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

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**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.

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

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**INTRODUCTION**

*Dioscorea hispida* Dennst. (locally known as *Ubi Gadong*) is a climber plant with edible tubers and trifoliate 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 *kuih putri mandi*, *kuih 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 tendril 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). Cleaning 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 Doyles (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*

<table>
<thead>
<tr>
<th>Accession No.</th>
<th>District</th>
<th>Site</th>
<th>Coordinate</th>
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<tbody>
<tr>
<td>DH 1</td>
<td>Hulu</td>
<td>Kg. Pangkalan Ajal</td>
<td>E 103.0670360°, N 4.9817190°</td>
</tr>
<tr>
<td>DH 18</td>
<td>Besut</td>
<td>Kg. Padang Buloh</td>
<td>E 102.4885277°, N 5.6586667°</td>
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<tr>
<td>DH 27</td>
<td>Setiu</td>
<td>Kg. Buloh</td>
<td>E 102.7469166°, N 5.5277778°</td>
</tr>
<tr>
<td>DH 35</td>
<td>Kuala</td>
<td>Kg. Pondok</td>
<td>E 103.0192223°, N 5.3930277°</td>
</tr>
<tr>
<td>DH 54</td>
<td>Marang</td>
<td>Kg. Gondang</td>
<td>E 103.1275460°, N 5.180790°</td>
</tr>
<tr>
<td>DH 56</td>
<td>Kemaman</td>
<td>Kg. Payoh</td>
<td>E 103.3991944°, N 4.3819445°</td>
</tr>
<tr>
<td>DH 65</td>
<td>Dungun</td>
<td>Kg. Lubuk Cermin, Jerangau</td>
<td>E 103.1990000°, N 4.8413611°</td>
</tr>
</tbody>
</table>

Note: Kg. = Kampong (village)

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

<table>
<thead>
<tr>
<th>Dioscorea spp.</th>
<th>Accession Number in the NCBI GenBank</th>
<th>References</th>
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<td><em>Dioscorea japonica</em></td>
<td>EU817835.1</td>
<td>Gao et al., 2008</td>
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<td>Gao et al., 2008</td>
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<td>AB679371.1</td>
<td>Fuse et al., 2011</td>
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<td><em>Dioscorea elephantipes</em></td>
<td>FJ215767.1</td>
<td>Merckx et al., 2008</td>
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<td><em>Dioscorea communis</em></td>
<td>EU186223.1</td>
<td>Merckx et al., 2008</td>
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<td><em>Dioscorea caucasia</em></td>
<td>FJ215769.1</td>
<td>Merckx et al., 2008</td>
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<td>Gao et al., 2008</td>
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</table>
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 penyalarense*) 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.

<table>
<thead>
<tr>
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<td>N 5° 00’ 07.3”, E 103° 04’ 15.1”</td>
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Table 3. Locality of *Dioscorea hispida* collected in the study (continued).

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

<table>
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<td>Kg. Dendang</td>
<td>N 4º 49’ 26.1”, E 103º 10’ 51.9”</td>
</tr>
<tr>
<td>DH 61</td>
<td>Dungun</td>
<td>Kg. Dendang</td>
<td>N 4º 49’ 26.5”, E 103º 10’ 51.8”</td>
</tr>
<tr>
<td>DH 62</td>
<td>Dungun</td>
<td>Kg. Tokkah</td>
<td>N 4º 48’ 27.9”, E 103º 21’ 31.2”</td>
</tr>
<tr>
<td>DH 63</td>
<td>Dungun</td>
<td>Kg. Tokkah</td>
<td>N 4º 48’ 28.2”, E 103º 21’ 30.9”</td>
</tr>
<tr>
<td>DH 64</td>
<td>Dungun</td>
<td>Kg. Tokkah</td>
<td>N 4º 48’ 27.8”, E 103º 21’ 31.0”</td>
</tr>
<tr>
<td>DH 65*</td>
<td>Marang</td>
<td>Kg. Bukit Payung</td>
<td>N 5º 13’ 08.6”, E 103º 05’ 34.5”</td>
</tr>
<tr>
<td>DH 66</td>
<td>Marang</td>
<td>Kg. Bukit Payung</td>
<td>N 5º 13’ 08.6”, E 103º 05’ 34.5”</td>
</tr>
<tr>
<td>DH 67</td>
<td>Marang</td>
<td>Kg. Bukit Payung</td>
<td>N 5º 13’ 08.9”, E 103º 05’ 34.6”</td>
</tr>
</tbody>
</table>

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).

