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Genetic distance and diversity of breadfruit (*Artocarpus altilis*) based on morphological characters in Bawean Island, Indonesia

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Abstract. *Rahmah W, Waluyo B. 2019. Genetic distance and diversity of breadfruit* (Artocarpus altilis) *based on morphological characters in Bawean Island, Indonesia. Biodiversitas 20: 3284-3291.* One of the ways to preserve species germplasm is by identifying the genetic distance and intraspecific diversity. The aims of this study was to study the distribution and cultivation status, genetic distance, and diversity of breadfruit plants, Artocarpus altilis (Parkinson ex F.A.Zorn) Fosberg , in Bawean Island, Gresik, Indonesia based on morphological characters. The study made 30 accessions of breadfruit plants spread throughout Bawean Island, which were found to be neither cultivated intensively nor used optimally by the inhabitants. The genetic distance showed that breadfruit in Bawean Island was clustered into 6 groups with similarity coefficient of around 0.9984 to 0.9999. The diversity index value of 1.48 indicated medium diversity of breadfruit on this island.

Keywords: Artocarpus altilis, Bawean, diversity, genetic distance, morphological characters

INTRODUCTION

Breadfruit [*Artocarpus altilis* (Parkinson ex F.A.Zorn) Fosberg] is a horticulture plant having similar nutritional composition to that of rice, therefore it is sometimes seen as substitute for rice in some parts of the world (Ijarotimi and Aroge 2005; USDA 2018a,b). Breadfruit trees do not only have the ability to adapt, but also to thrive in conditions where other crops could not survive (NTBG 2019), besides being a prolific producer of nutritious fruits. This plant is a staple food in Pacific Belt and an important element in the socio-cultural rituals and system of medicine (Langton and Lincoln 2018).

Breadfruit has global distribution and its grown in more than 90 countries (Badrie and Brooms 2010) with varied vernacular names viz. it is called sukun (Indonesia), kapiak (Papua New Guinea), breadfruit (English), bakri-chajar (Hindi, India), Nagadamini (Sanskrit, India), rimas (Philippines), kulur/curor (Malaysia), brotfruchtbaum (Germany), arbre à pain (French), árbol del pan (Spain), uto/ kulu (Fiji), bia/nimbalu (Solomon Island), beta (Vanuatu), ulu (Hawaii, Samoa), uru (Tahiti), kuru (Cook Island), and lemai (Mariana Islands) etc. (Ragone 1997). Breadfruit are known to occur throughout Indonesia, however, according to the variety release database (Pusat Perlindungan Varietas Tanaman dan Perizinan Pertanian 2019), there are only 3 released varieties and 4 varieties that have been registered. One of the released varieties of breadfruit is Manis Variety which is registered under the government of Tanah Datar, West Sumatra. Nevertheless, the said registration database shows that the breadfruit varieties were obtained originally from Bawean Island (Sistem Informasi Database Varietas Tanaman Kementerian Pertanian R.I. 2019). Therefore, it indicated that Bawean Island to be breadfruit diversity rich area in Indonesia.

Bawean is one of the small islands located in the north of Java Island under the administrative control of Gresik, East Java. Although breadfruits are distributed throughout this island, the breadfruit varieties like Manis which is native to this Island have neither been registered as local variety nor brought under breeding programs Primary reason behind such negligence could be due to lack of proper management of breadfruit germplasm by the local people of Bawean Island as well the issue less prioritized by the local government of Gresik.

If timely germplasm management and conservation initiatives are not taken, the breadfruit germplasm may get threatened in Bawean Island. However, to understand what we are going to manage, beforehand we need to know what resource(s) we have left for management. One of the ways to conserve the breadfruit germplasm is by identifying the genetic distance and diversity of breadfruit in Bawean Island. The identification is expected to be an early stage in the release of local varieties and varieties of breeding yield of Bawean breadfruit so that breadfruit in Bawean Island can be utilized optimally by the inhabitants.

