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Utilization of STMS markers to verify admixture in clonal progenies of *Acacia* mapping populations and relabelling using assignment tests

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ABSTRACT: Clonal propagation is widely used for *Acacia* breeding and commercial planting. When a large number of clones are handled, problems with mixings are commonly confronted. Detection of admixture in *Acacia* clones based on morphology particularly at seedling stage is not feasible. However, molecular markers are commonly used to test the genetic fidelity of planting materials. This paper reports the detection of mislabelling in *Acacia* clonal progenies using a sequence tagged microsatellite (STMS) genetic marker system. Progenies from two mapping populations were clonally propagated and field planted for phenotypic and genotypic evaluation at three locations in Malaysia: (a) Forest Research Institute Malaysia field station at Segamat, Johor, (b) Borneo Tree Seeds and Seedlings Supplies Sdn, Bhd. (BTS) field trial site at Bintulu, Sarawak and (c) Asiaprima RCF field trial site at Lancang, Pahang. During field planting mislabelling was reported at Segamat, Johor and similar was suspected for Bintulu, Sarawak. Screening revealed mislabelling events in both populations. A total of 18.52% mislabelling incidences were detected from both sites, of which 17.39% of mislabelling was detected for fibre length cross and 20% for wood density cross. The assignment test efficiently reestablished the mislabelled ramets to the respective clones. Future studies should be focused on the utilization of a higher number of markers, e.g. SSR or SNPs to increase a discrimination power. A high number of SNPs can be generated within a short period of time compared to SSR, but SNPs could be cost inhibitory. Multiplexing microsatellite combinations along with sample bulking will further reduce the processing time when screening large populations. The use of assignment test would efficiently assign mislabelled individuals to the respective clones. It is concluded that checking for mislabelling is imperative for future breeding and for analyses such as QTL mapping where a correlation between genotypic and phenotypic data is determined.

Keywords: tree breeding; DNA markers; quantitative trait loci (QTL); linkage mapping

Acacias are an important global resource, more than 3.5 million hectares are grown in Asia, Africa and South America (Midgley 2014). They are mainly used for timber, fuel wood, tanning, soil improvement and agroforestry. However, ever increasing demand for paper coupled with declining fibre supply from the forests of the world is forcing the pulp and paper industry to find technically and economically viable fibre sources to supplement forest-based resources (Jahan et al. 2008). It has been demonstrated that *Acacia* species like *A. mangium*, *A. auriculiformis*, *A. crassicarpa* and (*A. mangium* × *A. auriculiformis*) hybrid are suitable for timber and pulp production (Hardiyanto 2014). In Malaysia, high demand for the consumption of pulp and paper products has prompted the government to plan for the establishment of local pulp and paper industry through domestic production and also by ensuring steady supply

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of raw materials from sustainably managed forests as well as through development of commercial forest plantations. Development of 375,000 ha of forest plantations by planting of 25,000 ha per year reflects ambitious efforts achieving new goals under the Forest Plantation Program (Anonymous 2009). High priority is given to the planting of *Acacia* hybrid as the realization of its potential uses in various applications is increasing.

Acacias have displayed excellent growth but they have also been affected by various diseases e.g. root rot and wilt (Potter et al. 2006). For example, *A. mangium* is highly susceptible to heart rot disease. The conversely crooked, twisted trunk of *A. auriculiform* makes it unsuitable for timber production (Kojima et al. 2009). *Acacia* hybrids although prove superior than parents still carry inferior parental traits such as high lignin content, low wood density, small fibre length and problems associated with production of viable seeds and recalcitrant germination either naturally or through controlled pollination (Kijar 1992; Wickneswari, Norwati 1992). Hybrid seed production and their germination, and development of large hybrid populations for breeding have been resolved by establishing improved hybridization techniques (Sedgley et al. 1992). Similarly, an efficient micropropagation system to mass-produce *Acacia* hybrid clones has been established to support commercial planting and breeding programs (Aziah et al. 1999). The breeding of elite *Acacia* planting materials that have superior characteristics like growth, wood properties, low lignin content, straight stem form, adaptation to different soil types, resistance to pests and diseases and high pulp yield is important for the success of the local forest industry (Hardiyanto 2014).

