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Munirah Samsuddin *et al.*
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The Distribution of *Dioscorea hispida* Dennst. Germplasm in Terengganu and Phylogenetic Relationships of *Dioscorea* spp. using Internal Transcribed Spacer (ITS)

Munirah Samsuddin¹, Nashriyah Mat¹, * Nur Fatihah Hasan Nudin¹,
Nor-Zuhailah Mazlan¹, Nalini Arumugam¹, Muhamad Fahmi Abu Bakar²,
Muhamad Azhar Abdul Wahid¹ and Abdul Manaf Ali²

¹School of Plant Sciences; ²School of Biotechnology,
Faculty of Agriculture, Biotechnology and Food Sciences, Universiti Sultan Zainal Abidin (UniSZA),
Tembila Campus, 22200 Besut, Terengganu Darul Iman, MALAYSIA.

*Corresponding author; E-mail: fatihah@unisza.edu.my

ABSTRACT

Dioscorea hispida Dennst. is a source of carbohydrates and is also important for medicinal purposes. However, the distributions and phylogenetic relationships of *D. hispida* were not well documented in the state of Terengganu. *D. hispida* germplasms were collected from the seven districts of Terengganu between the months of April 2012 to June 2012. During the samplings and survey, a few of the eco-physiological parameters were measured such as the intensity of light, humidity and temperature as well as chlorophyll content. The locations of the distribution of *D. hispida* were mapped using the DIVA-GIS software. Subsamples of seven accessions were used for DNA fingerprinting. In phylogenetic analysis, these *D. hispida* germplasms were subjected to PCR amplification using DNA barcode such as internal transcribed spacer (ITS). The results of this study showed the distribution of *D. hispida* was mostly located near the rivers whilst the generated marker ITS discovered was not a suggested marker for the phylogenetic analysis of *Dioscorea* spp.

Keywords: *Dioscorea hispida*, distribution, phylogenetic analysis

ABSTRAK

Dioscorea hispida Dennst. adalah merupakan sumber karbohidrat dan juga penting untuk tujuan perubatan. Bagaimanapun, taburan dan hubungan filogenetik bagi tumbuhan *D. hispida* tidak didokumentasikan dengan baik di negeri Terengganu.

Germplasm *D. hispida* telah dikumpulkan daripada tujuh daerah di Terengganu di antara bulan April 2012 hingga Jun 2012. Ketika persampelan dan survei dilakukan, beberapa parameter eko-fisiologi telah diukur seperti keamatan cahaya, kelembapan, suhu serta kandungan klorofil. Lokasi taburan *D. hispida* telah dipetakan dengan menggunakan perisian DIVA-GIS. Tujuh aksesori daripada tujuh daerah telah digunakan dalam pengecapjarian DNA. Dalam analisis filogenetik, tujuh aksesori germplasm *D. hispida* tersebut telah didedahkan kepada amplifikasi PCR menggunakan barkod DNA, iaitu *Internal Transcribed Spacer* (ITS). Hasil kajian menunjukkan taburan *D. hispida* banyak terdapat berhampiran sungai, manakala penanda molekul ITS yang dihasilkan tidak dicadangkan sebagai penanda yang sesuai untuk digunakan dalam analisis filogenetik *Dioscorea* spp.

Kata kunci: *Dioscorea hispida*, taburan, analisis filogenetik

INTRODUCTION

Dioscorea hispida Dennst. (locally known as *Ubi Gadong*) is a climber plant with edible tubers and trifoliolate leaves (Burkill, 1951). The starchy tuber of *D. hispida* has become the most important source of dietary energy compared to other tuber crop species (Sato, 2011). According to an ethnobotanical study carried out by Nashriyah *et al.* (2012), the local Malay villagers in Besut, Marang, and Setiu districts use *D. hispida* as their food source for making the popular traditional local food such as *kuib putri mandi*, *kuib onde-onde* and *pengat*. The tubers of *D. hispida* are excellent sources of carbohydrates but contain a toxin that is called dioscorine (Webster *et al.*, 1984). It grows wildly and has become an exotic food nowadays. The tendrils of this yam is used for de-worming medicine while the corm is used to decrease blood glucose (Nashriyah *et al.*, 2011).

Dioscorea hispida is distributed worldwide in many tropical and subtropical regions (Sharma and Bastakoti, 2009; Nashriyah *et al.*, 2011). Terengganu is a state in Peninsular Malaysia that is located in the tropical region. However, Terengganu is unique because its beaches are located throughout this state from Besut in the north to Kemaman in the south (Malaysia State Department Information Terengganu, 2010), whereas it covers various ecosystems such as lake, swamps, inland forest, seawater and forest. However, *D. hispida* is a neglected species nowadays due to its toxicity (Leete and Michelson, 1989; Banaag *et al.*, 1997; Majumdar *et al.*, 2009). Clearing and clearing the forests as well as human habitation can be the main cause that has put significant pressure on certain species (Salmah *et al.*, 2013). Before this species becomes a vulnerable species like *Dioscorea rosei* R. Knuth and *Dioscorea longicuspis* R. Knuth (IUCN, 2012), a study needs to be conducted to document the germplasm of *D. hispida* so that the *in-situ* and *ex-situ* conservations can be done. Moreover, the phylogenetic relationships of this species and the efficiency of the generated ITS marker needs to be investigated. To date,

there is no study about the generated ITS as a DNA barcode in *Dioscorea* spp. It is important in the knowledge development of their taxonomic field.

