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Phenolic Heterogeneity in Mangosteen

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Abstract : Leaf extracts obtained from 52 trees of mangosteen found growing in two agroclimatic zones of Sri Lanka were chromatographed to study the pattern of distribution of phenolic constituents. Using aliquots of 400 λ to 450 λ of extracts, a total of 34 different spots were recognised among the 52 samples. All spots were not found in every tree. There was intra-location and inter-location variability suggesting that there is some genetic heterogeneity in spite of the fact that mangosteen is asexually propagated by the development of non-zygotic seeds.

1. Introduction

The tropical fruit mangosteen *Garcinia mangostana* L. is propagated by seed. Since the seeds are non-zygotic, their adventitious embryos develop from the epithelium of the inner integument of the ovule and the seedlings come true to type.[5,6,7] All cultivated mangosteens around the world therefore are believed to belong to a single seed propagated clonal population which look remarkably alike in vegetative morphology. The only variations that could be observed are differences in reproductive characteristics such as time of flowering, fruit ripening, intensity of pigmentation of the skin and fruit flavour which are mostly influenced by the environment.

Chemical characterization has helped to understand systematic and evolutionary relationships among plant species.[2,9] These techniques have not been used in mangosteen to determine whether the morphological homogeneity of the species is supported by chemical homogeneity. To test it, the nature of phenolic patterns in chromatograms was examined to understand the genetic stability of the species.

2. Materials and Methods

Leaf samples of 52 mature well formed trees growing in two agro-climatic zones of Sri Lanka where mangosteen is mostly found, namely, the wet midlands and wet lowlands, were used for this study. The trees were from 14 different locations (Table 1). Young and mature leaves from the outer canopy were collected in polythene bags and stored in the refrigerator. The following day a mixed sample of 2g of leaves from both types of leaves was heated in methanol for 10 mins, then homogenized in a Waring blender and extracted in a soxhlet for 8 to 10 hrs. in warm methanol.

TABLE 1. Location of trees tested from two agroclimatic zones.

Agroclimatic zone and location	Code No. of trees	Overland distance from University (Km)	Date of sampling
1. *Midland wet zone*			
1.1 Peradeniya	M_{61}, M_{67}	0.5	1977-09-06
(Botanical	M_{54}, M_{55}	,,	1977-09-08
Garden)	M_{53}, M_{56}, H_{125}	,,	1977-09-08
	H_{222}, H_{224}	,,	1977-10-05
1.2 Pilimatalawa	P_1, P_2, P_3, P_4	7	1977-10-18
1.3 Naranwela	N_1	10	1977-10-05
1.4 Meegammana	M_1	17	1977-10-18
2. *Lowland wet zone*			
2.1 Nittambuwa	NI_1, NI_2	60	1978-03-12
2.2 Mirigama	MI_1, MI_2, MI_3	70	1977-11-17
	MI_4, MI_5, MI_6	70	1977-11-17
2.3 Dunagaha			
Kadawala	DK_1, DK_2, DK_3	73	1977-11-17
2.4 Dunagaha	DU_1, DU_2 ,DU_4	75	1977-11-19
2.5 Divulapitiya	D_1, D_2, D_3, D_4 ,D_5	67	1977-10-10
2.6 Kalutara North	KA_1, KA_2, KA_3	106	1978-03-11
2.7 Neboda	NE_1, NE_2, NE_3	96	,,
2.8 Matugama	MA_1, MA_2, MA_3	100	,,
2.9 Agalawatta	AG_1, AG_2, AG_3	97	,,
2.10 Bellana	BA_1, BA_2, BA_3	96	,,
2.11 Morapitiya	MO_1, MO_2, MO_3	92	,,

The extract was concentrated in a vacuum at room temperature and the final volume was prepared in the proportion of 1 ml for every 0.07g fresh weight of leaves. The extracts were spotted on Whatman No. 1 paper at 400 λ and 450 λ after preliminary tests using volumes/aliquots ranging from 50 λ to 450 λ revealed these to be the best concentrations. Descending chromatography was used for developing the papers in the first direction in butanolacetic acid-water (4 : 1 : 2.2 v/v/v) and in the second direction in 2% acetic acid.