Acacia breeding and commercial planting require large-scale production and cultivation of clonal progenies. The ability to achieve large genetic gains in a short time has made clonal propagation a widely accepted means for large-scale plantation programs of *Acacia*. The main aim of clonal propagation is to retain the genetic integrity of the propagated plants with respect to the parent tree so that the desirable traits of the parents are maintained. Large-scale planting requires the transportation of planting materials from production sites to planting areas. This process may result in mislabelling and inadvertent mixing of planting materials. Mislabelling and misplanting are common problems in forest plantation even with proper management and involvement of experienced workers (Harju, Muona 1989; Wheeler, Jech 1992; Kawauchi, Goto 1999). Early detection of plant mislabelling will provide simple solutions to the problem such as replanting the plants or simply correcting the label. However, late detection could lead to development of inevitably time-consuming solutions and unnecessary cost increase especially when the plants are used for future breeding programs. Thus, it is important to develop rapid and cost-effective strategies for assessing mislabelling. As it is difficult to detect the genetic identity of ramets in clonal seed orchards by visual inspections, more reliable tools are needed for this purpose. Morphological identification of *Acacia* is impossible at seedling stage due to phenotypic plasticity of clones. Molecular markers have proved to be very useful in distinguishing between related genotypes. Different markers both protein and DNA have successfully been reported for clonal verification in different tree species such as isozyme (Nuray, Kani 2009), RAPD (Scheepers et al. 1997), AFLP (Tripathi et al. 2006), SNPs (Takrama et al. 2014) and microsatellite markers (Moriguchi et al. 2005). Microsatellites are markers of choice due to high reproducibility, abundance, codominant nature, multiallelic, high power to discriminate, effectiveness and ease of scoring and amenable to automation (Butcher et al. 1998; Butcher et al. 2000; Ng et al. 2005; Liew 2007). Microsatellites have successfully been used for the identification of pollen parents (Jones et al. 2008), for clonal identification (Liesebach, Schneck 2007), cultivar identification (Moriya et al. 2010), and paternity analysis (Millar et al. 2008). In this paper, we describe a case study of mislabelling of *Acacia* hybrid progenies using STMS markers planted on two sites, i.e. Segamat, Johor and Bintulu, Sarawak in Malaysia. Screening for mislabelling in breeding and commercial plantations involves a large number of individuals, therefore assigning mislabelled individuals to their correct clones is difficult to achieve manually. Assignment tests have been extensively used to assign unknown individuals to the population of origin. The method was first implemented by Petkau et al. (1995) and has been used successfully in population and conservation biology studies to assign individuals to specific source populations (Primmer et al. 2000). The details of this method have been extensively reviewed by Cornuet and Luikart (1996); Davies et al. (1999); Pritchard et al. (2000). Here, we introduce assignment tests as a tool in *Acacia* mislabelling management to efficiently assign mislabelled individuals to relative clones.

MATERIAL AND METHODS

Sample collection. This study was focused on two *Acacia* mapping populations developed for wood density and fibre length traits derived from an inter-

Table 1. List of samples for wood and fibre length mapping populations collected from two locations, i.e. Segamat, Johor and Bintulu, Sarawak (description of clones and ramets screened for mislabelling and identities originally used for field planting)

