Assessing the extent of diversity among noni *(Morinda citrifolia* L.*)* genotypes of Morobe Province, Papua New Guinea

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ABSTRACT

*The extent of morphological variation among extant noni (*Morinda citrifolia*: Rubiaceae) genotypes was assessed using 58 polymorphic traits. A total of 39 mature noni trees were sampled from five sites within the vicinity of Lae, Morobe Province, Papua New Guinea. Cluster analysis identified five homogenous clusters, and was able to separate the three known botanical varieties namely,* M. citrifolia *var.* citrifolia*,* M. citrifolia *var.* bracteata *and* M. citrifolia *var.* potteri *as distinct morphotypes. Ordination of the data revealed traits such as young shoot pigmentation, stem diameter, angle of insertion of primary branch on main stem, stipule shape, heterostyly, occurrence of pistillate florets, fruit shape, occurrence of floral bracts, fruit width, and peduncle positioning at maturity as having greater contributions to the observed variation. Although the genetic nature of these traits is yet to be elucidated, occurrence of floral bract and fruit branching were observed to be transitional between the botanical varieties, and may shed light on their origins. The 58 descriptor states showed varying levels of polymorphism, however, the significant (P<0.01) correlations observed between numerous traits provide an element of caution in the development of a descriptor list, particularly when considering stability of the traits and the sample size. The results obtained in this study provided useful information for the standardisation of the developed descriptor list comprising of 49 polymorphic descriptor states, and for future diversity studies in noni.*

Keywords*: Noni,* Morinda citrifolia*, genetic diversity, characterization, descriptor list.*

1 INTRODUCTION

Noni, *Morinda citrifolia* (L.): Rubiaceae (syns. *M. bracteata* Roxb., *M. citrifolia* var. *bracteata* (Roxb.) Hook f.; *M. indica* L.), is a plant species with numerous medicinal properties (Petards 1972). Traditionally, noni has been used as a treatment for diseases and natural maladies throughout Southeast Asia, the Pacific Islands, and also in some parts of India, Africa and the Caribbean Islands (Morton 1992). Reviews on its medicinal uses (Dixon *et al*. 1999; McClatchey 2002; Chan-Blanco *et al*. 2006) surmised that its popularity seem to hinge on a combination of its traditional uses, development and distribution of modern products, and a mixture of factual and fanciful information provided directly by manufacturers and indirectly by academic researchers e.g. Heinicke (1985). So far, the most important compounds identified in noni fruits are phenolics, such as damnacanthal and scopoletin, organic acids (caproic and caprylic acid), vitamins (ascorbic acid and provitamin A), amino acids such as aspartic acid, and minerals (Wang *et al*. 2002; Chan-Blanco *et al*. 2006).

Noni is a small evergreen tree that bears cauliflorous compound fruit with a pronounced "rancid cheese" odor when ripe (Cribb and Cribb 1975). Seeds have large air sacs and pits in the cells of the seed testa that give them their buoyancy (Guppy 1917; Hayden and Dwyer 1969). These adaptation features of noni seeds have enhanced their natural dispersion inland through streams and rivers, and using ocean currents, it was able to colonize coastal ecosystems in the tropics and sub-tropics (Guppy 1917). Secondary dispersal is probably aided by fruit-eating birds and other animals, or may have been intentionally distributed as a medicinal plant by migrating humans who colonized the Pacific Islands (Whistler 1992; Abbot 1992). Noni is postulated to have originated in Southeast Asia (Morton 1992) and was subsequently distributed to the islands of the western Pacific by various dispersal mechanisms (Johansson 1994; McClatchey 2002). Noni has now become naturalized in the tropic and sub-tropic Atlantic islands and shores of the American continent (Morton 1992).

Three botanical varieties of noni have been identified: *M. citrifolia* var. *citrifolia*; *M. citrifolia* var. *bracteata*; and *M. citrifolia* var. *potteri.* These botanical varieties are differentiated based on various morphological features. *M. citrifolia* var. *bracteata* and *M. citrifolia* var. *potteri* are distinguished from *M. citrifolia* var. *citrifolia* by their conspicuous floral bracts and green-white leaf variegations, respectively. The latter is considered to be the typical variety (McClatchey 2003), and is widely used for commerce (Cambie and Ash 1994). Nevertheless, *M. citrifolia* is recognized as being a morphologically diverse species with no clear sub-populations bearing unique characteristics (Smith 1988).

Apart from the works of Smith (1988), Morton (1992), Johansson (1994), and McClatchey (2003), there is limited information on the extent of variation in noni. This may be attributed to it being a new crop whose increasing popularity was based on the drive for natural pharmaceuticals, the inaccessibility of researchers to diverse germplasm, and the lack of a standardized descriptor lists for characterizing noni germplasm. This highlights the need for the development of a descriptor list based on stable polymorphic traits that could be used to assess the extent of genetic diversity within the species, and for cultivar identification.

The present study is a first attempt to assess the extent of morphological variation among existing germplasm to identify potential polymorphic traits that may be useful in the development of a descriptor list.

2 MATERIALS AND METHODS

2.1 MATERIALS

A total of 39 mature Noni trees were sampled from five sites within the vicinity of Lae, Morobe Province, Papua New Guinea (PNG), were assessed (Table 1). Lae is

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geographically located at 6°S, 146°E with altitude ranging from sea level up to 100m. It receives an average annual rainfall in excess of 3000mm, and an average daily temperature of about 30°C.

At each site, the plantations comprised of segregating progenies of several unknown families. The samples studied included genotypes of all the three existing botanical varieties: a) *M. citrifolia* var. *citrifolia*; b) *M. citrifolia* var. *bracteata*; and c) *M. citrifolia* var. *potteri*. Characterisation was based on one sample per genotype. No clones were used due to limited time for propagation. *M. citrifolia* var. *bracteata* and *M. citrifolia* var. *potteri* had one samples each as they are rare, and time limitations for generation of data, while *M. citrifolia* var. *citrifolia* formed the bulk of the genotypes studied (Table 1).

Table 1 Visited sites and description of noni, *Morinda citrifolia* (L.), trees that were sampled in this study.

