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# Characterization of Roselle (*Hibiscus sabdariffa* L.) and Kenaf (*Hibiscus cannabinus* L.) Accessions from Different Origins Based on Morpho-agronomic Traits

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## ABSTRACT

The evaluation of morpho-agronomic traits was carried to identify the genetic relationship between two species of *Hibiscus*, roselle (*Hibiscus sabdariffa* L.) and kenaf (*Hibiscus cannabinus* L.). Significant differences for morphological and agronomic characteristics were observed among roselle and kenaf accessions. The kenaf accessions showed better agronomic performance for most of the plant characteristics than roselle. Accession G4 had the tallest plants (213.0 cm) and number of branches/plant (24.83) among all accessions. 'Noonsoon' produced the highest stem diameter (8.61 cm) whereas 'Khoken' had the highest number of fruits/plant (226). Dendogram generated using morpho-agronomic characters were classified sixteen accessions into two main groups A and B. Five kenaf accessions included in group A whereas nine roselle accessions and two kenaf accessions were included in second group B. Roselle accessions were forming a group with a range of similarity coefficients of 0.5333 to 0.8780. 'Bengkalis' was close to roselle accessions although it was from different *Hibiscus* sp. 'Noonsoon' was very close to roselle accessions having a range of similarity coefficients of 0.5000 to 0.6326. Morphological characters would not be sufficient source for identification of relationship between roselle and kenaf varieties. In some cases, it was difficult to distinguish two species on the basis of morphological characters.

**Keywords:** beverage, fiber crop, genetic diversity, kenaf (*H. cannabinus* L.), Malvaceae, mestha, roselle (*H. sabdariffa* L.)

## INTRODUCTION

*Hibiscus* L. is the genus under the tribe Hibisceae of the family Malvaceae (Borssum-Waalkes 1966). The genus contains about 300 species that grow in tropical and subtropical regions throughout the world (Anderson and Pharis 2003). It includes both annual and perennial herbaceous and woody plants. Some of the species are economically important as sources of food and medicines and others species are useful as ornamentals (Wilson and Menzel 1964). The morphology and cytology of some of the species, especially in the section Furcaria, have been much investigated (Skovsted 1935, 1941).

One species of *Hibiscus*, known as roselle (*Hibiscus sabdariffa*) is used as a vegetable and to make herbal teas and jams. Another kenaf (*Hibiscus cannabinus*) is extensively used in fiber making. It is an annual or perennial plant belonging to the large family Malvaceae and is cultivated in tropical and subtropical regions for many useful purposes (Wilson and Menzel 1964). The crop is native to West Africa but it has been introduced to other countries and newly into Malaysia in early 1990s. Roselle is currently an important cash crop grown in the East Coast of Malaysia especially in Terengganu and Kelatan (Mohamad *et al.* 2002, 2005). It is locally known as "asam kumbang", "asam susur" and, "asam paya". It is grown mainly for its red acid succulent calyces that can be made into a drink or to make jams or jellies (Mahadevan *et al.* 2009; Foline *et al.* 2011). The red coloring makes it a popular ingredient of commercial herbal teas. In some places its leaves are also used as a vegetable and its stem has a fiber that is sometimes used for domestic purposes. Seeds of roselle are the source of nutrition, protein and other beneficial elements (Ismail *et al.* 2008). The seeds contain oil, which is good as a lubricant fuel, and used for making soap.

Another species, kenaf (*Hibiscus cannabinus* L.) is an annual herbaceous crop of the Malvaceae family, which is known for both its economic and horticultural importance. It is a fiber plant native to east central Africa where it has been grown for several thousand years for food and fibre (LeMahieu *et al.* 2003; Banerjee *et al.* 2007). According to Dempsey (1975), kenaf is a short-day, annual herbaceous plant cultivated for the soft bast fibre in its stem. Kenaf grows in tropical and temperate climates and thrives with abundant solar radiation and high rainfall. Under good conditions kenaf will grow to a height of 5–6 m within 6–8 months (Wood 2003). Kenaf is composed of various useful components and within each of these plant components there are usable portions (e.g. fibres and fibre strands, proteins, oils, and allelopathic chemicals). The combined attributes of these components provide ample potential product diversity to continue use and development of this crop (Webber and Bledsoe 2002). Much research has been done in kenaf, and a large number of varieties have been developed to meet the demands of high-fiber-yielding and disease-resistant kenaf in the recent decades (Dempsey 1975; Bitzer *et al.* 2000).