SPL Segamat, Johor				BTS Nursery, Bintulu, Sarawak			
Wood density		fiber length		wood density		fiber length	
Clone number	sample code	clone number	sample code	clone number	sample code	clone number	sample code
Parent ♀	AA 6	parent ♀	AA3	parent ♀	AA 6	parent ♀	AA3
Parent ♂	AM20	parent ♂	AM22	parent ♂	AM20	parent ♂	AM22
Clone 12	138/2-A	clone 30	42/2-B	clone 27	329/9-A	clone 1	A-A
Clone 12	138/2-B	clone 30	42/2-C	clone 27	329/9-B	clone 1	A-B
Clone 12	138/2-C	clone 30	42/2-D	clone 27	329/9-C	clone 1	A-C
				clone 27	329/9-D	clone 1	A-D
				clone 27	329/9-E		
				clone 27	329/9-F		
Clone 16	247/2-A	clone 31	105/5-A	clone 28	309/4-A	clone 4	384/2-B
Clone 16	247/2-B	clone 31	105/5-B	clone 28	309/4-B	clone 4	384/2-C
Clone 16	247/2-C	clone 31	105/5-C	clone 28	309/4-C	clone 4	384/2-D
		clone 31	105/5-D	clone 28	309/4-D		
		clone 31	105/5-E	clone 28	309/4-E		
				clone 28	309/4-F		
Clone 21	237/4-A	clone 32	111/1-B			clone 5	B-A
Clone 21	237/4-B	clone 32	111/1-C			clone 5	B-B
Clone 21	237/4-C	clone 32	111/1-E			clone 5	B-C
Clone 23	231/4-A					clone 6	204/1-A
Clone 23	231/4-A					clone 6	204/1-B
Clone 23	231/4-A					clone 6	204/1-C
Clone 24	141/1-A					clone 14	212/6-A
Clone 24	141/1-B					clone 14	212/6-B
Clone 24	141/1-C					clone 14	212/6-B
Clone 24	141/1-D						
Clone 25	134/2-A					clone 16	384/1-A
Clone 25	134/2-B					clone 16	384/1-B
Clone 25	134/2-C					clone 16	384/1-C
Clone 26	180/1-A					clone 17	111/5-A
Clone 26	180/1-B					clone 17	111/5-B
Clone 26	180/1-C					clone 17	111/5-C
Clone 26	180/1-D						
						clone 23	169/4-A
						clone 23	169/4-B
						clone 23	169/4-C
						clone 26	375/2-C
						clone 26	375/2-D
						clone 26	375/2-B
						clone 27	201/1-A
						clone 27	201/1-B
						clone 27	201/1-C
						clone 28	114/1-A
						clone 28	114/1-B
						clone 28	114/1-C
						clone 28	114/1-D
Total = 7	total = 23	total = 3	total = 11	total = 2	total = 12	total = 11	total = 35

specific cross between *A. mangium* × *A. auriculiformis*. Three years old parents for both mapping populations were crossed at a FRIM field station at Bidor, Perak, Malaysia. Fresh leaf samples from both parents, i.e. AA6 × AM20, for wood density and AA3 × AM22, for fibre length mapping popu-

Table 2. Sequences of both forward and reverse primers, microsatellite motif, expected product size (bp) and annealing temperature (°C) for microsatellite markers Am465, AH2_1, AH16, and AH18

Microsatellite marker	Type of primer	Primer sequence (5'–3')	Microsatellite motif	Expected product size (bp)	Annealing temperature (°C)
Am465	forward reverse	TGGGTATCACTTCCACCATT AGGCTGCTTCTTTGTGCAGG	$(CT)_{12}$	154–194	57
AH2_1	forward reverse	GACAGAGGGAGCATTTTGTA CAGACAAGACCAGAGAATGAC	$(CT)_{23}$	142–162	50
AH16	forward reverse	GAGGGTAATGCTTCAAGTAGAC TGCGTGTCTCCCCACTACTC	$(GA)_{16}$	105–113	50
AH18	forward reverse	GGCGCAACTCTCTCTCTCT TTGGTCACTTAGCGCATGCC	$(CT)_6(CA)_6$	150–154	54

lations, were collected from marcots maintained at FRIM Kepong, Malaysia and stored at 4°C.

Study site I, located at a FRIM Field Station at Segamat, Johor where sample mislabelling was originally reported. There were 3–4 clonal progenies called ramets, derived from hybrid seed progenies for each mapping population available on this location. A total of 7 clones represented by 23 ramets from wood density cross and 3 from fibre length cross with 11 ramets were collected (Table 1). All leaf samples were thoroughly washed using distilled water, dried and subsequently drenched with 70% ethanol. Leaf tissue was ground to powder and DNA was extracted using a QIAGEN DNeasy Plant Mini Kit® (QIAGEN, Hilden, Germany) according to manual instructions. DNA was suspended in 100µl elution buffer and stored at –20°C.

To evaluate hybrid performance under different environmental conditions both mapping populations, i.e. wood density and fibre length, were planted on study site II located at Bintulu, Sarawak. For mislabelling analysis a total of 2 clones comprising 12 ramets from wood density cross and 11 clones comprising 35 ramets from fibre length cross were randomly collected (Table 1). Genomic DNAs were extracted as above mentioned.