The objectives of this study were to investigate the distribution of *Dioscorea hispida* in Terengganu and the phylogenetic relationships between *D. hispida* and the other species of *Dioscorea* according to their internal transcribed spacer (ITS) regions, as well as to identify the efficiency of the generated ITS as the DNA barcode in *Dioscorea* spp. Other ecological parameters such as light intensity, humidity, temperature and chlorophyll content were also recorded in this study.

MATERIALS AND METHODS

Identification, Documentation and Sample Collection

Dioscorea hispida plants were collected from various locations throughout the seven districts of the state of Terengganu. The global positioning systems (GPS) location and ecological parameters were also recorded. The collected plants were planted in the Nursery of the Faculty of Agriculture, Biotechnology and Food Sciences, Universiti Sultan Zainal Abidin (UniSZA), Gong Badak Campus, Kuala Terengganu. The samplings of *D. hispida* were done from April to June 2012 (corresponding to the hottest season in Terengganu in 2012). For phylogenetic studies, a total of seven accessions of *D. hispida* (Table 1) germplasm were selected.

Extraction, Amplification and Purification of Products

Fresh leaves were extracted using the method of Doyle and Doyle (1987). Then, the samples were subjected to PCR-amplification following guidelines from the plants' working group (Munirah *et al.*, 2012) and the amplified products were sent to a commercial company (1st Base Laboratory, Malaysia) for DNA sequencing.

Phylogenetic Analysis

Raw DNA sequences data were edited using Chromas software (Hall, 1994) and Bioedit software. Sequences were compared with NCBI database by using the Basic Local Alignment Search Tool (BLAST) software and other *Dioscorea* spp. were identified (Table 2). The species were analyzed and the trees were generated by using PAUP*version 4.0b10 software (Swofford, 2002), using maximum parsimony (MP) by performing an exhaustive search using 100 replicates with the factory settings of bisection-reconnection (TBR) branch swapping. The gaps were treated as the missing value. Bootstrap analysis was performed with 1,000 replicates of simple taxon addition and TBR swapping in order to test support for each clade. For bootstrap value, the percentage of 50-70% is considered weak, 71-85% is

moderate and above 85% is strong (Kress *et al.*, 2002). Neighbour-joining (NJ) analysis was performed to estimate the tree distances. Graphic outputs were illustrated by using the TreeViewX software.

Table 1. The samples that were used to study the phylogenetic relationships of *Dioscorea hispida*

Accession No.	District	Site	Coordinate
DH 1	Hulu Terengganu	Kg. Pangkalan Ajal	E 103.0670360 °, N 4.9817190 °
DH 18	Besut	Kg. Padang Buloh	E 102.4885277 °, N 5.6586667 °
DH 27	Setiu	Kg. Buloh	E 102.7469166 °, N 5.5277778 °
DH 35	Kuala Terengganu	Kg. Pondok Lalang	E 103.0192223 °, N 5.3930277 °
DH 54	Marang	Kg. Gondang	E 103.1275460, N 5.1807900 °
DH 56	Kemaman	Kg. Payoh	E 103.3991944 °, N 4.3819445 °
DH 65	Dungun	Kg. Lubuk Cermin, Jerangau	E 103.1990000 °, N 4.8413611 °

Note: Kg. = *Kampung* (village)

Table 2. GenBank accession numbers of the various ITS sequences of *Dioscorea* spp. used in this analysis.

<i>Dioscorea</i> spp.	Accession Number in the NCBI GenBank	References
<i>Dioscorea japonica</i>	EU817835.1	Gao <i>et al.</i> , 2008
<i>Dioscorea alata</i>	EU817833.1	Gao <i>et al.</i> , 2008
<i>Dioscorea alata</i>	AB679371.1	Fuse <i>et al.</i> , 2011
<i>Dioscorea elephantipes</i>	FJ215767.1	Merckx <i>et al.</i> , 2008
<i>Dioscorea communis</i>	EU186223.1	Merckx <i>et al.</i> , 2008
<i>Dioscorea caucasia</i>	FJ215769.1	Merckx <i>et al.</i> , 2008
<i>Dioscorea sylvatica</i>	FJ215768.1	Merckx <i>et al.</i> , 2008
<i>Dioscorea polystachya</i>	EU817837.1	Gao <i>et al.</i> , 2008
<i>Dioscorea polystachya</i>	EU817836.1	Gao <i>et al.</i> , 2008
<i>Dioscorea polystachya</i>	EU817838.1	Gao <i>et al.</i> , 2008
<i>Dioscorea alata</i>	EU817831.1	Gao <i>et al.</i> , 2008

RESULTS AND DISCUSSION

The distributions of *Dioscorea hispida* were mapped by using the DIVA-GIS software. Figure 1 shows the distribution of *D. hispida* in seven districts of Terengganu which shows the locations of the distributions that are near to riverine areas. According to the Terengganu Forestry Department, *D. hispida* can be found in abundance near rivers but is very limited in forested areas (Salmah *et al.*, 2013), while Saikia *et al.* (2011) showed that the genus *Dioscorea* has been found along the edges of forests, forest tracts, on the banks of streams and rivulets. However, Majumdar *et al.* (2009) stated that *D. hispida* is distributed in the shady forest floor of semi-evergreen to deciduous forest.

