Genetic Diversity of Seed Storage Protein in Selected Melastomataceae and Fagaceae from Tasik Kenyir

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Abstract This paper presents the results of a study on genetic diversity from seed storage proteins of six species of plants from two families using sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE). The two families were; Melastomataceae (Senduduk); *Melastoma malabathricum, Clidemia hirta* and *Lijndenia laurina* and Fagaceae (Berangan, chesnut or timber); *Lithocarpus wallichianus*, *Castanopsis lucida* and *Castanopsis schefferiana.* The total numbers of polypeptide bands of Melastomataceae which resolved in 14% gel SDS were 33 bands, where three bands (9%) were monomorphic, while 30 bands (91%) were polymorphic with the size ranging from 12.89 to 95.24 kDa. In Fagaceae a total of 55 polypeptides bands were produced. Out of these one band (2%) was monomorphic among all three species and 54 (98%) were polymorphic. Cluster analysis for seed storage protein clearly distinguished between six species. Seed storage protein profiling of Melastomatecea and Fagaceae from this study highlighted a high degree of genetic diversity within the families. The high polymorphism shown by each family revealed that all the species are genetically variable. Therefore, it is recommended that the species should be conserved in order to establish in their natural habitat.

Keywords Fagacea · Melastomatacea · Genetic diversity · Seed storage protein · Tasik Kenyir

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Introduction

Genetic diversity is the heritable variation within and between population of organisms and the conservation of plant genetic diversity is essential for present and future human wellbeing (Rao and Hodgkin 2002). The loss of genetic diversity of plants can effect in the loss of valuable and desirable traits and reduce options to use unexplored resources for food production, industry and medicine (Falk et al. 2001). A better understanding of genetic diversity of plant and its distribution is essential for its conservation and use. At the same time will improve our understanding of the taxonomy and origin and evolution of plant species of interest, therefore, some population need a sufficient genetic diversity to survive to a new condition for adaptation (Rao and Hodgkin 2002; Falk et al. 2001).

Morphological trait can be used for assessing genetic diversity in plants but it is often influenced by the environmental factors (Siddique and Naz 2009). Biochemical markers such as proteins can served as genetic markers as they are direct products of active genes and are quite polymorphic, heritable and highly independent of environmental fluctuations (Chittora and Purohit 2012; Gepts 1990). In order to study the genetic diversity of plant, sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) is one of the technique can be used to analyse the variation of polypeptide band of seeds storage protein (Ungureanu et al. 2007). In this study, two plant families have been chosen in order to study their genetic diversity; (a) Melastomataceae and (b) Fagaceae. Melastomataceae plants originate in the tropic and subtropic regions, with a total of more than 4000 species in the world and 22 species found in Southeast Asian region including Malaysia (Joffry et al. 2011). It is ubiquitous, species-rich, and dominant in the forest area (Silveira et al. 2013) either as herbs or shrubs or tress and the leaf lamina is dorsiventral or centric (Watson and Dallwitz 1992). Three species of Melastomataceae that have been chosen in this research were *Melastoma malabathricum* (*Mm*, Senduduk Ungu), *Clidemia hirta* (*Ch*, Senduduk Bulu) and *Lijndenia laurina* (*Ll*, Nipis Kulit). *M. malabathricum* has ethno-pharmacological value where certain parts of the plant can be used in medicinal treatment such as the leaves, shoots, barks, seeds and roots (Joffry et al. 2011). *M. malabathricum* (Fig. 1a) has gained herbal status and have been used to treat diarrhoea, dysentery, haemorrhoids, cuts and wounds, toothache, and stomach-ache (Joffry et al. 2011) while *C. hirta* (Fig. 1b) also has been used as medicinal treatment for traditional folk medicine to treat some bacterial infection (Dianita et al. 2011), treat for skin infection (Franca et al. 1996), and treat for venom fever (Latiff and Mat-Salleh 2002). Whereas, there is no known study on the medicinal status of *L. laurina* (Fig. 1c).