The quality and quantity of the genomic DNA were determined using both agarose gel electrophoresis and NanoDrop 1000 Spectrophotometer wavelength readings (Thermo Fisher Scientific, USA). For gel electrophoresis 3 µl of genomic DNA was mixed with 2 µl of loading dye (Qiagen, Germany), DNA was loaded in 0.8% agarose gel and ran using 1 × TAE buffer for 3 h at 60 V. A DNA size marker λ *Hind III* (100 $\mu g \cdot ml^{-1}$) was used to determine DNA fragment size. The gel was stained with ethidium bromide solution (10 $\mu g \cdot ml^{-1}$) for 5 sec and destained using distilled water for 30 min before visualizing under UV light and documented by using an AlphaImager ™ 2200 (Alpha Innotech, USA) documentation system.

PCR amplification and microsatellite DNA analysis. Samples with high quality DNA were PCR amplified by using a set of four microsatellite markers, Am465, AH2-1, AH16 and AH18 (Table 2). PCR reaction was run in a total volume of 12.5 µl, containing 1.25 µl of 10 × PCR buffer, 0.375 µl $MgCl_2$ (1.5 mM), 1.25 µl of dNTPs (0.2 mM), 2.0 µl (0.4 µM) of forward and reverse primers, respectively, 0.10 µl of *Taq* polymerase (0.5 units, Intron Biotechnologies), and 1 ng/µl DNA template. PCR amplification profile was based on hot start with 1 cycle of denaturation at 94°C for 2 min followed by 35 cycles as follows: denaturation at 94°C for 30 s, annealing (57°C for Am465, 54°C for AH18 and 50°C for both AH2_1 and AH16 primer set) for 30 s, elongation step at 72°C for 20 s and a final extension at 72°C for 10 min. PCR reaction was stopped and stored at 10°C.

PCR reaction success was determined by running 1.5% agarose gel electrophoresis. For automated marker fragment analysis the forward primer was labelled with fluorescent dye and 1 µl of PCR product was mixed with 0.20 µl of standard indicator, i.e. GeneScan™-500 Lze™ (Applied Biosystems, USA). 8.80 µl of Hi_Di™ formamide solution (Applied Biosystems, USA) was added to give a total volume of 10 µl. The mixture was collected by centrifugation and denatured at 95°C for 4 min before loading and the fragment was analysed using an ABI PRISM 3100 genetic analyser (Applied Biosystems, USA). The fragment analysis data were analyzed using GeneMapper™ software version 4.0 (Applied Biosystems, USA).

Data analysis. Genotypes were scored based on a chromatograph analysis, which included allele sizes and composition, i.e. homozygous vs. heterozygous. Allele sizes were determined by comparison with an internal DNA marker reference standard.

Ramet identification and assignment to standard clones. Two levels of mislabelling were stud-

Table 3. Genotypes of parents and clonal progenies of wood density cross from SPL, Segamat Johor and Bintulu, Nursery Sarawak

Samples	Sample Code	Microsatellite markers		
		Am465	AH2_1	AH16
Segamat, Johor				
Parent ♀	AA 6	158/158	153/153	105/113
Parent ♂	AM20	190/190	162/164	111/111
Clone 12	138/2-A	158/190	153/164	105/111
	138/2-B	158/190	153/162*	111/113*
	138/2-C	158/190	153/164	105/111
Clone 16	247/2-A	158/190	153/164	105/111
	247/2-B	158/190	153/164	105/111
	247/2-C	158/190	153/164	105/111
Clone 21	237/4-A	158/190	153/162	105/111*
	237/4-B	158/190	153/162	111/113
	237/4-C	158/190	153/162	111/113
Clone 23	231/4-A	158/190	153/162	111/113
	231/4-A	158/190	153/162	111/113
	231/4-A	158/190	153/162	111/113
Clone 24	141/1-A	158/190	153/162	111/113
	141/1-B	158/190	153/162	111/113
	141/1-C	158/190	153/162	111/113
	141/1-D	158/190	153/162	111/113
Clone 25	134/2-A	158/190	153/164	111/113
	134/2-B	158/190	153/164	111/113
	134/2-C	158/190	153/162*	105/111*
Clone 26	180/1-A	158/190	153/162	111/113
	180/1-B	158/190	153/162	111/113
	180/1-C	158/190	153/162	111/113
	180/1-D	158/190	153/162	111/113
Bintulu, Sarawak				
Clone 29	329/9-A	158/190	153/164	111/113
	329/9-B	158/190	153/164	111/113
	329/9-C	158/190	153/162*	111/113
	329/9-D	158/190	153/164	105/111*
	329/9-E	158/190	153/164	111/113
	329/9-F	158/190	153/164	111/113
Clone 30	309/4-A	158/190	153/164	111/113
	309/4-B	158/190	153/164	111/113
	309/4-C	158/190	153/164	111/113
	309/4-D	158/190	153/164	111/113
	309/4-E	158/190	153/162*	105/111*
	309/4-F	158/190	153/164	105/111*

