Identification of the invasive weeds, camel melon, prickly paddy melon and colocynth in Australia—a morphological and molecular approach

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Summary Camel melon (*Citrullus lanatus* (Thunb.) Matsum. & Nakai var. *lanatus*), colocynth (*Citrullus colocynthis* (L.) Schrad.), and prickly paddy melon (*Cucumis myriocarpus* L.), are summer growing invasive weeds found throughout Australia. They infest both natural and agricultural ecosystems and are noxious weeds in some areas of Australia. Camel melon and prickly paddy melon are annuals, while colocynth is a perennial. Camel melon and prickly melon belong to different genera but as they share similar morphology and life history they are often misidentified at the vegetative stage. In this study, a molecular and morphological approach was used to facilitate identification of these melon species. For molecular taxonomic identification, two chloroplast genes *mat*K and *ycf*6–*psb*M intergenic spacer and a nuclear gene, *G3pdh* were used to identify these invasives. The sequences of *G3pdh* and *ycf*6–*psb*M identified camel melon as *C. lanatus* var. *citroides* and the colocynth as *C. colocynthis*, in direct contrast to the current widely published nomenclature (as above). Australian prickly paddy melon sequences at *mat*K revealed it to be *Cucumis myriocarpus.* For morphological characterisation, populations of each species from selected Australian states were grown in a glass house over a 4-month period in 2011. Each species exhibited distinct leaf lobation, branching of tendrils, floral, fruit and seed attributes, all of which are presented as useful identifying features. This study found that camel melon, colocynth and prickly paddy melon possessed unique morphological characteristics. In addition, each weed was identified to species level using multi locus DNA sequence analysis, demonstrating the utility of this approach for resolving nomenclatural errors and taxonomic mis-identifications.

Keywords Cucurbitaceous invasive weeds, camel melon, prickly paddy melon, colocynth, molecular taxonomy, morphological identification, *ycf*6–*psb*M, *G3pdh*, *mat*K, DNA sequence analysis, *Cucumis myriocarpus*, *Citrullus lanatus*, *Citrullus colocynthis*.

INTRODUCTION

Camel melon, prickly paddy melon and colocynth are summer growing invasive melons native to Africa and introduced into Australia in the mid 1800s. Camel melon and prickly paddy melon are annual weeds while colocynth is a perennial (Parsons and Cuthbertson 2001). These species are major weeds of broadacre crops and fallows and are declared noxious weeds in parts of Australia (Buckley 1981, Leys *et al*. 1990, Felton *et al.*1994, Michael *et al.* 2010). Prickly paddy melon is also a problematic invasive weed in southern Europe and California (Grubben and Denton 2004), while the global invasiveness of camel melon and colocynth is currently not well described. Camel melon is distributed across Europe, S. America and Africa (GBIF data portal 2012), while a similarly described melon referred to as a 'citron melon', (*Citrullus lanatus* var. *citroides*) is reported to be widely distributed weed in Texas (Grichar *et al.* 2001). Colocynth is found in parts of Asia, Europe and California (GBIF data portal 2012). Colocynth is a perennial and possesses a rootstock which perennates during winter and is capable of vegetative growth; it grows during summer months across Australia. In Australia, camel melon and prickly melon are summer growing annuals. As the three melons share similar morphology and life history, they are often confused in Australia (Michael *et al.* 2010). Their taxonomic identity remains inconsistent across Australian herbaria (B. Lepschi, personal communication, 2011). In order to successfully manage these weeds in Australian croplands, correct identification is critical.

Our recent studies with non voucher identified samples suggested relatively low genotypic diversity in geographically distinct populations of Australian camel melon and prickly paddy melon at *G3pdh* gene (Shaik *et al*. 2011). However, further studies with additional polymorphic genes and reassessment using identified voucher samples as references are required to substantiate those findings. A perusal of the literature (Dane *et al.* 2007) suggested that a

chloroplast gene region (*ycf*6–*psb*M) was also sufficiently informative to detect intraspecific variation within the genus *Citrullus*. Hence, this paper presents the sequence analysis at *G3pdh*, *mat*K and *ycf*6–*psb*M with additional samples (including voucher samples obtained from various Australian herbaria).

