matching (SM) coefficient, and a dendrogram was constructed using Unweighted Pair Group Method with Arithmetic Average (UPGMA) method. The results showed that 36 accessions of sorghum in East Nusa Tenggara varied in 17 morphological characters. At the similarity coefficients of 48%, all sorghum accessions were separated into 2 groups based on the presence or absence of aleurone layer. Group I consisted of 12 accessions with no aleurone layer, while group II consisted of 24 accessions has aleurone layer. The genetic diversity of sorghum of East Nusa Tenggara is low ( $I=0.62-0.71$  and  $h=0.37-0.42$ ). The genetic diversity between populations ( $H_T=0.4203$ ) is higher than within populations ( $H_S=0.3961$ ). This study provides the first complete information about sorghum diversity in East Nusa Tenggara that will be useful as basic information for sorghum development in this area in the future.

### How to Cite

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## INTRODUCTION

Sorghum is the fifth important cereal crop in the world after wheat, rice, corn, and barley that is used mostly for food, feed, industry, and energy source (Dahlberg *et al.*, 2011). Physiologically, sorghum has wide adaptability to abiotic stress, and able to grow well on marginal land such as dry land (Dahlberg *et al.*, 2011).

Over the last 50 years, sorghum development has been increased in the world, and the United States is the largest sorghum producer (Etuk *et al.*, 2012). Many improved sorghum cultivars have also been released in Asia including Indonesia. Indonesia has released 13 improved sorghum cultivars, 2 pure lines selected from local landraces and the others are from direct introductions (Deb *et al.*, 2004). The development of sorghum in Indonesia is now rising to support the food diversification program. Sorghum becomes one of non-rice food which has been developed on many dry areas in Indonesia included in East Nusa Tenggara (Sirappa, 2003).

Sorghum developed in East Nusa Tenggara consisted of many diverse local cultivars, but their diversity had not been described and documented yet. The diversity of local cultivar of sorghum showed high genetic diversity (Morojele & Mbewe, 2015), unfortunately, the information was still unavailable. Information of genetic diversity is important for improvement, conservation, and survival of sorghum (Gerrano *et al.*, 2014; Amelework *et al.*, 2016). Previous studies reported that based on morphological character, the genetic diversity of sorghum in North Shewa and South Welo Ethiopia is high (0.32-0.98) (Abdi *et al.*, 2002). Morphological character is more practice and easy to be observed when it is used for genetic diversity analysis (Gerrano *et al.*, 2014). The selected morphological characters should be a qualitative character because it is relatively stable from environment influence. The aims of this study were to (1) describe the variation of sorghum morphology of local cultivars in East Nusa Tenggara, (2) analyze the relationships between, and (3) assess the genetic diversity of local cultivars of East Nusa Tenggara based on their morphological characters.

## METHOD

### Plant Materials

A total of 36 accessions of local sorghum cultivars were collected from 11 districts in three islands (Figure 1), i.e. Timor (Kupang, North Central Timor, South Central Timor and Belu

districts), Sumba (East and Southwest Sumba districts), and Flores (East Flores, Sikka, Ende, Nagekeo, and Ngada districts) with the diverse local names (Table 1). The seeds of each local cultivar was collected and the name was given based on the district name and collection number. A local cultivar of one location was treated as an accession. All accessions then were grown in a greenhouse of Bogor Agricultural University.



**Figure 1.** Location map for exploration and sample collection. 1=Kupang, 2=South Central Timor, 3=North Central Timor, 4=Belu, 5=East Sumba, 6=Southwest Sumba, 7=East Flores, 8=Sikka, 9=Ende, 10=Nagekeo, 11=Ngada.

### Sorghum Planting

Each accession of sorghum was planted in a 30x30 cm polybag with 5 replications and 2 plants on each polybag. The polybags was placed randomly. Fertilization was given twice, on the 30<sup>th</sup> and 60<sup>th</sup> day after planting. Pest and disease control were conducted using standard practices. The sorghum flowers were encased before the pollen riped to prevent cross-breeding between accessions.