Site	Location	Botanical variety	Age of plant (years)	No. of sample	Genotype
Unitech Farm	6° 39' S, 146° 59' E	*citrifolia*	3	30	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
Yanga	6° 42' S, 147° 1' E	*citrifolia*	3	5	34, 36, 37, 38, 39
Malabu Settlement	6° 40' S, 146° 59' E	*citrifolia*	3	2	32, 33
Bundi Camp	6° 42' S, 146° 59' E	*bracteata*	2	1	35
Nasuapum	6° 34' S, 146° 49' E	*potteri*	4	1	31

2.2 CHARACTERISATION

In developing a working descriptor list, the coffee (*Coffea* spp: Rubiaceae) descriptor list (Anthony and Dussert 1996) was used as a template. Initially, 70 vegetative, floral, fruit and seed traits were identified based on observable levels of polymorphism at the visited sites. The quantitative traits were measured using either continuous or ordinal scales, while qualitative traits were assessed using either nominal or binary scales.

2.3 STATISTICAL ANALYSIS

The characterisation data set was collated using Microsoft Excel® spreadsheet. The data was then standardized before subjecting it to Cluster and Principal Component (PCA) Analyses using the software program Genstat Discovery Version, 2nd Edition (VSN 2005). Cluster analysis was performed to assess the level of similarity among the population based on the traits measured. A similarity matrix based on Euclidean distance coefficient was generated and clustered using the Group Average method (Sokal and Michener 1958).

Additionally, PCA was performed where principal components (PC) with latent roots ≥ 1.0 were considered important and were selected, as proposed by Jeffers (1967). The traits that were considered to be influential in determining the observed variation under the respective PCs were also assessed. Those traits with correlation coefficient ≥ 0.6 were seen to have greater contributions in explaining the observed variation (Matus *et al.* 1996). The level of diversity expressed by individual genotypes was determined based on the sum of squares of PC scores of the important components.

Pearson correlation was also performed on the standardised data using Minitab Release 13.31 (Minitab 2000) to assess which two traits are linearly related.

3 RESULTS

After omission of traits that were monomorphic and those that had missing data, a final descriptor list consisting of 58 polymorphic descriptor states was then used to generate the 58 x 39 data matrix for this study (Table 2 and 3). The monomorphic traits were noted in this study but were omitted from the list of descriptors include; phyllotaxy (clockwise-branching) leaf undulation and corolla tube and lobe color (creamy-white). Variable proportions of the sampled genotypes (0–97.4 %) were observed to express the qualitative traits (Table 2). A range of variation was also observed for the quantitative traits (Table 3). Most obvious were the high CV for plant height to the primary branch (58.8 %) and fruit weight (67.47 %).

Cluster analysis was useful in identifying unique groups of individuals (Figure 1). Individuals in cluster I, II and III were identified as those from variety *citrifolia*, and while clusters IV and V were of varieties *potteri* and *bracteata*, respectively. Cluster I had the highest number of individuals (25), followed by cluster III (7), cluster II (5) and the least were clusters IV and V each with a single individual. With the exception of varieties *potteri* and *bracteata*, the diversity groups were also found to pool several individuals of variety *citrifolia* from the various sites having similar traits. For instance, in cluster I, genotype 37 from Yanga occurs along with predominantly Unitech farm genotypes, while cluster III comprised of individuals from Yanga, Malabu settlement, and Unitech farm.

Although a total of 16 PCs had latent root >1.0 and cumulatively accounted for 85.9 % of the total variation (Table 4), only the first four PCs detected the most influential traits with correlation coefficient >0.6 (Table 5). The most important traits with greater contributions to the observed variation include; plant shape, internode length,

Table 2 Proportions and ranges (measured traits) of descriptor states in the studied noni, *Morinda citrifolia* (L.), germplasm.

Trait code.	Descriptor state	Proportion (%)	Trait code	Descriptor state	Proportion (%)
GOP	Plant shape		CFO	Colour of floral eye outline	
	a) Elongated conical	25.6		a) Pink	0.0
	b) Pyramidal	43.6		b) Green	66.7
	c) Bushy	25.6		c) Yellowish green	15.4
	d) Circular	2.6		d) Whitish green	2.6
	e) Droopy	2.6		e) Creamy green	15.4
OSH	Orthotropic shoot		SFE	Sloppiness of floral eye outline	
	a) Present	66.7		a) Raised sloppy outline	53.8
	b) Absent	33.3		b) Flat outline	23.1
YSP	Young shoot pigmentation			c) Sunken outline	15.4
	a) No pigmentation	87.2		d) Irregular	7.7
	b) Reddish pigmentation	12.8	FST	Fruits skin texture	
	c) Pinkish pigmentation	0.0		a) Rough (shallow cracks on almost all fruit eyes)	59.0
AIP	Angle of insertion of primary branches on main stem			b) Smooth (no cracks to few shallow cracks)	33.3
	a) Erect (≤ 45 degrees)	15.4		c) Very rough (deep cracks on fruit flesh and eyes)	7.7
	b) Semi-erect (> 45 degrees ≤ 90 degrees)	69.2	FSH	Fruit shape	
	c) Drooping (> 90 degrees)	15.4		a) Round	28.2
SSH	Stipule shape on lateral shoot			b) Obovate wide (egg-shaped)	51.3
	a) Round	25.6		c) Obovate elongata (carrot-shaped)	10.3
	b) Ovate	43.6		d) Oblong narrow	7.7
	c) Triangular	12.8		e) Round to obovate	2.6
	d) Ovate with truncate apex	17.9	OFB	Occurrence of floral bract on fruits	
	e) Ovate with cuspidate apex	0.0		a) No bract	94.9
SCL	Stipule color on lateral shoot			b) Single floral bract	2.6
	a) Green	53.8		c) Two to three floral bracts	0.0
	b) Light green	33.3		d) More than three bracts	2.6
	c) Dark green	5.1	PPF	Peduncle positioning at flowering	
	d) Pale green	5.1		a) Erect stout	38.5
	e) Reddish green	2.6		b) Erect long	48.7
PCR	Leaf petiole color			c) Semi-drooping	10.3
	a) Light green	38.5		d) Drooping	2.6
	b) Whitish green	2.6	PPM	Peduncle positioning at maturity	
	c) Reddish green	2.6		a) Erect stout (or sessile)	35.9
CLM	Color of upper side of leaf mid rib			b) Erect long	15.4
	a) Whitish green	97.4		c) Drooping	48.7
	b) Pinkish red	2.6	FBU	Fruit bunching	
CUM	Color of under side of leaf mid rib			a) Present	20.5
	a) Whitish green	97.4		b) Absent	79.5
	b) Pinkish red	2.6	FBR	Fruit branching	
YSC	Young shoot (foliage) color			a) Present	2.6
	a) Green	33.3		b) Absent	97.4
	b) Light green	66.7	FSE	Fruit segmenting	
LLC	Leaf lamina color			a) Present	38.5
	a) Green	82.1		b) Absent	61.5
	b) Light green	10.3	PMF	Peduncle insertion on matured fruits	
	c) Dark green	5.1		a) Exserted	0.0
	d) White-green variegation	2.6		b) Lightly inserted	43.6