Therefore, this study aims to determine: the distribution and cultivation status of breadfruit in Bawean Island, to determine the genetic distance based on morphological characters, and to determine the diversity of breadfruit plants in Bawean Island based on morphological characters.

MATERIALS AND METHODS

Study area

This study was conducted from January to April 2019 in Bawean Island (Latitude 5°40' to 5°50', Longitude 112°3' to 112°36') which is a small island located between Java and Kalimantan Islands and is under administrative area of Gresik District, East Java Province, Indonesia; 19 villages in two subdistricts, i.e. Sangkapura and Tambak were surveyed in this research (Table 1, Figure 1). The island has an elevation from 0 to 655 m asl. and has temperatures varying around 23°C to 33°C. Average humidity in the Island ranges from 67% to 95% (BMKG 2019).

Sampling and data collection

This study was gradually carried out by survey method (plant observations and semi-structured interviews). Initially, information from the local residents was sought about the existence of breadfruit plants in Bawean Island. Breadfruit plants were sampled with convenience sampling method. Convenience sampling is one of the sampling methods based on the probabilities put forward in several practical criteria such as easy accessibility, geographical proximity, or time schedule in accordance with the research objectives (Etikan et al. 2016). In the current study, the locations were divided into 4 areas, then \geq 3 plant samples were observed plants for each area. The details of the locational are as follows: (i) First area: Kota Kusuma, Sungaiteluk, Patar Selamat, Bululanjang, Pudakit Barat, Pudakit Timur, Lebak, Kumalasa, Suwari and Deket Agung, (ii) Second area: Sawah Mulya, Daun, Sungairujing, Gunung Teguh, Balikterus, Kebun Teluk Dalam and Sidogedung Batu, (iii) Third area: Teluk Jatidawang, Gelam, Sukaoneng, Kelumpanggubug, Sukalila, Tambak and Grejeg, (iv) Fourth area: Tanjungori, Paromaan, Dipongo, Kepuhteluk and Kepuh Legundi

In the next step, plant observations were made to note down morphological features (both qualitative and quantitative) and semi-structured interviews with owners of the plants were undertaken to find out information about the cultivation status of breadfruit in Bawean Island.

For plant observations, breadfruit plant descriptors according to Ragone and Wiseman (2007) and Jones et al. (2013) were followed and observations were made by inspecting the qualitative and quantitative characters in the leaf, flower, and fruit of breadfruit plants. Observation of the leaves was done on the fully expanded leaves located three points from the distal end of branch. Observation of the flowers was done on the mature male flower. Similarly, mature but not yet ripe fruits were observed for characteristics. The details of the qualitative and quantitative characters of breadfruit plants of Bawean Island are as follows: (i) Qualitative characters: crown shape, leaf dissection, leaf colour, leaf surface, shape of leaf apex, shape of leaf base, vein colour, fruit shape, skin texture, skin colour, scabbing between skin section, scabbing in center of skin section, scabbing colour between skin section, scabbing colour in center of skin section, flesh colour and colour of latex. (ii) Quantitative characters: leaf length, leaf width, number of lobes, length of lobes, width of lobes, sinus depth, petiole length, petiole diameter, flower length, flower diameter, fruit weight, fruit length, fruit width at middle, fruit width at top, fruit width at bottom, core length, core diameter and peduncle length.

Data analysis

Analysis of breadfruit distribution was done by recording the coordinates of the breadfruit plant location by Avenza Maps application version 3.7.1 and then presented as maps made by Desktop ArcGIS application (version 10.3). Cultivation status of breadfruit in Bawean Island was explained descriptively based on the results of interviews with breadfruit tree owners. Genetic distance analysis was performed by using agglomerative hierarchical clustering (AHC) based on similarities with the unweighted pair group method with the arithmetic average (UPGMA) (Mohammadi and Prasanna 2003). Analysis of the genetic distance of breadfruit plants was carried out by XLSTAT software (version 2014.5.03). The results of genetic distance analysis were presented in the form of dendrogram. Analysis of breadfruit diversity was conducted by using the Shannon-Wiener diversity index (*H'*) (Magurran 1988):

 $H' = -\Sigma(pi)(\ln pi)$

Where: H'= Diversity index value, and pi = proportion of each species. Value classification of Shannon-Wiener index is as follows: (i) H' < 1.0 = Low diversity, (ii) 1.0 < H' < 3.322 = Medium diversity, (iii) H' > 3.322 =High diversity.