Identification of roselle and kenaf varieties and understanding of genotypic characteristics and relationships between roselle and kenaf germplasm is important (Falusi 2008), which significantly promote their effective utilization and conservation. Traditionally, identification was based only on morphological and agronomical features. Since it is difficult to identify cultivars based entirely on these features, it is important to find an effective method to accurately identify roselle and kenaf varieties to meet our needs. This

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study was aimed to contribute the basic knowledge on roselle and kenaf in the aspect of their phylogenetic relationships and intraspecific diversity. This research will also contribute the additional knowledge regarding morpho-agronomic traits of roselle and kenaf.

## MATERIALS AND METHODS

Nine roselle and seven kenaf accessions from different origin were used in this study (**Table 1**). Seeds of each accession were germinated in a different tray in the glass house 1, Plant House Complex, Faculty of Science and Technology, Universiti Kebangsaan Malaysia, Bangi. After a week, the geminated seeds were transferred into a small poly bag and left to grow for four weeks. Seedling plantation in the field was the next step that was done after one month from growing in poly bag. All recommended intercultural operations were performed to grow a good stand of crops. The parameters of evaluation include; morpho-agronomic traits. Evaluation of morpho-agronomic traits of roselle and kenaf accessions was conducted at Terrace S, experimental plot of Plant House Complex, UKM. The traits in this evaluation were divided into two categories (i) plant characteristics (leaf shape, stem color, flower color and fruit color) and (ii) agronomic characteristics (plant height, stem diameter, number of branches/plant, number of fruits/plant). Evaluation of the morpho-agronomic plant is plant height (cm), stem diameter (cm), number of branches/plant, number of fruits/plant.

The selection of morphological characters was made by applying the IPGRI *Hibiscus* spp descriptor (Villatoro *et al*. 2009). These parameters are measured and analyzed to determine whether there is significance difference between roselle and kenaf. Every unit of experiment for each variety contained 10 plants (2 replications × 5 plants). The total number of samples of roselle and kenaf accessions was 90 (2 replications × 5 plants × 16 accessions). Analysis of variance (ANOVA) was conducted to analyze the data using SAS software version 9.1.3 (2008). Tukey's test was used to compare significant features of each roselle and kenaf accessions at 5% level of probability. A dendrogram was constructed using Numerical Taxonomy and Multivariate Analysis System (NTSYS version 2.11s; Rohlf 2000) and similarity coefficients were calculated by Simple Matching produced by UPGMA (Rohlf 2000).

**Table 1** Plant materials used in the present study.

| Roselle | Kenaf |
|---|---|
| UKMR1 (Malaysia) | Thai Nung (Cameroon) |
| UKMR2 (Malaysia) | Cuba |
| Accession 6 (Malaysia) | G4 (Cameroon) |
| Accession 3 (Sudan) | G393 (Cameroon) |
| Accession 8 (Sudan) | V36 (Cameroon) |
| Accession 12 (Sudan) | Kho ken (Cameroon) |
| Accession 21 (Arabian Peninsula) | Noonsoon (Cameroon) |
| Benkalese (Sudan) | |
| Nigeria | |

**Table 2** Morphological characters of nine roselle accessions.

| Accession | Stem color | Leaf shape | Flower color | Fruit color |
|---|---|---|---|---|
| UKMR 1 | Smooth reddish | 3-5 lobed | Red | Red |
| UKMR 2 | Smooth reddish | 3-5 lobed | Light red | Dark red |
| Bengkalis | Smooth green | 3-5 lobed | Yellow | Green |
| Accession 3 | Reddish | 3-5 lobed | Red | Red |
| Accession 6 | Reddish | 3-5 lobed | Red | Red |
| Accession 8 | Reddish | 3-5 lobed | Yellow | Red |
| Accession 12 | Red | 3-5 lobed | Yellow | Red |
| Accession 21 | Dark red | 3-5 lobed | Red | Dark red |
| Nigeria | Red | 3-5 lobed | Red | Dark red |