Table 2 continued

Trait code.	Descriptor state	Proportion (%)	Trait code	Descriptor state	Proportion (%)
LGS	Leaf glossiness			c) Deeply inserted (not obvious)	56.4
	a) Glossy		SCR	Seed color	
	b) Semi-glossy	12.8		a) Black	0.0
	c) Not glossy	79.5		b) Brown	33.3
LAS	Leaf apex shape	7.7		c) Reddish brown	61.5
	a) Acute			d) Dark brown	5.1
	b) Acuminate	82.1		e) Silver brown	0.0
LVN	Leaf venation number	17.9	SSE	Seed shape	
	a) 6-veins			a) Narrowly ovate	25.6
	b) 7-veins	28.2		b) Widely ovate	74.4
	c) 8-veins	25.6	SBS	Seed base shape	
	d) 6-7 veins	0.0		a) Pointed	41.0
	e) 6-8 veins	20.5		b) Sharply pointed	30.8
	f) 7-8 veins	7.7		c) Pointed but curved tip	28.2
	g) 9-8 veins	7.7	SCS	Surface curvature of seed apex	
	h) 9-veins	2.6		a) Smooth	
CLN	Corolla lobe number per floret	7.7		b) Rough	
	a) 5-lobed			c) Mildly rough	
	b) 6-lobed	28.2	SSA	Shape of seed air sac	
	c) 5-6-lobed	7.7		a) Globular	12.8
AIC	Anther insertion on corolla	64.1		b) Semi-globular	76.9
	a) Included (below the corolla surface)			c) Flattened	10.3
	b) Excluded (above the corolla surface)	94.9	TSB	Texture of seed back	
HET	Heterostyly	5.1		a) Smooth	5.1
	a) Tall style			b) Mildly rough (sandpapery feel)	74.4
	b) Equal height position	71.8		c) Wrinkled	20.5
	c) Short style	23.1	OEW	Outward extension of wing-like structure along seed vertical edges	
ANF	Anther number per floret	5.1		a) Narrowly extended	56.4
	a) Five			b) Widely extended	38.5
	b) Six	33.3		c) No extension	5.1
	c) Five to six	2.6	TWE	Termination of wing-like structure extension relative to seed apex	
NOF	Number of fully opened florets on flowering head at a time	64.1		a) Terminating slightly below apex	12.8
	a) One			b) Terminating well below apex	7.7
	b) Two	20.5		c) Terminating gradually at the apex	64.1
	c) Three	0.0		d) Terminating abruptly at the apex	15.4
	d) One to two	0.0	FVT	Formation of vertical trench between air sac and wing-like structure	
	e) One to three	33.3		a) Deep	46.2
OPF	Occurrence of pistillate florets	46.2		b) Shallow	20.5
	a) Present			c) Very shallow	33.3
	b) Absent	66.7	FLA	Flap-like appendage at the lower side of the air sac	
FCL	Fruit color	33.3		a) Highly increased	
	a) Yellowish green			b) Highly reduced	59.0
	b) Whitish green	12.8		c) Sharp increase	30.8
	c) Green	79.5			10.3
		7.7			

Table 3 Variation observed in quantitative traits of noni, *Morinda citrifolia* (L.)[†].

Trait code[§]	Trait	Mean ± SE (m)	Minimum (m)	Maximum (m)	CV (%)
PHP	Plant height to first primary branch (m)	0.64±0.06	0.20	2.30	58.80
PHT	Plant height to terminal bud (m)	2.70±0.13	1.59	5.23	31.04
PSN	Plant span (m)	2.44±0.10	1.20	3.60	25.85
SDR	Stem diameter (cm)	6.02±0.25	3.60	9.62	25.43
ILT	Internode length (cm)t	10.64±0.40	6.42	19.60	23.59
LLT	Leaf length (cm)	24.18±0.43	17.90	29.18	11.14
LWH	Leaf width (cm)	12.30±0.33	7.90	17.00	16.91
PLT	Petiole length (cm)	1.34±0.06	0.80	2.86	29.22
CTL	Corolla tube length (cm)	0.96±0.02	0.80	1.19	10.43
SLT	Style length (cm)	1.12±0.02	0.90	1.40	12.75
FLT	Fruit length (cm)	5.83±0.19	4.14	9.33	20.15
FWH	Fruit width (cm)	4.31±0.11	3.00	6.45	15.63
FWT	Fruit weight (g)	36.00±3.89	17.06	153.80	67.47
SEL	Seed length (cm)	0.86±0.02	0.72	1.11	11.90
SWH	Seed width (cm)	0.45±0.01	0.35	0.53	9.27
STS	Seed thickness (cm)	0.22±0.00	0.19	0.28	8.77

[†]SE = Standard error, CV = Coefficient of variation; and [§]Trait codes continues from Table 2.

Table 4 Variation accounted for by each principal component (PC).