### Observation of Morphological Characters

As many as 20 morphological characters of generative organ were observed. The observed characters were adapted from sorghum descriptor (IBPGR & ICRISAT 1993), i.e. inflorescence (panicle) compactness, panicle shape, panicle stands; shape, base and apex bracts color of seed with bracts (glume), presence and location of pubescent on bracts, angle on palea, awn presence, and percentage of seed covered by bracts; shape, base and apex color of seed without bracts (grain), seed lustre, seed dimple, aleuron presence and color, endosperm color, and endosperm texture.

### Data Analysis

The morphological characters were used for phenetic and genetic diversity analysis. The selected morphological characters were arranged in a matrix data and then scored. The sorghum

**Table 1.** Identities and local names of 36 accessions of sorghum of East Nusa Tenggara

Island	District	Accession code
Timor	Kupang	<i>Pen mina 1, pen mina 2, pen mina 3, pen mina 4</i>
	South Central Timor	<i>Buka 1, buka 2</i>
	North Central Timor	<i>Sain 1</i>
	Belu	<i>Batar ainaruk 1, batar ainaruk 2, batar ainaruk 3, batar ainaruk 4</i>
Sumba	East Sumba	<i>Watar 1</i>
	Southwest Sumba	<i>Watar pia 1, watar pia 2</i>
Flores	East Flores	<i>Watar holo 1, watar holo 2, watar holo 3, wara blong mera 1, watar nae eke 1, riang kemie 1, riang kemie 2, watar holo 4, watar holo 5</i>
	Sikka	<i>Watar 2, watar 3, watar 4, watar 5, watar 6</i>
	Ende	<i>Lolo 1, lolo 2, lolo 3</i>
	Nagekeo	<i>Latung 1, rolo 1, olo wolo viki 1, olo wolo 1</i>
	Ngada	<i>Sae lewa 1</i>

phenetic was analyzed using Simple Matching (SM) similarity index and a dendrogram was constructed using *Unweighted Pair Group Method with Arithmetic Average* (UPGMA) method. Phenetic analysis were performed with Numerical Taxonomy and Multivariate Analysis System (NTSYS) software version 2.11a (Rohlf, 2001). *Population Genetic Analysis* (Popgene) version 32 (Yeh *et al.*, 1999) software was used to analyze ten genetic diversity parameters: number of observed alleles ( $N_a$ ), number of effective alleles ( $N_e$ ), genetic diversity ( $h$ ), Shannon's Information Index ( $I$ ), number of polymorphic loci, percentage of polymorphic loci, genetic diversity between population ( $H_T$ ), genetic diversity within population ( $H_S$ ), genetic differentiation between population ( $G_{ST}$ ), and gene flow ( $N_m$ ).

## RESULT AND DISCUSSION

### Diversity of Morphological Characters of Local Sorghum Cultivar from East Nusa Tenggara

A total of 20 generative characters were observed in 36 sorghum accessions. Based on the overall characters observed, 17 characters are varied, while 3 characters are not. All accessions have the same vertical stems, ellipse panicles, and white (FFFFFF) endosperm. The variation characters of sorghum can be seen in Table 2. The variations have association with inflorescence (panicle) compactness, shape of seed with bracts, base and apex bracts color, location of pubescent on bracts, angle on palea, percentage of seed covered by bracts; shape, base and apex color of seed without bracts, seed lustre, seed dimple, aleurone color, and endosperm texture (Figure 2 and 3). The presence of pubescent on bracts, angle on

palea, awn on seeds, seed dimple and aleurone layer were found in some accessions.

Most sorghum of East Nusa Tenggara are characterized by open branches, oval-shaped seed, black base and apex bracts, pubescent on bracts, with no angle on palea, no awn on seed apex, 100% seed covered by bracts, brown base and apex of seed without bracts, no seed dimple, dull seed, with dark brown aleurone, and intermediate endosperm texture.

