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Population study of *Cucurbita moschata* based on morphological characters and isozyme banding patterns in Bima District, West Nusa Tenggara, Indonesia

MUHAMMAD FURQAN[1], SURANTO[1,2,♥], SUGIYARTO[1,2]

[1]Bioscience Graduate Program, Universitas Sebelas Maret. Jl. Ir. Sutami 36A, Surakarta 57126, Central Java, Indonesia
[2]Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Sebelas Maret. Jl. Ir. Sutami 36A, Surakarta 57126, Central Java, Indonesia. Tel.: +62-271-646994 ♥email: surantoak@yahoo.com

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Abstract. *Furqan M, Suranto, Sugiyarto. 2023. Population study of* Cucurbita moschata *based on morphological characters and isozyme banding patterns in Bima District, West Nusa Tenggara, Indonesia. Biodiversitas 25: 30-38.* Pumpkin is a tropical plant that benefits from its lipid, protein, carbohydrate, and mineral contents. The morphological characters of pumpkins can be influenced by their genetics and environment. This research aimed to examine the relationship among 9 accessions of *Cucurbita moschata* Duchesne from 9 locations in Bima district, West Nusa Tenggara (NTB) province, Indonesia. The morphological characters of stems, leaves, and flowers were analyzed qualitatively and quantitatively and an ANOVA was used to look at the difference of leaf and flower characters. Polyacrylamide gel electrophoresis (PAGE) with peroxidase and esterase was used to detect isozyme banding patterns. Morphological characters and isozyme data were analyzed using a UPGMA method with NTSYS version 2.0 software to construct a dendrogram. The results show evidence of the varied morphological appearance of *C. moschata* from the different locations. A total of 12 esterase bands and 10 peroxidase bands were detected from Wera2. An undetected band with Rf value of 0.567 was recorded, while a unique band with Rf value of 0.133 (peroxidase) was not detected. Undetected bands with Rf values of 0.645 and 0.726 of esterase were also recorded. A dendrogram showed three clusters of 9 accessions studied, in which Wera2 and Wera3 each stood alone, and a third large group was formed from the 7 remaining accessions. These results confirm the uniqueness of the Wera2 and Wera3 accessions. These results would be good information in determining a management strategy for improving the quality of pumpkins in the future.

Keywords: Bima district, *Cucurbita moschata*, isozyme banding pattern, morphological characters, pumpkin

INTRODUCTION

Cucurbita moschata Duchesne, known commonly as pumpkin, is a tropical plant that is beneficial for consumption due to its content of lipids, proteins, carbohydrates, and minerals, which are considered useful for human beings (Suranto et al. 2015). The fruit contains secondary metabolites (phenols and flavonoids), amino acids, and vitamins, such as A, B, C, E and α-tocopherol, β-carotene (Tamer et al. 2010; Jacobo-Valenzuela et al. 2011). Pumpkin has several varieties of usefulness for human beings, not only as a staple food, but also for the diet of people with diabetes millitus (DM). This usefulness is related to the low carbohydrate content and it is easy to set (Suranto et al. 2015; Hidayati et al. 2018). According to Nagar et al. (2018) and Quintana et al. (2018), the genotype of pumpkins resulted in their different mineral nutrients, in which the highest percentage of carbohydrate content was detected from the pulps.

Cultivated pumpkins in Indonesia are varied based on their morphological characteristics Pumpkin is easily grown in various parts of Indonesia, including West Nusa Tenggara (NTB), a region with varied environmental conditions. The occurrence of plant variations can be easily observed by looking at the appearance of morphological characters, such as leaf shape and flower color. However, when the complexity of morphological appearance within one species is evident, other plant characters may need to be tested to determine whether the varied characters are due to environmental or genetic influences. When such a phenomenon occurs, an experimental approach such as a transplant experiment can be applied to see whether environmental factors or plant plasticity are found to play a part in the plant life cycle (Suranto 2002). One method that can be used to detect genetic influences is molecular experiments to test the DNA, protein, or isozymes of plants (Suranto et al. 2023). Molecular genetics is found to be more stable and are not usually affected by unfavorable environmental conditions (Indriani et al. 2008).

One useful marker that is often used in the study of plant variation is plant isozyme data. These data can be used to identify individual plants within a population and to classify plant varieties of certain species (Na'iem 1996; Bhandari et al. 2016; Suranto et al. 2017). The appearance of isozyme banding patterns of plants within a single species may indicate polymorphism. The presence of such data can be interpreted as genetic variability (Cahyarini et al. 2004; Mahfudz et al. 2010). The use of plant isozyme data for the identification and classification of plants has been employed in *Ranunculus nanus* (Suranto 2002), *Cassia auriculata* (Siva and Khrisnamurthy 2005), *Oryza sativa* (Widiyanti et al. 2008), almond (Colic et al. 2010),

Amorphophallus (Anil et al. 2014), and *C. moschata* (Jiang et al. 2023). Accordingly, studies of *C. moschata* using genetic markers were conducted by Lee et al. (2021) and Putri et al. (2023) testing their genetic structure and genetic diversity respectively.

Based on the complexity of morphological characteristics of pumpkin varieties in Indonesia, mainly in Wes, it is highly crucial to prove whether these varied features are actually based on genetic (isozyme banding pattern). This research aimed to examine the relationship among 9 accessions of *Cucurbita moschata* from 9 locations in Bima District, West Nusa Tenggara (NTB), Indonesia.

MATERIALS AND METHODS

Source of plant materials

All plants used in this study were collected from Bima district, West Nusa Tenggara province, Indonesia. A total of 27 plants were sampled from 9 locations within the 3 subdistricts, namely Bolo, Langgadu, and Wera (Figure 1). Thus, 3 plants were selected from each location. Environmental conditions, such as altitude, light intensity, air temperature, relative humidity, and soil pH in 3 subdistricts were documented (Table 1). All the data were taken between January and March 2018.

Plant materials

In order to ensure a similar level of maturity and development of the plant samples, only flowering plant leaves were used for morphological observations. For isozyme banding patterns analysis, the seeds obtained from 3 subdistricts of Bima district were planted in the greenhouse of the Department of Biology, Universitas Sebelas Maret, Surakarta, Indonesia from July to September 2018. A total of 45 polybags were used which contained soil and compost with a ratio of 3:1. Seeds were sown from 9 different locations of pumpkin cultivated in Bima district. The seeds were watered once every 2 days and fertilizer was applied twice a month. When the plants had reached the 5-leaf foliage phase, the samples were ready to be harvested for electrophoretic purposes. The seedling experiment was conducted to ensure that isozyme samples were always ready for the electrophoretic process throughout the project.