<table>
<thead>
<tr>
<th>Accession No.</th>
<th>Light Concentration (µmol)</th>
<th>Humidity (%)</th>
<th>Temperature (°C)</th>
<th>Chlorophyll Content (CCI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DH01</td>
<td>30.58</td>
<td>73.20</td>
<td>32.70</td>
<td>11.30</td>
</tr>
<tr>
<td>DH02</td>
<td>30.58</td>
<td>74.14</td>
<td>32.62</td>
<td>9.50</td>
</tr>
<tr>
<td>DH03</td>
<td>58.98</td>
<td>72.72</td>
<td>32.19</td>
<td>9.10</td>
</tr>
<tr>
<td>DH10</td>
<td>167.33</td>
<td>73.73</td>
<td>28.27</td>
<td>5.70</td>
</tr>
<tr>
<td>DH11</td>
<td>42.48</td>
<td>75.03</td>
<td>31.44</td>
<td>7.20</td>
</tr>
<tr>
<td>DH13</td>
<td>28.03</td>
<td>61.88</td>
<td>31.74</td>
<td>9.60</td>
</tr>
<tr>
<td>DH14</td>
<td>111.82</td>
<td>65.55</td>
<td>32.17</td>
<td>10.00</td>
</tr>
<tr>
<td>DH15</td>
<td>28.04</td>
<td>61.88</td>
<td>31.74</td>
<td>11.30</td>
</tr>
<tr>
<td>DH22</td>
<td>56.91</td>
<td>85.13</td>
<td>28.40</td>
<td>10.30</td>
</tr>
<tr>
<td>DH23</td>
<td>67.13</td>
<td>74.13</td>
<td>31.40</td>
<td>11.40</td>
</tr>
<tr>
<td>DH24</td>
<td>264.10</td>
<td>41.36</td>
<td>41.13</td>
<td>7.80</td>
</tr>
<tr>
<td>DH31</td>
<td>43.56</td>
<td>83.37</td>
<td>27.90</td>
<td>7.80</td>
</tr>
<tr>
<td>DH32</td>
<td>10.07</td>
<td>80.83</td>
<td>28.53</td>
<td>17.60</td>
</tr>
<tr>
<td>DH33</td>
<td>14.02</td>
<td>29.04</td>
<td>29.04</td>
<td>9.50</td>
</tr>
<tr>
<td>DH34</td>
<td>13.38</td>
<td>30.16</td>
<td>30.16</td>
<td>10.50</td>
</tr>
<tr>
<td>DH35</td>
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<td>30.18</td>
<td>30.18</td>
<td>16.20</td>
</tr>
<tr>
<td>DH36</td>
<td>9.61</td>
<td>30.80</td>
<td>30.80</td>
<td>15.00</td>
</tr>
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<td>DH40</td>
<td>35.17</td>
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<td>32.90</td>
<td>7.20</td>
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<tr>
<td>DH41</td>
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<td>31.03</td>
<td>31.03</td>
<td>10.60</td>
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<tr>
<td>DH42</td>
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<td>31.13</td>
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<td>29.29</td>
<td>29.29</td>
<td>10.30</td>
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<td>23.00</td>
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<tr>
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<td>23.09</td>
<td>23.09</td>
<td>9.90</td>
</tr>
<tr>
<td>DH60</td>
<td>13.20</td>
<td>22.57</td>
<td>22.57</td>
<td>10.10</td>
</tr>
<tr>
<td>DH61</td>
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<td>24.25</td>
<td>24.25</td>
<td>16.10</td>
</tr>
<tr>
<td><strong>Range</strong></td>
<td><strong>1.93-264.10</strong></td>
<td><strong>22.57-85.13</strong></td>
<td><strong>22.57-41.13</strong></td>
<td><strong>5.70-17.60</strong></td>
</tr>
</tbody>
</table>
Table 5. The eco-physiological parameters that were measured during samplings of *Dioscorea hispida* in the midday (12-2 PM).