### The relationships of Sorghum Local Cultivars in East Nusa Tenggara

Phenetic analysis of 36 sorghum accessions of East Nusa Tenggara were done based on 17 morphological characters which were qualitative and constant characters. All accessions were grouped into one large group with a similarity coefficient of 45% (Figure 4). In similarity coefficient of 48%, all sorghum accessions were separated into 2 groups based on the presence or absence of aleurone layer. Group I consisted of 12 accessions without aleurone layer, while group II consisted of 24 accessions with aleurone layer. The analysis also classified some accessions that have similar characters in the percentage of seed covering by bracts and the color of bracts apex. In addition, there are similar accessions with similarity coefficient about 100%. It means that, some accessions which have same morphological characters also spreaded on other islands, but they have different local names.

All accessions in group I do not have aleurone layer in the seed, but they have semi-open branches and open inflorescence branches, rounded and oval shape of seed with bracts, goldenrod, goldenrod with black pattern, orange, brown, red, maroon, and black of base and apex bracts color.

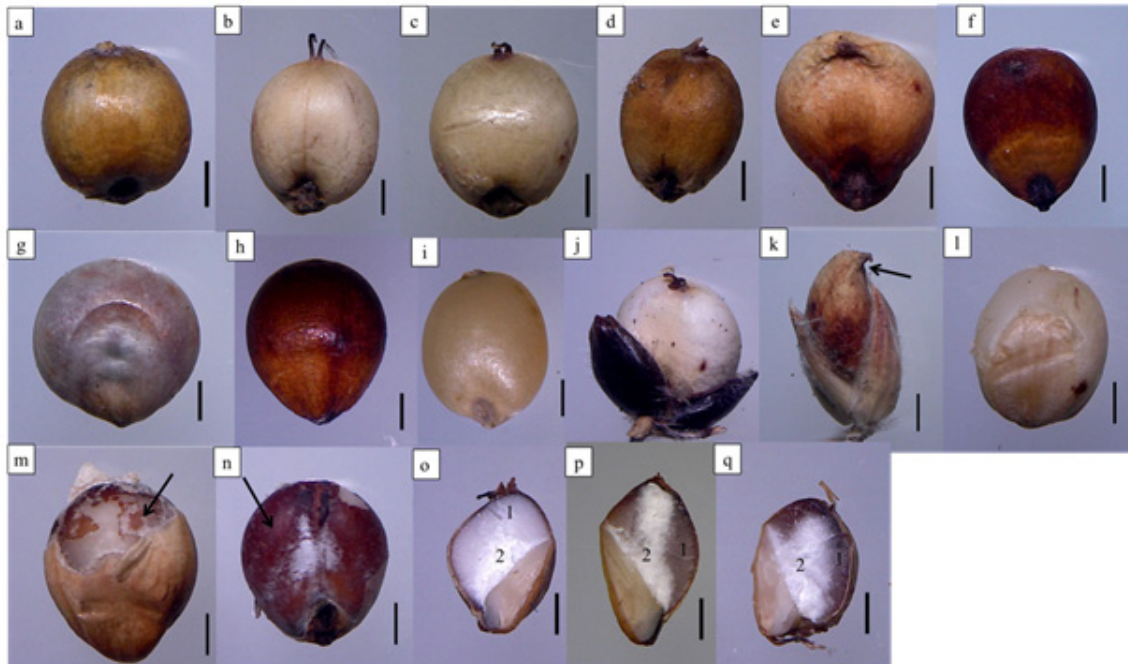
**Table 2.** Variation character of local sorghum cultivar of East Nusa Tenggara

