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Amplified fragment length polymorphism (AFLP) analysis of genetic variation in *Moringa oleifera* Lam.

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Abstract

***Moringa oleifera* is an important multipurpose tree introduced to Africa from India at the turn of this century. Despite limited knowledge of the levels of genetic diversity and relatedness of introduced populations, their utilization as a source of seed for planting is widespread. In order to facilitate reasoned scientific decisions on its management and conservation and prepare for a selective breeding programme, genetic analysis of seven populations was performed using amplified fragment length polymorphism (AFLP) markers. The four pairs of AFLP primers (*PstI*/*MseI*) generated a total of 236 amplification products of which 157 (66.5%) were polymorphic between or within populations. Analysis of molecular variance (AMOVA) revealed significant differences between regions and populations, even though outcrossing perennial plants are expected to maintain most variation within populations. A phenetic tree illustrating relationships between populations suggested at least two sources of germplasm introductions to Kenya. The high levels of population differentiation detected suggest that provenance source is an important factor in the conservation and exploitation of *M. oleifera* genetic resources.**

Keywords: amplified fragment length polymorphism, conservation, genetic variation, *Moringa oleifera*, Moringaceae

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Introduction

Moringa oleifera (syn. *M. pterygosperma* Gaertn., annual drumstick) belongs to a monogeneric family of shrubs and trees, the Moringaceae (Ramachandran *et al*. 1980; Jahn *et al*. 1986). Although the species is indigenous to northwest India, it is now cultivated throughout the Middle East, and in almost the whole tropical belt (Ramachandran *et al*. 1980; Jahn *et al*. 1986; Kantharajah & Dodd 1991). *M. oleifera* seed powder contains cationic polypeptides used as a natural coagulant for low-cost water purification with potential for use on a large scale for treatment of turbid waters in tropical developing countries (Jahn 1984; Gassenschmidt *et al*. 1995; Muyibi & Evison 1995; Ndabigengesere *et al*. 1995). The seeds also contain antimicrobial substances capable of reducing the concentration of bacteria in treated water (Jahn 1984; Sutherland *et al*. 1989), and edible oil which can also be used in cosmetics and soap production (Khan *et al*. 1975; Ramachandran *et al*. 1980; Nautiyal & Venkataraman 1987; Mayer & Stelz 1993). Other uses of this species are for vegetables and fodder (Jahn *et al*. 1986; Jahn 1991; Mayer & Stelz 1993), medicines (Caceres *et al*. 1991; Jahn 1991), ornamentals, live fencing, gum, food spices, rayon and paper pulp (Jahn *et al*. 1986; Nautiyal & Venkataraman 1987; Babu & Rajasekaran 1991).

M. oleifera is an outcrossing diploid ($2n = 28$), with some trees flowering throughout the year while others flower in two distinct seasons (Ramachandran *et al*. 1980; Jyoth *et al*. 1990). Although detailed studies of the distribution of genetic variability are limited, considerable variation in quantitatively inherited traits has been reported in natural

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populations from India (Ramachandran *et al*. 1980) and introduced populations in Kenya (G. M. Muluvi *et al*. our unpublished data), indicating considerable potential for improvement. The species was introduced to Kenya from India (exact location unknown) at the beginning of this century (Jahn 1991). However, little information is available on the genetic base of Kenyan populations and their genetic relationships to Indian populations. Such information would facilitate tree improvement programmes and the conservation and exploitation of *Moringa* genetic resources.

Molecular markers have proved to be powerful tools in the assessment of genetic variation both within and between plant populations by analysing large numbers of loci distributed throughout the genome (Powell *et al*. 1995). We chose amplified fragment length polymorphism (AFLP: Vos *et al*. 1995) for this study because the technique has previously been used successfully in diversity studies in other species (Maughan *et al*. 1996; Sharma *et al*. 1996; Travis *et al*. 1996; Ellis *et al*. 1997; Hongtrakul *et al*. 1997; Paul *et al*. 1997; Arens *et al*. 1998; Winfield *et al*. 1998). AFLP assays require no previous sequence knowledge and can detect 20–100 loci per assay (Maughan *et al*. 1996; Powell *et al*. 1996). The technique has not been extensively applied to tropical trees and, to our knowledge, no molecular assays have previously been utilized to analyse genetic variation in *Moringa* species. Our objectives in this study were to examine the levels of genetic differentiation between natural populations of *M. oleifera* to define genetically distinct units for conservation purposes, and to quantify levels of genetic diversity within populations to optimize sampling strategies for the efficient maintainance of variability in the species.