Comparative growth studies of the three species under controlled environmental conditions are necessary for further evaluation of the morphology of camel melon, prickly paddy melon and colocynth. Therefore, a combined molecular and morphological approach was used to further characterise and distinguish Australian populations of these three species, and provide clarity in identification. Specifically, the objectives of this study were: 1) to investigate and describe the distinguishing morphological characteristics of camel melon, prickly paddy melon and colocynth and 2) use informative gene regions to characterise the molecular taxonomy of each melon species in the Australian population using DNA sequence analysis.

MATERIALS AND METHODS

The molecular sequence analysis studies were conducted on geographically distinct samples of camel melon, prickly paddy melon and colocynth collected from across Australia in 2010 and 2011. Representative populations from NSW, VIC, ACT, SA, WA, TAS and NT were obtained for camel melon and prickly paddy melon, and WA and NT populations for colocynth. Glasshouse studies described herein did not include populations from ACT, VIC, or TAS, as insufficient seed was collected. After collection, seed was stored in a dark, dry location at ambient temperatures. GPS coordinates were noted for each collection site. For prickly paddy melon, a sample size of 30 (16 non vouchered (nv), 14 vouchered (v)) were analysed at *mat*K gene. Similarly, for camel melon, the sample size was 28 (20 nv, 8 v) at *G3pdh* gene and 27 (10 nv, 17 v) at *ycf*6–*psb*M gene. The colocynth had a sample size of 2 (1 nv, 1 v) at *ycf*6–*psb*M, 3 (2 nv, 1 v) at *G3pdh* and 2 (1 nv, 1 v) at *mat*K gene.

Molecular studies Samples of the three melons (voucher identified specimens from each Australian state herbaria, as well as samples sent by colleagues from around Australia) were used for molecular evaluation. DNA extraction, PCR, agarose gel electrophoresis, primers and methods of data analysis used in this study were identical to those used in Shaik *et al*. (2011). In addition, *ycf*6–*psb*M intergeneric spacer region was amplified using primer pairs: *ycf*6F (CTT GGG CTG CTT TAA TGG) (Dane *et al.* 2007) *psb*M-r1 (5' GTA AAT ATT CTY GCA TTT ATT GC) (Heinze 2007).

Glasshouse studies Following seed collection, a total of 93 plants (36 plants of camel melon from SA and NSW; 36 plants of prickly paddy melon from NT, WA, NSW, and SA; and 21 plants of colocynth from WA, NSW and SA) were grown in controlled glasshouse conditions during December 2011–May 2012. Seed dormancy was overcome by removal of the seed coat, followed by germination at 28°C. Seedlings were initially established in containers of formed sphagnum peat moss (*Jiffy*® Denmark) and after 30 days were transplanted into large plastic pots. The potting mix comprised a 1:1 ratio of standard potting mix and sterile sand. Six plant replicates from a representative population of each melon location were arranged in a randomised split plot design. The vines were staked and were kept adequately watered and fertilised throughout the experimental period. Data on leaf and floral characteristics were collected on leaves at the 5th node of each plant and the flowers at the 8th node. Fruit were assessed when they were fully formed and mature. The morphological descriptors were noted based on IPGRI and modified UPOV cucurbit descriptors as suggested by Solmaz and Sari (2009) and Křístková *et al.* (2003).