Characters	Characters states (the percentage of character states)
Inflorescence (panicle) compactness	compact (22%), semi-compact (3%), semi-open branches (3%), open branches (72%)
Shape of seed with bracts	rounded (3%), oval (83%), ellipse (14%)
Base bracts color	goldenrod (DAA520) (17%), goldenrod with black pattern (DAA520+000000), (6%), brown (D2691E) (22%), orange red (FF4500) (3%), red (FF0000) (8%), maroon (800000) (3%), black (000000) (41%)
Apex bracts color	goldenrod (17%), goldenrod with black pattern (6%), orange (FFA500) (6%), brown (19%), red (8%), maroon (3%), black (41%)
Presence of pubescent on bracts	absent (17%), present (83%)
Location of pubescent on bracts	only on edge (40%), on edge and apex (60%)
Presence of angle on palea	absent (17%), present (83%)
Presence of awn	absent (97%), present (3%)
Percentage of seed covered by bracts	25% (6%), 50% (27%), 75% (17%), and 100% (50%)
Shape of seed without bracts	round (6%), oval (94%)
Base color of seed without bracts	white (39%), brown (55%), dark brown (8B4513) (3%), burly wood (DEB887) (3%)
Apex color of seed without bracts	white (39%), white brown (FFDEAD) (3%), brown (41%), dark brown (14%), burly wood (3%)
Seed dimple	absent (97%), present (3%)
Seed lustre	dull (94%), lustre (6%)
Presence of aleurone	absent (33%), present (67%)
Aleurone color	dark brown (96%), sienna (A0522D) (4%)
Endosperm texture	corneus dominant (8%), intermediate (42%), starch dominant (50%)

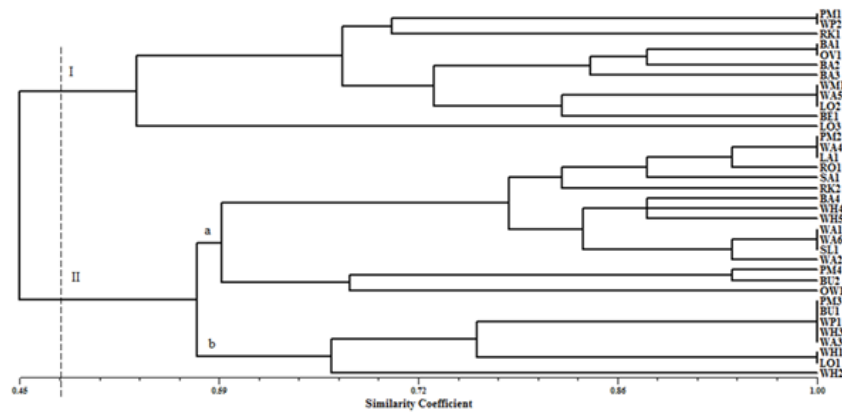


**Figure 2.** Variations of inflorescence (a-d) and seed with bracts (e-u). Inflorescence compactness: a. compact, b. semi-compact, c. semi-open branches, d. open branches. Shape: e. rounded, f. oval, g. ellipse. Presence of awn: h. without awn, i. with awn. Base and apex bracts color: j. goldenrod, k. goldenrod with black pattern, l. brown, m. base orange-red, apex orange, n. red, o. maroon, p. black. Presence of angle on palea: q. without angle, r. with angle. Presence and location of pubescent pubescent on bractea: s. without pubescent, t. pubescent on edge (arrow), u. pubescent on edge and apex (arrows). Percentage of seed covered by bracts: v. 25%, w. 50%, x. 75%, y. 100%. e-y: bar scale = 1mm.





**Figure 3.** Variations of seed without bracts. Shape: a. round, b. oval. Base and apex color: c. base and apex white, d. base and apex brown, e. base dark brown, apex white brown, f. base brown, apex dark brown, g. base and apex burlywood. Seed lustre: h. dull, i. lustre. Seed dimple: j. without dimple, k. with dimple (arrow). Aleurone presence and color: l. without aleurone, m. aleurone with dark brown color (arrow), n. aleurone with sienna color (arrow). Endosperm texture: o. corneus dominant, p. intermediate, q. starch dominant, 1. corneus, 2. starch. Bar scale=1mm.