RESULTS AND DISCUSSION

Distribution, cultivation status and utilization of breadfruit in Bawean Island

During the study, 30 accessions of breadfruit samples were obtained from Bawean Island (13 accessions from Sangkapura Subdistrict and 17 accessions from Tambak Subdistrict (Table 1, Figure 1).

It was found that the most favorable factor for the sustenance and spread of breadfruit plants in Bawean Islands (as noticed and revealed through interviews) was the environmental conditions of the Island (BMKG 2019). It was corroborated with the other studies which also suggest that the breadfruit plants can grow at altitudes 0-1550 masl with a temperature of 5°C to 40°C (Ragone 2006), and has good growth in the temperature range of 21°C to 32°C (Roberts-Nkrumah 2015). It was observed that the cultivation of breadfruit plants in the Island was not much in practice and also the 30 sampled accessions were found to be growing without special treatments like fertilization, irrigation or pest and disease management. The majority of the fruits obtained were consumed by the tree owners and given to their neighbors but not put forth for selling and only fruits from 8 accessions were harvested (when mature but not ripe) by the owners to the middlemen. It was also noticed that breadfruit plants in Bawean Island were used only used as snacks such as

chips, boiled breadfruit, cakar-cakar and fried breadfruit, whereas as the name suggests. Breadfruit has huge potential of its processing into flour which can further be processed into variety of food items. Olaoye et al. (2007) explained that the addition of breadfruit flour in biscuit production can improve the quality of biscuits, especially its crude fiber content. According to Tamegnon et al. (2017), breadfruit from Benin is rich in fiber that can be used as porridge, donuts, croquettes, soups, and others. Besides, breadfruit contains phenolic compounds including triterpenes and flavonoids which have antimicrobial properties and hence provide medicinal benefits to those consuming it (Badrie and Broomes 2010).

Morphological variation

Breadfruit plants in Bawean Island have morphological variations. These variations were recorded from the observations on qualitative and quantitative characters in 30 accessions of sampled plants. The observations showed that crown of breadfruit in Bawean Island was variously shaped like pyramidal, wide pyramid, elliptical and irregular (Figure 2.A-D).

Breadfruits in Bawean Island had glossy to dull, green and dark green leaves with moderately dissected and deeply dissected leaves (Figure 3). However, all accessions observed have a diamond shape of leaf apex with no variation. But the observation of the leaf base shows variations in form of acute, rounded and cuneate shapes. Observations on the vein color show that breadfruit plants in Bawean Island have green, yellow-green or yellow veins.

The results of observations on the quantitative character of the leaves also indicate the variations. Breadfruit plants in Bawean Island have leaf length ranging 37 cm to 78 cm, leaf width ranging 28 cm to 64 cm. Breadfruit leaves in Bawean Island have lobes with a total of 7 to 9 lobes with lobe length reaching 32.2 cm and lobe width reaching 14.5. The depth of the breadfruit sinus in Bawean Island is between 1 cm and 4 cm. Leaf breadfruit Bawean Island has a leaf stalk with a length ranging from 3.5 cm to 9 cm and the width of the petiole ranges from 0,5 cm to 2 cm.

Observations of flower and fruit showed that the breadfruit plants in Bawean Island did not fruit and flower at the same time. From 30 plant accessions observed, only 16 accessions were fruiting and 1 accession was flowering. The flowering accessions are TJO accessions with a length of 20 cm and a width of 3 cm.