The dried papers were examined in visible light and under ultraviolet light (254 mμ and 360 mμ) before and after spraying with 10% sodium carbonate. They were next examined after spraying with sulphanilic acid (9g sulphanilic acid + 90 ml conc. HCl in 1*l* of water) and 5% sodium nitrate, in the ratio of 1:5.

3. Results

The chromatographic pattern of suspected phenolic spots are shown in Figure 1 and the detection methods and appearance of spots are given in Table 2. In this study the chemical nature of the spots were not identified. Thirty four spots in all were recognised. They were not found in every tree (Table 3). In both agro-climaitc zones there were intra-location variability (eg. Mirigama and Peradeniya) and inter-location variability in all trees (eg. spots 4, 14 and 18). Only nine spots (2, 4, 12, 16, 20, 25, 28, 29 and 30) were found in over half of the trees examined.

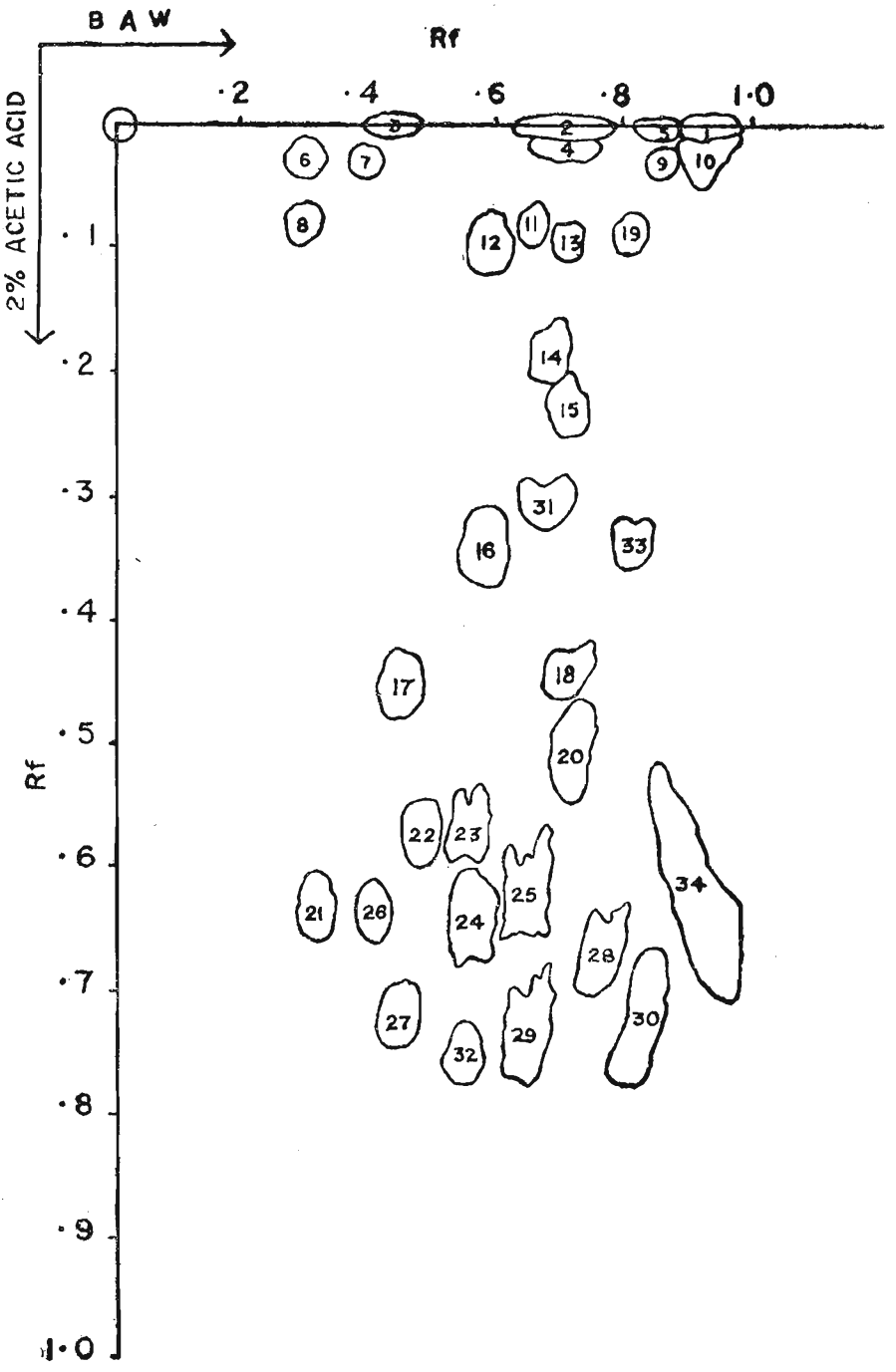

Figure 1. Composite chromatogram of mangosteen trees.

TABLE 2. Colour reaction of spots on dry chromatograms of mangosteen

Detection Procedure	Appearance	Spot Number
Visible light	Green	1
UV light (254 mμ)	Light yellow	4, 5, 6, 7, 8, 11, 13, 32
	Yellowish green	10
	Light purple	1, 2, 3, 11, 16, 26
	Purple	12
	Pink	14, 27
	Light blue	19, 21, 23, 24, 25, 29
	Blue	31, 33
UV light (360 mμ)	Dark purple	2, 3, 15
	Purple	1, 11, 13
	Blue	21, 23, 24, 25, 29, 31
	Light blue	26
	Light yellow	6,7, 8, 10, 12
	Pink	14
After Na_2CO_3 Spray		
Visible light	Light yellow	12
	Light pink	14
	Light brown	16
UV light (254 mμ)	Yellow	12
	Pink	1, 14, 18
	Brown	16
	Blue	9, 26, 28, 30, 32
	Purple	17
	Dark Purple	20
	Fluorescent green	25, 29
UV light (360 mμ)	Brown	16. 22
	Fluorescent green	25, 29
	Dark purple	34
	Dark blue	28, 30
After sulphanilic acid spray		
Visible light	Light brown	16, 20, 22
	Light pink	25*, 28*, 29*
