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CHARACTERIZATION OF LOCAL GARLIC (*Allium sativum* L.) IN NORTH CENTRAL TIMOR REGENCY BASED ON PHENOTYPIC AND GENOTYPIC CHARACTERS

KARAKTERISASI BAWANG PUTIH LOKAL EBAN (Allium sativum L.) DI KABUPATEN TIMOR TENGAH UTARA BERDASARKAN KARAKTER FENOTIP DAN GENOTIP

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ABSTRACT

Phenotypic and genotypic characters a source of superior character in the framework of plant breeding programs, especially in increasing local food yields and productivity. In this study, local cultivars of garlic from Eban, North Central Timor (TTU) were used. A total of 15 phenotypic characters were observed, and threeISSR primers were used to identify the phenotypic and genotypic characters. The phenotype characterization showed the similarities in most of the characters observed. The tuber characters showed some differences in tuber diameter (2.5 cm and 1.9 cm), the number of cloves (14 and 10 cloves), tuber weight (11.28 g and 5.18 g), weight of cloves (0.89 g and 0.45 g) for local garlic from Saenam and Fatuneno, respectively. A total of ninepolymorphic bands and 17 monomorphic bands from threeprimers Inter Simple Sequence Repeat (ISSR) were detected. The coefficient of similarity of two groups based on genotypic characters was 0.65% - 1%. This study concluded that the two local Eban garlic varieties were different based on morphology and ISSR data. However, phenotypically there were differences in the size of the tubers and cloves, the number of cloves per tuber, and the weight of the cloves.

Keywords:*genotype, ISSR, local garlic, phenotype*

ABSTRAK

Karakter fenotip dan genotip berperan penting sebagai sumber karakter unggulan dalam rangka program pemuliaan tanaman, khususnya peningkatan hasil dan produktifitas pangan lokal. Pada penelitian ini digunakan 2 kultivar lokal bawang putih asal Eban Kabupaten Timor Tengah Utara (TTU). Sebanyak 15 karakter fenotip diamati dan tiga primer ISSR digunakan untuk mengidentifikasi karakter fenotip dan genotip. Karakterisasi fenotip menunujukkan persamaan pada sebagian besar karakter yang diamati. Karakter umbi menunjukkan beberapa perbedaan pada diameter umbi (2.5 cm dan 1.9 cm), jumlah siung (14 siung dan 10 siung), berat umbi (11.28 g dan 5.18 g), berat siung (0,89 g dan 0,45 g) pada bawang putih lokal asal Saenam dan Fatuneno berturut-turut. Koefisien kemiripan dari dua grup berdasarkan karakter genotip 0.65% – 1%. Kesimpulan hasil penelitian ini adalah, kedua kultivar bawang putih lokal Eban memiliki perbedaan pada berdasarkan karakter morfologi dan analisis ISSR. Namun, secara fenotip ada perbedaan pada ukuran umbi dan siung, jumlah siung per umbi, dan berat siung.

Kata kunci: *genotip, ISSR, bawang putih lokal, fenotip*

INTRODUCTION

Garlic (*Alliumsativum* L.) is a seasonal herb suitable for being planted in mountainous areas with sufficient sunlight. Eban local garlic is well known by the people of North Central Timor (TTU) because of its benefits for daily consumption. Market demand continues to increase in line with the rate of population growth, improving economic development, and increasing public knowledge about the importance of nutritional needs. At present, local garlic varieties produced from the center region and supplied at the wholesaler level are still very limited. This is due to a massive flow of imported garlic into the Indonesian garlic market. Therefore, exploration and characterization of germplasm arevery important in an effort to support the improvement of the quality of garlic varieties in the future.

The use of garlic in everyday life is not only well known as a seasoning for cooking but also as an antidote to various diseases. Garlic can treat common ailments, such as coughs and fever. Garlic can also maintain the immune system and has been shown to be effective in dealing with opportunistic infections such as herpes anti-bacterial and antimicrobial [1].