The breadfruit shape characters observed were spherical, broadly ovoid, oval, oblong, ellipsoid and heartshape (Figure 4). From the observation of the fruit skin texture, it was found that the breadfruit plants had smooth and flattened pebbly skin texture fruit. Observations on skin color of fruit also showed variations. The variation was light green and yellow-green. Observations on the surface of the breadfruits skin showed a variety of scab characters both in the scabbing character between the skin section and the scabbing character in center of the skin section. On the skin surface of the fruit drawn many fields in the form of 5 to 7 square structures. The scab which is between the square plane is called scabbing between skin sections. While the scab in the middle of each field is called the scabbing in center of skin section. The variations of scabbing between skin sections were no scabbing, few, medium and many. Observation on scabbing in the center of the skin section shows that breadfruit skin has a scabbing in the center of the skin section with few, medium and many traits. The next observed character is the scabbing color between the skin section and the scabbing color in center of the skin section. Observations on the scabbing color between skin sections show variations of green and brown scabbing between sections. While observations on the scabbing color in center of skin section shows there is no variation. Scabbing color in the center of skin section has a brown color.

Observations on the character of the fruit flesh color also showed variations. Breadfruit in Bawean Island has a creamy or light yellow flesh color of fruit. Breadfruit also has a variety of latex colors. The latex color of breadfruit observed during the survey was reddish and white.

Breadfruit observed had 625 grams to 2486 grams weight. It has a length of 24 cm and the width of the fruit reaches 18 cm in the middle of the fruit and reaches 12.5 cm at the top and bottom of the fruit. In the center of the breadfruit, there is a core that has a length of 6.5 cm to 15 cm and a core width of 2.5 to 4 cm. Breadfruits have a peduncle length of around 5 cm to 11.5 cm.

 Table 1. Geographic distribution of the studied 30 breadfruit

 sample accessions in Bawean Island, Gresik, Indonesia

Acc.	Village	Subdistrict	I atituda	Longitudo	
no.	vinage	Subulstifict	Latitude	Longitude	
DN 1	Daun	Sangkapura	-5.83551	112.70337	
DN 2	Daun	Sangkapura	-5.83408	112.70455	
KBG	Klumpanggubug	Tambak	-5.75044	112.64651	
SKO	Sukaoneng	Tambak	-5.75857	112.63097	
DPG 1	Diponggo	Tambak	-5.73568	112.69972	
DPG 2	Diponggo	Tambak	-5.73390	112.69936	
PTS	Patar Selamat	Sangkapura	-5.84183	112.65657	
GLM	Gelam	Tambak	-5.76195	112.62456	
KTD 1	Kebun teluk dalam	Sangkapura	-5.81559	112.69797	
KTD 2	Kebun teluk dalam	Sangkapura	-5.81188	112.69469	
KTD 3	Kebun teluk dalam	Sangkapura	-5.81227	112.69457	
PKL 1	Pekalongan	Tambak	-5.74210	112.64670	
PKL 2	Pekalongan	Tambak	-5.74206	112.64637	
SKL	Sukalila	Tambak	-5.75032	112.64469	
GJG 1	Grejeg	Tambak	-5.75243	112.66771	
GJG 2	Grejeg	Tambak	-5.75161	112.66786	
PRM	Paromahan	Tambak	-5.77059	112.68084	
TJO	Tanjung ori	Tambak	-5.72936	112.67943	
KPT 1	Kepuh Teluk	Tambak	-5.75489	112.72386	
KPT 2	Kepuh Teluk	Tambak	-5.75369	112.72373	
KPT 3	Kepuh Teluk	Tambak	-5.75370	112.72359	
KPL 1	Kepuh Legundi	Tambak	-5.76023	112.72368	
KPL 2	Kepuh Legundi	Tambak	-5.76298	112.72391	
PDT 1	Pudakit Timur	Sangkapura	-5.81645	112.61844	
PDT 2	Pudakit Timur	Sangkapura	-5.81985	112.61889	
DKA 1	Deket Agung	Sangkapura	-5.80853	112.58294	
DKA 2	Deket Agung	Sangkapura	-5.80780	112.57570	
SDGB	Sidogedung Batu	Sangkapura	-5.80132	112.72025	
GTH	Gunung Teguh	Sangkapura	-5.83750	112.66138	
SWR	Suwari	Sangkapura	-5.81314	112.60636	