	Light yellow	12

*Colour reaction on wet paper.

TABLE 3. The types of spots recognised in different trees from 15 locations in 2 agroclimatic zones

Tree Number	Spot Number																																
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34
1. Midland wet zone																																		
1.1. Peradeniya																																		
M_{53}	+			+								+				+				+					+			+	+	+				
M_{54}	+	+	+													+				+		+			+			+	+	+	+			
M_{55}	+		+	+										+						+					+			+	+	+	+			
M_{56}	+			+								+		+		+				+		+			+			+	+	+				
M_{61}	+		+	+								+		+						+					+			+	+	+	+			
M_{67}	+	+	+	+										+							+				+			+	+	+	+			
H_{125}	+			+							+			+		+				+	+	+			+			+	+	+				
H_{222}	+															+				+		+			+			+	+	+				
H_{224}	+			+							+			+		+		+		+	+	+						+	+	+				
1.2 Pilimatalawa																																		
P_1	+				+							+				+				+	+	+			+			+	+	+				
P_2	+				+									+		+				+	+				+			+	+	+				
P_3	+				+							+		+		+	+	+	+	+	+	+			+				+	+				
P_4	+				+									+		+		+		+	+	+			+			+	+	+				
1.3 Naranwela																																		
N_1	+				+	+	+				+	+		+		+	+	+		+		+			+	+		+	+	+				
1.4 Meegammana																																		
M_1	+				+									+		+	+	+					+		+			+	+	+				
2. Lowland wet zone																																		
2.1 Nittambuwa																																		
NI_1	+	+	+	+								+	+			+		+		+	+				+			+	+	+				
NI_2	+	+	+	+			+							+		+				+	+	+			+			+	+	+				
2.2 Mirigama																																		
MI_1	+	+		+								+				+				+					+				+	+	+	+	+	+
MI_2	+	+		+				+				+				+				+					+				+	+		+		+
MI_3	+	+										+				+									+				+	+				+
MI_4	+	+										+								+					+				+	+			+	+
MI_5	+	+		+								+								+					+				+	+				
MI_6	+	+		+								+				+				+					+				+	+		+		+