Garlic (*Allium sativum* L.) has a unique smell and taste so that it possesses economic value. Moreover, garlic having active compounds played important dietary and medicinal roles throughout history [2,3].According to Food and Environmental Research Agency [4], every planting material,especially the commercial, should have a published description showing its different morphological characters with other varieties. Therefore. Genetic resources should be characterized to improve theiruse.

Garlic has a fairly high variation, especially in terms of ripening/harvesting, tuber size, shape and colour, number of cloves per tuber, size of cloves, aroma, flowering ability, and leaf characteristics. One of the efforts to maintain local garlic cultivars is to explore and collect cultivars as well as the characterization in production centerareas which will be replanted as germplasm [5].

The morphological characterization of three accessions and four varieties of garlic collection of East Java Agriculture Technology Assessment Center and variability level of quantitative character has been documented. However, the collection of germplasm that isnot characterized properly and systematically will lead to the duplication of accessions or too many unique types [6]. Therefore, the researchers will conduct the exploration and the characterization for all local varieties of Eban garlic in the TTU Regency to provide a database and avoid duplication of local garlic accessions.

The phenotypic and genotypic characters of Eban local garlic in the TTU Regency have the potential to be a source of superior character in overcoming various obstacles to local garlic cultivation. The information on these characters is important for local garlic breeding. With the limited superiority of the garlic varieties, identification andcharacterization of local garlic genetic resources (SDG) areimportant. This characterization can identify local accessions that can be developed directly or have the potential to improve existing varieties [7]. Genetic variation analysis can be done by identifying morphological and molecular characters. The identification of plant morphological characters is the identification of external characters, both qualitatively and quantitatively.

The identification of morphological characters has a weakness;namely, the appearanceis often influenced by environmental factors. Agroclimatic conditionscan vary morphological characteristics and adds complexity to the characterization of garlic clones. Therefore, the identification of molecular characters is needed to complement the morphological information in determining the parents used in plant breeding and selection variety.

The study of genetic diversity, phylogeny, gene tagging, genome mapping, and evolutionary biology in various plants was using Inter Simple Sequence Repeat (ISSR). The results show that ISSR markers are high polymorphism in maize [8]. ISSR would be classified as *Alliummonanthum* and *A. grai* [9].ISSR was using fordifferent species, varieties, and cultivars of *Allium*[10]. Sai et al.[11]reportedgenetic similarity and diversity using molecular markers of RAPD and ISSR in nine onion cultivars. ISSR technique wasused on24 individual accessions and species of *Allium*[12]. In the genus *Allium*, Gehan et al. [13] reported genetic diversity of 16 SSR and 3 ISSR markers on some species of *Allium* L.

This study aimed to characterize morphological/phenotypic and genotypic using the ISSR (Inter Simple Sequence Repeat) marker of local Eban varieties of garlic from Seanam village and Fatuneno village.

METHODS

Material. The materials used in this study were garlic leaves, 70% alcohol, DNA extraction kit (Nucleon Phytopure), cold chloroform, cold isopropanol, 70% cold ethanol, sterile aquabides, TE buffer, distilled water, 10X TBE buffer (0.89 MTris Borec Acid, 0.02 M EDTA Disodium, pH 8.4), agarose powder, DNA ladder (Vivantis 100 bp), loading dye, DNA Staining flourosafe (Sybr Safe DNA Gel Stain Invitrogen), DNA PCR Kit (2x My Taq HS Red Mix Bioline), 23 gell ice, and ISSR Primer (Table 1).

Phenotype characterization. The phenotypic characters observed included tuber shape, tuber colour, tuber weight per plant, tuber diameter, clove colour, number of cloves per tuber, weight of cloves, plant height, stem diameter, leaf colour, leaf orientation, number of leaves, leaf width, leaf length, and harvesting age.