Materials and methods

Plant material

One-hundred and forty genotypes of *Moringa oleifera* from seven different populations (20 trees per population) were used: two from Tamil Nadu (South India), one from Ex-Nsanje region (Southern Malawi) and four from Kenya. One of the Indian samples was a natural population while the second (PKM1) has been the subject of selection. The four populations from Kenya represent the only populations of *M. oleifera* in the country. They are distributed from the coast westwards and each separated by a distance of at least 150 km (Fig. 1).

Fig. 1 Map showing the location of the seven *Moringa oleifera* populations studied.

DNA isolation

Genomic DNA isolation was performed according to the method of Edwards *et al*. (1991). Additional steps of chloroform:isoamyl alcohol (24:1) purification and RNase A (10 mg/mL) treatment were incorporated.

AFLP protocol

The AFLP method was carried out following the standard procedure described by Vos *et al*. (1995). High-quality genomic DNA (0.5 μg) was digested with a pair of restriction enzymes (*Pst*I/*Mse*I) then ligated to double-stranded *Pst*I and *Mse*I adapters. The ligate was pre-amplified with nonselective primers and selective amplification carried out using pairs of 2 bp and 3 bp selective primers (Table 1). The products were separated on polyacrylamide gels using an M13 sequencing ladder as a size standard.

Data analysis

Amplification products were scored as discrete character states (present/absent). The data were transformed into band frequencies and diversity values based on phenotype frequency (phenotypes being the band patterns produced by individual primer pairs) were calculated using Nei's unbiased statistic (1987):

$$\hat{H} = \frac{n(1 - \sum[p_i^2])}{n - 1}$$

where n = number of individuals analysed and p_i is the frequency of the ith phenotype. Total diversity values were averaged across loci. Monomorphic bands were included in diversity calculations.

A matrix of genetic distances between genotypes based on the number of shared amplification products was calculated using the metric of Nei & Li (1979). Average genetic distances within populations were calculated from the distance matrix, which was also used to construct a neighbour-joining phenogram using the NEIGHBOUR and DRAWTREE options in the PHYLIP package (version 3.57c: Joe Felsenstein, University of Washington, USA). Finally, the distance matrix was used to perform a hierarchical analysis of molecular variance (AMOVA: Excoffier *et al*. 1992) using the ARLEQUIN software (Schneider *et al*. 1997).

Table 1 Sequences of AFLP adaptors and primers used in this study

*Pst*I adaptors	5' – CTCGTAGACTGCGTACATGCA – 3'	
	5' – TGTACGCAGTCTAC – 3'	
*Pst*I primers	P00	5' – GACTGCGTACATGCAG – 3'
	P12	P00 + AC
	P14	P00 + AT
	P15	P00 + CA
	P17	P00 + CG
*Mse*I adaptors	5' – GACGATGAGTCCTGAG – 3'	
	5' – TACTCAGGACTCAT – 3'	
*Mse*I primers	M00	5' – GATGAGTCCTGAGTAA – 3'
	M51	M00 + CCA