**Table 3** Morphological characters of seven kenaf accessions.

| Accession | Stem color | Leaf shape | Flower color | Fruit color |
|---|---|---|---|---|
| Thai nung | Rough green | 3-7 lobed | Cream | Green |
| Kho ken | Rough red | 3-7 lobed | Cream | Red |
| Cuba | Rough green | 3-7 lobed | Cream | Green |
| G393 | Rough green | 3-7 lobed | Yellow-red | Green |
| G4 | Smooth green | 3-7lobed | Yellow-red | Green |
| V36 | Rough green | 3-7 lobed | Yellow | Green |
| Noon soon | Smooth green | 3-7 lobed | Yellow | Green |

**Table 4** Means for plant characteristics; plant height, Stem diameter, number of branches per plant, number of fruits per plant.

| Accessions | Plant height (cm) | Stem diameter (cm) | Number of branches/ plant | Number of fruits/plant |
|---|---|---|---|---|
| UKMR1 | 65.33 d | 4.96 cd | 4.00 e | 22.50 f |
| UKMR2 | 92.00 cd | 6.26 abcd | 6.66 de | 39.50 def |
| Accession 3 | 120.00 bc | 7.11 abc | 8.66 cde | 109.83 bcd |
| Accession 6 | 122.32 bc | 7.10 abc | 8.23 de | 96.50 bcd |
| Accession 8 | 168.00 ab | 7.05 abc | 10.16 cde | 97.67 bcd |
| Accession 12 | 125.33 bc | 6.66 abcd | 7.66 de | 96.33 bcde |
| Accession 21 | 121.00 bc | 6.48 abcd | 5.33 e | 55.00 cdef |
| Nigeria | 78.00 cd | 4.30 d | 4.66 e | 18.00 f |
| Bengkalis | 80.00 cd | 4.40 d | 4.78 e | 20.00 f |
| Cuba | 193.33 a | 7.30 abc | 10.83 bcde | 152.83 b |
| V36 | 212.50 a | 8.00 ab | 17.50 b | 81.83 bcdef |
| Noonsoon | 200.17 a | 8.61 a | 18.00 a | 109.83 bcd |
| Thai nung | 200.00 a | 6.18 abcd | 8.66 cde | 44.17 def |
| Kho ken | 180.33 a | 8.48 a | 15.50 bc | 226.33 a |
| G4 | 213.00 a | 7.46 abc | 24.83 a | 23.00 f |
| G393 | 178.33 a | 7.33 abc | 13.83 bcd | 124.17 bc |
| *F*-test | * | * | * | * |
| Mean | 146.85 | 6.73 | 10.58 | 82.34 |

Means with the same letter within a column are not significantly different at $P < 0.05$

## RESULTS AND DISCUSSION

### Morpho-agronomic characterization

The evaluation of morpho-agronomic traits was carried out for 9 roselle and 7 kenaf accessions. The traits were divided into two categories, firstly, morphological characters (leaf shape, stem color, flower color and fruit color) and secondly, agronomic characters (plant height, stem diameter, number of branches/plant and the number of fruits/plant). Variations among morphological characters in roselle and kenaf accessions are summarized and presented in **Tables 2** and **3**. Considerable morphological differences in characters, such as stem character, leaf shape, and flower color were observed between kenaf and roselle. The maturity of roselle was much later than that of kenaf accessions. However, morphological variation among the roselle and kenaf accessions was small. Most of the roselle accessions had red stems, red flowers and lobed leaves. On the other hand, kenaf accessions had red or green stems, entire or palmate leaves, and cream flowers. From these morphological characters, there were similar characters shared between the two species roselle and kenaf in aspect of the color. The results of analysis of variance which carried out on a plant height, stem diameter, numbers of branches/plant, number of fruits/plant showed significant variation among roselle and kenaf accessions (**Table 4**).