**Figure 4.** Dendrogram of 36 accessions sorghum based on morphological characters using UP-GMA method. PM=*pen mina*, BU=*buka*, SA=*sain*, BA=*batar ainaruk*, WA=*watar*, WP=*watar pia*, WH=*watar holo*, WM=*wara blong mera*, BE=*batar nae eke*, RK=*riang kemie*, LO=*lolo*, LA=*latung*, RO=*rolo*, OV=*olo wolo viki*, OW=*olo wolo*, SL=*sae lewa*.

Their seed are covered by bracts ranged from 25-100%, white of base and apex of seed color without bracts, while awn and dimple on seed are absence. Seed lustre was found in two accessions (*pen mina 1* and *watar pia 2*), while the others are dull. There are three groups of similar accessions, i.e. *pen mina 1* and *watar pia*; *batar ainaruk 1* and *wolo viki 1*; *watar blong mera 1*, *watar 5*, and *lolo 2*.

Group II consisted of accessions with dark brown and sienna aleurone color. All sorghum ac-

cessions in this group have oval and ellipse shape of seed with bracts, goldenrod, orange, brown, orange red, and black base and apex of bracts color, also 50-100% of seed covering by bracts. Awn on seed was found in *riang kemie 1*, while the seed dimple was found in *olo wolo 1*. All sorghum accessions have dull seed with white, white brown, brown, dark brown, and burly wood of base and apex color of seed without bracts. In similarity coefficient of 59%, all sorghum accessions were

separated into 2 groups based on inflorescence compactness. Group a, consisted of 16 accessions with semi-compact and open branches of inflorescence, while group b has compact inflorescence. In this group, there are four groups of similar accessions with similarity coefficient of 100%, i.e. *pen mina 2, watar 4, and latung 1; watar 1, watar 6, and sae lewa; pen mina 3, buka 1, watar pia 1, watar holo 3, and watar 3; watar holo 1 and lolo 1*.

Sorghum var. *bicolor* was grouped into six races, namely *bicolor, caudatum, durra, guinea, cafir* and *nervosum* (OGTR, 2017). Based on panicle compactness and spikelet characters, all observed accessions were classified into five races, *bicolor, caudatum, guinea, cafir* and *nervosum*. The *bicolor* race consisted of 17 accessions, *pen mina 2, sain 1, batar ainaruk 4, watar 1, watar blong mera 1, batar nai eke, watar holo 4, watar holo 5, watar 2, watar 4, watar 5, watar 6, lolo 2, lolo 3, latung 1, rolo 2, and sae lewa 1*. This race is characterized by having seed covered by 100% of bracts and open branched panicle. *Riang kemie 2* accession has ellipse seed. It is grouped into the *nervosum* race. *Riang kemie 1* and *olo wolo 1* accessions were grouped into the *caudatum* race since they have semi-compact and semi-open branched panicle, and their seed are covered by bracts less than 100%. Eight accessions with compact panicle are *pen mina 3, open 1, watar pia 2, watar holo 1, watar holo 2, watar holo 3, watar 3, and lolo 1*, were grouped into the *cafir* race. Other accessions with open branched panicle and percentage of seed covered by bracts of 25% to 75%, *pen mina 1, pen mina 4, open 2, batar ainaruk 1, batar ainaruk 2, batar ainaruk 3, watar pia 2* and *olo wolo viki 1*, were classified into the *guinea* race.