*Mislabelled ramets

ied. The first level was based on the presence of any mislabelling or mixing between mapping populations. The second level was based on mislabelling that occurred within the population. Mislabelling between mapping populations was determined based on the presence of progenies having wrong genotypes compared to the parents. Whereas mislabelling between ramets within each clone was observed by comparing the genotype of the ramets within each clone.

Mislabelled individuals were assigned by using GENECLASS2 software (Piry et al. 2004). The Bayesian model (Rannala, Mountain 1997) was used in the analysis with 'leave-one-out' option (Cornuet et al. 1999). Genotype likelihood ratios were calculated to evaluate statistical support (value > 1.0) and a threshold value of $P < 0.10$ was chosen for population assignment. Ramets with 99% probability of similarity were assigned to their reference clone.

RESULTS

A total of 35 ramets for wood density cross and 46 ramets for fibre length cross from both sites were screened for mislabelling testing (Tables 3 and 4). For wood density cross 23 ramets from SPL Segamat Johor and a total of 12 ramets from Bintulu, Sarawak, were genotyped. Similarly, for fibre length cross 11 ramets from Segamat, and 35 ramets from Bintulu were genotyped.

Screening was initially done using four microsatellite markers, i.e. Am465, AH2_1, AH16 and AH18. PCR products from three markers were successfully resolved based on chromatographs except AH18. AH18 produced only a single high intensity peak of allele size 150 bp whereas other allele could not be analysed due to the low intensity and ambiguous peak resolution. Therefore the fragment analysis data on AH18 marker was not used for further mislabelling analysis. Three SSR markers analysed were polymorphic in both parents used for mapping populations (Table 3).

Parental genotyping for wood density cross with Am465 locus revealed both female (AA6) and male parents (AM20) that were homozygous with allele sizes of 158/158 bp and 190/190 bp, respectively. The number of alleles ranged from 2 for Am465 followed by 3 each for AH2_1 and AH16, respectively (Table 5). The AH2_1 locus was homozygous for female parent (AA6) with 153/153 bp and heterozygous for male parent (AM20) with allele size of 162/164 bp. The third locus AH16 produced a heterozygous genotype for female parent (AA6) with allele size of 105/113bp and a homozygous genotype for male parent (AM20) with allele size of 111/111 bp (Table 3). Ramet genotypes segregated in Mendelian fashion. The Am465 locus was monomorphic and failed to identify any mislabelling event among ramets of wood density cross from both sites. AH2_1 and AH16 clearly demonstrat-

Table 4. Genotypes of parents and clonal progenies of fibre length cross from both SPL Segamat Johor and BTS, Nursery Sarawak