### Plant height

The means of plant height showed significant difference among roselle and kenaf accessions (**Table 4; Figs. 1, 2**). The accession G4 had the highest plant height (213.0 cm) and UKMR-1 had the lowest plant height (65.33 cm). Moreover, the results also indicated that there was no significant difference among kenaf accessions for height of the plant. Among the roselle accessions UKMR-1 produced the shortest plant height (65.33 cm) whereas accession 8 produced the tallest plant (168.00 cm). Short plants are preferred in breeding programs, such as rice and wheat crops, because short plants can reduce the problems of collapse and can respond well to fertilizer. However, Cheng (2004) found that roselle plants with a higher main stem are stronger and do not fall easily in production levels compared to short plants.

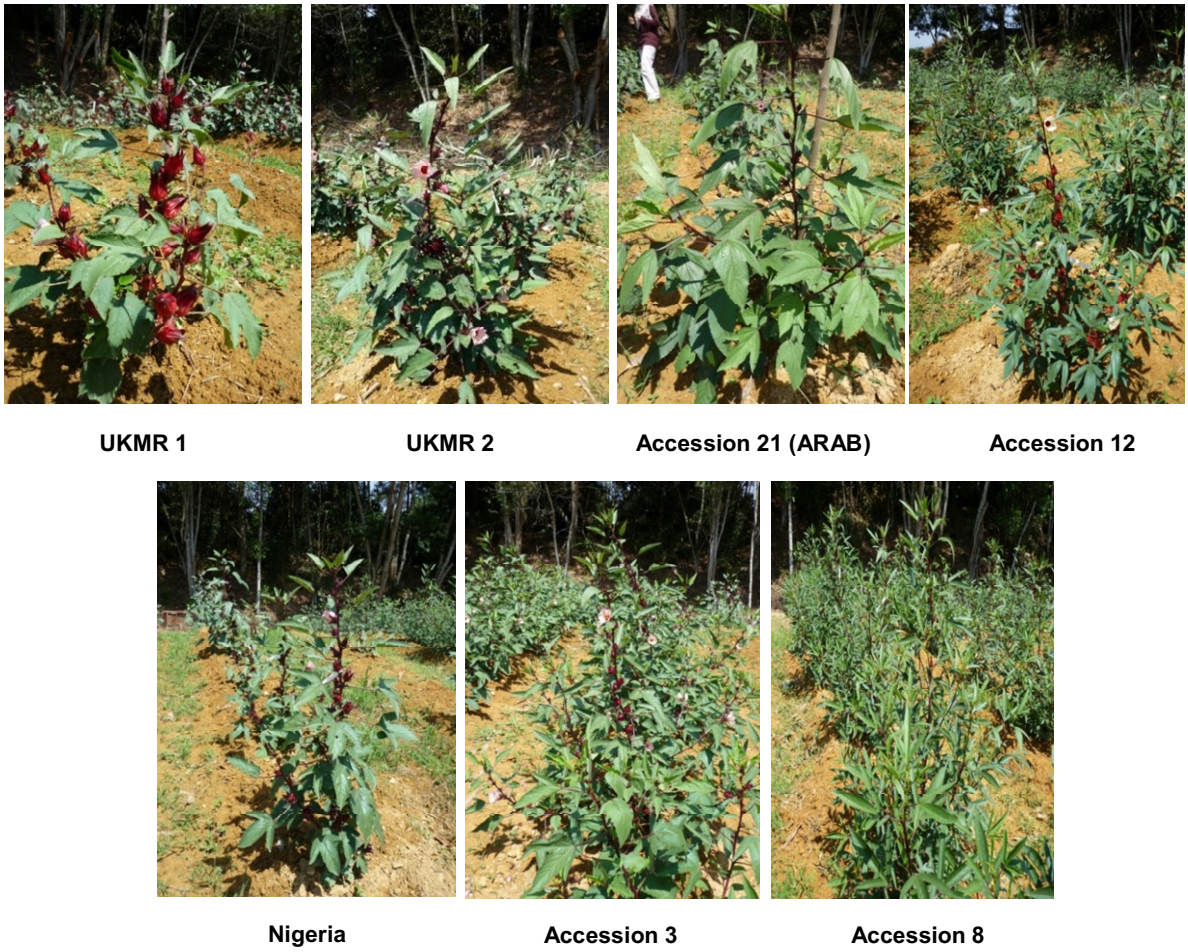


**Fig. 1 Plant architecture of seven roselle accessions.**

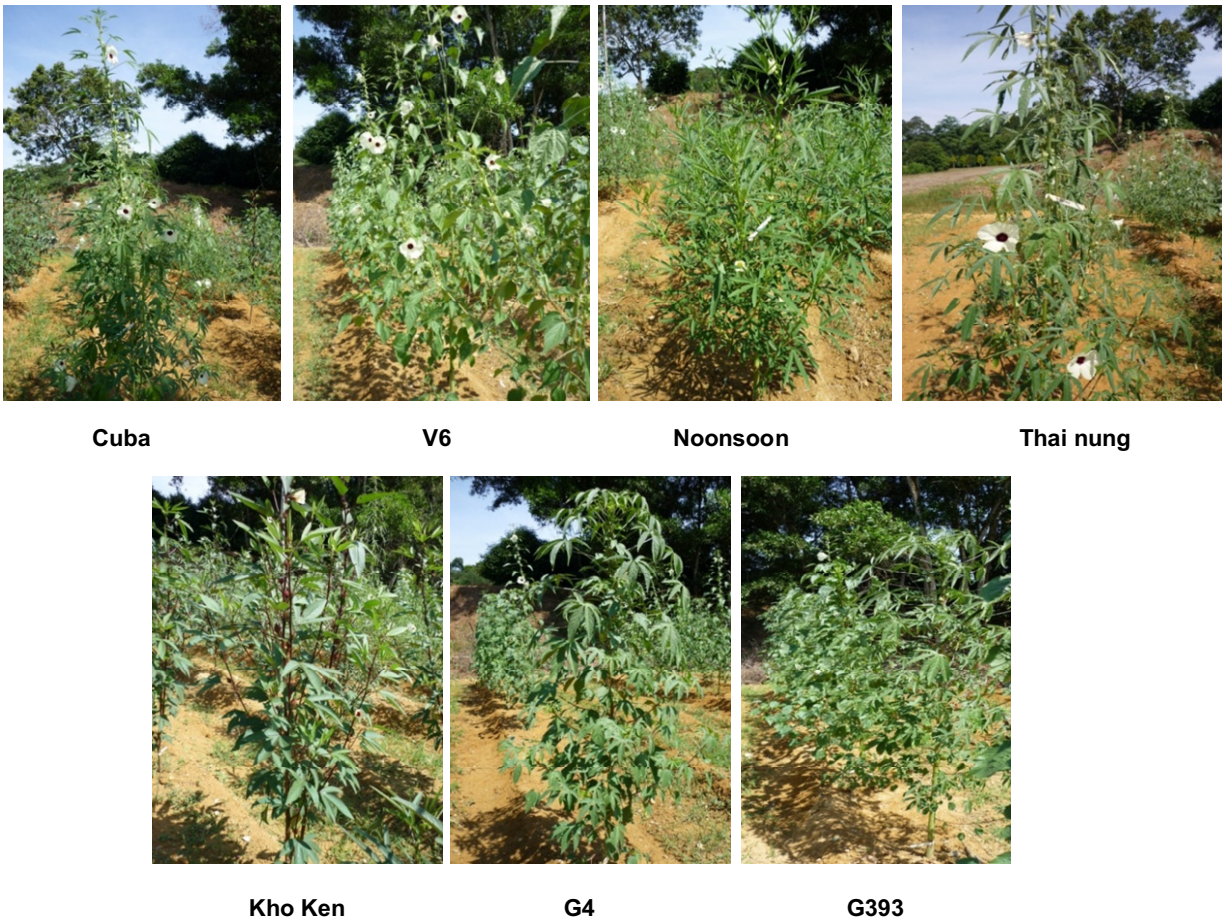


**Fig. 2 Plant architecture of seven kenaf accessions.**

## Stem diameter

ANOVA showed that stem diameter varied significantly among roselle and kenaf accessions (**Table 4**). Stem diameter for kenaf accessions was a little wider compare to roselle accessions. Among all accessions, 'Noonsoon' had the widest stem with diameter 8.61 cm. On the other hand, Nigeria had the lowest stem diameter (4.30 cm). Among roselle varieties, accessions 3, 8 and 12 had the widest diameter: 7.11, 7.05 and 6.66 cm, respectively. There were significant differences between kenaf and roselle accessions.