At the race category, sorghum from East Nusa Tenggara showed high differences in characteristics among all accessions, so that, one more category under race namely forma was formed. Classification on forma level were based on bracts color. Based on the characters, there are five forma within *bicolor* race and three forma within *guinea* race. The forma within the *bicolor* race are *brown* forma (*batar ainaruk 4, watar 1, watar holo 5, watar 2, watar 6, and sae lewa 1*), *red orange* forma (*watar holo 4*), *red* forma (*watar blong mera 1, watar 5, and lolo 2*), *maroon* forma (*lolo 3*), and *black* forma (*pen mina 2, sain 1, batar nai eke 5, watar 4, latung 1, and rolo 1*). The *guinea* race consists of three forma, *goldenrod* forma (*pen mina 1, pen mina 4, buka 2, and watar pia 2*), *brown* forma (*batar ainaruk 2* and *batar ainaruk 3*), and *goldenrod pattern black* forma (*batar ainaruk 1* and *olo wolo viki 1*).

### Genetic Diversity of Sorghum Local Cultivar in East Nusa Tenggara

The genetic diversity of local cultivar sorghum was varied among three islands. The number of observed alleles ranged from 2.35–3.12, while the number of effective alleles is 1.91–2.04. The percentage of polymorphic loci ranged between 76.47–94.12% and the highest percentage was on Flores island (Table 3). Since Shannon Information Index of local cultivar sorghum ranged between 0.62–0.71 ( $I < 1$ ) and Nei's genetic diversity of sorghum in East Nusa Tenggara is 0.37–0.42, the diversity of sorghum species in East Nusa Tenggara is categorized low. Genetic diversity of species is considered high when Shannon Information Index is more than three, intermediate if  $1 \leq I \leq 3$ , and low if  $I < 1$  (Odum, 1993). The genetic diversity is also categorized low when Nei's genetic diversity ranged between 0.1–0.4, intermediate if  $0.5 \leq h \leq 0.7$ , and high if  $0.8 \leq h \leq 1$  (Nei, 1987).

Based on the genetic analysis result, genetic diversity between populations ( $H_T = 0.4203$ ) is higher than within populations ( $H_S = 0.3961$ ) (Table 4). Genetic differentiation between populations value ( $G_{ST} = 0.0575$ ) indicates that genetic differentiation between sorghum populations is intermediate, but there is a high gene flow among populations ( $N_m = 8.2008$ ).

The low value of Nei's genetic diversity and Shannon Information Index of sorghum of East Nusa Tenggara is suspected because there are many similar accessions found in different islands. The comparison of genetic diversity of three islands showed that the highest genetic diversity ( $h$ ) was found in Sumba island ( $H_T = 0.42$ ). It is suspected that the observed accessions on Sumba Island have different characteristics one another. In contrast to the Sumba island, there are several similar accessions in Flores island, such as: *watar blong mera 1, watar 5* and *lolo 2; watar 6* and *sae lewa 1; watar holo 3* and *watar 3*; and *watar holo 1* and *lolo 1*. As a result, compared to the other island, the Shannon Index value ( $I = 0.71$ ) of Flores island is the highest, but its genetic diversity ( $h = 0.40$ ) is lower than Sumba island. Timor island has the lowest genetic diversity ( $h = 0.37$ ) and Shannon information index ( $I = 0.62$ ). Similar to Flores island, there are two similar accessions in Timor island, *pen mina 3* and *buka 1*. It is not surprising that this island has lower genetic diversity. The small accession number is suspected to influence the genetic diversity of a population. Since small number of accession or species in a population caused the low opportunity for cross pollination, thus, it may decrease the genetic di-

**Table 3.** Genetic diversity parameters and polymorphic loci among population in ENT

Population	Total Accession	Na	Ne	h	I	J	P (%)
Timor	11	2.35	1.92	0.37	0.62	13	76.47
Sumba	3	2.12	2.04	0.42	0.65	13	76.47
Flores	22	3.12	1.91	0.40	0.71	16	94.12
Average		2.53	1.96	0.40	0.66	14	82.35

Na= number of observed alleles; Ne= number of effective alleles; h= Nei's genetic diversity; I= Shannon information index, J= number of locus polymorphic, P= percentage of locus polymorphic

**Table 4.** Analysis of the genetic structure of 36 sorghum accessions of ENT

Value	$H_T$	$H_S$	$G_{ST}$	$N_m$
Average	0.4203	0.3961	0.0575	8.2008
Standard Deviation	0.0600	0.0537		

$H_T$ =genetic diversity between accessions in total populations;  $H_S$ =genetic diversity in population;  $G_{ST}$ =genetic divergence between populations;  $N_m$ =gene flow

versity of population.