Table 1. Environmental condition of plant materials used in the study

Locations (subdistrict)	Environmental parameters				
	Altitude (m asl.)	Light intensity (lux)	Air temperature (°C)	Relative humidity (%)	Soil pH
Bolo	18	150300	40.2	42	7
	38	106000	37.3	49	7
	78	88040	34.2	60	7
Langgudu	59	119300	38.2	45	7
	72	103800	36.3	55	7
	82	90380	35.4	58	7
Wera	124	83910	32.6	70	7
	127	70160	32.2	72	7
	149	56500	30.8	75	7

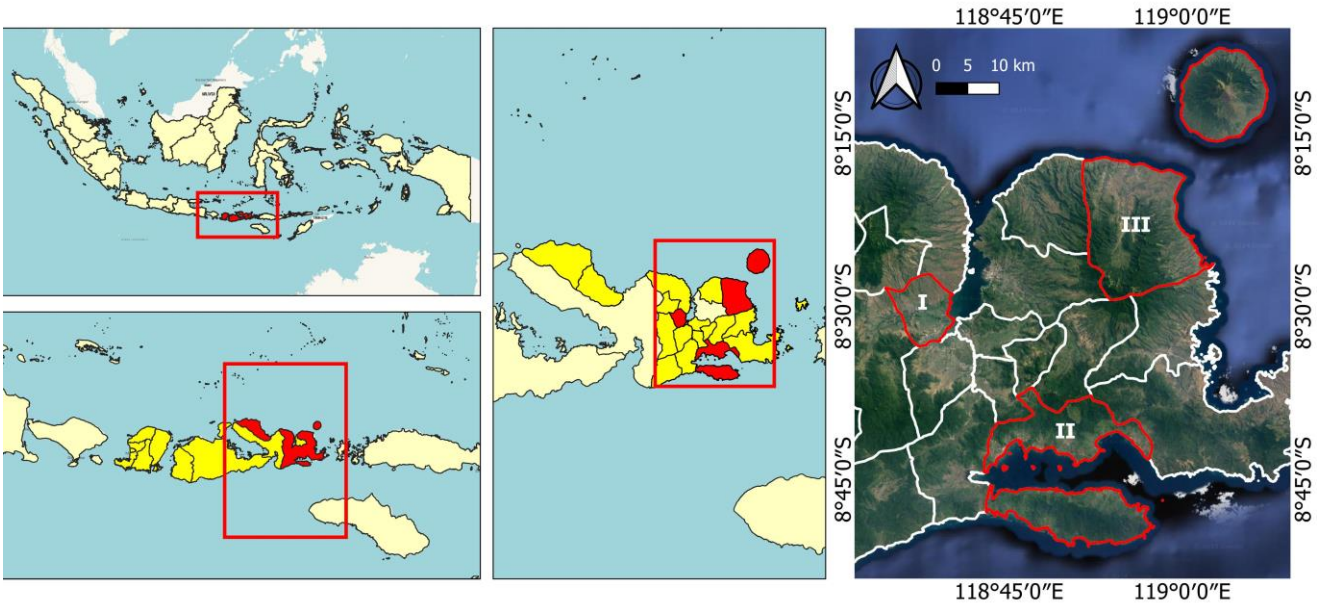

Figure 1. Source of plant materials in 3 subdistricts of Bima District, West Nusa Tenggara (NTB) province, Indonesia. (Note: Subdistricts: I: Bolo, II: Langgudu, III: Wera)

Procedures

The research procedures of morphological observation and isozyme banding pattern analysis are explained as follows:

Morphological observation

The field observation of morphological characters was carried out in 3 subdistricts of Bima district. The observations of stems, leaves, and flowers were carried out between January and March 2018. Stem observations consisted of color, shape, stripe surface, hairness, length and diameter of stem; leaf observations included shape, apex, margin, basal, venation, color, length and width of leaves, length and diameter of petiole; and flower morphological characters consisted color, total number of calyx and petals, and length of pedicels.

Isozyme banding patterns analysis

Electrophoretic observationsAll the procedures for analyzing the isozyme banding patterns and preparing the solution for the electrophoresis were adopted from Suranto (2001). Peroxidase and esterase were employed for analyzing the banding patterns of isozymes. Vertical electrophoresis was chosen and polyacrylamide gel (PAGE) was used. Two buffer solutions were prepared. A tank buffer was used for electrophoretic purposes and an extraction buffer was prepared for extracting the samples. The tank buffer was made by diluting 14.4 g of boric acid and 31.5 g of boric powder in 2 L of distilled water. Meanwhile, the extraction buffer was prepared by adding 0.018 g of cysteine, 0.021 g of ascorbic acid, and 5 g of sucrose to 20 ml of pH 8.4 tank buffer. The extraction buffer was kept in a cold room while not in use.

Two sock solutions were prepared. Stock A solution was prepared by diluting 4.5 g of TRIS (Hydroxymethyl) Methylamine (PURISS), 0.51 g of citric acid in 500 mL of distilled water, and stock B solution was made by mixing 30 g of acrylamide, 0.80 g N N'-methylene-Bis-Acrylamide with 100 mL of distilled water. The two solutions were kept in a cold room for making the acrylamide gel. The gel casting was done by mixing 5 mL of stock solution A and 2 mL of stock solution B with 5 µL TEMED and 7 µL APS (10%), then shaking gently before pouring the solution into the casting gel.

To obtain the best quality sample, 1 g of fresh leaf was crushed on a mortar containing 1 mL of extraction buffer. The sample was then centrifuged for 3 minutes at 4000 rpm. Supernatants were used for the electrophoretic purposes. During this experiment, the mortar was kept in a cold condition to avoid enzyme degradation. For running the PAGE, 7 µL of the resulting supernatants were loaded in each well of acrylamide gel. A constant voltage of 80 volts was used for 60 minutes. The power supply was turned off when the dye solution reached 5 mm above the basic line of the gel.