Table 1. ISSR Primers

No	Primer	Primer Sequence 5'-3'	Base Amount
1	UBC-836	AGAGAGAGAGAGAGACYA	18
2	UBC-825	ACACACACACACACACT	17
3	UBC-812	GAG AGA GAG AGA GAG AA	17

DNA isolation. The plant DNA isolation was carried out using the Nucleon Phytopure Kit and following the Nucleon Phytopure Kit protocol with slight modifications. DNA isolation was carried out by first weighing the garlic plant leaves that had been collected as much as 0.3 grams. The leaves were then crushed with a mortar and grinder until smooth, and 600 µl of Phytopure I reagent was added. The mixture was then given 200 µl of Phytopure II reagent and inverted several times until homogeneous. The mixture was then incubated at 65 ºC for 10 minutes, then transferred to an icebox for 20 minutes. After that, 500 µl of cold chloroform and 100 µl of Phytopure Resin were added to the mixture, then shookfor 30 minutes. Supernatant and pellets were divided by centrifuge at 10000 rpm for 10 minutes. Next, isopropanol was added with the same volume as the volume of the supernatant, and then it is strongly inverted several times. The supernatant and isopropanol mixture was then centrifuged at a minimum speed of 10.000 rpm for 10 minutes.

Then the tube containing the pellets was washed with 100µl of 70% ethanol and centrifuged again at 10.000 rpm for 5 minutes. The remaining ethanol was removed, and the pellet was allowed to air dry for about 10 minutes. The isolated DNA was then resuspended with the addition of 50 µl TE 1X and stored in cold conditions (-200 ºC).

ISSR-PCR analysis. PCR was done by first making a PCR mix according toMyTaqRedmix (Bioline) PCR kit protocol. Agarose gel 2% was used to amplification the product and separated using electrophoresis before the DNA sample was inserted into the gel well. The 6 µl DNA sample was piped and then inserted into the well on the minigel. The electrodes were connected to a 50 volt "power supply" within 50 minutes. DNA visualization was carried out by placing the electrophoretic gel into the UV transilluminator. The observations could be made using a digital camera or by connecting them to the Optilab.

Data analysis. The phenotypic data obtained were analyzed using Microsoft Excel (.xls) to determine the average value of each phenotypic character. The average results were then tabulated and matched with the IPGRI book [14].

Genotypic or molecular data based on ISSR markers is based on the presence or absence of bands. The size of the DNA band was determined by measuring the distance of each band formed using the Image Raster 3 computer program compared to the distance and size of the DNA ladder and then analyzed using Microsoft Excel (.xls). Scoring, Cluster Analysis, and Dendrogram Construction Data in the form of DNA strands are converted into 0-1 matrices (binary state scoring) by means of visible DNA bands coded using number 1 while invisible DNA bands are coded using number 0. Relationship between garlic was analyzed using MVSP 3.1 UPGMA (Unweighted Pair Group Method with Arithmetic).

RESULTS AND DISCUSSION

Exploration. Exploration activities in several villages in the subdistrict of Miomafo Barat (Eban) have been carried out, and twoaccessions of garlic were collected from Saenam Village and Fatuneno Village (Figure 1). Local garlic wasthe centerof Eban's local garlic production, which hasbeen marketed, while the rest of the villages are only for daily consumption.

Figure 1. The tuber of local Eban garlic from Saenan (a) and Fatuneno (b)

The center of local garlic production in the TTU Regency was in Eban (West Miomafo sub-district). The exploration in fivevillages (Saenam, Fatuneno, Suanae, Lemun, Fatunisuan) resulted in only twocultivars being identified, i.e., from Saenam and Fatuneno Villages. This indicatedthat local garlic was rarely found. It was found in the exploration that farmers were reluctant to grow local garlic because as they assumed that the local garlic loses competitiveness with China's garlic with a better tuber quality of the tubers but was sold at a low price. In fact, however, the quality of local garlic was superior in terms of taste. This could be seen from the very strong smell of local garlic as compared to imported garlic. Content of active compound aliin and organo S in local garlic were responsible for the strong smell. Therefore, the utilization of local garlic as raw material for medicines was very promising.