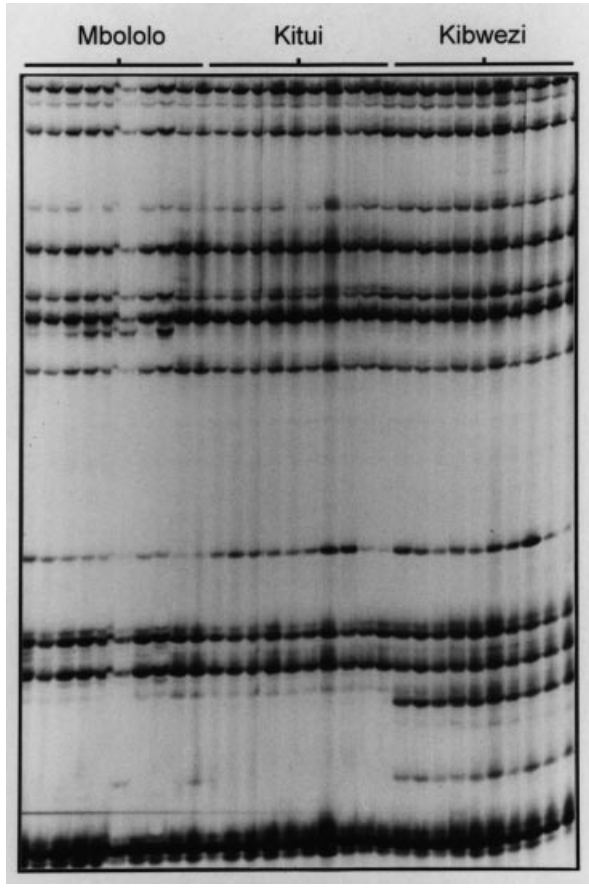

Fig. 2 An example of the part of the variation detected in *Moringa oleifera* using primers M51 and P15. An M13 sequencing ladder was used to accurately size fragments.

Results

Levels of diversity within and between populations of Moringa oleifera

Each of the four primer combinations revealed between one and 30 polymorphic loci in individual populations and between 16 and 60 polymorphic loci across all populations. A section of a typical gel is shown in Fig. 2. In total, 157 polymorphic loci were generated, with between 19 (Kitui) and 61 (India) amplified in individual

populations. Diversity values for primer pairs in individual populations ranged from 0.006 (P17/M51 in Kitui and Mbololo) to 0.145 (P14/M51 in Kibwezi), with average values for all loci ranging from 0.026 in Kitui to 0.099 in India. Values of Nei's average diversity within populations ranged from 0.040 in Kitui to 0.122 in the PKM1 population (Table 2). Monomorphic bands were included in calculations.

Partitioning the variation within and between populations using an analysis of molecular variance (AMOVA) showed that 59.15% of the genetic variability existed as variation between populations ($\Phi_{ST} = 0.5915$; $P < 0.00001$: Table 3). A comparison of African and Indian populations showed that 18.59% of the variation existed between the two groups, but this was nonsignificant ($P = 0.0538$). Approximately 14.44% of the variation was partitioned between the Kenyan populations and the rest ($\Phi_{ST} = 0.1444$; $P = 0.0342$).

Genetic relationships in M. oleifera

Figure 3 shows the neighbour-joining phenetic tree based on Nei and Li's genetic distance (Nei & Li 1979). The India and PKM1 populations form single, distinct clusters which are separate from the four Kenyan populations. The individuals from the Malawi population do not form a single group but are dispersed, with two individuals (Malawi 9 and Malawi 10) associated with the India population. The remaining Malawi individuals are closer to the PKM1 population than they are to the Kenyan populations, which is consistent with the Malawi population being a recent introduction from India. The Kenyan populations form single, monophyletic clades and cluster in two groups, the first containing the Likoni and Mbololo populations and the second containing the Kitui and Kibwezi populations.

Discussion

In the present study, we have used AFLPs to study genetic diversity within and between seven geographically isolated populations of *Moringa oleifera*. Significant levels of population differentiation were found and, although high levels of between-population differentiation have previously been reported in tropical tree species using random amplified polymorphic DNA (RAPDs) (Chalmers *et al*. 1992 in *Gliricidia sepium*; Gillies *et al*. 1997 in *Cedrela odourata*; Cardoso *et al*. 1998

Table 2 Diversity statistics for four AFLP primer combinations (primer given in table plus primer M51) in seven populations of *Moringa oleifera* (*n* = number of polymorphic bands; *H* = genetic diversity; *D* = Nei and Li average genetic distance)

	P12		P14		P15		P17		All loci		
Population	*n*	*H*	*n*	*H*	*n*	*H*	*n*	*H*	*n*	*H*	*D*
India	30	0.144	13	0.098	15	0.111	3	0.046	61	0.099	0.115
PKM1	16	0.108	18	0.109	16	0.084	5	0.074	55	0.094	0.122
Malawi	17	0.068	8	0.056	17	0.078	3	0.023	45	0.056	0.074
Likoni	11	0.055	3	0.041	18	0.077	8	0.073	40	0.061	0.083
Mbololo	19	0.104	5	0.041	7	0.043	1	0.006	32	0.049	0.064
Kitui	10	0.057	4	0.023	4	0.018	1	0.006	19	0.026	0.040
Kibwezi	13	0.087	12	0.145	22	0.090	1	0.011	48	0.083	0.102
All	48	0.089	33	0.073	60	0.072	16	0.034	157	0.067	0.085

Table 3 Analysis of molecular variance (AMOVA)