Principal component	Latent roots	Variability (%)	Accumulated variability (%)
PC1	8.0	13.8	13.8
PC2	7.0	12.1	25.9
PC3	5.3	9.1	35.0
PC4	5.0	8.6	43.6
PC5	3.8	6.5	50.1
PC6	3.0	5.2	55.3
PC7	2.8	4.8	60.1
PC8	2.5	4.4	64.5
PC9	2.2	3.8	68.3
PC10	1.9	3.2	71.5
PC11	1.8	3.0	74.5
PC12	1.7	2.8	77.3
PC13	1.4	2.4	79.7
PC14	1.3	2.3	82.0
PC15	1.2	2.0	84.0
PC16	1.1	1.9	85.9

Table 5 Correlation coefficients of each trait with respect to each principal component

Trait code[§]	Principal components (PC)				Trait code	Principal components (PC)			
	PC1	PC2	PC3	PC4		PC1	PC2	PC3	PC4
PHP	0.11	0.32	0.25	-0.05	OPF	0.47	0.74[†]	-0.10	-0.27
PHT	0.39	0.50	-0.14	0.23	FCL	-0.37	0.10	-0.17	0.24
PSN	0.09	0.40	-0.22	0.06	CFO	-0.11	-0.07	-0.08	0.33
GOP	-0.62[†]	0.22	-0.01	-0.14	SFE	-0.46	-0.20	-0.36	-0.50
OSH	-0.24	-0.16	0.07	0.51	FST	-0.61[†]	0.29	-0.40	0.41
SDR	-0.17	0.79[†]	0.02	-0.07	FSH	0.64[†]	0.18	-0.17	-0.19
YSP	0.21	0.62[†]	-0.18	-0.11	OFB	0.68[†]	-0.20	-0.18	-0.07
AIP	0.46	0.78[†]	-0.11	-0.29	FLT	0.21	0.05	0.28	0.03
SSH	0.46	0.62[†]	-0.11	-0.29	FWH	0.39	0.26	0.69[†]	-0.39
SCL	0.47	-0.23	-0.54	-0.01	FWT	-0.09	0.37	0.49	-0.05
ILT	-0.61[†]	0.35	0.05	-0.03	PPF	-0.39	0.03	0.42	0.18
LLT	-0.23	0.13	-0.51	0.44	PPM	-0.02	0.34	-0.05	0.72[†]
LWH	0.33	0.14	-0.40	-0.48	FBU	-0.05	0.48	-0.13	0.03
PLT	0.26	0.32	0.24	0.12	FBR	-0.41	0.35	-1.03	0.09
PCR	-0.32	0.43	0.04	0.00	FSE	0.51	0.27	-0.09	-0.37
CLM	0.05	0.12	0.31	-0.28	PMF	0.03	-0.03	-0.72[†]	-0.19
CUM	-0.53	0.28	0.29	-0.32	SEL	-0.49	-0.24	-0.21	-0.36
YSC	-0.46	0.10	0.33	-0.30	SWH	-0.13	0.12	0.09	-0.27
LLC	-0.04	0.17	0.41	-0.17	STS	-0.25	-0.21	-0.64[†]	-0.06
LGS	0.03	0.31	-0.36	0.18	SCR	0.02	0.16	0.20	0.27
LAS	0.10	0.33	-0.20	0.45	SSE	-0.59	-0.04	-0.10	0.18
LVN	-0.58	0.42	-0.22	-0.41	SBS	0.17	-0.39	-0.19	-0.15
CLN	0.31	0.03	0.19	-0.30	SCS	-0.15	-0.19	-0.37	-0.56
AIC	-0.49	-0.01	0.24	-0.49	SSA	-0.31	0.46	0.48	0.38
HET	0.67[†]	0.14	0.10	-0.15	TSB	-0.24	0.57	0.26	-0.30
ANF	-0.14	0.13	0.09	-0.09	OEW	0.13	0.36	0.43	0.35
CTL	-0.16	0.39	-0.12	0.04	TWE	-0.68[†]	-0.05	-0.24	-0.45
SLT	-0.42	0.06	0.20	-0.03	FVT	0.20	0.51	-0.46	-0.31
NOF	-0.15	0.11	-0.18	-0.18	FLA	-0.38	0.46	-0.18	-0.24

[†] Relevant traits when explaining the component; and [§]Trait codes continues from Table 2.

Table 6 Diversity ranking of the studied genotypes based on the principal component scores.

Rank	Genotype	Diversity score	Rank	Genotype	Diversity score
1	35	242.3	21	14	40.6
2	31	190.0	22	12	34.1
3	34	123.6	23	15	31.4
4	33	65.3	24	24	30.9
5	11	63.5	25	3	30.3
6	32	60.6	26	5	29.4
7	38	57.6	27	27	26.8
8	13	54.6	28	1	26.7
9	7	54.5	29	17	26.1
10	16	53.9	30	21	25.0
11	6	53.2	31	36	25.0
12	39	51.5	32	10	24.3
13	23	46.3	33	9	23.7
14	22	44.8	34	18	21.8
15	37	44.4	35	8	21.2
16	20	43.1	36	26	17.6
17	28	41.9	37	4	16.5
18	25	41.4	38	30	16.2
19	29	41.1	39	2	13.0
20	19	41.0			

Table 7 Correlation coefficients of noni, *Morinda citrifolia*(L.), traits showing significant associations.

Trait code[§]	PHT	THT	PSN	SDR	LPL	LPC	CTL	CUL	LLC	CLN	AIC	FLT	FWT
THT	**0.73****												
PSN	0.18	0.57**											
SDR	0.32*	**0.69****	**0.80****										
LPL	0.53**	**0.60****	-0.04	0.08									
LPC	0.07	0.22	-0.16	-0.08	0.36*								
CTL	0.07	0.26	-0.22	-0.2	**0.64****	**0.60****							
CUL	0.07	0.26	-0.22	-0.2	**0.64****	**0.60****	**1.00**						
LLC	-0.04	-0.03	-0.09	0.05	-0.02	0.08	-0.07	-0.07					
CLN	0.03	0.15	0.06	-0.01	0.18	-0.01	0.12	0.12	0.17				
AIC	0.33*	0.33*	0.33*	0.47**	0.18	0.07	-0.05	-0.05	-0.12	0.21			
FLT	0.46**	0.33*	0.02	0.11	0.2	0.29	-0.18	-0.18	0.23	0.11	0.07		
FWT	0.55**	0.45**	0.12	0.34*	0.36*	0.12	-0.1	-0.1	**0.60****	-0.13	0.11	**0.62****	
SWD	-0.12	0.04	-0.11	0.04	0.22	0.22	0.28	0.28	0.06	0.03	0.31*	-0.07	0.11

[§]Trait codes are defined in Tables 2 and 3; Superscripts *** = significant association at P<0.05 and P<0.01 respectively; and correlation coefficients in bold typescripts are considered as important linear associations.

Table 8 Polymorphic morphological traits selected to form the descriptor list for noni, *M. citrifolia*.