Samples	Sample code	Microsatellite markers		
		Am465	AH2_1	AH16
Segamat, Johor				
Parent ♀	AA 3	158/158	153/153	105/113
Parent ♂	AM22	155/194	142/162	109/111
Clone 1	A-A	155/158	142/153	105/111
	A-B	158/194	142/153	105/109*
	A-C	158/194	142/153	105/109*
	A-D	155/158	142/153	105/111
Clone 4	384/2-B	155/158	142/153	111/113
	384/2-C	155/158	142/153	111/113
	384/2-D	155/158	142/153	111/113
Clone 5	B-A	158/194	142/153*	105/111
	B-B	158/194	153/162	105/111
	B-C	158/194	153/162	105/111
Clone 6	204/1-A	155/158	142/153	105/111
	204/1-B	155/158	142/153	105/111
	204/1-C	155/158	142/153	105/111
Clone 14	212/6-A	155/158	142/153	105/111
	212/6-B	155/158	142/153	105/111
	212/6-B	155/158	142/153	105/111
Clone16	384/1-A	155/158	142/153	105/111
	384/1-B	155/158	142/153	105/111
	384/1-C	155/158	153/162*	105/111
Clone 17	111/5-A	155/158	153/162	109/113
	111/5-B	155/158	153/162	109/113
	111/5-C	155/158	153/162	109/113
Clone 23	169/4-A	158/194*	142/153*	105/109
	169/4-B	155/158	153/162	105/109
	169/4-C	155/158	153/162	105/111*
Clone 26	375/2-B	158/194	153/162	111/113
	375/2-C	158/194	153/162	111/113
	375/2-D	158/194	153/162	105/111*
Clone 27	201/1-A	158/194	142/153	105/111
	201/1-B	158/194	142/153	105/111
	201/1-C	158/194	142/153	105/111
Clone 28	114/1-A	155/158	153/162	105/109
	114/1-B	155/158	153/162	105/109
	114/1-C	155/158	153/162	105/109
	114/1-D	155/158	153/162	105/109
Bintulu, Sarawak				
Clone 30	42/2-B	155/158	142/153	105/111
	42/2-C	155/158	142/153	105/111
	42/2-D	158/194*	153/162*	105/111
Clone 31	105/5-A	155/158	142/153	105/111
	105/5-B	155/158	142/153	105/111
	105/5-C	155/158	142/153	105/111
	105/5-D	155/158	142/153	105/111
	105/5-E	155/158	142/153	105/111
Clone 32	111/1-B	155/158	153/162	109/113
	111/1-C	155/158	153/162	109/113
	111/1-E	155/158	153/162	109/113

*Mislabelled ramets

ed cases of mislabelling from both sites (Table 3). A total of 20% mislabelling was detected for wood density cross, of which 13.04% from Segamat Johor and 20% from Bintulu, Sarawak (Table 5). The genotypic comparison of both populations did not show any mislabelling as no cases of cross population alleles were observed. All three populations planted with clonal materials are located in different geographical locations, which limited the chances of cross population contamination and mislabelling. Therefore mislabelling occurred due to misplacing and change of the label tags.

Screening of the fibre length mapping population using three markers revealed that both parents were polymorphic. The number of alleles observed ranged from three each for Am465 and AH2_1 loci and four for Ah16 (Table 5). Parental genotypes for the AM22 locus were homozygous for female parent (AA3) with allele sizes of 158/158 bp and heterozygous for male parent (AM22) with allele sizes of 155/194 bp. Parental genotypes for AH2_1 locus were homozygous with allele size of 153/153 bp for female parent whereas heterozygous for male parent with allele size of 142/162 bp. Both parents were heterozygous for AH16 loci with female parent allele sizes of 105/113 bp and male parent 109/111 bp. A total of 17.39% mislabelling was observed for fibre length cross on both sites, of which 20% was observed on site I and 9.09% on site II. High numbers of mislabelled individuals were observed for wood density mapping population followed by fibre length mapping population.

Detected mislabelled individuals were assigned to the reference clones using an assignment test based on multilocus genotypic data. For this wood density population from Segamat and fibre length population from Bintulu were tested due to larger sample sizes. One mislabelled ramet from Segamat wood density cross was assigned to four different clones with more than 95% probability whereas two other ramets could not be assigned to any of the respective clones (Table 6). Similar efforts were made to assign 7 mislabelled ramets from the fibre length cross mapping population. Four ramets were successfully assigned to the putative clones with more than 95% confidence probability and three ramets could not be assigned to any clones (Table 7). Two of four clones were assigned to more than one clone (Table 7). Ramets failed to be assigned to any clone attributed to the sample size used in this study. Similarly, mislabelled ramets were assigned to more than one clone due to the low exclusion power achieved due to a low number of screened markers.