		Variance component		
Source of variation	d.f.	Variance	% variation	Probability
Between provenances	6	5.87	59.15	$P < 0.00001$
Between groups (India vs. Africa)	1	2.04	18.59	$P = 0.05376$
Between provenances within groups	5	4.89	44.53	$P < 0.00001$
Within provenances	133	538.80	36.88	$P < 0.00001$
Between groups (Kenya vs. Rest)	1	1.53	14.44	$P = 0.03421$
Between provenances within groups	5	4.99	47.23	$P < 0.00001$
Within provenances	133	538.80	38.33	$P < 0.00001$

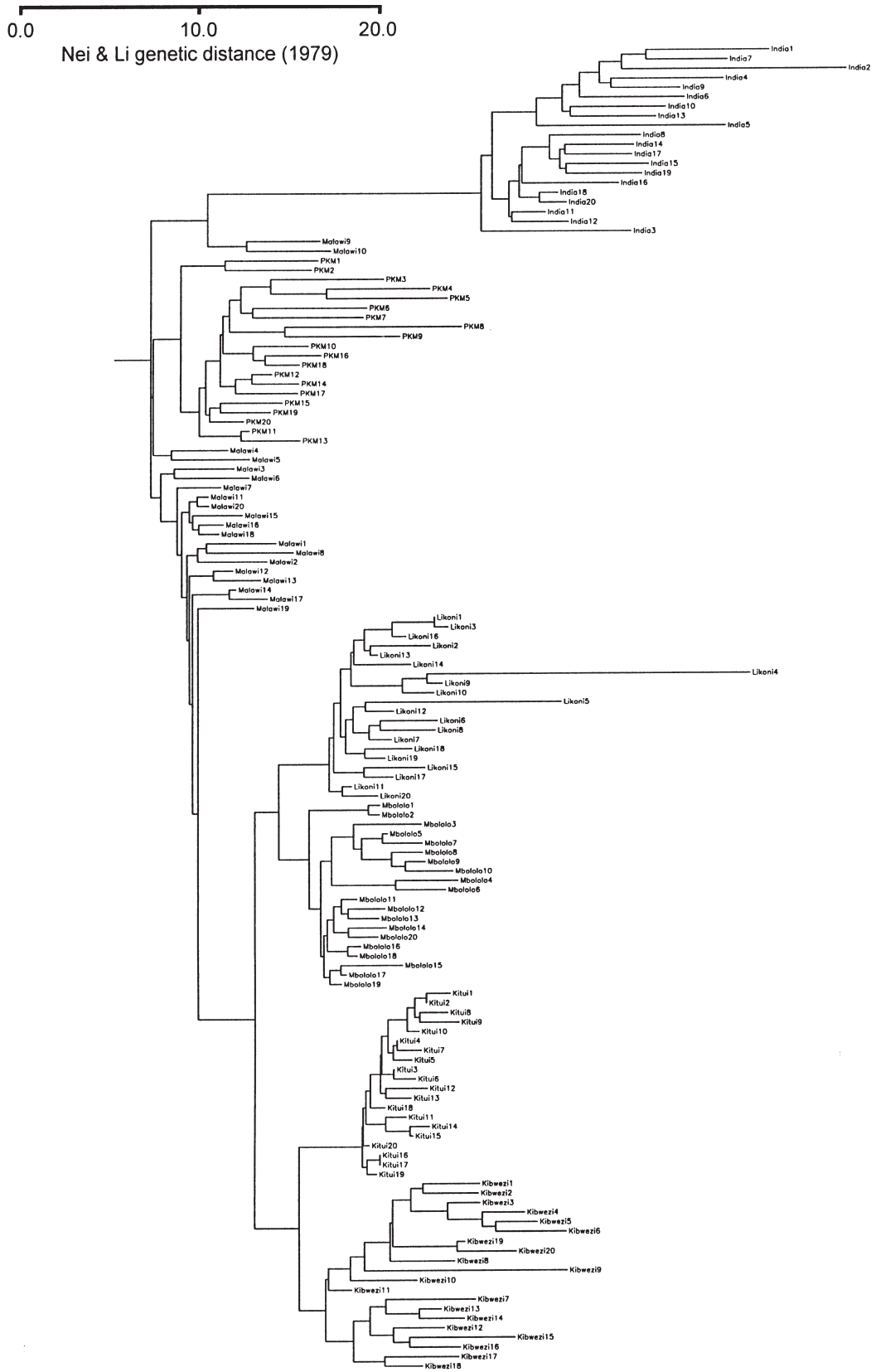

Fig. 3 Neighbour-joining phenetic tree of relationships between *Moringa oleifera* individuals based on Nei and Li's genetic distance Nei & Li (1979).