Staining the gel

The peroxidase isozyme was made by diluting 0.0125 g of O-Dianisidine in 2.5 mL of acetone solution, and then adding 20 mL of acetate buffer (pH 4.5). Before pouring the solution into the gel, 2 drops of H_2O_2 were added. The gel was soaked for 15 minutes until the band pattern appeared. The gel was then rinsed using distilled water and the pictured band was photographed. The esterase staining was carried out by diluting 0.025 g of 1-naphthyl acetate, and 0.0125 g of fast blue BB salt in 2.5 mL of acetone solution before adding 20 mL of phosphate buffer pH 6.5. The gel was soaked in the esterase enzyme solution for 1 to 3 hours until the band pattern appeared, and the gel was then rinsed using distilled water.

Data analysis

Morphological character data were analyzed both qualitative and quantitative. The qualitative analysis was conducted by observation or descriptive explanation, while the quantitative analysis was performed by analysis of variance (ANOVA). ANOVA was performed by Duncan's multiple range test ($p<0.05$) using SPSS 20.0 version software for measured quantitative morphological characters data to test the significance of variation among accessions (Suratman et al. 2016; Pitoyo et al. 2018; Muzzazinah et al. 2021; Suratman et al. 2022).

The movement of bands was calculated based on the Rf (Retardation factor) (Lehmann et al. 1989). In detecting the isozyme banding patterns, the presence and absence of the bands were observed, as well as the thickness of the bands. A similarity dendrogram of the accessions was constructed based on the morphological characters and isozyme banding patterns by applying an Unweighted Pair Group Method with Arithmetic Averages (UPGMA) cluster analysis using Numerical Taxonomy and Multivariate Analysis System (NTSYS) Version 2.00 (Rohlf 1998).

RESULTS AND DISCUSSION

Morphological characters

Stems

The slightly diverse microclimate conditions in each of the sampling locations showed small variations in the morphological appearance of *C. moschata*. This can be interpreted as evidence that microclimate contributes to morphological appearance, although many other factors may also influence this condition. In general, a pentagonal stem shape was recorded in all the sample locations, although there were slight variations in stem color and stem surfaces. A bright green color was observed in plant stems from subdistricts of Bolo and Langgudu, while plant stem from subdistrict of Wera showed a dark green color. Hairness of the stem surface was recorded as very dense, with very smooth hairs, from subdistrict of Wera, while rough, sparse hairs were observed on the stems from Bolo and Langgudu subdistricts (Table 2). Significant differences in stem diameter and stem length were also found (Table 3). The longest stem of 19.100 cm was recorded from subdistrict of Wera and the shortest of 10.667 cm from the subdistrict of Bolo, while the largest diameter of 1.883 cm and the smallest diameter of 1.250 cm were obtained from subdistricts of Wera and Bolo, respectively.

Table 3. ANOVA on quantitative morphological characters of *C. moschata* stem from 3 different locations in Bima district

Location (Subdistrict)	Stem characters (cm)	
	Length	Diameter
Bolo	10.667[a]±1.641	1.250[a]±0.087
Langgudu	11.530[a]±0.606	1.350[a]±0.126
Wera	19.100[b]±1.735	1.883[b]±0.073

Note: Based on the Duncan Multiple Range Test (DMRT), the numbers followed by the different letters in the same row are significantly different, P<0.05

Leaves

The leaf morphology of pumpkin was quite uniform (Figure 2). There were no significant differences in shape, apex, lateral or basal leaves, or leaf venation. However, variations were observed in leaf color. The leaf shape of this species is orbicularis with 5 lobes, apex acuminatus, margin serratus, basal leaf emarginate, and venation palminervis. Light green leaves were observed from Langgudu subdistrict, while dark green leaves were found from Wera subdistrict. In addition, light green leaves with small white dots on the venation were detected from Bolo subdistrict (Table 4).

The length and width of leaves varied slightly, but no variations were observed in length and diameter of petiole (Figure 2). The longest leaf of 13.200 cm and the widest leaf were recorded from subdistrict of Wera, while the shortest leaf of 10.733 cm and the smallest width of 11.333 cm both came from subdistrict of Bolo. The longest leaf petiole of 11.067 cm and the biggest diameter of 0.833 cm were from subdistrict of Wera, while the shortest petiole was recorded from subdistrict of Bolo and the smallest diameter of petiole was accession from subdistrict of Langgudu (Table 5).

Table 2. Qualitative morphological characters of *C. moschata* stem from 3 different locations in Bima district

Location (Subdistrict)	Stem characters			
	Color	Shape	Stripe surface	Hairness
Bolo	Light green	Pentagonal	Clear	Rough, short, seldom
Langgudu	Light green	Pentagonal	Clear	Rough, short, seldom
Wera	Dark green	Pentagonal	Not really clear	Smooth, short, dense