## Number of branches/plant

Results based on ANOVA showed significant differences between roselle and kenaf accessions for number of branches/plant at $P = 0.05$ (**Table 4**). Kenaf accessions were significantly different from roselle varieties for this trait (**Table 4**). The mean number of branches/plant in this study ranged from 4.0 to 24.83 among the accessions studied. G4 had the highest number of branches/plant (24.83) followed by 'Noonsoon' (18.00). However, UKMR-1 and Nigeria had the lowest number, 4.00 and 4.67, respectively. Arivazhagan and Manivannan (2010) reported higher number (>13.93) of primary branches/plant in roselle.

**Table 5** The similarity coefficient by NTSYSpc 2.10e software between nine roselle and seven kenaf accessions.

| | Acc3 | Acc6 | Acc8 | Acc12 | Acc21 | UKMR 1 | Nigeria | UKMR 2 | Bengkalis | Cuba | V36 | Noonsoon | Thainung | Khoken | G4 | G393 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Acc3 | 1.0000 | | | | | | | | | | | | | | | |
| Acc6 | 0.8409 | 1.0000 | | | | | | | | | | | | | | |
| Acc8 | 0.6170 | 0.6808 | 1.0000 | | | | | | | | | | | | | |
| Acc12 | 0.7200 | 0.7800 | 0.7500 | 1.0000 | | | | | | | | | | | | |
| Acc21 | 0.7500 | 0.8125 | 0.7083 | 0.8775 | 1.000 | | | | | | | | | | | |
| UKMR 1 | 0.7954 | 0.7446 | 0.6382 | 0.8125 | 0.8478 | 1.0000 | | | | | | | | | | |
| Nigeri | 0.6888 | 0.6458 | 0.6818 | 0.6800 | 0.7083 | 0.7111 | 1.0000 | | | | | | | | | |
| UKMR 2 | 0.7674 | 0.7173 | 0.6444 | 0.7500 | 0.8222 | 0.8780 | 0.7209 | 1.0000 | | | | | | | | |
| Bengkalis | 0.5348 | 0.5333 | 0.6000 | 0.5744 | 0.6000 | 0.5952 | 0.5609 | 0.6000 | 1.0000 | | | | | | | |
| Cuba | 0.2978 | 0.2800 | 0.3111 | 0.3018 | 0.3673 | 0.3191 | 0.3720 | 0.3409 | 0.4000 | 1.0000 | | | | | | |
| V36 | 0.3469 | 0.3800 | 0.3913 | 0.4230 | 0.4400 | 0.5958 | 0.4883 | 0.4222 | 0.3846 | 0.6896 | 1.0000 | | | | | |
| Noonsoon | 0.5652 | 0.5625 | 0.5555 | 0.6326 | 0.5918 | 0.6222 | 0.6279 | 0.6279 | 0.5000 | 0.3095 | 0.4634 | 1.0000 | | | | |
| Thainung | 0.4200 | 0.4230 | 0.4680 | 0.4905 | 0.5098 | 0.4693 | 0.5333 | 0.5000 | 0.5128 | 0.6363 | 0.7878 | 0.5116 | 1.0000 | | | |
| Khoken | 0.4583 | 0.3673 | 0.5111 | 0.5294 | 0.4901 | 0.4489 | 0.5454 | 0.4468 | 0.4871 | 0.2926 | 0.4141 | 0.6410 | 0.4651 | 1.0000 | | |
| G4 | 0.3913 | 0.3673 | 0.3478 | 0.3584 | 0.4000 | 0.3829 | 0.4418 | 0.4090 | 0.4444 | 0.7407 | 0.7333 | 0.3488 | 0.7272 | 0.3658 | 1.0000 | |
| G393 | 0.3404 | 0.3469 | 0.3555 | 0.3148 | 0.3529 | 0.3333 | 0.4186 | 0.3555 | 0.4166 | 0.7692 | 0.6451 | 0.2954 | 0.6000 | 0.3414 | 0.7500 | 1.0000 |