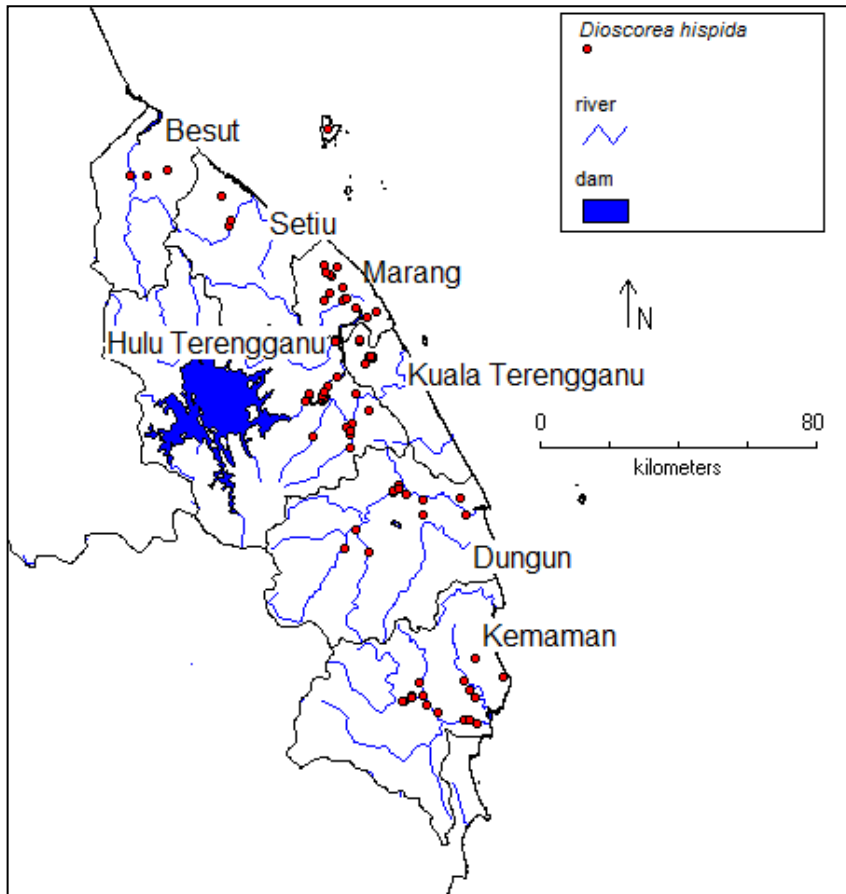


Fig. 1. Distribution of *Dioscorea hispida* in Terengganu

The distribution of *Dioscorea hispida* which is near to rivers can be due to human migration and exchange of genetic materials. These factors are the main causes for the wide distribution of certain plants around the world (Zakri, 1994). However, there is no detailed report until today that the distributions of *D. hispida* are due to these factors. Note that the distributions of *D. hispida* are less, especially near the dam, in the district of Hulu Terengganu (Fig. 1). It may be due to the low population of people that stay in the area since there is a smaller population of people who live in Hulu Terengganu compared to other districts of Terengganu (Department of Statistics Malaysia, 2010). According to Amat *et al.* (2013) in their study of Mediterranean high-mountain plants (*Erysimum penyalarens*) among different areas in Cuerda Larga, Spain, the difference in abundance of plants is due to human activity. Animal disturbance and human use are negatively related to the plant's abundance, whereas human disturbance is positively related to plant abundance. The details of the location of *Dioscorea hispida* are as stated in Table 3 with the latitude and longitude coordinates.

Table 3. Locality of *Dioscorea hispida* collected in the study.

Accession No.	District	Site	GPS
DH 1*	Hulu Terengganu	Kg. Pangkalan Ajal	N 4° 59' 46.5", E 103° 03' 34.3"
DH 2	Hulu Terengganu	Kg. Pangkalan Ajal	N 4° 59' 46.5", E 103° 03' 34.3"
DH 3	Hulu Terengganu	Kg. Pangkalan Ajal	N 4° 59' 46.5", E 103° 03' 34.3"
DH 4	Hulu Terengganu	Kg. Kuo	N 5° 00' 07.3", E 103° 04' 16.9"
DH 5	Hulu Terengganu	Kg. Kuo	N 5° 00' 07.3", E 103° 04' 15.1"
DH 6	Hulu Terengganu	Kg. Kuo	N 5° 00' 07.3", E 103° 04' 16.9"
DH 7	Hulu Terengganu	Kg. Kuala Ketil	N 5° 07' 36.1", E 103° 02' 09.6"
DH 8	Hulu Terengganu	Kg. Kuala Ketil	N 5° 07' 36.1", E 103° 02' 09.6"
DH 9	Hulu Terengganu	Kg. Kuala Ketil	N 4° 59' 46.5", E 103° 03' 34.3"
DH 10	Besut	Kg. Jabi	N 5° 40' 22.6", E 102° 35' 16.0"
DH 11	Besut	Kg. Jabi	N 5° 40' 22.6", E 102° 35' 16.0"
DH 12	Besut	Kg. Jabi	N 5° 04' 22.6", E 102° 35' 16.0"
DH 13	Besut	Kg. Jabi	N 5° 04' 22.6", E 102° 35' 16.0"
DH 14	Besut	Kg. Jabi	N 5° 40' 22.6", E 102° 35' 16.0"
DH 15	Besut	Kg. Jabi	N 5° 40' 22.6", E 102° 35' 16.0"

Table 3. Locality of *Dioscorea hispida* collected in the study (continued).