Code No.	Trait	Code No.	Trait
1.0	Vegetative characters	2.7	Number of fully opened florets on flowering heads at one time
1.1	Plant height	2.8	Presence of pistillate florets
1.2	Crown diameter	2.9	Occurrence of floral bracts
1.3	Plant shape	3.0	Fruit characters
1.4	Presence of orthotropic shoot	3.1	Fruit colour
1.5	Trunk diameter	3.2	Colour of floral eye outline
1.6	Young shoot pigmentation	3.3	Floral eye position relative to bract or rudimentary bract
1.7	Growth habit of primary branch	3.4	Fruit skin texture
1.8	Interpetiolar stipule apex shape on lateral shoot	3.5	Fruit shape
1.9	Stipule colour on lateral shoot	3.6	Presence of parthenocarpic florets
1.10	Internode length	3.7	Fruit length
1.11	Leaf length	3.8	Fruit width
1.12	Leaf width	3.9	Fruit weight
1.13	Leaf petiole length	3.10	Peduncle positioning at flowering
1.14	Leaf petiole colour	3.11	Peduncle positioning at maturity
1.15	Young shoot (foliage) colour	3.12	Fruit bunching
1.16	Leaf lamina colour	3.13	Fruit branching
1.17	Leaf glossiness	3.14	Fruit segmentation
1.18	Leaf apex shape	3.15	Fruit base shape on mature fruit
1.19	Number of lateral veins of leaf	4.0	Seed characters
2.0	Floral characters	4.1	Average number of seeds per fruit
2.1	Number of corolla lobes per floret	4.2	Seed length
2.2	Length of filament	4.3	Seed width
2.3	Heterostyly	4.4	Seed thickness
2.4	Anther number per floret	4.5	Seed colour
2.5	Corolla tube length	4.6	Presence of pulp plates on seed coat
2.6	Style length		

Source: Waki *et al.* (2008).

young shoot pigmentation, stem diameter, angle of insertion of primary branch on main stem, stipule shape, heterostyly, occurrence of pistillate florets (Figure 2), fruit shape, occurrence of floral bracts on fruits (Figure 3), fruit width, and peduncle positioning at maturity. Based on the diversity score generated from PCA (Table 6), the most diverse varieties were identified to be 34, 35, and 31, respectively.

In addition, correlation based on the standardised data set showed numerous associations between the various

traits (Table 7). Considering the genetic nature of the samples, the sample size and the CV observed on some metric traits, only the highly significant ($P<0.001$) correlations were considered.

Using these results and confirmatory field observations, the descriptor list for noni was rationalised by eliminating redundant descriptor states and reducing the levels of those that were too finely defined e.g. leaf venation number, LVN (Table 2). Firstly, the descriptor state levels of plant shape and occurrence of floral bracts were appropriately reworded to avoid ambiguity. Similarly, filament length was found to be either conspicuous or inconspicuous and was so rephrased. Further field observations revealed that the number of the first five or more, but not greater than 10, florets were observed to be expressing parthenocarpy and was therefore included as a new descriptor state. Moreover, closer examination of the seed (Figure 4) also revealed that the grooves on the seed testa were actually pulp plates that may be found on the air sac, the embryo sac, and between the spine and the air sac, and thus, makes redundant eight descriptor states, namely SBS, SCS, SSA, TSB, OEW, TWE, FVT, and FLA (Table 2), that described the same structure.

The correlated traits were also considered for exclusion to eliminate redundancy in the descriptor list. Although an absolute association was observed for color of midrib of top and underside of leaf (r=1.00), both were also linearly associated with petiole color and so were replaced by the latter. Plant height from ground level to the terminal bud was also found to be linearly associated with plant height to the first primary branch (r=0.73) and leaf length (r=0.60) and so was omitted. Rationalisation of the descriptor list resulted in the selection of 49 polymorphic descriptor states (Table 8) that could be used for characterising noni germplasm.

4 DISCUSSIONS

Noni is naturally propagated from seed. The present study has shown variable levels of diversity amongst the characterised genotypes. This may be indicative of a higher level of heterozygosity in noni. Although it has been viewed that noni is a self-pollinating species (Nelson 2003), the expression of heterostyly and pistillate florets suggests possibility of out-crossing in the species. It was observed that some genotypes that had pistillate florets (Figure 2) and/ or florets with tall styles, that is, those that exceeded the height of the anthers on the corolla tube, tend to exhibit premature fruit fall, when completely isolated.

Expressions of traits common to the three botanical varieties were also noted. These traits were variably expressed by the three most diverse genotypes, namely, genotypes 34, 35 and 31. Firstly, occurrence of floral bract was observed to be present on all the florets of the inflorescences of genotype 35 (var. *bracteata*), while it was only observed in the first three florets of some inflorescences of genotype 34 (var. *citrifolia*) and genotype 31 (var. *potteri*). Secondly, fruit branching (Figure 5) was noted to be predominant in genotype 31, while it was expressed in some fruits of genotype 34, but was not observed in genotype 35. The white-leaf variegation, however, was unique to variety *potteri*. Occurrence of floral bract and fruit branching were observed to be transitional between the botanical varieties.

Although the genetic nature of these traits is yet to be elucidated, their random expressions provide circumstantial evidence on the involvement of mutations. For varieties *bracteata* and *potteri*, their distinct morphological features, that is, floral bracts and white-leaf variegation, respectively, may have been the consequences of such mutations enhanced by isolation and non-random mating. Natural barriers such as land and sea (ocean) together with floral characteristics that facilitate self-pollination may have fostered the development of these unique morphotypes. More elaborate studies on wild communities of these morphotypes may provide useful clues to shed light on the breeding system of the species.

Characterisation based on the 58 morphological descriptor states was able to separate the 39 genotypes studied as different morphotypes. However, caution should taken when interpreting these results, as their genetic background as segregating progenies, the environment, and more so plant age may have had some influence on the observed variation, particularly on the quantitative traits measured. The high level of variation observed for plant height (from ground level) to the first primary branch (CV = 58.80%) and fruit weight (CV = 67.47%) may be indicative of such influence. It is, therefore, crucial that samples comprising of several clones of the same age are grown in one or more locations to counteract the variations due to plant age, the environment and the interaction between the genotype and the environment.

The level of polymorphism expressed by young shoot pigmentation, fruit shape, stem diameter, angle of insertion of primary branch on main stem, fruit width, stipule shape, heterostyly, pistillate florets, occurrence of floral bracts and peduncle positioning at maturity were able to explain the observed variation. However, growth-dependent traits such as stem diameter and fruit width must be treated with reservations unless the assessment in done on plant samples of the same age. Additionally, data obtained in this study was based a single sample per genotype, and so, the stability of the descriptor states still needs to be established.