Another plant family have been studied was Fagaceae or chestnut family which have four genera and 64 species were found in the Malay Peninsula (Ng 1991) either as trees or shrubs and the leaf lamina is dorsiventral to bifacial (Watson and Dallwitz 1992). Many species of Fagaceae have important economic uses such as oak, chestnut, and beech are commonly used as timber for floors, furniture, cabinets, and wine barrels and it is widespread in the Northern hemisphere, with a centre

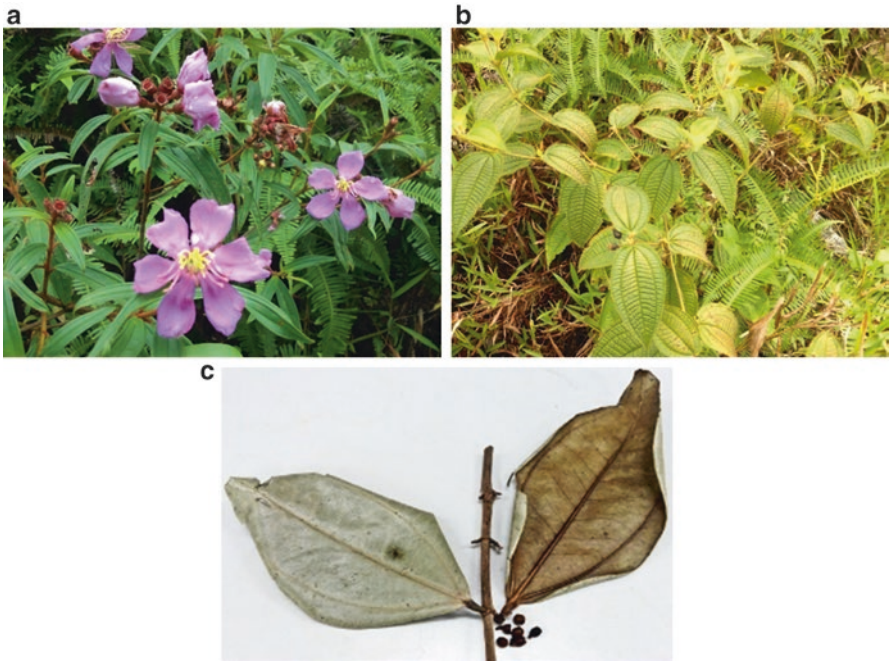

Fig. 1 *Melastoma malabathricum, Mm* (**a**), *Clidemia hirta, Ch* (**b**) and *Lijndenia laurina, Ll* (**c**)

of diversity found in tropical Southeast Asia (Nixon 1993; Manos et al. 2001). To understand more about the genetic diversity of Fagaceae, there were three species of Fagaceae have been studied in this research such as *Castanopsis lucida (Cl)* (Fig. 2a), *Castanopsis schefferiana (Cs)* (Fig. 2b) and *Lithocarpus wallichianus (Lw)* (Fig. 2c) were used in this study.

Information regarding to the application of molecular technique in genetic study of Melastomatacea and Fagacea is very little. Therefore, this study was conducted to assess the genetic diversity of this two plants by using sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) with two different protein buffers; (a) Phosphate buffer and (b) Tris-HCL buffer (Chittora and Purohit 2012). The polypeptide bands were analyzed by using Dendrogram Unweighted Pair Group Method with Arithmetic (D-UPGMA) software.

Polymorphism of Polypeptide Bands

In this study, the protein samples were extracted by using two different buffers which were phosphate buffer and Tris-HCl extraction buffer. The range of the protein concentration from 0.1 to 2.0 mg/ml. Based on Jaccard similarity matrix for binary data, the relationships among species have been determined. Figure 3 shows