Accession No.	District	Site	GPS
DH 16	Besut	Kg. Oh	N 5° 39' 40.5", E 102° 32' 01.3"
DH 17	Besut	Kg. Oh	N 5° 39' 40.5", E 102° 32' 01.3"
DH 18*	Besut	Kg. Oh	N 5° 39' 40.5", E 102° 32' 01.3"
DH 19	Besut	Kg. Padang Buloh	N 5° 39' 31.2", E 102° 29' 18.7"
DH 20	Besut	Kg. Padang Buloh	N 5° 39' 31.4", E 102° 29' 19.2"
DH 21	Besut	Kg. Padang Buloh	N 5° 39' 31.6", E 102° 29' 19.1"
DH 22	Setiu	Kg. Guntung Dalam	N 5° 36' 17.4", E 102° 43' 37.2"
DH 23	Setiu	Kg. Guntung Dalam	N 5° 36' 18.7", E 102° 43' 36.6"
DH 24	Setiu	Kg. Guntung Dalam	N 5° 36' 18.1", E 102° 43' 36.8"
DH 25	Setiu	Kg. Buloh	N 5° 31' 40.0", E 102° 44' 48.9"
DH 26	Setiu	Kg. Buloh	N 5° 31' 40.6", E 102° 44' 49.0"
DH 27*	Setiu	Kg. Buloh	N 5° 31' 40.6", E 102° 44' 48.5"
DH 28	Setiu	Kg. Bukit Kemudu	N 5° 32' 32.9", E 102° 45' 18.8"
DH 29	Setiu	Kg. Bukit Kemudu	N 5° 32' 33.1", E 102° 45' 18.9"
DH 30	Setiu	Kg. Bukit Kemudu	N 5° 32' 32.6", E 102° 45' 19.3"
DH 31	Kuala Terengganu	Kg. Jeram Sungai	N 5° 20' 01.7", E 103° 03' 14.4"
DH 32	Kuala Terengganu	Kg. Jeram Sungai	N 5° 19' 42.5", E 103° 02' 51.4"
DH 33	Kuala Terengganu	Kg. Jeram Sungai	N 5° 19' 42.6", E 103° 02' 51.3"
DH 34	Kuala Terengganu	Kg. Pondok Lalang	N 5° 23' 35.0", E 103° 01' 09.2"
DH 35*	Kuala Terengganu	Kg. Pondok Lalang	N 5° 23' 34.9", E 103° 01' 09.2"
DH 36	Kuala Terengganu	Kg. Pondok Lalang	N 5° 23' 34.8", E 103° 01' 09.4"
DH 37	Kuala Terengganu	Kg. Lingai	N 5° 25' 13.5", E 102° 59' 58.0"
DH 38	Kuala Terengganu	Kg. Lingai	N 5° 25' 13.8", E 102° 59' 58.0"
DH 39	Kuala Terengganu	Kg. Lingai	N 5° 25' 15.3", E 102° 59' 58.0"
DH 40	Marang	Kg. Gondang	N 5° 10' 34.8", E 103° 07' 45.9"
DH 41	Marang	Kg. Gondang	N 5° 10' 32.8", E 103° 07' 47.2"
DH 42	Marang	Kg. Gondang	N 5° 10' 32.8", E 103° 07' 47.2"
DH 43	Marang	Kg. Lubuk Batu	N 5° 09' 44.6", E 103° 06' 37.2"
DH 44	Marang	Kg. Lubuk Batu	N 5° 09' 44.4", E 103° 06' 37.4"
DH 45	Marang	Kg. Lubuk Batu	N 5° 09' 44.2", E 103° 06' 36.9"
DH 46	Marang	Kg. Lubuk Batu	N 5° 09' 43.9", E 103° 06' 36.9"

Table 3. Locality of *Dioscorea hispida* collected in the study (continued).

Accession No.	District	Site	GPS
DH 47	Kemaman	Kg. Dadong	N 4° 16' 57.9", E 103° 13' 51.1"
DH 48	Kemaman	Kg. Dadong	N 4° 16' 57.7", E 103° 13' 51.1"
DH 49	Kemaman	Kg. Dadong	N 4° 16' 57.7", E 103° 13' 50.6"
DH 50	Kemaman	Kg. Seberang Tayor	N 4° 17' 08.0", E 103° 15' 31.1"
DH 51	Kemaman	Kg. Seberang Tayor	N 4° 17' 08.3", E 103° 15' 31.3"
DH 52	Kemaman	Kg. Seberang Tayor	N 4° 17' 08.4", E 103° 15' 31.5"
DH 53	Kemaman	Kg. Payoh	N 4° 22' 55.0", E 103° 23' 57.1"
DH 54*	Kemaman	Kg. Payoh	N 4° 22' 55.2", E 103° 23' 57.3"
DH 55	Kemaman	Kg. Payoh	N 4° 22' 55.5", E 103° 23' 57.2"
DH 56*	Dungun	Kg. Lubuk Cermin, Jerangau	N 4° 50' 28.9", E 103° 11' 56.4"
DH 57	Dungun	Kg. Lubuk Cermin, Jerangau	N 4° 50' 28.7", E 103° 11' 56.3"
DH 58	Dungun	Kg. Lubuk Cermin, Jerangau	N 4° 50' 28.4", E 103° 11' 56.4"
DH 59	Dungun	Kg. Dendang	N 4° 49' 32.7", E 103° 10' 47.0"
DH 60	Dungun	Kg. Dendang	N 4° 49' 26.1", E 103° 10' 51.9"
DH 61	Dungun	Kg. Dendang	N 4° 49' 26.5", E 103° 10' 51.8"
DH 62	Dungun	Kg. Tokkah	N 4° 48' 27.9", E 103° 21' 31.2"
DH 63	Dungun	Kg. Tokkah	N 4° 48' 28.2", E 103° 21' 30.9"
DH 64	Dungun	Kg. Tokkah	N 4° 48' 27.8", E 103° 21' 31.0"
DH 65*	Marang	Kg. Bukit Payung	N 5° 13' 08.6", E 103° 05' 34.5"
DH 66	Marang	Kg. Bukit Payung	N 5° 13' 08.6", E 103° 05' 34.5"
DH 67	Marang	Kg. Bukit Payung	N 5° 13' 08.9", E 103° 05' 34.6"

Note: Kg. = *Kampung* (village)

(*) Accessions used in the phylogenetic analysis.