Significant correlations were also noted between several of the studied traits, such that considerable redundancy in the descriptor list was revealed. As such, it was essential to rationalise the descriptor list is in order to avoid diminishing marginal returns to the use of increasing number of descriptor list as noted in taro, *Colocasia esculenta* (L.) Schott (Okpul *et al*. 2005).

The results obtained in this study have provided useful information enabling the development of the descriptor list for noni comprising of 49 polymorphic descriptor states (Table 8, Waki *et al.* 2008). This descriptor list will be useful in accessing variation within and between noni populations, and may serve as a basis for further improvement in future. Future work should look at a larger sample size both clonal and genotypes, of all the three botanical varieties, originating from several sites where they are endemic. It would also be appropriate to complement agro-morphological data with geographical, chemical and molecular traits in improving the current descriptor list. Diversity assessment at molecular levels for noni would no doubt be an added advantage, as it will enable assessment at the genotype level.

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6 REFERENCES

Abbot, I.A. 1992. *La'au Hawaii: Traditional Hawaiian uses of plants*. Bishop Museum Press, Honolulu, Hawaii, USA.

Anthony, J., Dussert, S. 1996. *Descriptors for Coffee (*Coffea *spp. and* Psilanthus *spp.)*. International Plant Genetic Resource Institute, Rome, Italy.

Cambie, R.C., Ash, J. 1994. *Fijian medicinal plants*. CSIRO, Canberra, Australia.

Chan-Blanco, Y., Vaillant, F., Perez, A.M., Reynes, M., Brillouet, J., Brat, P. 2006. The noni fruit (*Morinda citrifolia* L.): A review of agricultural research, nutritional and therapeutic properties. *Journal of Food Composition and Analysis* **19**, 645-654.

Cribb, A.B., Cribb, J.W. 1975. *Wild foods in Australia*. William Collins, Pty. Ltd., Sydney, Australia.

Dixon, A.R., McMillen, H., Etkin, N.L. 1999. Ferment this: The transformation of noni, a traditional Polynesian medicine (*Morinda citrifolia*, Rubiaceae). *Economic Botany* **53(1)**, 51-68.

Guppy, H.B. 1917. *Plants, Seeds and Currents in the West Indies and Azores*. Williams and Norgate, Covenant Garden, London, UK.

Hayden, M.V., Dwyer, J.D. 1969. Seed morphology in the tribe Morindeae (Rubiaceae). *Bulletin of Torrey Botany Club* **96(6)**, 704-710.

Heinicke, R.M. 1985. The pharmacologically active ingredient of noni. *Pacific tropical botanical bulletin* **15**, 10-14.

Jeffers, J.N.R. 1967. Two case studies in the application of principal component analysis. *Application of Statistics* **16**, 225-236.

Johansson, J.T. 1994. The genus *Morinda* (Morindeae, Rubioideae, Rubiaceae) in New Caledonia: taxonomy and phylogeny. *Opera Botanica* **122**, 1-67.

McClatchey, W. 2002. From Polynesian healers to health food stores: changing perspectives of *Morinda citrifolia* (Rubiaceae). *Integrative Cancer Therapies* **1(2)**, 110-120.

McClatchey, W. 2003. Diversity of growth forms and uses in the *Morinda citrifolia* L. complex. In: *Proceedings of the 2002 Hawaii Noni Conference*, S.C. Nelson (ed.), University of Hawaii at Manoa, Hawaii, USA, 5-10.

Matus, I.M., Gonzales, G., Del Poso, A. 1996. Evaluation of phenotypic variation in the Chilean collection of garlic (*Allium sativum*, L.) clones using multivariate analysis. *Plant Genetic Resources Newsletter* **117**, 31-36.

Minitab. 2000. *Minitab Statistical Software*, Minitab Inc. Http://www.minitab.com

Morton, J.F. 1992. The Ocean-going Noni, or Indian Mulberry (*Morinda citrifolia,* Rubiaceae) and some of its colourful relatives. *Economic Botany* **46**, 241-256.

Nelson, S.C. (ed.). 2003. *Proceedings of the 2002 Hawaii Noni Conference*. University of Hawaii CTAHR-Cooperative Extensive Service Proceedings P-1/03, University of Hawaii, Honolulu, Hawaii.

Okpul, T., Mace, E.S., Godwin, I.D., Singh, D., Wagih, M.E. 2005. Evaluation of variability among breeding lines and cultivars of taro (*Colocasia esculenta* (L.) Schott) in Papua New Guinea using ISSR fingerprinting and agro-morphological characterisation. *Plant Genetic Resource Newsletter* **143**, 8-16.

Petards, J. 1972. *Raau Tahiti. The use of Polynesian medicinal plants in Tahitian medicine*. South Pacific Commission, Noumea, New Caledonia.

Smith, A.C. 1988. *Morinda* Pacific Tropical Botanical Garden, Lawai, Hawai'i. *Flora Vitiensis Nova* **4**, 332-341.

Sokal, R. R. and Michener, C. D. 1958. A statistical method for evaluating systematic relationships. Univ Kansas Science Bull. **38**, 1409-1438.

VSN. 2005. Genstat Discovery Version (2nd edition). Http: // www.vsn-intl.com.

Waki, J. 2005. *Morphological variation among noni (Morinda citrifolia) landraces in Morobe Province, Papua New Guinea*. Unpublished Postgraduate Diploma thesis. Agriculture Department, PNG University of Technology, Lae, Papua New Guinea.

Waki, J., Okpul, T. and Komolong, M. 2008. A descriptor list for morphological characterisation of Noni (*Morinda citrifolia* L). *South Pacific Journal of Natural Science* **10**, 61-66.

Wang, M.Y., West, B.R., Jensen, C.J., Nowicki, D., Su, C., Palu, A.K., Anderson, G. 2002. *Morinda citrifolia* (noni): A literature review and recent advances in noni research. *Acta Pharmacologica Sinica* **23**,1127-1141.

Whistler, W.A. 1992. *Polynesian herbal medicine*. National Tropical Botanical Gardens, Lawai, Kaua'i, Hawaii, USA.