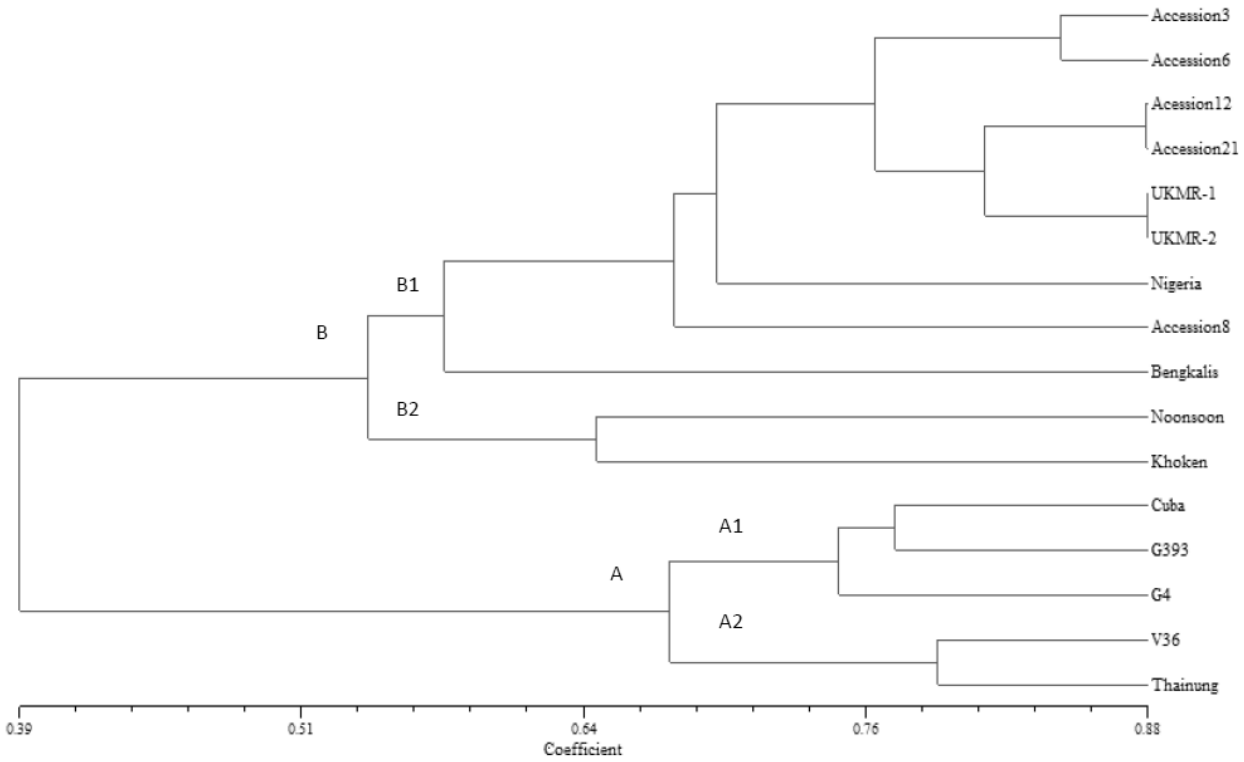


**Fig. 3 Dendrogram showing the relationship among nine roselle and seven kenaf accessions revealed by UPGMA cluster analysis based on morpho-agronomical characters.**

**Table 6** Clustering groups of all roselle and kenaf accessions based on the dendrogram.

| Group | Subgroup | Accession |
|---|---|---|
| A | A1 | Cuba, G393, G4 |
| | A2 | Thainung, V36 |
| B | B1 | Accession 3, Accession 6, Accession 12, Accession 21, UKMR 1, UKMR 2, Nigeria, Accession 8, Bengkalis |
| | B2 | Noonsoon, Khoken |

### Number of fruits/plant

ANOVA for number of fruits/plant showed significant differences among the accessions studied (**Table 4**). The number of fruits/plant was maximum in Kho ken (226.33) and showed the best performance among the accessions for this trait. On the other hand, Cuba produced the second highest number of fruits/plant (152), both kenaf accessions. 'Noonsoon' and G4 produced 44.17 and 23.0 fruits/plant, respectively. Two accessions of roselle, UKMR-1 and Nigeria, also showed significant differences with other roselle varieties in terms of fruit numbers. Arivazhagan and Manivannan (2010) obtained maximum number of fruits/plant in roselle accession HS-6 (137.93) followed by HS-1 (132.83) and the lowest number of fruits in HS-7 (42.87).