During the samplings of *Dioscorea hispida*, the ecological parameters such as light intensity, humidity, chlorophyll content and temperature were recorded. The parameters were taken in the morning, afternoon and evening during the hottest season of the year in Terengganu (April 2012 to June 2012). The ecological parameters were measured during sampling in the morning (before 12 PM) in Table 4, midday (12-2 PM) in Table 5 and afternoon (after 2 PM) in Table 6.

Table 4. The eco-physiological parameters that were measured during samplings of *Dioscorea hispida* in the morning (before 12 PM).

Accession No.	Light Concentration (μmol)	Humidity (%)	Temperature ($^{\circ}\text{C}$)	Chlorophyll Content (CCI)
DH01	30.58	73.20	32.70	11.30
DH02	30.58	74.14	32.62	9.50
DH03	58.98	72.72	32.19	9.10
DH10	167.33	73.73	28.27	5.70
DH11	42.48	75.03	31.44	7.20
DH13	28.03	61.88	31.74	9.60
DH14	111.82	65.55	32.17	10.00
DH15	28.04	61.88	31.74	11.30
DH22	56.91	85.13	28.40	10.30
DH23	67.13	74.13	31.40	11.40
DH24	264.10	41.36	41.13	7.80
DH31	43.56	83.37	27.90	7.80
DH32	10.07	80.83	28.53	17.60
DH33	14.02	29.04	29.04	9.30
DH34	13.38	30.16	30.16	10.50
DH35	2.06	30.18	30.18	16.20
DH36	9.61	30.80	30.80	15.00
DH40	35.17	32.90	32.90	7.20
DH41	203.40	31.03	31.03	10.60
DH42	70.08	31.13	31.13	8.80
DH47	6.35	29.29	29.29	10.30
DH48	9.55	29.21	29.21	12.20
DH49	5.06	29.52	29.52	12.50
DH56	1.93	23.00	23.00	16.90
DH57	4.76	24.04	24.04	9.60
DH58	4.19	24.28	24.28	17.40
DH59	15.49	23.09	23.09	9.90
DH60	13.20	22.57	22.57	10.10
DH61	15.88	24.25	24.25	16.10
Range	1.93-264.10	22.57-85.13	22.57-41.13	5.70-17.60

Table 5. The eco-physiological parameters that were measured during samplings of *Dioscorea hispida* in the midday (12-2 PM).

Accession No.	Light Concentration (μmol)	Humidity (%)	Temperature ($^{\circ}\text{C}$)	Chlorophyll Content (CCI)
DH04	130.86	71.53	33.12	6.30
DH05	152.53	61.17	33.74	4.70
DH06	39.60	65.87	33.50	7.40
DH25	121.20	72.51	32.57	9.40
DH26	49.04	78.69	31.83	5.50
DH27	128.04	81.41	31.84	8.10
DH28	93.87	76.47	32.08	16.30
DH29	51.90	67.00	34.79	16.20
DH30	36.85	61.90	35.59	9.60
DH37	1005.80	32.62	32.62	10.30
DH38	108.70	37.45	37.45	9.90
DH39	704.60	33.28	33.28	5.80
DH43	274.70	34.30	34.30	5.40
DH44	866.20	44.04	44.04	4.40
DH45	232.00	35.87	35.87	7.20
DH46	300.90	37.22	37.22	8.10
DH50	10.80	29.62	29.62	9.50
DH51	18.18	29.10	29.10	19.30
DH52	15.42	29.47	29.47	14.20
DH53	104.27	28.15	28.15	15.80
DH54	5.64	29.99	29.99	10.10
DH55	20.30	30.53	30.53	11.80
DH62	3.63	24.09	24.09	11.00
DH63	25.12	24.31	24.31	11.40
DH64	24.61	25.43	25.43	17.00
Range	3.63-1005.80	24.09-81.41	24.09-44.04	4.40-19.30

Table 6. The eco-physiological parameters that were measured in samplings of *Dioscorea hispida* in the afternoon (after 2 PM).

Accession No.	Light Concentration (μmol)	Humidity (%)	Temperature ($^{\circ}\text{C}$)	Chlorophyll Content (CCI)
DH07	77.14	54.69	38.59	6.70
DH08	19.94	60.38	35.80	11.30
DH09	4.82	64.93	33.10	14.90
DH19	200.10	61.51	30.12	7.60
DH20	197.81	74.80	34.20	12.00
DH21	146.92	65.25	35.40	14.70
DH65	60.64	26.64	26.64	13.20
DH66	1.12	26.36	26.36	15.20
DH67	11.28	27.10	27.10	13.20
Range	1.12-200.10	26.36-74.80	26.36-38.59	6.70-15.20

The different sampling time was unavoidable due to the time constraints during sampling. Nevertheless, the results showed that *D. hispida* can widely grow in various light concentration between 1.12 μmol to 1005.8 μmol with various humidity which ranged from 22.57% to 85.13%. Saikia *et al.* (2011) stated that the genus of *Dioscorea* has been found along the edges of forest where more light penetrates with thinly shaded areas. The results showed that *D. hispida* can be grown in wide-ranging light concentrations and humidity conditions. In addition, the temperatures ranged from 22.57 $^{\circ}\text{C}$ to 44.04 $^{\circ}\text{C}$. Because Terengganu is located in the tropical region, the temperature of the environment is according to the standard temperature in the tropic. Moreover, the location of *D. hispida* was recorded within moderate altitudinal range (Saikia *et al.*, 2011).