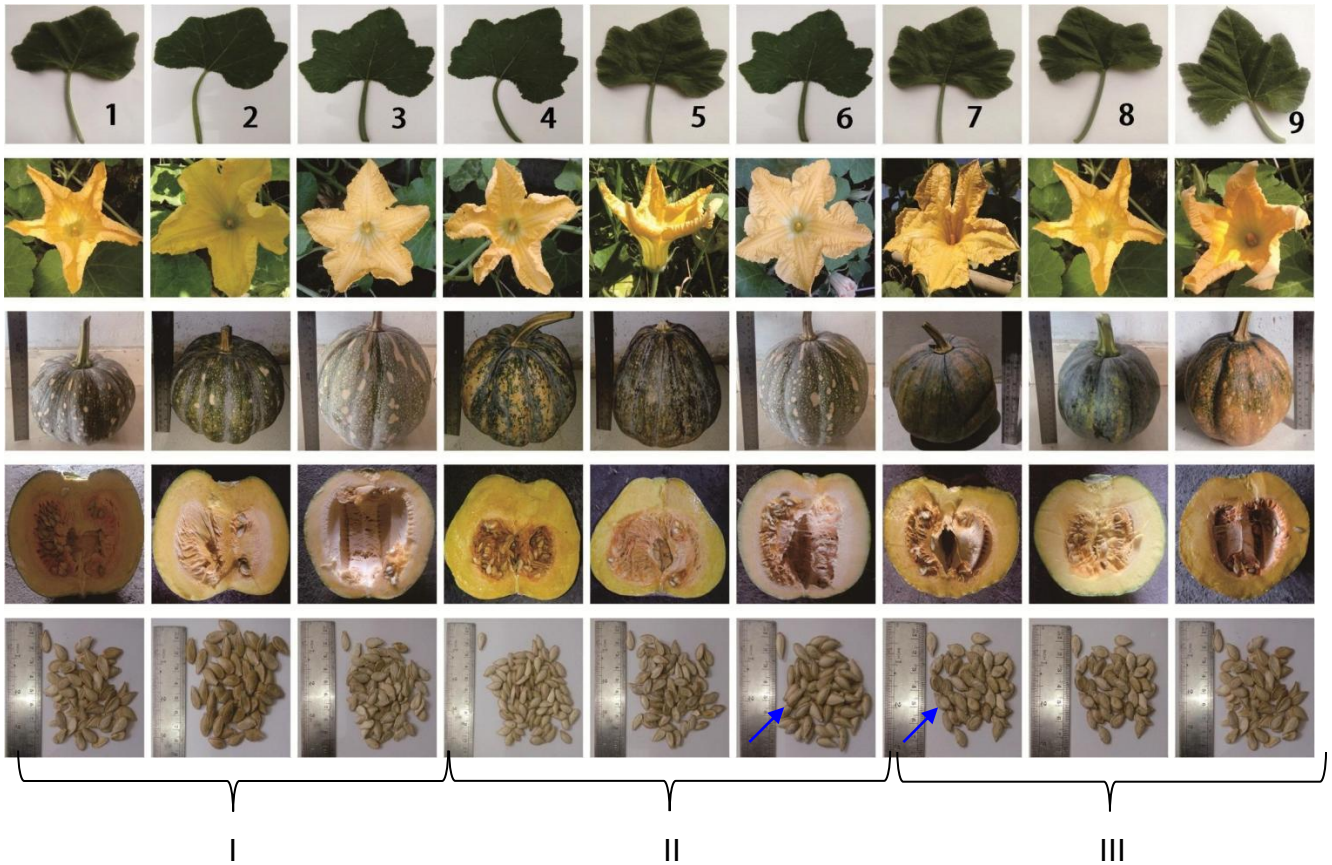

Figure 2. Morphological characters of C. *moschata* from 3 different locations in Bima district. Notes: I: Bolo, II: Langgudu, III: Wera

Table 4. Qualitative morphological characters of *C. moschata* leaf from 3 different locations in Bima district

Location (Subdistrict)	Parameters of morphological character of leaf					
	Shape	**Apex**	**Margin**	**Basal**	**Venation**	**Color**
Bolo	Orbicularis	Acuminatus	Serratus	Emarginate	Palminervis	Light green leaves with small white dots
Langgudu	Orbicularis	Acuminatus	Serratus	Emarginate	Palminervis	Light green
Wera	Orbicularis	Acuminatus	Serratus	Emarginate	Palminervis	Dark green

Table 5. ANOVA on quantitative morphological characters observation of *C. moschata* leaf from 3 different locations in Bima district

Location (Subdistrict)	Parameter (cm)			
	Length	**Width**	**Length of petiole**	**Diameter of petiole**
Bolo	10.733a±0.404	11.333a±0.291	9.900a±0.069	0.817a±0,.033
Langgudu	12.333bc±0.601	15.367bc±0.536	10.000a±0.681	0.783a±0.017
Wera	13.200bc±0.231	15.967bc±0.788	11.067a±1.601	0.833a±0.044

Note: Based on the Duncan Multiple Range Test (DMRT), the numbers followed by the different letters in the same row are significantly different, P<0.05

Table 6. ANOVA on quantitative morphological characters of *C. moschata* flowers from 3 different locations in Bima district

Location (subdistrict)	Parameter (cm)		
	Length of petal	**Diameter of flower**	**Length of pedicel**
Bolo	7.867^{a}±0.176	13.267^{a}±0.569	8.067^{a}±0.961
Langgudu	7.867^{a}±0.240	13.200^{a}±0.808	6.267^{a}±0.696
Wera	7.400^{a}±0.642	12.733^{a}±1.157	6.567^{a}±1.189

Note: Based on the Duncan Multiple Range Test (DMRT), the numbers followed by the different letters in the same row are significantly different, P<0.05

Flowers

The observation of 3 flower characters, namely length of petal, diameter of flower, and length of pedicel, showed no significant differences (Figure 2). However, the longest petal of 7.86 cm was recorded from Bolo subdistrict. The biggest flower diameter of 13.267 cm and the longest flower pedicel of 8.067 cm were also found at this location. Conversely, the shortest petal of 7.400 cm and the smallest flower diameter of 12.733 cm were recorded from the subdistrict of Wera, while the shortest length of pedicel of 6.267 cm was recorded from Langgudu subdistrict.

No significant differences were observed in the appearance of flower characters, especially the total number of sepals and petals. The only difference noted was the orange flower color in the sample collection from subdistrict of Wera, while flowers from the other two subdistricts of Bolo and Langgudu were characterized by a yellow and yellow to orange color. This attractive orange flower color needs to be examined further to determine whether this accession could be a potential subspecies. Further details of the morphological characters of leaves, flowers, fruits, and seeds are presented in Figure 2.

Isozyme banding patterns

The results of peroxidase isozyme analysis showed slight differences in the banding patterns formed in 3 subdistricts in Bima district (Figure 3). There were Rf value differences in samples collected from different locations. Ten isozyme bands of peroxidase were identified. The bands ranged from Rf values of 0.017 to 0.567. The typical common Rf bands for all the locations sampled were 0.017, 0.033, 0.067, 0.100, 0.433, and 0.567. However, variations in band thickness were observed. It was interesting to note that the band appearance from the different altitudes varied in thickness with lower altitudes showing a more intense band thickness than higher altitudes. The subdistrict of Bolo which has a lower altitude, and Langgudu with a medium altitude presented thicker bands compared with the highest altitude of Wera (Table 1). A unique appearance was also recorded for the band with Rf value of 0.133 which was absent (not detected) in the population from Bolo subdistrict.

The results of peroxidase isozyme analysis recorded much better and clearer bands than the esterase analysis, particularly for accessions of 3, 5, and 6 (Figure 4). Thirteen bands were recorded for the esterase isozymes, with a range between 0.016 and 0.758. Common bands were found at all sample locations (Rf: 0.016; 0.097; 0.113; 0.226; and 0.565) with varied thicknesses. Certain bands were found to be absent in particular locations. Bands with an Rf value of 0.387 were not detected in the subdistrict of Wera, while bands with Rf values of 0.516 and 0.758 were absent in Bolo subdistrict. In addition, the bands in different altitudes showed variations in thickness. Bands in lower altitude (Bolo) and medium altitude (Langgudu) showed thicker bands than in the highest altitude (Wera). This may lead to the speculation that plant altitude can potentially influence the expression of enzyme activities.