Phenotypic character. The phenotypic characters observed were tuber shape, tuber colour, tuber weight per plant, tuberdiameter, number of cloves per tuber, clove colour, the weight of the cloves, leaf colour, leaf orientation, number of leaves, leaf width, length of leaf, height of the plant, stemdiameter, and harvesting age.

Based on the phenotypic characterization of local Eban garlic (Table 2 and Figure 2, 3, 4, 5), it was obtained that phenotypically the local Eban garlic was not much different. The coefficient of

similarity of two groups based on genotypic characters is 0.65% - 1%. Two local Eban garlic varieties were different based on morphology and also ISSR data. Some differences in the number of cloves and tuber size were due to differences in environmental growing conditions in the cultivation areas. This was in accordance with Hardiyanto et al. [15], who observed that garlic clones did not show a significant difference in tuber's shape, tubers and cloves color, leaf color, and leaf orientation. The phenotype character was influenced by genotype and environment interaction. The more environmental factors involved, the more variation wouldemerge. The Eban region hadaverage uniform weather, resulting in the same phenotype ofthe local garlic explored in this study. This wasevidenced by molecular characterization and the results, which showed a high degree of similarity.

The difference was observed in some growth characteristics and at harvest. In general, the characteristic of Saenam local garlic hadthicker and wider leaves than Fatuneno local garlic, which hadthinner and narrow leaves. In this study, tuber size was smaller than the original habitat. Influences of changeable environmental factors and ecological variability couldcause vegetatively reproducing plant species such as garlic. Bulb induction and development in garlic were influenced by temperature and day length [16]. Maximum tuber weight (11.28 g), tuber diameter (2.5 cm), leaf length (30 cm), and leaf width (1.1 cm) hadbeen recorded in garlic genotypes collected from Saenam village. Height of plant ranged from 30 – 50 cm, leaf length ranged from 20 – 30 cm, leaf width from 0.65 – 1.1 cm, number of cloves from 10 – 14 cloves, and tuber weight from 5.18 – 11.28 g. The lowest plant height, 30 cm, and lowest blub weight, 0.45 g,hadbeen recorded in local garlic collected from Fatuneno village. Numerous research articles showed that the characters such as plant height, bulb size, and the number of cloves were correlated directly and/or indirectly to garlic yield [17]. Eventhough it was mostly vegetatively propagated, hence there was a strong interaction between genetic and environment, garlic couldshow wide variation in tuber size, plant height, number, and size of clove due to agroclimatic condition.

Table 2. Phenotypic characters of local Eban garlic

No	The plant characteristics	Unit	Saenam (S)	Fatuneno (F)
A	Leaf			
1.	Number of leaves	Blade	± 9 blade	± 7 blade
2.	Leaf width	Cm	± 1.1 cm	± 0.65 cm
3.	Leaf length	Cm	± 30 cm	± 20 cm
4.	Leaf orientation	upright, spread out, and semi-upright	spread out	spread out
5.	Leaf color	Green, dark green, grayish-green, and yellowish-green	Dark Green	Dark Green
B	Stem			
1.	Diameter of stem	Cm	± 0.4 cm	± 0.2 cm
C	Tuber			
1.	Tuber Shape	Globe dan Flat Globe	Globe	Globe
2.	Tuber Color	White, purplish-white, dark purple, purple, violet, and beige	White	White
3.	Clove color	White, purplish-white, dark purple, purple, violet, and beige	Beige	Beige
4.	Diameter of tuber	Cm	± 2.5 cm	± 1.9 cm
5.	Tuber weight per plant	G	± 11.28 g	± 5.18 g
6.	Number of cloves per plant	Clove	± 14 clove	± 10 clove
7.	Clove weight	G	± 0.89 g	± 0.45 g
D	Plant height	Cm	± 50 cm	± 30 cm
E	Harvest Age	HST	± 90 HST	± 90 HST

Figure 2. The growth phase of local Eban garlic: Fatuneno (Left), Saenam (Right)