RESULTS AND DISCUSSION

Molecular studies Gene sequences at matK confirmed Australian prickly paddy melon to be *C. myriocarpus*. G3pdh and ycf6–psbM gene sequences from Australian camel melon were identical to a globally widespread *C. lanatus* var. *citroides* genotype, and differed from overseas *C. lanatus* var. *lanatus* and *C. colocynthis* sequences. Phylogenetic relationships between the three melons at G3pdh were reconstructed using neighbor-joining analysis including a Genbank accession of *Praecitrullus fistulosus* sequence as an out group. Figure 1 clearly indicates that the three melons are distinct at the molecular level. Table 1 shows the variable sites between the Australian camel melon and colocynth at sequenced G3pdh gene portion. The colocynth samples from Australia were similar to *Citrullus colocynthis* at ycf6–psbM and G3pdh genes. Prickly paddy melon was identified as *Cucumis myriocarpus* at matK.

Morphology studies The three species were distinguished on the basis of stem, leaf, tendril, floral and fruit morphology (Table 2 and Figure 2). The tap root is a clear identifying characteristic for these melon species and is swollen in the case of colocynth. Tendrils were also an identifying feature at plant maturity. At maturity, tendrils were of two types in colocynth, i.e. unbranched/simple at the initial nodes and bifid (at later nodes). In the case of prickly paddy

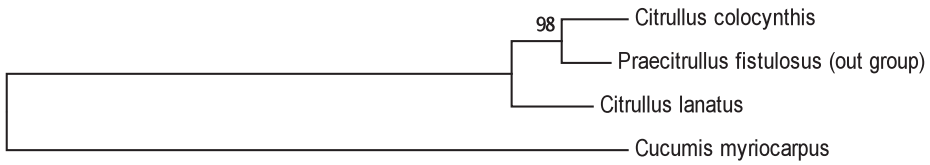

Figure 1. Neighbor-joining tree showing genetic distance relationships among camel melon, prickly paddy melon and colocynth sampled from Australia at the nuclear *G3pdh* gene. The value at node indicates the bootstrap supports from 1000 replications. The scale bar equals one percent genetic difference.

Table 1. Characterisation of Australian colocynth (*Citrullus colocynthis*) and camel melon (*Citrullus lanatus*) observed at nuclear *G3pdh* gene.

	Variable sites at *G3pdh* gene between *Citrullus colocynthis* and *Citrullus lanatus*											
	113	169	188	254	255	316	357	367	417	424	427	432
C. colocynthis	C	A	G	A	C	C	C	T	A	G	A	C
C. lanatus	T	G	C	G	T	A	G	C	G	A	G	T

Table 2. Morphological distinguishing characteristics of camel melon (*Citrullus lanatus*), colocynth (*Citrullus colocynthis*) and prickly paddy melon (*Cucumis myriocarpus*) in Australia.

Morphological character	*Citrullus colocynthis*	*Citrullus lanatus*	*Cucumis myriocarpus*
Stem shape	angular	angular	cylindrical
Tendril dissection	unbranched at lower nodes and bifid at later nodes	all bifid or bifid at lower nodes and trifid at later nodes	unbranched
Leaf lobation	nine lobes	seven lobes	five lobes
Mature leaf colour	dark green	green	light green
Leaf marginal undulation	high	low	medium
Tap root form	swollen	not swollen	not swollen
Pistillodium	absent	absent	present
Androecium colour	predominantly greenish	bright yellow	light yellow
Ovary hairiness	sparse, short hairs	very dense, long hairs	not hairy but has prickles
Stigma colour	greenish yellow	bright yellow	light greenish yellow
Petalloid sepals	present	absent	absent
Fruit shape	spherical	globose or oblong	subglobose
Fruit rind pattern	mottled / mosaic	spotted or striped	striped
Seed shape	ovate	ovate	elliptic
Seed coat patterns	absent	mostly present	absent
Seed coat colour	greenish grey	mostly brown	cream

melon, tendrils were simple, and in camel melon they were branched. Plants with either all bifid or plants with bifid and trifid (at later nodes) tendrils were noted. Mature leaves of colocynth were characterised by nine major lobes, while they were seven-lobed in camel melon and five-lobed in the case of prickly paddy melon.