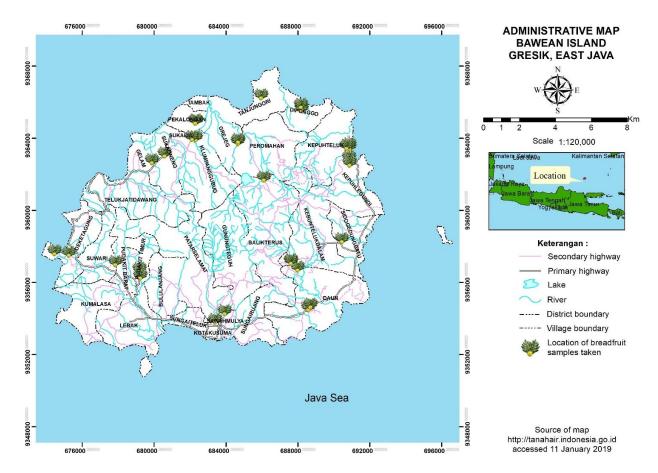


Figure 1. Geographic distribution of the studied 30 breadfruit sample accessions in Bawean Island, Gresik District, East Java, Indonesia

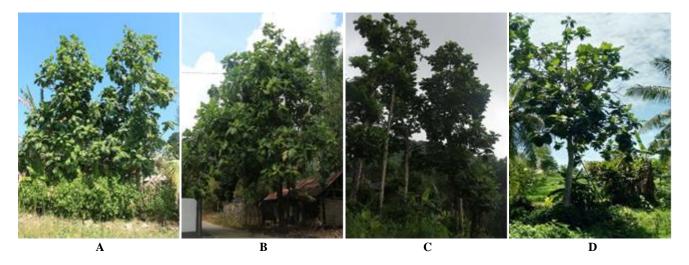


Figure 2. Crown shape variation of breadfruit. A. Pyramidal (DN2); B. Wide pyramid (KBG); C. Elliptical (PRM); D. Irregular (KTD1)

Based on the data of varieties descriptions from (Pusat Perlindungan Varietas Tanaman dan Perizinan Pertanian 2019), some characteristics of Bawean breadfruit has similar characteristics with the 3 released breadfruit varieties and 4 breadfruit varieties that have been registered. Some others are different. While the comparison of the characteristic between Bawean breadfruit and varieties of breadfruit that have been released and registered are as follows (Table 2). Padaidi, Manis, and Tado'puli are the breadfruit varieties that have been released. Puloseribu, Bangsyamlan, Iriana, and Tengah-Tengah are the breadfruit varieties that have been registered.



Figure 4. Fruit shape of breadfruit. A. Spherical (DN1); B. Broad ovoid (KPL1); C. Oval (KPT2); D. Oblong (KTD2); E. Ellipsoid (KTD1); F. Heart-shape (GJG1)

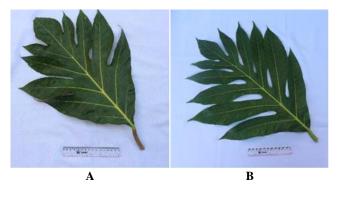


Figure 3. Leaf Dissection variation of breadfruit. a. moderately dissected (PTS); b. deeply dissected (GLM)