in *Caesalpina echinata*), such results are in contrast to expectations for woody, perennial, predominantly outcrossed species which maintain most variation within populations (Hamrick 1989). Although *M. oleifera* is insect (bee) pollinated (Puri 1941; Chand *et al*. 1994) and adapted to a mixed mating system (Jyoth *et al*. 1990), founder effects and restricted gene flow between populations due to geographical isolation (Schaal *et al*. 1998) may account for the significant differences between populations and regions.

The highest levels of genetic diversity were found within the Indian populations. Among the Kenyan populations, the highest level of genetic variation was observed among the widely dispersed Kibwezi population while the more geographically restricted Kitui populations had the lowest level of diversity. Distribution range and population size have been identified as major correlates of within-population genetic variation in tropical tree species with restricted populations showing significantly less variation than those with a broader distribution (Loveless 1992), while genetic variation varies directly with population size (Travis *et al*. 1996). The relatively low levels of genetic variation in the introduced populations may suggest that these populations were based on a small number of genetically related accessions at the time of introduction. Alternatively, strong selection pressures may also have contributed to the low levels of diversity.

The neighbour-joining phenogram suggests a direct relationship between populations and their geographical origin in agreement with similar work by Hormaza *et al*. (1994) and Pakniyat *et al*. (1997) in *Pistacia vera* cultivars and wild barley, respectively. The clustering of Kibwezi and Kitui populations and Mbololo with Likoni suggests two sources of introduction of Kenyan *M. oleifera*. Historical relationships have been found to contribute to the genetic structure of most plant populations with those having recent common ancestry being genetically more similar than those having more distant common ancestry (Schaal *et al*. 1998). Kitui and Kibwezi fall within the same climatic zone while Mbololo is intermediate between Likoni and Kibwezi. Loveless (1992) identified habitat heterogeneity to have an effect on population structure. Therefore, if the Kenyan populations were introduced from a common source, then climatic factors, short rotation and restricted gene flow may have played a role in influencing genetic differentiation between the two Kenyan groups. The clustering of the Malawi population close to the Indian populations suggests an Indian, rather than an African, origin for this population.

The present data on patterns of genetic differentiation suggest that the Kenyan populations should be considered distinct from each other for the purposes of seed collection, planting and management. In the case of conservation of genetic resources, seed collection should be done across the species range to ensure a more representative sampling of the genetic variation. The significant levels of genetic differentiation observed in this study may be related to adaptive variation, and structured progeny trials are required to assess the performance of the various populations for traits of interest. Although there is a risk in using neutral genetic markers for making inferences about adaptive processes unless selection is still acting or there is a very close linkage between the selected locus and the neutral marker (Palacios & Gonzales-Candelas 1997), work on *Cedrela odourata* by Gillies *et al*. (1997) found a correlation between molecular differences between populations and adaptive characters.

For nonindustrial agroforestry species, obtaining quick, accurate estimates of the distribution of genetic variation in a cost-effective manner is particularly important. To date, most studies of this nature have employed RAPDs. In the present study we have used AFLPs to detect variability in common genotypes, although a parallel study on a subset of the same material using RAPDs has shown that information on diversity levels and relationships between populations is congruent between both marker systems (Muluvi 1998). The AFLP technology is extremely robust and proficient at revealing intrapopulation diversity and estimating genetic distance between individuals and populations (Travis *et al*. 1996; Arens *et al*. 1998; Winfield *et al*. 1998). Furthermore, three times the number of data points (amplification products) were generated with AFLPs compared with RAPDs over an equivalent period of time (Muluvi 1998). These factors contribute to the conclusion that AFLPs provide a cost-effective procedure to monitor the extent and distribution of diversity in *Moringa* and other agroforestry species.

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References

Arens P, Coops H, Jansen J, Vosman B (1998) Molecular genetic analysis of black poplar (*Populus nigra* L.) along Dutch rivers. *Molecular Ecology*, **7**, 11–18.

Babu SC, Rajasekaran B (1991) Agroforestry, attitude towards risk and nutrient availability: a case study of south Indian farming systems. *Agroforestry Systems*, **15**, 1–15.