The chlorophyll content was also measured in this study. According to the results of this study, the range of chlorophyll content was between 17.6 CCI to 4.4 CCI. Jahan *et al.* (2008) stated that chlorophyll content is closely related to the nitrogen content in the soil. Other parameters that can be measured are elevation and annual rainfall. However, Saikia *et al.* (2011) reported that *D. hispida* is located in moderate altitudinal range with a mean of 2955 mm annual rainfall.

DNA sequencing results showed that the seven samples that were amplified using PCR did not show any evolution in their ITS regions except for an insertion and deletion (indel) in DH 35 and DH 27 as shown in Figure 2. However, according to Jakse *et al.* (2005), phylogenetic analysis of indels did not reveal a clear

relationship among elite onion populations and there was no agreement among trees generated by using indels.

The shortest phylogenetic tree containing 19 parsimony-informative characters with a minimum length of 1267 steps, a consistency index (CI) of 0.9858 and a retention index (RI) of 0.3077 were obtained (Fig. 3). The CI and RI values indicate the degree of “tree-likeness” in data for character change. When CI = 1, there was no homoplasy whereas when RI = 1, the character fits perfectly with the tree. The homoplasy index (HI) was 0.0142. *Stenomeris dioscoreifolia* Planch was designated as an outgroup, as it was placed in a sister-group relationship with *Dioscorea* spp. based on combined nuclear and mitochondrial data (Merckx *et al.*, 2006).

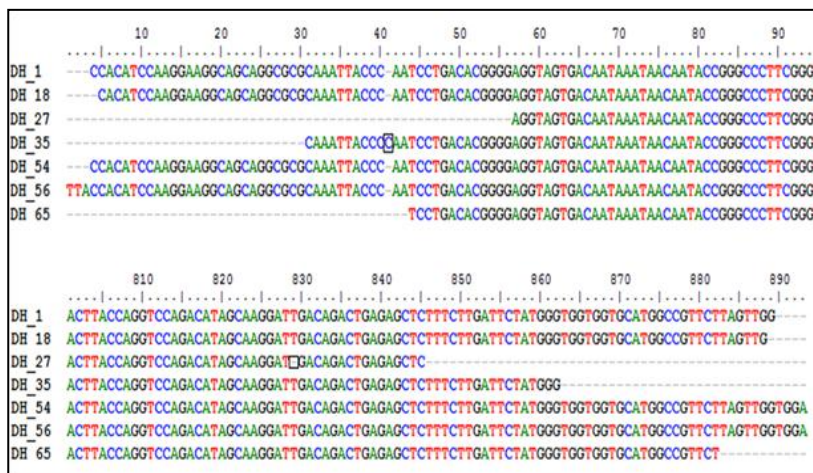


Fig. 2. The insertion and deletion (indels) on the sequences of the Internal Transcribed Spacer (ITS) regions of *Dioscorea hispida*.

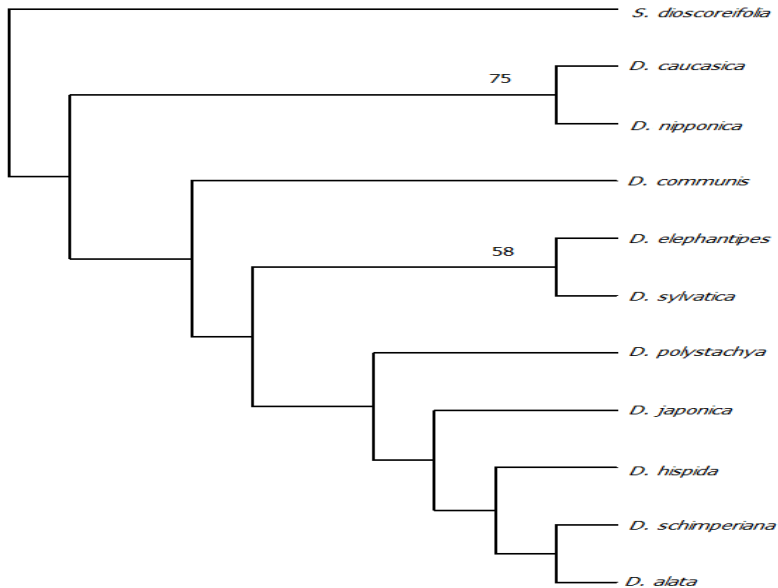


Fig. 3. Parsimony tree of *Dioscorea* spp. resulting from the Internal Transcribed Spacer (ITS) sequences. Numbers above the branches were bootstrap percentage value. Branches received less than 50% bootstrap support collapsed in the strict consensus tree.

According to Munirah *et al.* (2012), *Dioscorea japonica* Thunb. is closely related to *D. hispida* due to the similarity of the ITS sequences in Basic Local Alignment Search Tool-Nucleotide Blast (BLASTN) analysis. In this study, only *D. caucasica* Lipsky was found to be related to *D. nipponica* Makino with the moderate support of 75%, whilst the relationship between *D. elephantipes* Engl. and *D. sylvatica* Eckl. shows a weak support of 58%. Other *Dioscorea* spp. received extremely low bootstrap support and therefore, the relationships between these species were uncertain. These weak-moderate relationships suggest that the ITS barcode could not be widely used for the phylogenetic analysis of *Dioscorea* spp. as it appears to be not the best marker for the genus *Dioscorea*. Wilkin *et al.* (2005) reported that a combined large subunit of ribulose-biphosphate carboxylase and maturase K genes' sequence analysis could better infer the phylogenetic relationship in *Dioscorea*.