Genetic distance of breadfruit plants in Bawean Island

Analysis of genetic distance on qualitative characters showed that 30 accessions of breadfruit were found have similarity coefficient values clustered at 0.0168 to 0.9999 (Figure 5.a). Thirty breadfruit accessions were divided into 3 groups at a similarity coefficient of 0.3635. The first group consisted of 19 accessions having similarities in leaf dissection, shape of leaf apex, fruit skin texture and scabbing color in center of skin section. The second group consists of 2 accessions that have similarities in leaf surface character and shape of leaf base. The third group consisted of 9 accessions that has similarities in the shape of leaf apex, fruit skin texture, scabbing in center of skin section, scabbing color between skin section, scabbing color in center of skin section and fruit flesh color. While the genetic distance analysis on quantitative characters showed that 30 accessions of breadfruit were found have a similarity coefficient values clustered at 0.9987 to 0.9999 (Figure 5.b). Thirty breadfruit accessions are divided into 5 groups at a similarity coefficient of 0.9998. The separation of some groups occurred because of the differences in the character of fruit weight and fruit length.

Analysis of genetic distance on qualitative and quantitative characters showed different grouping results. Quantitative characters have a greater number of groups than those in the qualitative characters. It occurs because of other factors beyond genetic factors that affect a quantitative character. In addition, the qualitative character has a far genetic distance between accessions, while the quantitative character has a very close distance between accessions. It because the qualitative and quantitative characters are a matrix. This means that although it's different qualitatively, it can be similar or the same quantitatively.

Thirty accessions of breadfruit plants found have a similarity coefficient value clustered at 0.9984 to 0.9999 on a combination of qualitative and quantitative characters (Figure 6). Thirty breadfruit accessions are divided into 6 groups at a similarity coefficient value of 0.9998. Although the results of the analysis showed a grouping, the similarity coefficient values for the 30 accessions approaching the value of one indicate that the breadfruit accession was found to have a close kinship.

Characters	Bawean breadfruit (This study)	Released and registered varieties *)							
		Padaidi	Manis	Tado'puli	Pulo Seribu	Bangsyamlan	Iriana	Tengah-Tengah	
Crown shape	Pyramidal, wide pyramid, elliptical and irregular	Wide pyramid	Elliptical	Wide pyramid	-	-	Wide pyramid	Wide pyramid	
Leaf color	Green and dark green	Dark green	Dark green	Dark green	Dark green	Green	Green	Dark green	
Leaf surface	Glossy and dull	Glossy	Glossy	Glossy	-	Glossy	Glossy	Dull	
Shape of leaf apex	Diamond	Diamond	Diamond	Diamond	-	Diamond	-	-	
Shape of leaf base	Acute, rounded and cuneate	-	-	-	-	Acute	Rounded	-	
Fruit shape	Spherical, broadly ovoid, oval, oblong, ellipsoid and heart-shape	-	Broad ovoid	Oval	-	Oval	Oval	Oval	
Skin texture	Smooth and flattened pebbly	-	Smooth	Spiny with pointed flexible tip	-	Spiky with hard raised center point	Spiny with pointed flexible tip	Spiky with hard raised center point	
Skin color	Light green and yellow-green	Yellow-green	Brown	-	Yellow-green	Yellow-green	Yellow-green	Green	
Fesh color	Creamy and light yellow	Creamy	Creamy	Creamy	Creamy	Light yellow	-	-	
Leaf length (cm)	37-78	-	11-13.6	-	-	46-62	49-65	38.1-40.4	
Leaf width (cm)	28-64	-	4.6-5.5	-	-	34-47 cm	42-53.7 cm	40.4-60.4	
Number of lobes	7-9	-	-	-	-	-	7-9	-	
Petiole length (cm)	3.5-9	6-7	-	7-8	5	4.5-7	6.8-7.5	6.2-7.8	
Flower length (cm)	20	-	-	-	-	-	6.5-30	-	
Flower diameter (cm)	3	-	6-8	-	-	-	2.6-5.1	-	
Fruit weight (g)	625-2486	800-1100	1200-1500	900-1200	1500-2000	1200-1700	2000-4500	2200-2400	
Fruit length (cm)	11-24	-	7.5-9	-	-	18-24.7	20.3-27.0	19.6-22.3	
Fruit width at middle (cm)	11-18	-	-	-	-	11.5-14	16.5-18	-	
Core length (cm)	6.5-15	-	-	-	6	-	-	-	
Peduncle length (cm)	4-11.5	6-7	2-4	-	-	-	-	-	