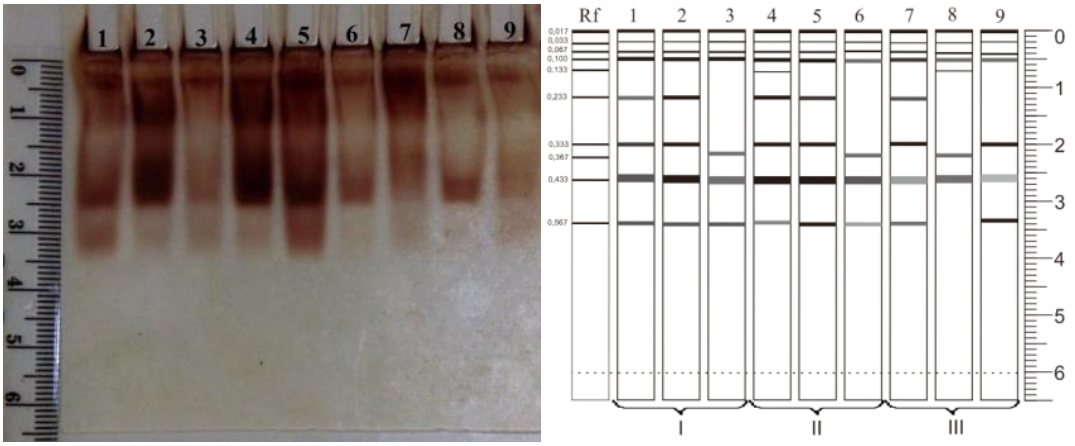

Figure 3. Peroxidase isozymes of *C. moschata* leaf samples collected from Bima district. Note: I. Bolo, II. Langgudu, III. Wera. The isozyme banding patterns presented in this picture were repeated at least 4 times during electrophoresis

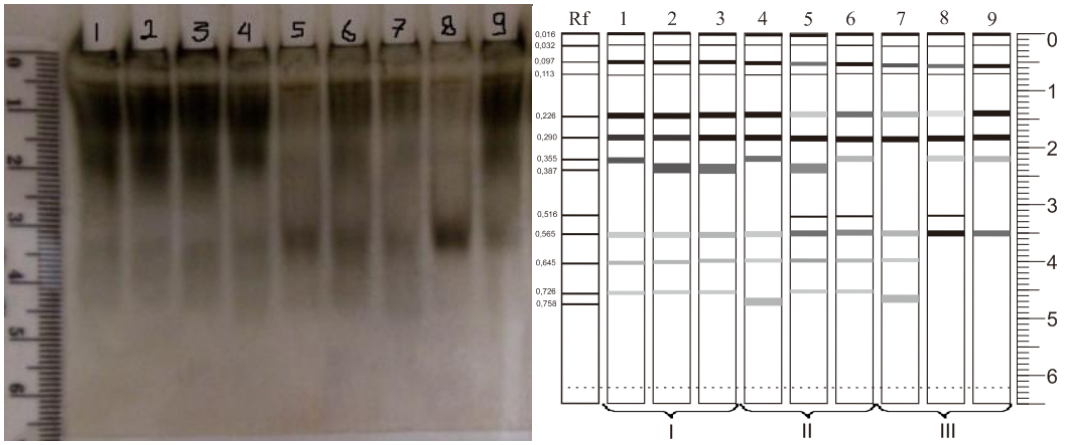

Figure 4. Esterase isozymes of *C. moschata* leaf samples collected from Bima district Note: I. Bolo, II. Langgudu, III Wera. There were 3 accessions in each subdistrict

Cluster analysis among *C. moschata* accessions

Combined morphological data and banding patterns obtained from peroxidase and esterase isozyme analysis were used to construct a dendrogram. Nine accessions from 3 subdistricts were analyzed to understand their relationships. In general, there were 3 clusters of pumpkins with a coefficient similarity of 0.660 (66.0%). The cluster I consisted of 7 accessions, Bolo1 and Bolo2 were grouped at the same position (0.83) followed by Langgudu1 (0.80), Langgudu2 (0.74), and Wera1 (0.73). Another subcluster was shown by Bolo3 and Langgudu3 with similarity coefficient of 0.785. Two other distinct clusters were occupied by Wera2 and Wera3 with a coefficient similarity of 0.70. From this dendrogram, it is clear that Wera3 stands alone at 0.66 compared with the other groups which consist of 8 accessions.

Discussion

The environmental conditions observed, such as altitude, light intensity, air temperature, and relative humidity varied widely, while pH remained stable (Table 1). The relative humidity was recorded as decreasing along with increasing light intensity. Meanwhile, light intensity was seen to decrease from the lower altitude to the higher altitude. Environmental conditions have been found to have a direct or indirect influence on the growth and development of plants. Gong et al. (2018) reported that changing the environmental conditions could cause plants to adapt both morphologically and physiologically. These adaptation processes have the potential to result in both qualitative and quantitative variations in the plant's morphological characters (Hidayati et al. 2018). According to Muthumani et al. (2013), altitude influences the degree of light intensity, temperature, rainfall, relative humidity, as well as wind velocity. Reducing the light intensity would cause a decreased temperature due to increasing altitudes. Meanwhile, increasing the relative humidity would by increasing altitudes (Hidayati et al. 2018). Besides, the concentration of oxygen and carbon dioxide in the air will also be influenced by the altitude as well as the type and condition of soils (Laily et al. 2012).