Sun *et al.* (2012) suggested that the *matK* is a strong candidate for *Dioscorea* identification. However, it is not a perfect candidate because it is ineligible for an ideal barcode. Although the intra-specific and interspecific divergences were mainly non-overlapping, there is no distinct barcoding gap found. According to Ward *et al.* (2005) and Lahaye *et al.* (2008), distinct gaps with no overlap are essential for the

ideal barcode. However, Sun *et al.* (2012) did not include the ITS region in their study due to low sequencing success even though this region was proposed to be the most promising universal DNA barcode in plants.

The distance tree (Fig. 4) was estimated by using the neighbour-joining (NJ) analysis based on the formulae of Kimura (1980). It was noted that although the topology was identical in both MP and NJ trees, the bootstrap supports were slightly different. The relationships between *D. caucasica* and *D. nipponica* (bootstrap value of 93%) as well as *D. elephantipes* and *D. sylvatica* (bootstrap value of 77%) were defined.

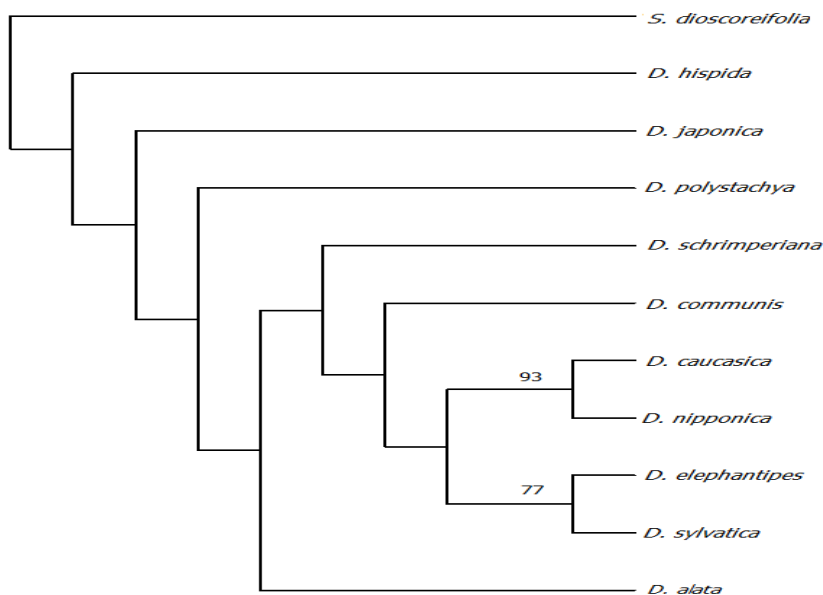


Fig. 4. Neighbour-joining tree of *Dioscorea* spp. resulting from the Internal Transcribed Spacer (ITS) sequences. Numbers above the branches were bootstrap percentage value. Branches received less than 50% bootstrap support collapsed in the strict consensus tree.

CONCLUSION

This research presented details of the germplasm locations for conservation and improvement programs for *Dioscorea hispida*. This study also revealed that *D. hispida* can be found near rivers and grows in variable environmental conditions. In addition, phylogenetic analysis did not suggest the ITS region as a useful marker to discriminate *Dioscorea* species.

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REFERENCES

- Amat, M. E., Vargas, P. & Gomez, J. M. 2013. Effects of human activity on the distribution and abundance of an endangered Mediterranean high-mountain plant (*Erysimum penyalarens*). *Journal for Nature Conservation* **21**: 262-271.
- Banaag, A., Honda, H. & Shono, T. 1997. Effects of alkaloids from yam, *Dioscorea hispida* Schluskel, on feeding and development of larvae of the diamondback moth, *Plutella xylostella* (Lepidoptera: Yponomeutidae). *Applied Entomology and Zoology* **32**: 119-126.
- Burkill, I. H. 1935. *Dioscorea hispida*. *A Dictionary of the Economic Products of the Malay Peninsula*. The Crown Agents for the Colonies, London.
- Department of Statistics Malaysia. 2010. Downloaded at <http://www.statistics.gov.my/portal/index.php?lang=en> [Accessed on 10th March 2013].
- Doyle, J. J. & Doyle, J. L. 1987. A rapid DNA isolation procedure for small quantities of fresh leaf tissue. *Phytochemical Bulletin* **19**: 11-15.
- Hall, T. A. 1999. BioEdit: A user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symposium Series* **41**: 95-98.
- IUCN. 2012. IUCN Red List of Threatened Species Version 2012.2. *The International Union for Conservation of Nature*. Downloaded at <http://www.iucnredlist.org> [Accessed on 6th May 2013].
- Jahan, M. S., Ogawa, K., Nakamura, Y., Shimoishi, Y., Mori, I. C. & Murata, Y. 2008. The deficient glutathione in guard cells facilitates abscisic acid-induced stomatal closure but does not affect light-induced stomatal opening. *Bioscience, Biotechnology, and Biochemistry* **72**: 2795-2798.
- Jakse, J., Martin, W., McCallum, J. & Harvey, M. J. 2005. Single nucleotide polymorphism, indels and simple sequence repeats for onion cultivar identification. *Journal of the American Society for Horticultural Science* **130**: 912-917.
- Kimura, M. 1980. A simple method for estimating evolutionary rate of base substitution through comparative studies of nucleotide sequences. *Journal of Molecular Evolution* **16**: 111-120.