Caceres A, Cabrera O, Morales O, Mollinedo P, Mendia P (1991) Phamacological properties of *Moringa oleifera*. 1: preliminary screening for antimicrobial activity. *Journal of Ethnopharmacology*, **33**, 213–216.

Cardoso MA, Provan J, Powell W, Ferreira PCG, de Oliveira DE (1998) High genetic differentiation among remnant populations of the endangered *Caesalpinia echinata* Lam. (Leguminosae – Caesalpinioideae). *Molecular Ecology*, **7**, 601–608.

Chalmers KJ, Waugh R, Sprent JI, Simons AJ, Powell W (1992) Detection of genetic variation between and within populations of *Gliricidia sepium* and *G. maculata* using RAPD markers. *Heredity*, **69**, 465–472.

Chand H, Singh R, Hameed SF (1994) Studies on the flowering period, density of Indian mustard and competing flora for honey bees in and around Pusa, Bihar (India). *Journal of Entomological Research*, **18**, 361–367.

Edwards K, Johnstone C, Thompson C (1991) A simple and rapid method for the preparation of plant genomic DNA for PCR analysis. *Nucleic Acids Research*, **19**, 1349.

Ellis RP, McNicol JW, Baird E *et al*. (1997) The use of AFLPs to examine genetic relatedness in barley. *Molecular Breeding*, **3**, 359–369.

Excoffier L, Smouse PE, Quattro JM (1992) Analysis of molecular variance inferred from metric distances among DNA haplotypes: application to human mitochondrial DNA restriction data. *Genetics*, **131**, 479–491.

Gassenschmidt U, Klans D, Jarry D, Taucher B, Neebergall H (1995) Isolation and characterization of a floculating protein from *Moringa oleifera* Lam. *Biochemica et Biophysica Acta*, **24093**, 477–481.

Gillies ACM, Cornelius JP, Newton AC *et al*. (1997) Genetic variation in Costa Rican populations of the tropical timber species *Cedrela ordorata* L. assessed using RAPDs. *Molecular Ecology*, **6**, 1133–1145.

Hamrick JL (1989) Isozymes and the analysis of genetic structure in plant populations. In: *Isozymes in Plant Biology* (eds Soltis DE, Soltis PS), pp. 87–105. Dioscorides Press, Portland, OR, USA.

Hongtrakul V, Huestis GM, Knapp SJ (1997) Amplified fragment length polymorphisms as a tool for DNA fingerprinting sunflower germplasm: genetic diversity among oilseed inbred lines. *Theoretical and Applied Genetics*, **95**, 400–407.

Hormaza JI, Dollo L, Polito VS (1994) Determination of relatedness and geographical movements of *Pistacia vera* L. (pistachio; Anacardiaceae) germplasm by RAPD analysis. *Economic Botany*, **48**, 349–358.

Jahn SAA (1984) Effectiveness of traditional flocculants as primary coagulants and coagulant aids for the treatment of tropical raw water with more than a thousand fold fluctuation in turbidity. *Water Supply, 2, Special Subject*, **6**, 8–10.

Jahn SAA (1991) The traditional domestication of a multipurpose tree *Moringa stenopetala* (Bak.f.) Cuf. in the Ethiopian Rift valley. *AMBIO*, **20**, 244–247.

Jahn SAA, Mussad HA, Burgstaller H (1986) The tree that purifies water. Cultivating multipurpose Moringaceae in the Sudan. *Unasylva*, **152**, 23–28.

Jyoth PV, Atluri JB, Reddi CS (1990) Pollination ecology of *Moringa oleifera* (Moringaceae), *Proceedings of Indian Academy of Sciences, (Plant Science)*, **100**, 33–42.

Kantharajah AS, Dodd WA (1991) Rapid clonal propagation of *M. oleifera* Lam., using tissue culture. *South Indian Horticulture*, **39**, 224–228.

Khan FW, Gul P, Malik MN (1975) Chemical composition of oil from *Moringa oleifera*. *The Pakistan Journal of Forestry*, 100–102.

Loveless MD (1992) Isozyme variation in tropical trees: patterns of genetic organization. *New Forests*, **6**, 67–94.

Maughan PJ, Maroof MAS, Buss GR, Huestis GM (1996) Amplified fragment length polymorphism (AFLP) in soybean: species diversity, inheritance, and near-isogenic line analysis. *Theoretical and Applied Genetics*, **93**, 392–401.