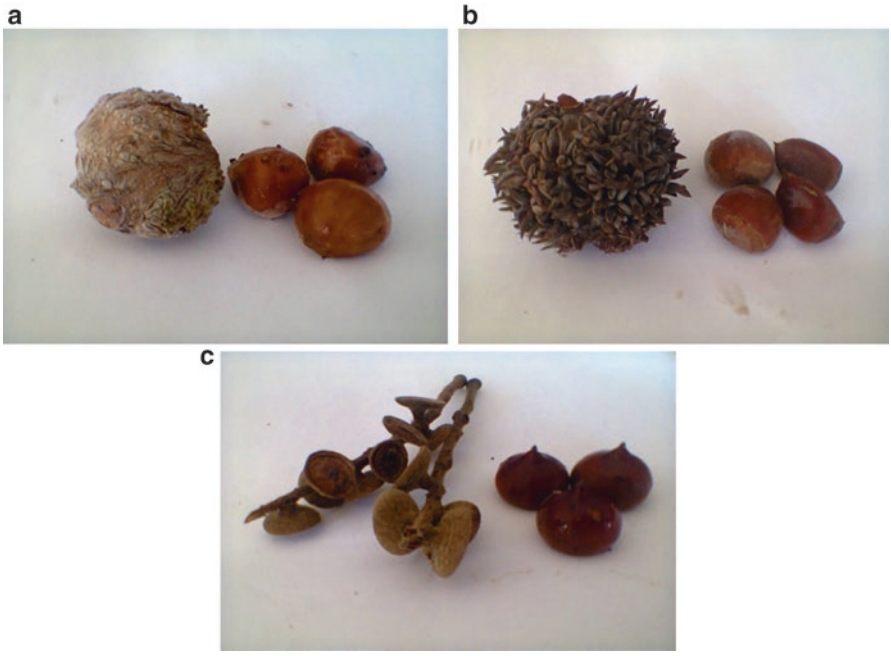

Fig. 2 *Castanopsis lucida, Cl* (**a**), *Castanopsis schefferiana, Cs* (**b**) and *Lithocarpus wallichianus, Lw* (**c**). (From Wan Bayani et al. 2013)

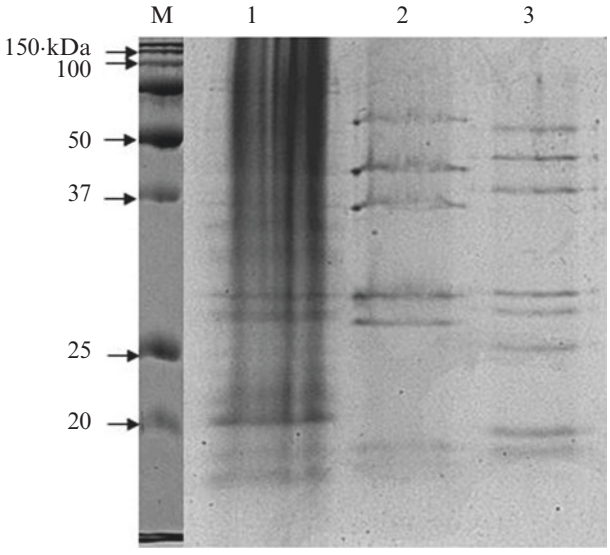

Fig. 3 Protein profile of 14% gel SDS-PAGE. Lane 1: *Mm*, Lane 2: *Ch*, Lane 3: *Ll*. M: protein marker

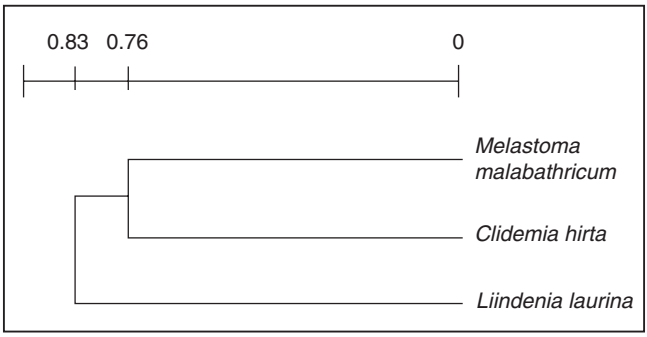

Fig. 4 Dendrogram obtained from polypeptide bands analysis using D-UPGMA showing genetic distance among three species of Melastomataceae

the protein profile of Melastomatacea. The total protein band of seed protein extracts of three species of Melastomataceae from both buffers was 33 protein bands (range from 12.9 to 95.2 kDa), 30 were polymorphic (91%) and only three bands were monomorphic (9%) or common (32.12 kDa, 17.09 kDa and 15.34 kDa) among three species of Melastomataceae. However, three bands have been shared between *Mm* and *Ch* (51.86 kDa, 45.49 kDa and 28.17 kDa), two shared bands between *Mm* and *Ll* (63.33 kDa and 29.48 kDa), whereas between *Ch* and *Ll* only shared one band (64.42 kDa). The cluster analysis was done by using Unweighted Pair-Group Method of Arithmetic (UPGMA) to produce dendrogram (Fig. 4). When the lower similarity, it is means that higher genetic distance among species. *Mm* and *Ch* were clustered in the same group which the genetic distance was 0.76 whereas the genetic distance between *Mm* and *Ch* with *Ll* was 0.83 (the highest genetic distance). The similarity between *Mm* and *Ch* was more than between *Mm* and *Ll* or the most closely related species were between *Mm* and *Ch,* may be from their morphology, *Mm* and *Ch* look similar to each other.

