GENERAL DISCUSSION

Since a large number of morphological characters is known for *Freycinetia* and *Pandanus* species, it appears useful to consider their use in identifying species of Pandanaceae from Java. Characters of leaf shape, leaf apex, the way of leaf auricle fragmenting, and the type of female inflorescence were found useful in delimitation and identification of Javanese *Freycinetia*, while characters of present or absent prop roots, the surface of stem, leaf shape, leaf apex, the armature of leaf apex, the texture of leaves in dry state, the amarture of leaf margin and midrib, the colour of leaf marginal and midrib teeth, the distinctness of tertiary cross vein, present or absent apical ventral pleats, the position of infructescence, phalange shapes, the stigma shape, the position of seed chamber, are proved useful for distinguishing species among Javanese *Pandanus*.

Systematic study of *Pandanaceae* in Java revealed that based on morphological characters, seven species of *Freycinetia* and sixteen species, two varieties and two cultivars of *Pandanus* can be recognized. This number is hardly different from that given by Backer and Bakhuizen van den Brink (1968) in their “Flora of Java”. Account of the *Pandanaceae* in “Flora of Java” of Backer and Bakhuizen van den Brink (1968), seven species of *Freycinetia* and fifteen species of *Pandanus* are reported from Java. The result of this study is almost similar with Stone’s treatment on *Pandanaceae* of Java (1972). The similarity of the latter study to the former is on the status of *P. furcatus* Roxb. Backer and Bakhuizen van den Brink (1968) treated the *P. bantamensis* Koord., *P. pseudolais* Warb., and *P. scabrifolius* Martelli were included in one species, i.e *P. furcatus* Roxb., whereas in this study and Stone’s treatment those three species were considered three different species.

Besides gross morphological characters, anatomical character of leaf are used as well. They have proved useful especially for distinguishing between closely related species, because anatomical characters can provide information and strengthening conclusion based on morphological characters. Some of these anatomical characters are so diagnostic, because the appearance of particular anatomical character seems sufficiently constant.
The tissue which furnish the characters are especially the epidermis, the stomatal complex, the hypodermis, the crystal cells, the chlorenchymatous fibers and fibrous states. Stomata in *Freycinetia* of Java is relatively uniform, the differentiation is only found in the quantitative manner, except in the stomata of *F. sumatrana* and in *Pandanus*. Stomata in *F. sumatrana* are arranged in neat longitudinal rows and alternating with a row of calcium oxalate crystals, while stomata in *Pandanus* were variable, and the variation largely involved papillae developed on subsidiary and neighbouring cells. The range of variation in the epidermal tissue including the stomata proves to be a great value in identification of *Pandanus* species in Java. Anatomical data are also of particular view who need to identify incomplete material.

Since morphological characters represent only a small portion of the plant genome and are influenced by environmental factors, they have limited utility for describing potentially complex genetic structure which may exist within and between taxa (Avise 1993), some of species description becomes insufficient for an accurate recognition of the species concerned, with the consequence that confusion sometimes occured, particularly for closely related species, such as *P. furcatus* complex and *P. tectorius* complex.

Various molecular approach have been devised to overcome these constraint (Soltis & Soltis 1998). The stability and the power of nucleic acid markers provide a clear advantage that has been exploited in different population studies of the family, therefore an effort has been made to redescribe these species in detail, using molecular data, i.e comparative sequence of *atpB-rbcL* IGS. These markers of genetic variation are generally independent of environmental factors and more numerous than phenotypic characters, thereby providing a clearer indication of the underlying variation in the genome (Avise 1993).

Anatomical data along with molecular data have been revolutionized taxonomic concept. A diverse array of molecular approaches is now available to the plant systematist, including comparative sequencing and various PCR-based techniques, such as ISSR (Soltis & Soltis 1998), and molecular data served to confirm taxonomic conclusion based on morphological and anatomical data since
taxonomist in general, aware of the need to examine many source of potential evidence.