Tree Number	Spot Number																															
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34
2.3 Dunagaha Kadawala																																		
DK_1	+	+										+				+									+				+	+			+
DK_2	+	+																							+				+			+		
DK_3	+	+		+						+	+					+				+					+				+			+	+	+
2.4 Dunagaha																																		
DU_1	+	+									+									+					+				+	+			+	+
DU_2	+	+		+					+		+	+				+				+					+				+	+			+	+
DU_3	+	+		+					+		+	+				+									+				+	+	+			+
2.5 Divulapitiya																																		
D_1	+			+							+			+		+		+		+			+	+	+			+	+	+				
D_2	+			+			+	+			+			+		+		+		+			+	+	+	+		+	+	+				
D_3	+			+			+	+				+		+		+				+			+	+	+	+		+	+	+				
D_4	+			+			+	+			+			+		+		+		+			+	+	+	+		+	+	+				
D_5	+			+							+	+				+							+	+	+			+	+	+				
2.6 Kalutara North																																		
KA_1	+	+	+	+												+		+		+	+				+		+	+	+	+				
KA_2	+	+	+				+			+				+						+	+				+			+	+	+				
KA_3	+	+	+				+					+		+	+	+		+		+	+	+	+	+	+			+	+	+				
2.7 Neboda																																		
NE_1	+	+	+	+									+							+	+				+			+	+	+				
NE_2	+	+	+				+	+		+		+	+							+	+				+			+	+	+				
NE_3	+	+			+											+				+	+	+	+	+	+			+	+	+				
2.8 Matugama																																		
MA_1	+	+	+	+								+								+	+				+			+	+	+		+		
MA_2	+	+	+							+		+								+								+		+				
MA_3	+	+	+	+		+										+	+			+		+	+	+	+			+		+				
2.9 Agalawatte																																		
AG_1	+	+	+																						+			+		+				
AG_2	+	+	+							+		+								+								+						
AG_3	+	+	+								+		+		+	+		+		+	+		+	+	+			+	+					
2.10 Bellana																																		
BA_1	+	+	+	+	+		+					+								+	+				+			+	+					
BA_2	+	+		+		+	+	+				+		+	+	+		+		+	+	+			+			+	+	+				
BA_3	+			+	+	+	+	+				+		+		+		+		+	+	+	+	+	+			+	+	+				
2.11 Morapitiya																																		
MO_1	+	+	+	+								+		+						+	+				+			+	+	+				
MO_2	+	+	+				+	+		+		+				+				+			+	+	+					+				
MO_3	+	+	+		+								+	+		+		+		+	+	+	+	+	+			+	+	+				

4. Discussion

Phenolic spots that are specific to species have been recognised in chromatograms of many plant genera such as *Baptisia*,[1,3] *Potentilla*,[4] *Psophocarpus*.[8] In such instances certain spots appear consistently in all samples and could be used as markers in distinguishing species and in recognising the origin of interspecific hybrids. In mangosteen, however, with the exception of the chlorophyll spot (No. 1), none of the other spots appeared in all the samples tested.

Variation of phenolic patterns between locations could be due to environmental differences. But the presence of intralocational variation suggests that even though mangosteen is a apomictic species with a unique system of vegetative reproduction through seeds, there appears to be genetic heterogeneity. This heterogeneity would have aided its survival in evolution and perhaps still helps to adapt itself to different agro-ecological niches both under cultivation and in natural stands. However, the exact ages of trees in this study are not known although it could be assumed that the small clusters of trees growing at each location would be more or less of the same age. Whether differences in age could have also contributed to the intra-locational variation of phenolic constituents is not known.

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