Figure 3. Tuber yield of local Eban garlic: Fatuneno (Left), Saenam (Right)

Figure 4. Cloves yield of Eban local garlic: Fatuneno (Left), Saenam (Right)

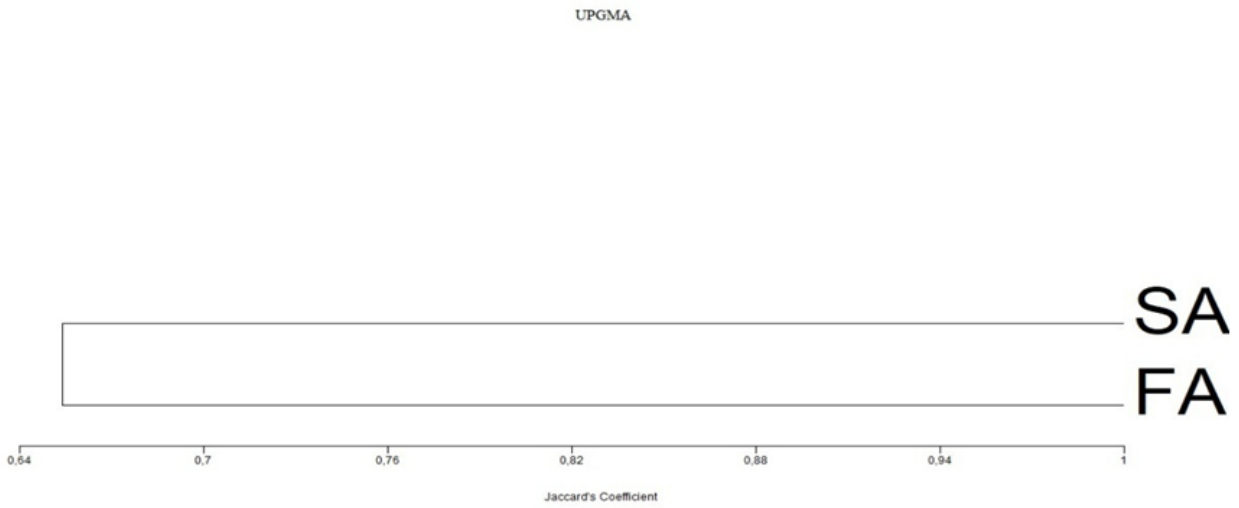

Figure 5. Dendrogram of 2 local Eban garlic cultivars based on ISSR markers.SA: Samples from Saenam Village, FA: Samples from Fatuneno Village

Genotype character. Based on the amplification of the 3 ISSR primers, the similarity of the twolocal cultivars of garlic ranged from 0.65–1% (Figure 5). The dendrogram construction using the MVSP program and the Jaccard coefficient revealed a very high level of similarity. The cultivars from Saenam Village (SA) and Fatuneno Village (FA) hada similarity level of 0.65%.

The results of the ISSR analysis used threeprimers, 20 amplified bands (Table 2 and Figure 6) consisting of ninepolymorphic and 17 monomorphic. Maximum polymorphic (4) and monomorphic (9) have been recorded in UBC-812. The lowest polymorphic (2) hadbeen recorded in UBC-836 while monomorphic same both of UBC 836 and UBC 825 (4). The size of the fragment ranged from 200 bp to 1200 bp. A high polymorphic level was also shown by Sai et al. [11], in which 6 ISSR primers generated 28 variable polymorphic bands. Shuxia et al.[18] screened 39 garlic genotypes using 17 ISSR primers, and Salar et al. [19] analyzed 31 garlic genotypes with sixISSR primers. Furthermore, the size of fragments ranged from 240 bp to 800 bp.