Morphological, anatomical and comparative sequence data of \( atpB-rbcL \) IGS were able to solve the taxonomical problem of \( P. \) furcatus complex and \( P. \) tectorius complex and as a result \( P. \) bantamensis Koord., \( P. \) pseudolais Warb., and \( P. \) scabrifolius Martelli were treated as three different species, and \( P. \) odoratissimus L.f and \( P. \) tectorius var. littoralis were treated as two different species.

In this study, ISSR (Inter Simple Sequence Repeat) has been used to determine genetic diversity among six species of \( Freycinetia \) and thirteen species of \( Pandanus \) in Java. The result showed that six species of \( Freycinetia \) and thirteen species of \( Pandanus \) from Java have high genetic diversity, although \( Freycinetia \) has a bit lower of genetic diversity than \( Pandanus \).

PCA (Principal Component Analysis) was conducted to view the clustering and species relationship in Javanese \( Pandanus \) and Javanese \( Freycinetia \). The result of PCA analysis for thirteen Javanese \( Pandanus \) showed different clustering pattern and species relationship compare to the cluster analysis, even though divided into three groups also. In PCA analysis, group I consisted of seven species, while in cluster analysis group I consisted of six species; group II in PCA analysis consisted of two species, while in cluster analysis group II consisted of six species; and group III in PCA analysis consisted of four species, while in cluster analysis only consisted of one species, but for those species which showed the highest affinity was the same for both analysis, i.e \( Pandanus \) nitidus and \( P. \) scabrifolius indicating the close relationship to each other. Some characteristics, linear leaf, coriaceous leaf, whitish leaf base, apex gradually long tapering to subulate apex, leaf margin armed with stout spine, smooth apical ventral pleat, straight peduncle and stigma on style placed \( P. \) nitidus and \( P. \) scabrifolius in the same line and high similarity between these species was observed in our analysis; whereas the result of PCA analysis for six Javanese \( Freycinetia \) showed similar clustering pattern and species relationship to the cluster analysis, but for those species which showed the highest affinity was different in both analysis. In cluster analysis, \( Freycinetia \) sumatrana showed the
highest affinity with *F. imbricata*, while in PCA analysis, *Freycinetia sumatrana* showed the highest affinity with *F. javanica*. The reason for this difference is that in cluster analysis, all band characters were used in calculating to build dendrogram, while in PCA analysis only a few band character were used, i.e only 62.2% in *Pandanus* and 87.5% in *Freycinetia* the variability contained in the original data support the division. Species relationship resulted from both cluster analysis and PCA analysis was not in accordance with species relationship resulted from morphological character. In cluster and PCA analysis it was shown that *P. nitidus* and *P. scabridolius* had close relationship, but from morphological character it was shown that *P. scabridolius* had close relationship either with *P. bantamensis* or *P. pseudolais*, and not with *P. nitidus*. This result indicated that ISSR marker must be used cautiously for estimating species relationship.

As interpreted by Warburg (1900) and most later authors, *P. tectorius* Parkinson is a polymorphic and widespread species, and numerous cultivars exist and are highly variable, commonly unbranched to branched, shrubs to trees, thornless or thorny, green or green striped leaves, e.g *Pandanus tectorius* cv. Sanderi. Many species or cultivars are important shrubs or trees in garden around houses in Java, viz. *P. dubius* Spreng., *P. spurius* Miq. cv. Putat, *P. tectorius* Parkinson. cv. Sanderi, and *P. utilis* Bory.

Generally, species with small geographically range tend to maintain less genetic diversity than that of geographically widespread species (Hamrick & Godt 1989). Based on this assumption, a low level of genetic diversity within species is expected in *Freycinetia*, that are lower than the genetic diversity in some wide geographical range of species of the genus *Pandanus*.

High genetic diversity is important. Beside as a safeguard against coevolving biotic factors such as pests and disease (Namkoong 1986), it is also allowing *Freycinetia* and *Pandanus* species to adjust to the ever-changing environment in Java, whether the changes are due to the natural or human factors (Chamberlain and Hubert 2001; Hedrick 2004). In Java, *Pandanaceae* are found in various forest type but mostly in hill forest and lower montane forest. Illegal and drastic destruction of hill forest into agriculture land and buildings have a
significant negative effect on the amount of available habitat, and may lead to the disappearance of *Pandanaceae* species from Java.