- Kress, W. J., Prince, L. M. & William, K. J. 2002. The phylogeny and a new classification of the ginger (Zingiberaceae): Evidence from molecular data. *American Journal of Botany* **89**: 1682-1696.
- Lahaye, R., Van, D. B. M., Bogarin, D., Warner, J., Pupulin, F., Gigot, G., Maurin, O., Duthoit, S., Barraclough, T. G. & Savolainen, V. 2008. DNA barcoding the floras of biodiversity hotspots. *Proceedings of the National Academy of Sciences USA* **105**: 2923-2928.
- Leete, E. & Michelson, R. H. 1989. The incorporation of 3-hydroxy-3-methylglutaric acid into the lactone ring of dioscorine in *Dioscorea hispida*. *Phytochemistry* **28**: 3325-3330.
- Majumdar, K., Saikia, B. & Datta, B. K. 2009. *Dioscorea hispida* Dennst. (Dioscoreaceae) - A new recorded for Tripura, India. *Pleione* **3**: 224-226.
- Malaysia State Department of Information Terengganu. 2010. Downloaded at <http://www.penerangan.gov.my/terengganu/index.php/artikel/peta-negeri> [Accessed on 8th November 2011].
- Merckx, V., Schols, P., Kamer, H., M., Maas, P., Huysmans, S. & Smets, E. 2006. Phylogeny and evolution of *Burmanniaceae* (Dioscoreales) based on nuclear and mitochondrial data. *American Journal of Botany* **93**: 1684-1698.
- Munirah, S., Nashriyah, M., Nor-Zuhailah, M., Siti Nor Indah, O., Salmah, T., Norhayati, N. & Ali, A. M. 2012. Internal Transcribed Spacer (ITS) as a molecular marker for identification of *Dioscorea hispida* Dennst. (Dioscoreaceae). *Journal of Agrobiotechnology* **3**: 47-55.
- Nashriyah, M., Nur Athiqah M. Y., Syahril Amin, H., Norhayati, N., Mohamad Azhar, A. W. & Khairil, M. 2011. Ethnobotany and distribution of wild edible tubers in Pulau Redang and nearby islands of Terengganu, Malaysia. *World Academy of Science, Engineering and Technology* **60**: 1832-1835.
- Nashriyah, M., Salmah, T., Nur Atiqah, M. Y., Siti Nor Indah, O., Muhamad Azhar, A. W., Munirah, S., Nornasuha, Y. & Ali, A. M. 2012. Ethnobotany and distribution of *Dioscorea hispida* Dennst. (Dioscoreaceae) in Besut, Marang and Setiu Districts of Terengganu, Peninsular Malaysia. *World Academy of Science, Engineering and Technology* **72**: 1083-1086.
- Salmah, T., Nashriyah, M., Abdul Ghani, Y. & Shamsul Bahri, A. R. 2013. Anatomical Study of *Dioscorea hispida* Dennst. (Dioscoreaceae) Stem, Leaf, Petiole, Tuber and Root by Using Optical Microscope, SEM and TEM. *Journal of Agrobiotechnology* **4**: 32-41.
- Saikia, B., Rawat, J. S., Tag, H. & Das, A. K. 2011. An investigation on the taxonomy and ecology of the genus *Dioscorea* in Arunachal Pradesh, India. *Journal of Frontline Research in Arts and Science* **1**: 44-53.
- Sato, H. 2011. The potential of edible wild yams and yam-like plants as a staple food resource in the African Tropical Rain Forest. *African Study Monographs* **26**: 123-134.
- Sharma, K., Mishra, A. K. & Misra, R. S. 2008. A simple and efficient method for extraction of genomic DNA from tropical tuber crops. *African Journal of Biotechnology* **7**: 1018-1022.

- Sun, X. Q., Zhu, Y. J., Guo, J. L., Peng, B. & Bai, M. M. 2012. DNA Barcoding the *Dioscorea* in China, a vital group in the evolution of monocotyledon: Use of *matK* gene for species discrimination. *PLoS ONE* **7**: e32057.
- Swofford, D. L. 2000. PAUP*. Phylogenetic Analysis Using Parsimony (*and other methods) Version 4. Sinauer Associates, Sunderland, Massachusetts.
- Ward, R. D., Zemplak, T. S., Innes, B. H., Last, P. R. & Hebert, P. D. N. 2005. DNA barcoding of Australia's fish species. *Philosophical Transactions of the Royal Society B: Biological Sciences* **360**: 1847-1857.
- Webster, J., Beck, W. & Ternai, B. 1984. Toxicity and bitterness in Australian *Dioscorea bulbifera* L. and *Dioscorea hispida* Dennst. from Thailand. *Journal of Agriculture and Food Chemistry* **32**: 1087-1090.
- Wilkin, P., Schols, P., Chase, M. W., Chayamarit, K., Furness, C. A., Huysmans, S., Rakotonasolo, F., Smets, E. & Thapayai, C. 2005. A plastid gene phylogeny of the yam genus, *Dioscorea*: Roots, fruits and Madagascar. *Systematic Botany* **30**: 736-749.
- Zakri, A. H. 1993. *Sumber Genetik Tumbuhan*. 1st edition. Dewan Bahasa dan Pustaka. p. 23-25. Kuala Lumpur, Malaysia.