In Fagacea a total of 55 polypeptide bands were recorded from three species by using both extraction buffers (range from 151 to 380 kDa). Figure 5 shows the protein profile of Fagacea. Fifty-four bands were polymorphic (98%) and only one band (190 kDa) was monomorphic (2%) or common among three species. Five bands have been shared between C*l* and *Cs* (330 kDa, 303 kDa, 180 kDa, 175 kDa and 166 kDa), four shared bands between *Cl* and *Lw* (196 kDa, 194 kDa, 193 kDa and 186 kDa), whereas between *Cs* and *Lw* shared two band (172 kDa and 182 kDa). The genetic distance between *Cl* and *Cs* was 0.77. Meanwhile the genetic distance between *Cl* and *Cs* to *Lw* was 0.88 (Fig. 6). The similarity between *Cl* and *Cs* was more than between *Cl* and *Lw* or the most closely related species were between *Cl* and *Cs* may be from the appearance of their seed, *Cl* and *Cs* look similar to each other.

As the comparison the genetic diversity by using SDS-PAGE in some plants indicated the following results; 5–11% polymorphism in three genotypes of *Abrus precatorius* (Chittora and Purohit 2012), 5–84% polymorphism in ten genotypes of

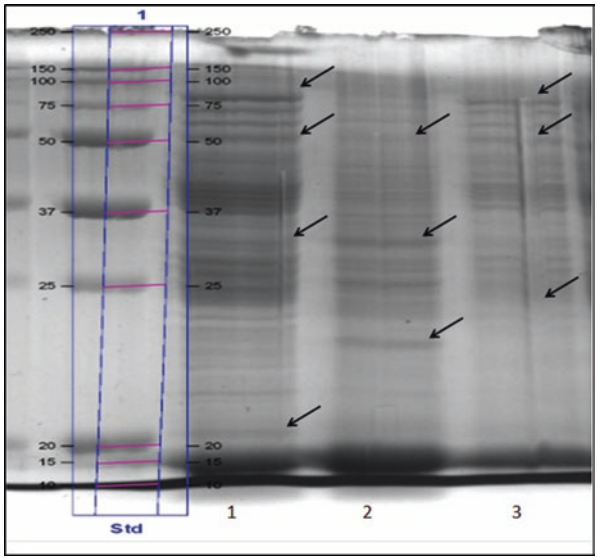

Fig. 5 Protein profile of 12% gel SDS-PAGE. Lane 1: *Cl*, Lane 2: *Cs*, Lane 3:*Lw*. Std: protein marker

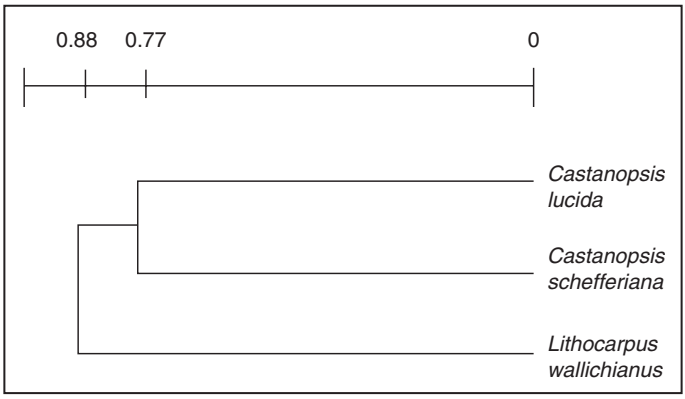

Fig. 6 Dendrogram of three species of Fagaceae. (From Wan Bayani et al. 2013)

wheat (Siddique and Naz 2009), 0–60% polymorphism in twenty genotypes of walnut (Khan et al. 2010), 0–80% polymorphism in eleven genotypes of *Oryza sativa* (Inamullah et al. 2010) and 0–100% polymorphism in nineteen genotypes of *Capsicum* (Akbar et al. 2010).

Conclusion

In conclusion, the genetic diversity of six species of two plants family were successfully determined from seed storage proteins by using SDS-PAGE. The seeds have an important genetic information of the plant and it must be conserved to avoid genetic loss by environmental factors or some diseases. This information will be useful as the basic genetic information for the species. The Melastomatacea is very useful as ethnomedicine since many races using this as their medicine and for Fagaceae, this family has important economic uses especially in timber industry.

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