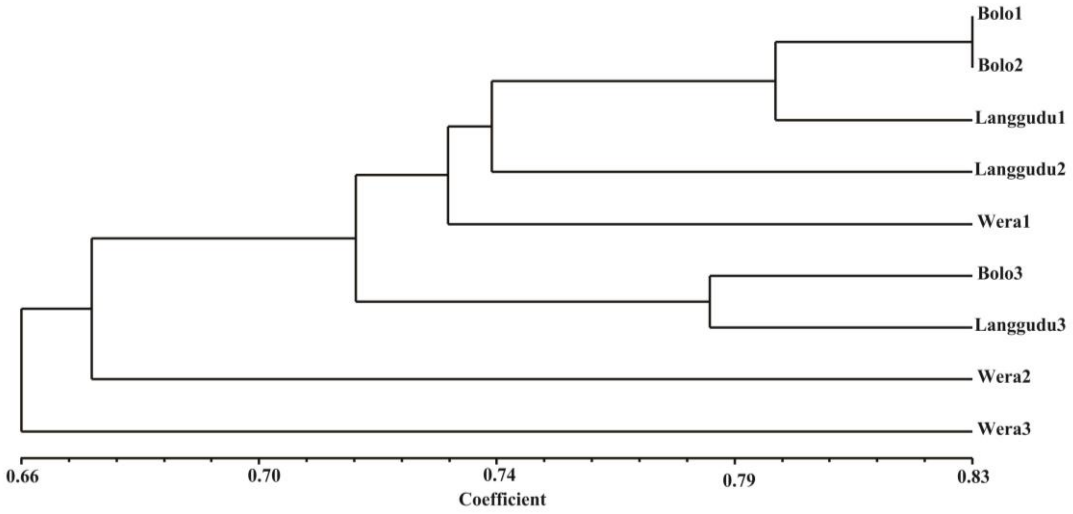

Figure 5. The dendrogram among 9 accessions of *C. moschata* from district Bima, West Nusa Tenggara (Indonesia) using morphological and isozyme markers

The stem diameter and internode length of pumpkins growing at the highest altitude showed the highest values compared with plants at the other altitudes. This may be due to the reduced light intensity of plants growing at a high altitude. According to Burkholder et al. (1936), plants growing at lower altitudes tend to produce more auxin to be distributed, as well as cell wall plasticity. The auxin hormone can promote the elongation of plant stems and cells.

In general, the size of leaves at the highest altitude was smaller than those at lower altitudes. This may be due to the slower photosynthetic process of plants in lower habitats and the greater surface volume of plant leaf area needed to catch the light intensity to maximize photosynthesis (Pan et al. 2013). This condition can affect the development of the leaf petiole, resulting in a smaller petiole size (Hidayati et al. 2018). In addition, plant leaves growing in low light intensity usually possesses a lighter green color. This may be caused by the presence of a+b chlorophyll which is used in the photosynthesis process (Majuakim et al. 2014). Low light intensity can cause the ratio of a/b chlorophyll to decrease, with a greater concentration of b chlorophyll becoming more evident. This encourages plants to improve their light efficiency by catching every single area of photosynthesis (Ye et al. 2017). The existence of chlorophyll can be interpreted as a sign of water absorbing the red, blue, and violet lights which are then reflected as green light, and transferred into the reaction center of the photosynthesis process (Ng'etich et al. 2012).

The flower characters, particularly petal length, flower diameter, and pedicel length appeared to show no significant difference. However, flower diameter and pedicel length tended to be smaller and shorter in plants growing at the highest altitude (Yaqoob and Nawchoo 2017). This may be caused by the fact that plants growing at a lower altitude tend to flower early, and therefore the process of cell division can occur more quickly than at other higher altitudes (Frei et al. 2014). The flower color observed at the highest altitude was brighter than at the other altitudes. This phenomenon could be due to light intensity, which affects the distribution of plant color pigment (Cruz et al. 2012).

The isozyme banding patterns of esterase, and peroxidase displayed polymorphisms. The esterase isozymes produced 12 bands, most of which were varied. Meanwhile, 10 bands of peroxidase were detected. In these examples, esterase could be recorded as useful for providing isozyme data of genetic variability, considering that polymorphism can occur in the wider spectrum (Tiwari and Bakshi 2015). This enzyme can be regarded as one of the crucial enzymes that is highly suitable for differentiating plants (Rakshit et al. 2011; Sumathi and Balamuragan 2014). As a genetic marker, isozymes can be considered highly important in polymorphic studies. The polymorphic validity of isozyme data in studies of plant biodiversity has been shown by Kumar et al. (2013). Moreover, a good point was made by Khan et al. (2010), who stated that isozymes are a useful marker for genetic polymorphism within the plant group structure. The differences between isozyme banding patterns can be interpreted as revealing the genetic diversity between varieties of plants (Suratman et al. 2016). A related finding was also reported by Suranto et al. (2019) in 10 cultivars of sweet potato using peroxidase isozymes. Accordingly, Restykania et al. (2019) also found isozyme variation among madeira vine (*Anredera cordifolia*). Meanwhile, Jiang et al. (2023) recorded that at least superoxide dismutase (SOD), catalase (CAT), and glutathione reductase (GR) could significantly enhance the activities of those antioxidant enzymes.

The dendrogram shows that each cluster consists of geographically related accessions. The grouping of accessions does not always show similarity of geographic origin, but may express genetic similarity. Diversity of genetic data from several *C. moschata* accessions from Asia, Africa, and America have been constructed, and the highest genetic diversity was found in Africa and South Asia, followed by the Mexico accessions. This finding may be useful in providing a good valuable information in

constructing the genetic structure of *C. moschata* germplasms (Lee et al. 2021).

Recent studies conducted by Putri et al. (2023) using ISSR markers employed several cultivars of *C. moschata* and found that only 18.6% polymorphism rate was recorded. The variability of pumpkin genetics can be partly explained by their biotic and abiotic environmental factors. Various conditions, such as geographic, climate, and reproduction differences may explain the related diversities observed. This can eventually be used to help maximize the efficiency of collecting germplasm in the conservation of pumpkin accessions (Suratman et al. 2013). One of the benefits of a cluster analysis is to predict genetic similarity between accessions and parental identification by conducting the proper hybridization selection, and to obtain better accessions by looking at the percentage of genetic similarity. For plant hybridization, two or more similar compatible characters of accessions can be used for the experiment (Prabha et al. 2010; Lombardi et al. 2014; Pitoyo et al. 2018).

In conclusion, the morphological characters and environmental conditions of plants growing in different habitats, together with their isozyme banding patterns, are considered to be useful as additional data in helping to understand the occurrence of genetic variability of pumpkin accessions. A dendrogram showed three clusters of 9 accessions studied, in which Wera2 and Wera3 each stood alone, and a third large group was formed from the 7 remaining accessions. These results confirm the uniqueness of the Wera2 and Wera3 accessions. This would be good information in determining a management strategy for improving the quality of pumpkins in the future.

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REFERENCES

Anil SR, Siril EA, Beevy SS. 2014. Diversity analysis in *Amorphophallus* using isozyme markers. Intl J Veg Sci 20 (4): 305-321. DOI: 10.1080/19315260.2013.803509.