### Genetic relationships between roselle and kenaf accessions

Similarity coefficients were calculated using Simple Matching produced by UPGMA (**Table 5**) and a dendrogram (**Fig. 3**) was also constructed with NTSYSpc 2.10 software to demonstrate a clear relationship between the two related species. The similarity coefficient between roselle and kenaf ranged from 0.2800 to 0.8780 showed a wide range of genetic identity. The mean genetic similarity value between roselle accessions was 0.7052 and between kenaf accessions was 0.5489 while similarity coefficients between both roselle and kenaf accessions studied was 0.5370. Based on this value, it was noticed that the relationship between roselle accessions was more closely related than between the kenaf accessions. The mean identity of the polymorphism of the entire sample was 0.5370, indicating that high levels of polymorphism for the roselle and kenaf accessions. This relationship was supported further by the results of cluster analysis. The dendrogram obtained by the UPGMA clustering method revealed that the roselle accessions with two kenaf accessions were clustered in one group and the remaining kenaf accession was in the other group. The first group (A) consists of 5 kenaf accessions (G393, Cuba, V36, Thainung and G4) whereas the second group (B) consisted of 8 roselle accessions (Accession 3, Accession 6, Accession 12, Accession 21, UKMR 1, UKMR 2, Nigeria, Accession 8), two accessions (Noonsoon and Khoken) of kenaf and one accession (Bengkalis) from other hibiscus species. Genetic linkage among the related species of hibiscus was present.

The cluster analysis also demonstrated a considerable divergence among the included accessions, particularly between kenaf and roselle. The first group (A) was divided into two subgroups (**Table 6**) namely (A1) and (A2) in which subgroup A1 consisted of the accessions Cuba, G393 and G4, while the other subgroup (A2) consisted of two accessions (V36 and Thainung). The second group (B) also consisted of two subgroups. The first subgroup, B1 comprised 9 roselle accessions (Accession 3, Accession 6, Accession 12, Accession 21, UKMR 1 and UKMR 2, Nigeria, Accession 8 and Bengkalis). Very low genetic diversity (0.91-0.98 similarities) was also observed among 94 roselle accessions by Hanboonsong *et al.* (2000). The second subgroup (B2) comprised of two kenaf accessions, namely 'Noonsoon' and 'Khoken' (**Table 6**).

Varietal identification of kenaf is always problematic and knowledge on genetic diversity of kenaf varieties is also limited, which significantly hindered effective utilization and conservation of kenaf germplasm. Results from morphological characters, such as middle stem diameter, whole stalk weight, and days to 50% flowering, are highly responsible for the variation of the kenaf varieties, but it is difficult to identify individual varieties merely by the morpho-agronomic characters. Cheng *et al*. (2002) concluded that accessions of same species should cluster in the same group in a dendogram. Kenaf and roselle are independent species with close relationships, and great genetic diversity was also detected among the kenaf accessions with different origins (Cheng *et al.* 2004). Referring to the dendogram, the kenaf accessions was grouped into cluster 1 (A) and roselle accessions were grouped into cluster 2 (B). On the other hand, Bengkalis accession in group B showed a high similarity coefficient with roselle accessions even though it was from different species (**Table 6**). Although 'Noonsoon' and 'Khoken' accessions are from kenaf species, their similarity coefficient showed these two accessions were more related to roselle than to kenaf. Variation between kenaf accessions was higher than that between roselle accessions.

## CONCLUSION

The present study on characterization of roselle and kenaf accessions using morpho-agronomic characters is very useful to understand intraspecific diversity. Knowledge from this study is very important for taxonomic classification of roselle and kenaf within a conservation and management program and for the development of varieties of these two species.

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