Polymorphic percentage of threeprimers namely UBC-812 (30.77%), UBC-836 (30%), UBC-825 (42.86%). Maximum polymorphic percentageswere recorded in UBC-825 (42.86%), and the lowest polymorphic was recorded in UBC-836 (30%). The total polymorphic percentage of the threeprimers was 103.65%. This suggested that the ISSR molecular markers had an extremely high ability to duplicate DNA bands. The ISSR markers use of certain plants such as Onions and corn also showed high polymorphism. Rakesh et al. [20] reported that fourISSR primers were used to assess genetic diversity in 131 accessions of Indian garlic producing 100% polymorphism. ISSR markers generated 103 DNA bands with 89.57% polymorphism in *Alliumcepa*[21]. A high polymorphic 85.4% of maize using ISSR markers hadbeen recorded by Idris et al.[22].

This study concluded that the two local Eban garlic cultivars hadhigh similarities. However, there were differences phenotypically in the size of the tubers and cloves, the number of cloves per tuber, and the weight of the cloves.

Table 3. Polymorphisms of the DNA bands from the ISSR amplification

Primer	Number of characters	Polymorphic	Monomorphic	% Polymorphism
UBC-812	13	4	9	30.77
UBC-836	6	2	4	30
UBC-825	7	3	4	42.86
Total	20	9	17	103.63

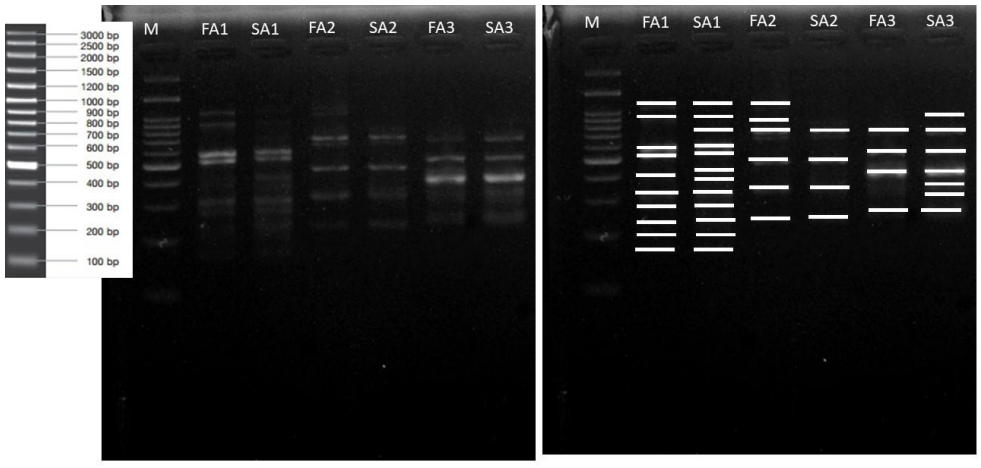

Figure 6. PCR results with threeprimers: 1.UBC-812, 2.UBC-836. 3. UBC-825. Left: electrophoresis result. Right: result visualization

CONCLUSION

Two accessions of garlic were collected from Saenam Village and Fatuneno Village. The difference was observed in some growth characteristics and at harvest. Plant height was found to range from 30 – 50 cm while leaf length ranged from 20 – 30 cm, leaf width from 0.65 – 1.1 cm, number of cloves from 10 – 14, and tuber weight from 5.18 – 11.28 g. The lowest plant height, 30 cm, and lowest blub weight, 0.45 g, had been recorded in local garlic collected from Fatuneno village. Maximum tuber weight (11.28

g), tuber diameter (2.5 cm), leaf length (30 cm), and leaf width (1.1 cm) hadbeen recorded in garlic genotypes collected from Saenam village. Numerous research articles showed that the characters such as plant height, bulb size, and number of cloves were correlated directly and/or indirectly to garlic yield. PCR results produced 20 amplified bands consisting of 9 polymorphic and 17 monomorphic bands. The coefficient of similarity of two groups based on genotypic characters is 0.65% - 1%. The conclusion of this study is that the two local Eban garlic varieties are different based on morphology and also ISSR data. However, phenotypically there were differences in the size of the tubers and cloves, the number of cloves per tuber, and the weight of the cloves.

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