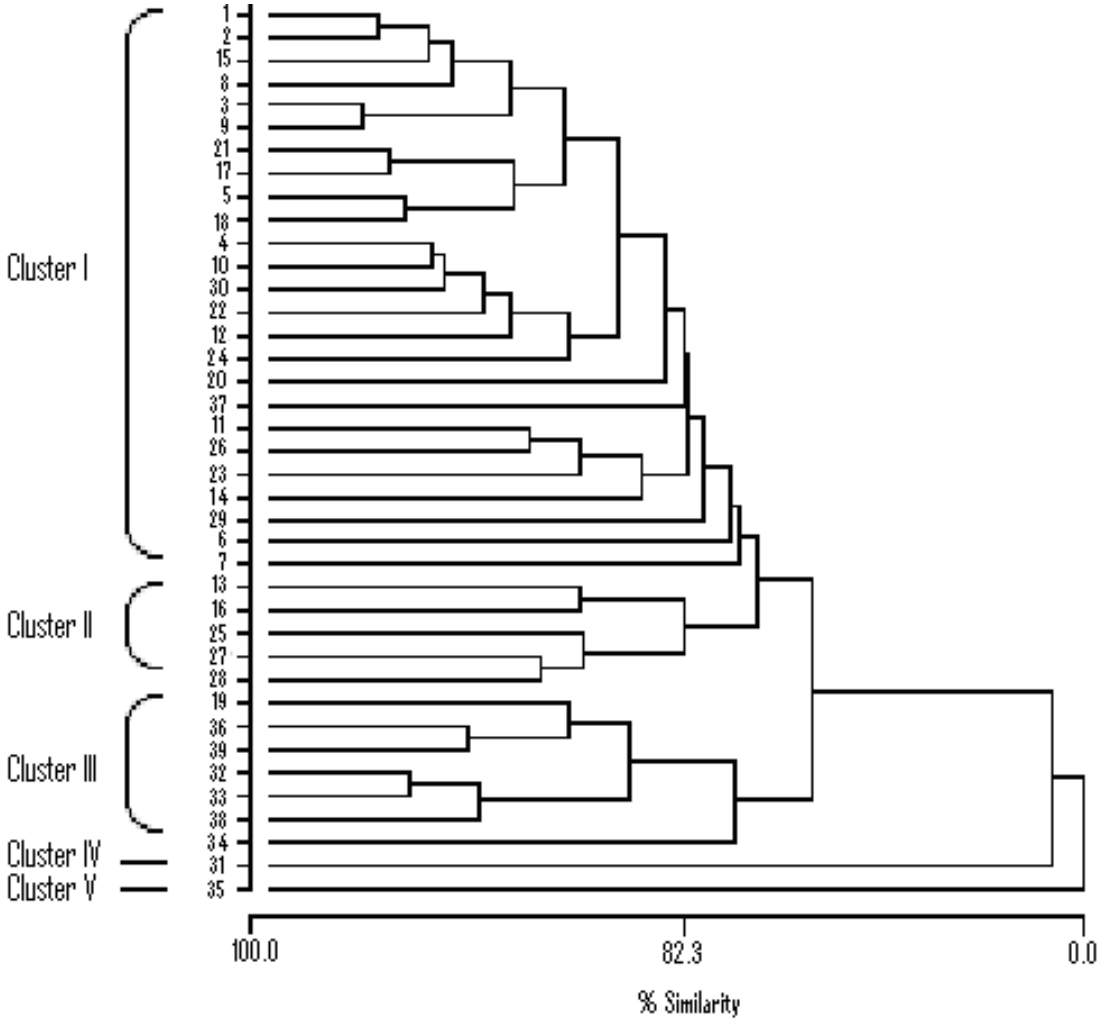


Figure 1 A dendrogram illustrating similarity based on Euclidean distance coefficient for 39 noni, *Morinda citrifolia* (L.), genotypes based on 58 morphological descriptor states.

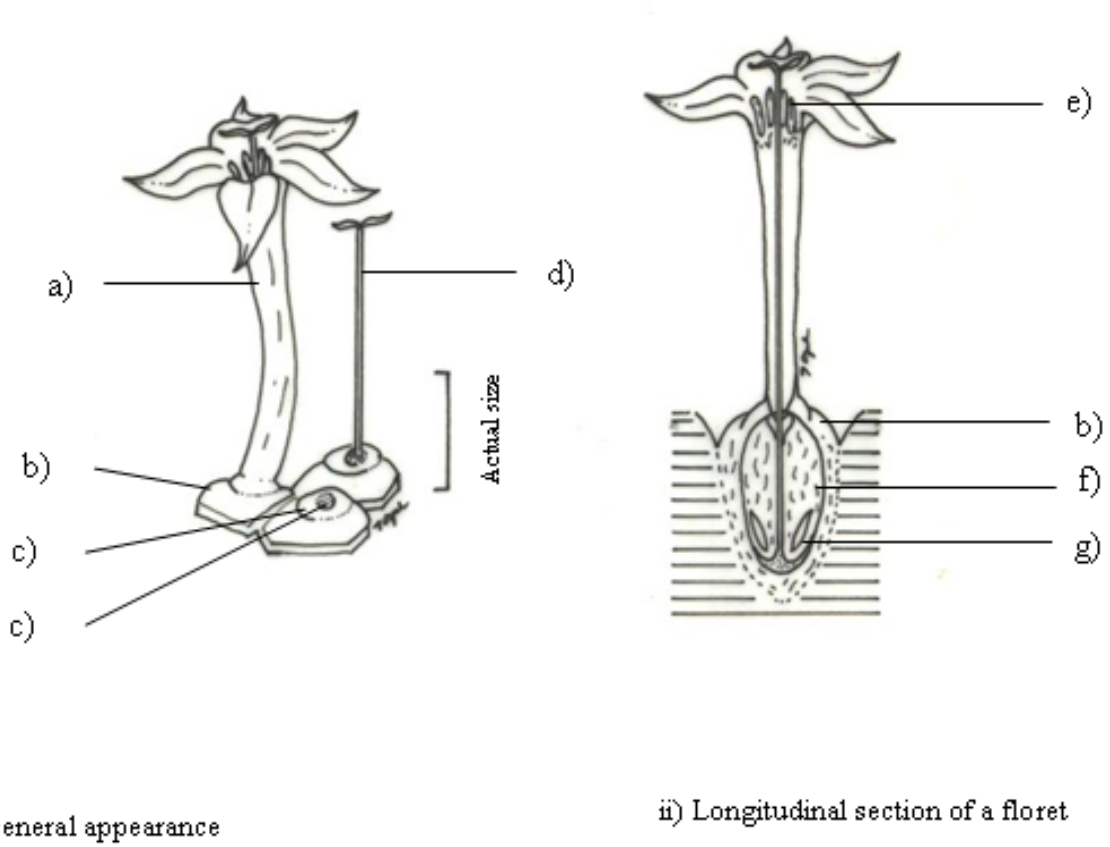


Figure 2 Floral characters: a) normal floret, b) rudimentary floral bract, c) floral eye, scar left by the corolla tube and style, d) pistillate floret with exposed pistil, e) stamen, f) placenta, and g) ovule (Waki *et al.* 2008).