Table 5. Allelic diversity and percentage of mislabelling between ramets of wood density and fibre length mapping populations collected from two sites, i.e. SPL Segamat, Johor and BTS Nursery, Sarawak

Type of Cross		Am465		AH2_1		AH16	
		wood density	fibre length	wood density	fibre length	wood density	fibre length
Site I, SPL Segamat, Johor							
Total number of	ramets screened	23	35	23	35	23	35
	parental alleles (bp)	158,190 (2)	155,158,194 (3)	153,162,164 (3)	142,153,162 (3)	105,111,113 (3)	105,109,111,113 (4)
	ramet alleles (bp)	158,190 (2)	155,158,194 (3)	153,162,164 (3)	142,153,162 (3)	105,111,113 (3)	105,109,111,113 (4)
	mislabelled ramets	0.0	1.0	2	3	3	4
Mislabelling (%)		0.0	2.86	8.7	8.6	13.04	11.4
Site II, Bintulu, Sarawak							
Total number of	ramets screened	12	11	12	11	12	11
	parental alleles (bp)	158,190 (2)	155,158,194 (3)	153,162,164 (3)	142,153,162 (3)	105,111,113 (3)	105,109,111,113 (4)
	ramet alleles (bp)	158,190 (2)	155,158,194 (3)	153,162,164 (3)	142,153,162 (3)	105,111,113 (3)	105,109,111,113 (4)
	mislabelled ramets	0.0	1	2	1	3	0
Mislabelling (%)		0.0	9.1	16.67	9.1	25.0	0.0

total mislabelled ramets (wood density cross, Segamat, Johor) = 3 (13.04%); total mislabelled ramets (wood density cross, Bintulu, Sarawak) = 4 (20.0%); total mislabelled ramets (fibre length cross, Segamat, Johor) = 7 (20.0%); total mislabelled ramets (fibre length cross, Bintulu, Sarawak) = 1 (9.09%); total wood density cross = 7 (20.0%); total fibre length cross = 8 (17.39%); total = 15 (18.52%)

DISCUSSION

Acacia hybrid is an emerging forest tree for pulpwood production in Southeast Asia due to its high growth rate, good fibre properties, disease resistance and high adaptability to various environments. However, there is a need to increase the productivity of forest plantation by selection of superior trees through breeding. Acacia breeding requires a large supply of breeding materials for selection and testing under different environmental conditions. However, recalcitrant hybrid seed germination hampers a large-scale testing of materials, thus it requires the development of a clonal

Table 6. Assignment probability of mislabelled ramets from Segamat wood density cross population based on multigenotypic data to the respective clones

Mislabelled Ramets	Clone 12 138/2-A	Clone 16 247/2-A	Clone 21 237/4-B	Clone 23 231/4-A	Clone 24 141/1-A	Clone 25 134/2-A	Clone 26 180/1-A
138/2-B (Clone 12)	0.0000	0.0000	0.9999	0.9999	0.9999	0.0000	0.9999
237/4-A (Clone21)	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
134/2-C (Clone25)	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000

Table 7. Assignment probability of mislabelled ramets from Bintulu Sarawak, fibre length cross population based on multigenotypic data to the respective clones

Mislabelled Ramets	Clone 5	Clone 6	Clone 14	Clone 17	Clone 23	Clone 26	Clone 27	Clone 28
A-B (Clone 1)	0.0000	0.9999	0.9999	0.9999	0.0000	0.0000	0.0000	0.0000
A-C (Clone 1)	0.0000	0.9999	0.9999	0.9999	0.0000	0.0000	0.0000	0.0000
B-A (Clone 5)	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.9999	0.0000
384/1-C (Clone 16)	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
169/4-A (Clone 23)	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
169/4-C (Clone 23)	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
375/2-D (Clone 26)	0.9999	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000

micropropagation system for large-scale production of clonal materials for commercial and breeding programs. Clonal propagation is also desirable to achieve large genetic gains in a short period of time by retaining parental characteristics and genetic integrity. Large-scale clonal production and processing may result in mislabelling and mixing of the planting materials. Plantation forest planning and propagation happen several years before wood consumption, therefore mislabelling can seriously affect the whole production process. Correct clonal identity also has important implications in breeding procedures where mislabelled clones can significantly affect the expected gains from breeding. Similarly, the quality control of large-scale Acacia clonal plantation operations is crucial, especially in vertically integrated production systems where wood is required from clones with specific wood properties. Clonal identification based on morphology is not feasible due to phenotypic plasticity, however molecular markers have been successfully used to solve several questions related to the management of genetic variation, identity and relationship in breeding and production populations.