<table>
<thead>
<tr>
<th>Accession No.</th>
<th>Light Concentration (µmol)</th>
<th>Humidity (%)</th>
<th>Temperature (°C)</th>
<th>Chlorophyll Content (CCI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DH04</td>
<td>130.86</td>
<td>71.53</td>
<td>33.12</td>
<td>6.30</td>
</tr>
<tr>
<td>DH05</td>
<td>152.53</td>
<td>61.17</td>
<td>33.74</td>
<td>4.70</td>
</tr>
<tr>
<td>DH06</td>
<td>39.60</td>
<td>65.87</td>
<td>33.50</td>
<td>7.40</td>
</tr>
<tr>
<td>DH25</td>
<td>121.20</td>
<td>72.51</td>
<td>32.57</td>
<td>9.40</td>
</tr>
<tr>
<td>DH26</td>
<td>49.04</td>
<td>78.69</td>
<td>31.83</td>
<td>5.50</td>
</tr>
<tr>
<td>DH27</td>
<td>128.04</td>
<td>81.41</td>
<td>31.84</td>
<td>8.10</td>
</tr>
<tr>
<td>DH28</td>
<td>93.87</td>
<td>76.47</td>
<td>32.08</td>
<td>16.30</td>
</tr>
<tr>
<td>DH29</td>
<td>51.90</td>
<td>67.00</td>
<td>34.79</td>
<td>16.20</td>
</tr>
<tr>
<td>DH30</td>
<td>36.85</td>
<td>61.90</td>
<td>35.59</td>
<td>9.60</td>
</tr>
<tr>
<td>DH37</td>
<td>1005.80</td>
<td>32.62</td>
<td>32.62</td>
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<td>DH38</td>
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<td>37.45</td>
<td>9.90</td>
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<tr>
<td>DH39</td>
<td>704.60</td>
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</tr>
<tr>
<td>DH43</td>
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<td>34.30</td>
<td>5.40</td>
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<tr>
<td>DH44</td>
<td>866.20</td>
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</tr>
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<td>9.50</td>
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<tr>
<td>DH51</td>
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<tr>
<td>DH52</td>
<td>15.42</td>
<td>29.47</td>
<td>29.47</td>
<td>14.20</td>
</tr>
<tr>
<td>DH53</td>
<td>104.27</td>
<td>28.15</td>
<td>28.15</td>
<td>15.80</td>
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<td>DH54</td>
<td>5.64</td>
<td>29.99</td>
<td>29.99</td>
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<tr>
<td>DH55</td>
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<td>30.53</td>
<td>30.53</td>
<td>11.80</td>
</tr>
<tr>
<td>DH62</td>
<td>3.63</td>
<td>24.09</td>
<td>24.09</td>
<td>11.00</td>
</tr>
<tr>
<td>DH63</td>
<td>25.12</td>
<td>24.31</td>
<td>24.31</td>
<td>11.40</td>
</tr>
<tr>
<td>DH64</td>
<td>24.61</td>
<td>25.43</td>
<td>25.43</td>
<td>17.00</td>
</tr>
</tbody>
</table>

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).

<table>
<thead>
<tr>
<th>Accession No.</th>
<th>Light Concentration (µmol)</th>
<th>Humidity (%)</th>
<th>Temperature (°C)</th>
<th>Chlorophyll Content (CCI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DH07</td>
<td>77.14</td>
<td>54.69</td>
<td>38.59</td>
<td>6.70</td>
</tr>
<tr>
<td>DH08</td>
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<td>60.38</td>
<td>35.80</td>
<td>11.30</td>
</tr>
<tr>
<td>DH09</td>
<td>4.82</td>
<td>64.93</td>
<td>33.10</td>
<td>14.90</td>
</tr>
<tr>
<td>DH19</td>
<td>200.10</td>
<td>61.51</td>
<td>30.12</td>
<td>7.60</td>
</tr>
<tr>
<td>DH20</td>
<td>197.81</td>
<td>74.80</td>
<td>34.20</td>
<td>12.00</td>
</tr>
<tr>
<td>DH21</td>
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<td>65.25</td>
<td>35.40</td>
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</tr>
<tr>
<td>DH65</td>
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<td>26.64</td>
<td>26.64</td>
<td>13.20</td>
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<tr>
<td>DH66</td>
<td>1.12</td>
<td>26.36</td>
<td>26.36</td>
<td>15.20</td>
</tr>
<tr>
<td>DH67</td>
<td>11.28</td>
<td>27.10</td>
<td>27.10</td>
<td>13.20</td>
</tr>
<tr>
<td><strong>Range</strong></td>
<td><strong>1.12-200.10</strong></td>
<td><strong>26.36-74.80</strong></td>
<td><strong>26.36-38.59</strong></td>
<td><strong>6.70-15.20</strong></td>
</tr>
</tbody>
</table>

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 °C to 44.04 °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*.](image)
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.

![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.](image)

**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.
ACKNOWLEDGEMENTS

The authors gratefully acknowledge the financial aid by the Unit Perancangan Ekonomi Negeri Terengganu (UPEN) or Economic Planning Unit of Terengganu State, Malaysia for sponsoring the research on *Dioscorea hispida*.

REFERENCES