Mayer FA, Stelz E (1993) *Moringa stenopetala* provides food and low cost water purification. *Agroforestry Today*, **5**, 16–18.

Muluvi GM (1998) *Molecular ecology and population genetics of two multipurpose tropical trees,* Moringa oleifera *Lam. &* Moringa stenopetula *(Bak. F.) Cuf.* PhD Thesis, University of Dundee, Dundee, UK.

Muyibi SA, Evison LM (1995) Optimising physical parameters affecting coagulation of turbid waters with *Moringa oleifera* seeds. *Water Research*, **29**, 2689–2695.

Nautiyal BP, Venkataraman KG (1987) *Moringa* (drumstick) – An ideal tree for social forestry: Growing conditions and uses – Part 1. *MYFOREST*, **23**, 53–58.

Ndabigengesere A, Narasiah KS, Talbot BG (1995) Active agents and mechanisms of coagulation of turbid waters using *Moringa oleifera*. *Water Research*, **29**, 703–710.

Nei M (1987) *Molecular Evolutionary Genetics.* Columbia University Press, New York, NY, USA.

Nei M, Li W-H (1979) Mathematical model for studying genetic variation in terms of restriction endonucleases. *Proceedings of the National Academy of Sciences of the USA*, **76**, 5269–5273.

Pakniyat H, Powell W, Baird E *et al*. (1997) AFLP variation in wild barley (*Hordeum spontaneum* C. Koch) with reference to salt tolerance and associated ecogeography. *Genome*, **40**, 332–341.

Palacios C, Gonzales-Candelas F (1997) Analysis of population genetic structure and variability using RAPD markers in the endemic and endangered *Limonium dufouri* (Plumbaginaceae). *Molecular Ecology*, **6**, 1107–1121.

Paul S, Wachira FN, Powell W, Waugh R (1997) Diversity and genetic differentiation among populations of Indian and Kenyan tea (*Camellia sinensis* (L.) O. Kuntze) revealed by AFLP markers. *Theoretical and Applied Genetics*, **94**, 255–263.

Powell W, Orozco-Castillo C, Chalmers KJ, Provan J, Waugh R (1995) Polymerase chain reaction-based assays for the characterisation of plant genetic resources. *Electrophoresis*, **16**, 1726–1730.

Powell W, Morgante M, Andre C *et al*. (1996) The comparison of RFLP, RAPD, AFLP and SSR (microsatellite) markers for germplasm analysis. *Molecular Breeding*, **2**, 225–238.

Puri V (1941) The life history of *Moringa* Lam. *Journal of the Indian Botanical Society*, **20**, 263–283.

Ramachandran C, Peter KV, Gopalakrishnana PK (1980) Drumstick (*Moringa oleifera*): a multipurpose Indian vegetable. *Economic Botany*, **34**, 276–283.

Schaal BA, Hayworth DA, Olsen KM, Rauscher T, Smith WA (1998) Phylogenetic studies in plants: problems and prospects. *Molecular Ecology*, **7**, 465–474.

Schneider S, Kueffer J-M, Roessli D, Excoffier L (1997) *Arlequin, Version 1.1: a Software for Population Genetic Data Analysis*. Genetics and Biometry Laboratory, University of Geneva, Switzerland.

Sharma SK, Knox MR, Ellis THN (1996) AFLP analysis of the diversity and phylogeny of *Lens* and its comparison with RAPD analysis. *Theoretical and Applied Genetics*, **93**, 751–758.

Sutherland JP, Folkard GK, Grant WD (1989) Seeds of *Moringa* species as naturally accuring flocculants for water treatment. *Science, Technology and Development*, **7**, 191–197.

Travis SE, Maschinski J, Keim P (1996) An analysis of genetic variation in *Astragalus cremnophylax* var. Cremnophylax, a critically endangered plant, using AFLP markers. *Molecular Ecology*, **5**, 735–745.

Vos P, Hogers R, Bleeker M *et al*. (1995) AFLP: a new technique for DNA fingerprinting. *Nucleic Acids Research*, **23**, 4407–4414.

Winfield MO, Arnold GM, Cooper F *et al*. (1998) A study of genetic diversity in *Populus nigra* subsp. *betulifolia* in the Upper Severn Area of the UK using AFLP markers. *Molecular Ecology*, **7**, 3–10.

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