Plant genetic diversity is caused by several factors, i.e., environmental variations, gene flow, and gene mutations. Environmental variety caused not only species diversity but also diversity within species (Muraya *et al.*, 2013). Genetic diversity within populations and between populations also occurred because of gene flow, such as seed or pollen dispersal. Seed and pollen dispersal are influenced by populations distance. When the distance is closer, pollen and seed dispersal will be better. Pollen and seed dispersal can occur through animal feed, fertilizer, animal, and human mobility (Epperson, 2004; Muraya *et al.*, 2013).

Pollen dispersal may trigger cross-pollination in sorghum. Sorghum is a self-pollination plant, but the potential for cross-pollination is high, especially in sorghum with open branched panicle of 30-60%. Even less than 10%, the opportunity to cross-pollination can also occur in compact panicle (Kumar, 2016). Sorghum in East Nusa Tenggara varied in panicle compactness. This variation is suspected causing high genetic diversity within population.

The distance between Timor, Sumba, and Flores islands causes low ability of pollen dispersal, thus, the cross-pollination between sorghum populations is low. Nevertheless, the value of genetic diversity between populations ( $H_T=0.4205$ ) is higher than within population ( $H_S=0.3961$ ). It occurs because gene flow between populations is high ( $N_m=8.2008$ ) due to the seed dispersal. According to Zongo *et al.* (2005) and Muraya *et al.* (2013), farmers can bring sorghum from one place to another and exchange seeds with nearby areas, so people movement may cause gene flow

among populations. The presence of similar accessions in different populations support that gene flow is present between populations located on different island.

Gene mutations in large loci can also affect genetic diversity in a population. Two alleles in a locus will be identical, only if the allele does not mutate from generation to generation, but allele frequency in nature is always diverse or not identical. The diversity of allele frequency will occur when the number of alleles observed is higher than the number of effective alleles (Sinha and Kumaravadivel, 2016; Riupassa *et al.*, 2015). Based on available data, the number of alleles observed is higher than the number of effective alleles, indicating that allele frequency on three islands was varied. According to Kumar *et al.* (2012), in addition to gene mutation, the diversity of allele frequency in populations can also be affected by evolution, natural selection, and migration.

The characteristic variation, relationship among accessions and genetic diversity of sorghum from East Nusa Tenggara are new data that have not been described in previous studies. Since the genetic diversity of sorghum from this area is low, all information of its morphological variation is very important to be used as basic information for developing sorghum in the future, especially in East Nusa Tenggara where sorghum is an important crop.

## CONCLUSION

Sorghum in East Nusa Tenggara varied in 17 qualitative characters of generative organ, i.e. inflorescence (panicle) compactness, shape

of seed with bracts, base and apex bracts color, location of pubescent on bracts, angle on palea, percentage of seed covered by bracts; shape, base and apex color of seed without bracts, seed lustre, seed dimple, aleurone color, and endosperm texture. The presence of pubescent on bracts, angle on palea, awn on seeds, seed dimple and aleurone layer were found in some accessions.

All accessions were grouped into one large group with a similarity coefficient of 45%. At the similarity coefficients of 48%, all sorghum accessions were separated into 2 groups based on the presence or absence of aleurone layer. Group I consisted of 12 accessions with no aleurone layer, while group II consisted of 24 accessions with aleurone layer.

The genetic diversity of sorghum of East Nusa Tenggara is low ( $I = 0.62-0.71$  and  $h = 0.37-0.42$ ). The genetic diversity between populations ( $H_T = 0.4203$ ) is higher than within populations ( $H_S = 0.3961$ ).

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