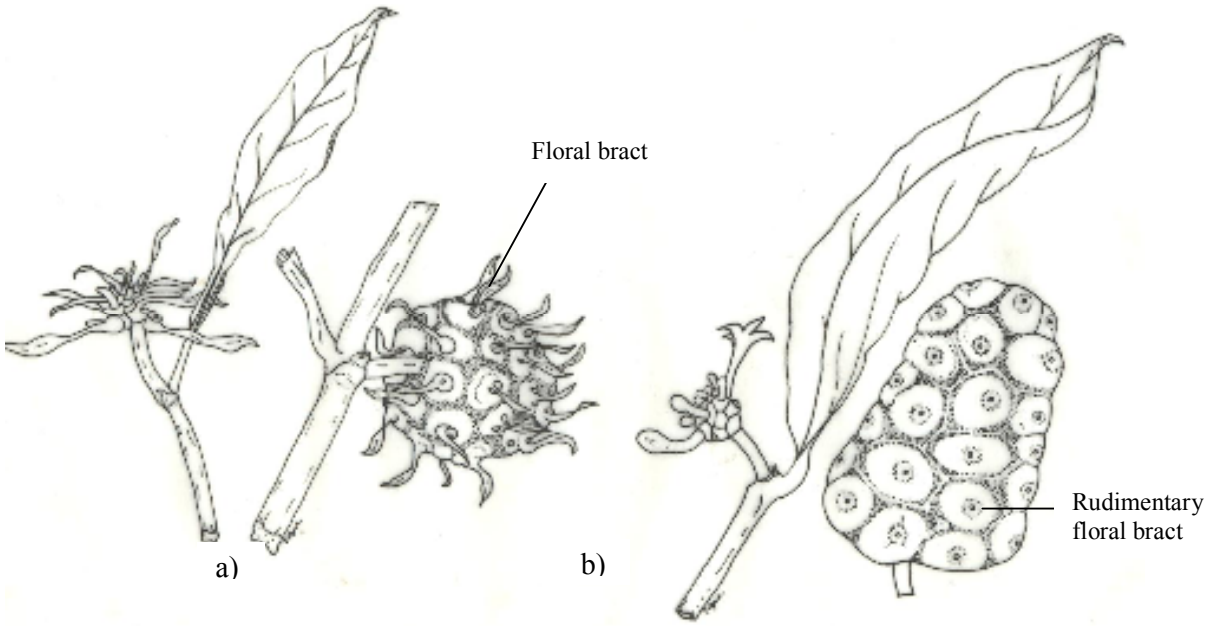


Figure 3 Inflorescences and fruits: a) variety *bracteata*, , showing floral bracts, and b) variety *citrifolia*, with rudimentary bracts (Waki *et al.* 2008).

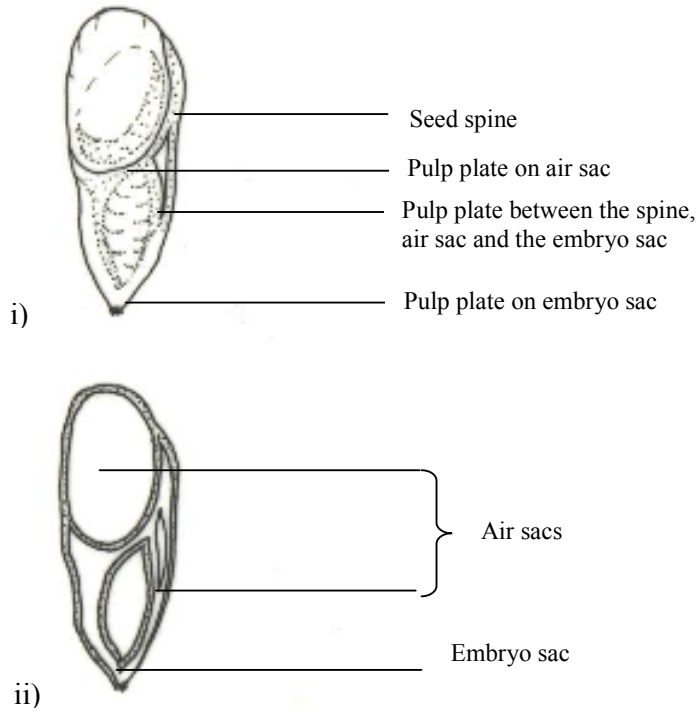
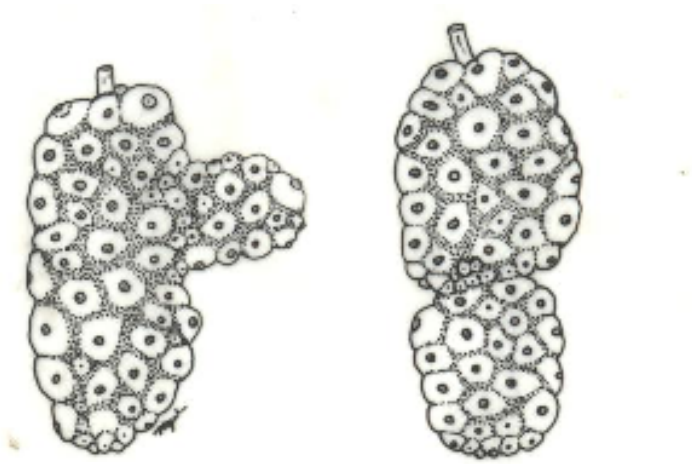


Figure 4 Seed characteristics: i) External features, and ii) a longitudinal section of a generalized noni seed (Waki *et al.* 2008).



a) Fruit branching b) Fruit segmentation

Figure 5 Fruit branching and segmentation (Waki *et al.* 2008).