This study was started to screen and identify mislabelling in *Acacia* breeding populations, i.e. wood density and fibre length cross, planted on three sites in Malaysia. Two populations screened with STMS markers revealed mislabelling in both populations and the extent of mislabelling ranges from 17.39 to 20% for fibre length cross and wood density cross, respectively. Mislabelling incidence observed on both sites, i.e. Segamat, Johor and Bintulu, Sarawak, is 18.52%. Mislabelling is however observed only within populations and not between populations. These populations are clonally propagated, therefore a possibility of any external contamination was rare and mislabelling incidences are a result of clonal material mishandling. Mislabelling in clonally propagated materials is a common problem. These problems have been reported in other forest species, e.g. 2–13% mislabelling reported in ramets of Douglas-fir (Adams 1983) by using allozyme markers. Similarly, Harju and Muona (1989) found 7–10% of mislabelled ramets in two Scots pine (*Pinus sylvestris* L.) orchards, 10% in two loblolly pine (*Pinus taeda* L.) orchards (Wheeler, Jech 1992) whereas Kawauchi and Goto (1999) found 19.9% of mislabelling ramets within *P. hunbergii* planting. In this study, 15 out of 81 trees (18.52%) were mislabelled. This level of mislabelling is comparable to that of previous reports and it is an indicator of the serious problem of mislabelling in Acacia breeding populations. A high percentage of mislabelled ramets could be due to the small sample size used in this preliminary study. Sample size also affected the probability of assigning the mislabelled individuals to their putative clones. However, it is also realized that the probability of finding the right genotype can be increased by increasing the number of polymorphic markers. According to Zhang et al. (2006), the use of a low number of microsatellite loci may have a low discriminative power. A high number of markers should be used to achieve a higher discriminative power. However, the optimum number of microsatellite loci suitable for checking mislabelling should be carefully determined. It is so because the higher number of used microsatellite loci will not only increase the checking cost and the time but also it may increase the genotyping error (Paetkau et al. 2004). Even though the genotyping error could be reduced (Taberlet, Luikart 1999; Bonin et al. 2004), it will still result in added cost and time consumed. Mislabelling studies involve either large commercial or breeding population plantations generally composed of a large number of testing materials. In applying DNA markers to a large number of test materials, the extraction of DNA is an especially laborious process (Åkerman et al. 1995). Initial screening using sample bulking can help minimize the sample size, cost and time required for processing (Goto et al. 2001). Multiplexing STMS markers and screening sample bulks could help reduce the screening time and cost. Recent development in next generation sequencing (NGS) has made SNP markers affordable and very effective for studies involving large-scale sample screening (Takrama et al. 2012). A large number of SNP markers can be generated in a short time and thus will increase a discrimination power. Similarly, finding a clonal match in a large population for mislabelled ramets based on several multilocus genotypes is very laborious and time consuming. We successfully and efficiently use assignment tests to find right clones for the mislabelled ramets. Assignment tests have successfully been used by Petkau et al. (1995), Cornuet, Luikart (1996), Davies et al. (1999) and Pritchard et al. (2000) in population and conservation biology studies to assign individuals to specific source populations.

It is concluded that correct labelling of Acacia clones is important especially when the planted trees are used for a further analysis such as QTL mapping. The right identity will ensure the reliability of the phenotypic data for a subsequent analysis. This study has provided the first step to identify mislabelling incidences by using DNA

markers and assignment tests. It is suggested that future studies should involve the use of a large number of markers like SNPs, and sample bulking along with utilization of assignment test to reduce the work load, time and screening costs. Checking for mislabelling could increase operational costs for the establishment of a mapping population for QTL analysis. However, this could be avoided by good field practices during transportation of materials from laboratory to nursery and to the field, handling of materials in the field nursery or holding area and transplanting of materials in the field. However, there is always a probability of mislabelling of materials, and it is imperative to randomly test the planting materials for such incidences by using DNA markers.

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