Table 2. Characteristics comparison between Bawean breadfruit and varieties of breadfruit that have been released and registered

Note: Pusat Perlindungan Varietas Tanaman dan Perizinan Pertanian (2019)

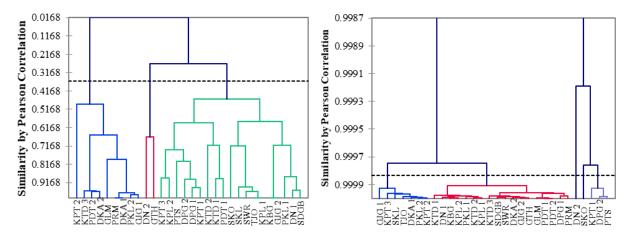


Figure 5. Dendrogram of 30 breadfruit accession analyzed by UPGMA. A. Based on qualitative characters; B. Based on quantitative characters

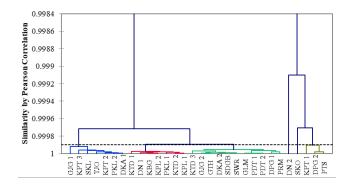


Figure 6. Dendrogram of 30 breadfruit accession analyzed by UPGMA based on a combination of qualitative and quantitative characters

This study is in accordance with the research of Ifah et al. (2018) who analyzed nine accessions of breadfruit plants from Yogyakarta. The study also showed that the majority of members in each group of the 9 accessions had a similarity coefficient value above 0.8, pointing towards close kinship. According to Ifah et al. (2018), the morphological variations may arise due to variations in environmental factors such as soil type and condition, rainfall, climate, whereas factors such as mutations or the human intervention are also known to influence the process of genetic variation.

Diversity of breadfruit plants in Bawean Island and their conservation status

Analysis of breadfruit plant diversity in Bawean Island showed Shannon-Wiener diversity index (H') value of 1.48 indicating medium diversity, even though, the dendrograms are showing 6 clusters and possibly 6 varietal lines of breadfruit in the Island. Two very interesting reasons behind the moderate breadfruit diversity in the Island may be due to (i) the common practice of local propagation of breadfruit plants in the Island in form of root cuttings (ii) and the breadfruit plants existing in the Island were seedless due to parthenocarpy. This was corroborated by the study of Zerega et al. (2005) who reported that Polynesian breadfruit has lower diversity than Micronesia and Melanesian breadfruit because the dominant Polynesian breadfruit is propagated vegetatively. As it was noticed that cross-pollinated flowers would also produce seedless fruits, therefore ways to initiate seeded fruits need to be found out to increase genetic diversity (Ragone 2001). According to Bhandari et al. (2017), evolutionary abilities such as selection, mutation, and migration can produce changes in allelic frequencies in a population and affect genetic diversity. As Bawean Island is seen as native area of the breadfruit plants in Indonesia, it needs to be thoroughly investigated through genetic markers to find out novel lines of breadfruit for their breeding and conservation of germplasm. Further, it was found that there was general lack of awareness among the local people of the Bawean Island regarding the role of breadfruit trees in food security at times of environmental degradation, eco-restoration of degraded sites, low maintenance - high nutrition crop and nurturing of public health (Langton and Lincoln 2018) as it was noticed that many breadfruit trees had been felled while others were in line. Therefore, it is thought necessary to aware and sensitizes locals regarding the importance, prospects, and methods of sustainable cultivation and utilization of breadfruits on the Island.

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