Bhandari K, Siddique KHM, Turner NC, Kaur J, Singh S, Agrawal SK, Nayyar H. 2016. Heat stress at reproductive stage disrupt leaf carbohydrate metabolism, impairs reproduction function, and severely reduces seed yield in Lentil. J Crop Improv 30 (2): 118-151. DOI: 10.1080/15427528.2015.1134744.

Burkholder PR. 1936. The Rôle of Light in the Life of Plants. I. Light and Physiological Processes. Bot Rev 2 (1): 1-52. DOI: 10.1007/BF02869924.

Cahyarini RD, Yunus A, Purwanto E. 2004. Identification of genetic diversity of several local soybean varieties in Java based on isozyme analysis. Agrosains 6 (2): 79-83. [Indonesian]

Colic S, Milatovic D, Nikolic D, Zec G. 2010. Isoenzyme polymorphism of almond genotypes selected in the region of northern Serbia. Hortic Sci (Prague) 37 (2): 56-61.

Cruz BP, Chedier LM, Peixoto PHP, Fabri RL, Pimenta DS. 2012. Effect of light intensity on the distribution of anthocyanins in *Kalanchoe brasiliensis* Camb. and *Kalanchoe pinnata* (Lamk.) Pers. An Acad Bras Cienc 84: 211-218. DOI: 10.1590/s0001-37652012000100022.

Frei ER, Ghazoul J, Pluess AR. 2014. Plastic responses to elevated temperature in low and high elevation populations of three grassland species. PLoS ONE 9 (6): e98677. DOI: 10.1371/journal.pone.0098677.

Gong J, Zhang Z, Zhang C, Zhang J, Ran A. 2018. Ecophysiological responses of three tree species to a high-altitude environment in the southeastern Tibetan Plateau. Forests 9 (2): 48. DOI: 10.3390/f9020048.

Hidayati NR, Suranto, Sajidan. 2018. Morphological characteristics and isozyme banding patterns of *Cucurbita moschata* at different altitudes. Biodiversitas 19: 1683-1689. DOI: 10.13057/biodiv/d190513.

Indriani FC, Sudjindro ANS, Soetopo L. 2008. Genetic diversity of *hibiscus cannabinus* L. and isozyme analysis in related species. Agritek 6 (9): 1793-1802. [Indonesian]

Jacobo-Valenzuela N, Marostica-Junior MR, Zazueta-Morales JdJ, Gallegos-Infante JA. 2011. Physicochemical, technological properties, and health-benefit of *Cucurbita moschata* Duchesne vs. Cehualca: A review. Food Res Intl 44 (9): 2587-2593. DOI: 10.1016/j.foodres.2011.04.039.

Jiang W, Zhang Y, Zeng J, Yao J, Lu A, Fang Z, Wang G, Wang W, Zhang Y. 2023. Composition analysis of acid hydrolysates from *Cucurbita moschata*. Duch. polysaccharides and their effect on oxidative stress resistance of *Caenorhabditis elegans* J. Food Sci Hum Wellness12 (3): 795-800. DOI: 10.1016/j.fshw.2022.09.014.

Khan SA, Ahmed H, Khan SM, Khan A, Ali S, Saeed M. 2010. Conformation of sunflower F_1 hybrids using SDS-PAGE analysis. Afr J Biotechnol 9 (29): 4516-4520. DOI: 10.5897/AJB10.1584.

Kumar OA, Tata SS, Kumar KP. 2013. Analysis of several popular cultivars of Madagascar periwinkle (*Catharanthus roseus* (L.) G.Don.) using biochemical markers. Not Sci Biol 5 (4): 458-461.

Laily AN, Suranto, Sugiyarto. 2012. Characterization of *Carica pubescens* in Dieng Plateau, Central Java based on morphological characters, antioxidant capacity, and protein band pattern. Nusantara Biosci 4 (1): 16-12. DOI: 10.13057/nusbiosci/n040104.

Lee H-Y, Jang S, Yu C-R, Kang B-C, Chin J-H, Song K. 2021. Population structure and genetic diversity of *Cucurbita moschata* based on genome - wide high - quality SNPs. Plants 10 (1): 56. DOI: 10.3390/plants10010056.

Lehmann PF, Kemker BJ, Hsiao C-B, Dev S.1989. Isoenzyme biotypes of *Candida* species. J Clin Microbiol 27 (11): 2514-2521. DOI: 10.1128/jcm.27.11.2514-2521.1989.

Lombardi M, Materne M, Cogan NOI, Rodda M, Daetwyler HD, Slater AT, Forster JW, Kaur S.2014. Assessment of genetic variation within a global collection of lentil (*Lens culinaris* Medik.) cultivars and landraces using SNP markers. BMC Genet 15: 150. DOI: 10.1186/s12863-014-0150-3

Mahfudz, Na'iem M, Sumardi, Hardiyanto EB. 2010. Mating system analysis of merbau (*Intsia bijuga* O.Ktze) in Papua by isoenzyme markers. Jurnal Pemuliaan Tanaman Hutan 4 (2): 109-116. DOI: 10.20886/jpth.2010.4.2.109-116. [Indonesian]

Majuakim L, Ng SY, Abu Bakar MF, Suleiman M. 2014. Effect of altitude on total phenolic and flavonoids in *Sphagnum junghuhnianum* in tropical montane forest of Borneo. Sepilok Bull 19&20: 23-32.

Muthumani T, Verma DP, Venkatesan S, Kumar RSS. 2013. Influence of altitude of planting on quality of south Indian black teas. J Nat Prod Plant Resour 3 (1): 18-23.

Muzzazinah, Suratman, Nurmiyati, Ariani SRD. 2021. Morphological and anatomical characters variation of *Indigofera* accessions from Java, Indonesia. Biodiversitas 22: 2104-2116. DOI: 10.13057/biodiv/d220459.

Na'iem M. 1996. Introduction to Isoenzyme Analysis and its Use in Cultivating Plants. Kanisius, Yogyakarta. [Indonesian]

Nagar A, Sureja AK, Kar A, Bhardwaj R, Krishnan SG, Munshi AD. 2018. Profiling of mineral nutrients and variability study in pumpkin (*Curcubita moschata*) genotypes. Agric Res 7: 225-231. DOI: 10.1007/s40003-018-0329-3.

