ABSTRACT

The purpose of this study was to characterize the variation pattern that exists in the wild *Musa acuminata* (AA) populations, widely distributed in Malaysia. Fourteen populations of *Musa acuminata*, which included subspecies *malaccensis*, *siamea* and *truncata* were collected from different locations including Selangor, Pahang, Perak, Kelantan, Kedah and Terengganu for studies on variation within and between populations, based on morphological characters, biochemical, ploidy and genomic DNA content. Techniques of numerical taxonomy were employed to determine the variation pattern and these included use of the clustering method and principal component analysis. Ploidy level and genomic DNA content were determined by flow cytometry (FCM). Anthocyanins of different wild banana bracts were determined through high performance liquid chromatography (HPLC). *Musa acuminata ssp. malaccensis* suckers were screened in the 'Hot Spot' for Fusarium wilt resistance studies. Similarly seed progenies from five *Musa acuminata ssp. malaccensis* populations were raised through zygotic embryo culture and screened for resistance to Fusarium wilt disease by using seedlings planted in a double tray technique. Seedlings that appeared to be resistant or susceptible were further characterized by using randomly amplified polymorphic DNA markers (RAPD). Finally a tetraploid was induced by colchicine treatment in *Musa acuminata ssp. malaccensis* characterized as resistant to *Fusarium oxysporum* f. sp. *cubense* Race 4.

The fourteen wild *M. acuminata* populations appeared very variable and morphological characters showed greater variability compared to ploidy, anthocyanin and genomic DNA content. The two main subspecific groups of these fourteen populations are *malaccensis* (lowland) and *truncata* (highland). These two groups could further be classified based on

anthocyanin composition and genomic DNA content. No variation was observed for ploidy level among *Musa acuminata* accessions (2n =22).

For the most variable *M. acuminata ssp. malaccensis*, further characterization was carried out to check its response to Fusarium wilt Race 4. Screening of the suckers in the Fusarium "Hot Spot" for two years revealed high resistance to *Fusarium oxysporum* f. sp. *cubense* Race 4. However seed progenies derived from *in vitro* zygotic culture of the *Musa acuminata ssp. malaccensis* were found to be segregating for disease resistance. Optimized *in vitro* zygotic culture resulted in more than 90% germination after 1-2 weeks. Seed progenies showed a large RAPD polymorphism. Four primers OPA-03, primer-24, primer-25 and primer-27 produced specific banding patterns of sizes 500bp, 300bp, 1.5Kbp, and 500bp respectively in susceptible plants. Primer-21 produced a band of 1.0Kbp consistently present in resistant progenies.

Polyploids were induced in seed progenies of *M. acuminata ssp. malaccensis* resistant to FOC Race 4, using 0.5% (w/v) colchicine with 2.0% (v/v) DMSO. Ploidy of the treated plantlets was determined through flow cytometry (FCM). Colchicine treatment resulted in 15% tetraploids (2n=4x=44). Tetraploids were further confirmed through chromosomal counting and based on morphological comparison between diploids, tetraploids and mixoploids.

ABSTRAK

Kajian ini bertujuan untuk mencirikan corak variasi yang wujud di dalam populasi pisang liar, *Musa acuminata* (AA) yang tersebar luas di Malaysia. Empat belas populasi meliputi subspesis *malaccensis, siamea* dan *truncata* telah dikumpulkan dari pelbagai lokasi untuk mengkaji variasi di dalam dan di antara populasi berdasarkan ciri-ciri morfologi, penanda biokimia dan kandungan genomik DNA. Teknik-teknik taksonomi numerikal digunakan untuk menentukan corak variasi termasuk kaedah klasifikasi dan analisis komponen prinsipal. Tahap ploidi dan kandungan genomik DNA ditentukan menggunakan `flow cytometry' (FCM). Komposisi anthocyanin di dalam jantung pisang liar ditentukan dengan `high performance liquid chromatography' (HPLC). Anak-anak pisang *Musa acuminata ssp malaccensis* diuji di `Hot Spot' untuk kajian kerintangan terhadap penyakit layu Fusarium. Begitu juga anak-anak benih yang dihasilkan melalui kultur zigot embrio daripada lima populasi *Musa acuminata ssp. malaccensis* diuji kerintangan menggunakan teknik `double tray'. Anak-anak pokok yang didapati sebagai rintang atau rentan kemudiannya dicirikan menggunakan amplifikasi rawak penanda-penanda polimorfik (RAPD). Seterusnya, suatu tetraploid yang terhasil dengan pengaruhan melalui rawatan *Musa acuminata ssp. malaccensis* dengan kolkisina, didapati mempunyai ciri-ciri kerintangan terhadap *Fusarium oxysporum* f. sp. *cubense* Ras 4.

Kesemua 14 populasi pisang liar *Musa acuminata* didapati mempunyai variasi dan ciri-ciri morfologi menunjukkan kepelbagaian yang tinggi berbanding tahap ploidi, kandungan anthocyanin dan kandungan genomik DNA. Dua kumpulan utama bagi populasi ini adalah *malaccensis* (tanah pamah) dan *truncata* (tanah tinggi). Kedua-dua kumpulan ini kemudiannya dikelaskan berdasarkan komposisi anthocyanin dan kandungan genomik DNA. Tiada variasi bagi tahap ploidi diperhatikan di kalangan sampel-sampel *Musa acuminata* (2n=22).

Bagi variasi *Musa acuminata ssp. malaccensis* tertinggi, pencirian seterusnya dilakukan untuk mengenalpasti tindakbalas terhadap penyakit layu Fusarium Ras 4. Ujian terhadap anak-anak pokok di `Hot Spot' selama 2 tahun menunjukkan kerintangan yang tinggi terhadap *Fusarium oxysporum* f. sp. *cubense* Ras 4. Bagaimanapun, anak-anak benih yang terhasil dari kultur zigot *Musa acuminata ssp. malaccensis* didapati bersegregasi terhadap kerintangan penyakit. Kultur zigot *in vitro* menghasilkan percambahan melebihi 90% selepas 1-2 minggu. Anak-anak benih ini juga menunjukkan polimorfism yang besar dalam penanda-penanda RAPD. Empat primer iaitu primer OPA-03, primer-24, primer-25 dan primer-27 dikenalpasti dapat menghasilkan corak jalur spesifik bersaiz 500bp, 300bp, 1.5Kbp, and 500bp masing-masing di dalam pokok-pokok yang rentan. Primer-21 menghasilkan jalur bersaiz 1.0kbp yang wujud secara konsisten di dalam progeni-progeni yang rintang.

Pengaruhan poliploidi progeni-progeni *M. acuminata ssp. malaccensis* yang rintang terhadap FOC Ras 4 dilakukan menggunakan campuran kolkisina pada 0.5% (w/v) dan DMSO pada 2.0% (v/v). Tahap ploidi pokok-pokok yang dirawat ditentukan menggunakan flow cytometer (FCM). Rawatan kolkisina menghasilkan 15% tetraploid (2n=4x=44). Tetraploid seterusnya disahkan melalui pengiraan kromosom dan perbandingan morfologi di antara diploid, tetraploid dan mixoploid.