Ng'etich OK, Aguyoh JN, Ogweno JO. 2012. Growth, yield and physiological responses of spider plant (*Cleome gynandra* L.) to calcium ammonium nitrate rates. Intl J Agron Plant Prod 3 (9): 346-355.

Pan S, Liu C, Zhang W, Xu S, Wang N, Li Y, Gao J, Wang Y, Wang G. 2013. The scaling relationships between leaf mass and leaf area of vascular plant species change with altitude. PLoS ONE 8 (10): e76872. DOI: 10.1371/journal.pone.0076872872.

Pitoyo A, Prameta AA, Marsusi, Suratman, Suranto. 2018. Morphological, anatomical and isozyme variability among taro (*Colocasia esculenta*) accessions from southeastern part of Central Java, Indonesia. Biodiversitas 19 (5): 1811-1819. DOI: 10.13057/biodiv/d190531.

Prabha D, Negi YK, Khanna VK. 2010. Morphological and isozyme diversity in the accessions of two cultivated species of barnyard millet. Academ Arena 2 (9): 29-34.

Putri ANR, Kusnanda PS, Agellya BF, Sartika D, Daryono BS. 2023. Genetic variation of butternut squash (*Cucurbita moschata* Duchesne) based on inter-simple Sequence Repeat. J Trop Biodivers Biotechnol 8 (1): 1-8. DOI: 10.22146/Jtbb.77228.

Quintana SE, Marsiglia, RM Machacon D, Machacon D, Torregroza E, Garcia–Zapateiro LA. 2018. Chemical composition and physicochemical properties of squash (*Curcubita moschata*) cultivated in Bolivar Department (Colombia). Contemp Eng Sci 11 (21): 1003-1012. DOI: 10.12988/ces.2018.8384

Rakshit A, Sarvari P, Rakshit S, Dadlani M. 2011. Characterization of hybrids and parental lines of maize using isozyme markers for DUS testing. Seed Res 39 (2): 156-160.

Restykania, Suratman, Pitoyo A, Suranto. 2019. Morphology and isozyme variation among madeira vine (*Anredera cordifolia*) accessions from southeastern part of Central Java, Indonesia. Biodiversitas 20: 3024-3032. DOI: 10.13057/biodiv/d201035.

Rohlf FJ. 1998. NTSYS-pc Numerical Taxonomy and Multivariate Analysis System. Exeter Software, New York.

Siva R, Khrishnamurthy KV. 2005. Isozyme Diversity in *Cassia auriculata* L. Afr J Biotechnol 4 (8): 772-775.

Sumathi S, Balamurugan P. 2014. Discrimination of oats (*Avena sativa* L.) cultivars using isozyme markers. Afr J Biotechnol 13 (29): 2930-2934. DOI: 10.5897/AJB2013.13158

Suratman, Pitoyo A, Mulyani S. 2013. Analysis of genetic variability in soursop (*Annona muricata* L.) populations from Central Java and East Java based on Random Amplified Polymorphic DNA (RAPD) marker. J Biol Res 19 (1): 15-19. DOI: 10.23869/132.

Suratman, Pitoyo A, Kurniasari S, Suranto. 2016. Morphological, anatomical, and isozyme variation among giant taro (*Alocasia macrorrhizos*) accessions from Central Java, Indonesia. Biodiversitas 17 (2): 422-429. DOI: 10.13057/biodiv/d170204

Suratman, Suranto, Muzzazinah, Purnomo. 2022. Leaf anatomical characters variation of *Strobilanthes* s.l. from Sumatra, Indonesia and its taxonomic implications. Biodiversitas 23 (7): 3706-3721. DOI: 10.13057/biodiv/d230748

Suranto. 2001. Studies of *Ranunculus* population: Isozyme pattern. Biodiversitas 2 (1): 85-91. DOI: 10.13057/biodiv/d020101.

Suranto. 2002. Cluster analysis of *Ranunculus* species. Biodiversitas 3 (1): 201-206. DOI: 10.13057/biodiv/d030106.

Suranto, Tedianto, Purwanto E, Setyono P, Mahadjoeno E. 2015. The relationship between altitudes and the contents of protein, carbohydrates, lipids of pumpkin (*Cucurbita moschata*). Agrivita J Agric Sci 37: 59-66. DOI: 10.17503/Agrivita-2015-37-1-p059-066.

Suranto, Arief A, Supyani. 2017. The use of electrophoretic isozymes to detect tungro-infected rice. Agrivita J Agric Sci 39 (2): 145-152. DOI: 10.17503/agrivita.v39i2.643.

Suranto, Wahyuni D, Purwanto E. 2019. Evidence of pollen features and peroxidase isozymes in their morphological complexity of ten local cultivars of sweet potato from Indonesia. Biodiversitas 20 (9): 2511-2518. DOI: 10.13057/biodiv/d200912.

Suranto, Hidayati NR, Furqan M, Mahadjoeno E, Sajidan. 2023. Flavonoid compound of *Cucurbita moschata* at three different altitudes. Biodiversitas 24 (3): 1853-1860. DOI: 10.13057/biodiv/d240361.

Tamer CE, İncedayi B, Parseker Yonel S, Yonak S, Çopur ÖU. 2010. Evaluation of several quality criteria of low calorie pumpkin dessert. Not Bot Hort Agrobot Cluj Napoca 38 (1): 76-80. DOI: 10.15835/nbha3813557.

Tiwari C, Bakshi M. 2015. Genetic diversity and phylogenetic relationship among accessions of *Drepanostachyum falcatum* (Nees) Keng f. from the Garhwal Himalayas. Proc 10th World Bamboo Congress. World Bamboo Organization, Seoul.

Widiyanti, Suranto, Sugiyarto. 2008. Studies on the variations of seed morphology, pollen features and isozyme banding pattern of local variety *Oryza sativa* (Raja lele). Bioteknologi 5 (1): 18-25. [Indonesian]

Yaqoob U, Nawchoo IA. 2017. Impact of habitat variability and altitude on growth dynamics and reproductive allocation in *Ferula jaeschkeana* Vatke. J King Saud Univ Sci 29 (1): 19-27. DOI: 10.1016/j.jksus.2015.10.002

Ye S, Shao Q, Xu M, Li S, Wu M, Tan X, Su L. 2017. Effects of light quality on morphology, enzyme activities, and bioactive compound contents in *Anoectochilus roxburghii*. Front Plant Sci 8: 857. DOI: 10.3389/fpls.2017.00857.