Male flowers of prickly paddy melon possessed a prominent 'Pistillodium' (a triangular solid swollen structure enclosed within the petal base/hypanthium). In comparison, a distinct pistillodium was not observed in either camel melon or the colocynth. The sepals, which grade into petals referred to as petalloid sepals, were observed in both male and female colocynth flowers, while they were not found in either camel melon or prickly paddy melon. The androecium and stigma lobes were bright yellow in camel melon, while they were predominantly greenish yellow in colocynth and light greenish yellow in prickly paddy melon. The ovary was covered by very dense, long silky hairs in camel melon. The hairs covering the ovary were sparse and short in the case of colocynth. In prickly paddy

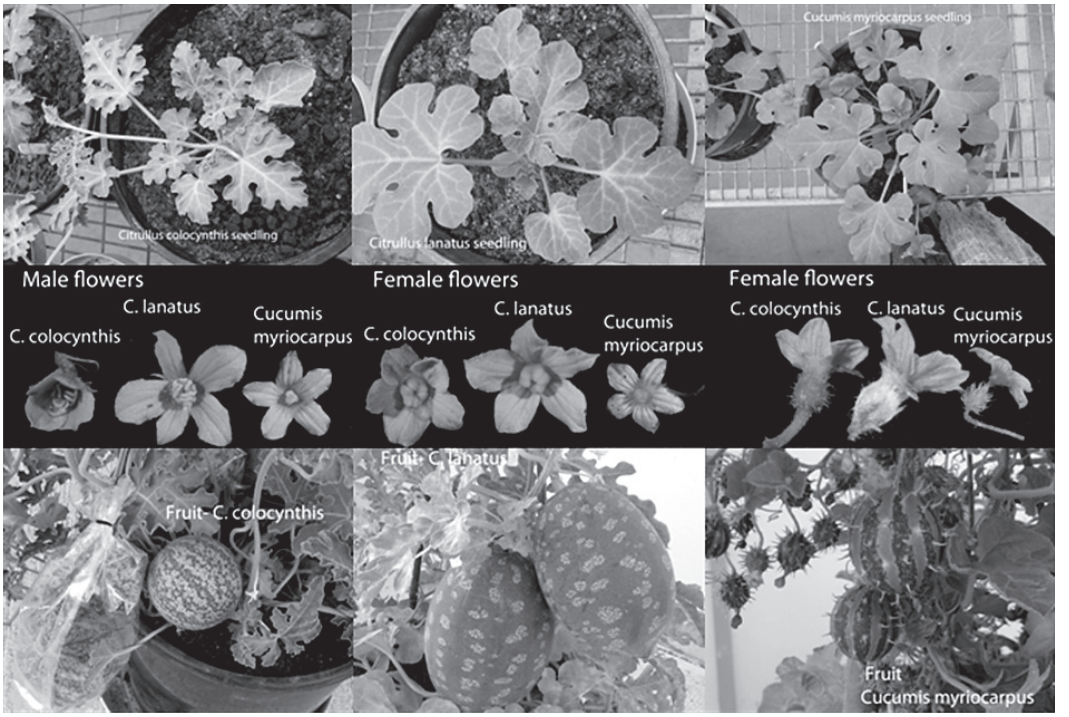

Figure 2. Morphological characteristics of colocynth (*Citrullus colocynthis*), camel melon (*Citrullus lanatus*), and prickly paddy melon (*Cucumis myriocarpus*) in Australia.

melon the ovary was covered with prickles instead of hairs (i.e. aculeate).

A comparison of the fruit shape suggested that camel melon produces either globose or oblong shaped fruits, while colocynth fruits were spherical. Prickly paddy melon fruits were obovate. The colocynth had a distinct mottled/mosaic fruit pattern. Both prickly paddy melon and camel melon fruits exhibited stripes, but some fruits of camel melon exhibited a spotted pattern instead of stripes.

Both colocynth and camel melon produced ovate seeds, while the prickly paddy melon seed was characterised by a spindle shape. The seed surface of camel melon was rough with black to brownish patterns. In comparison, the colocynth seed did not show patterning, and had a smooth surface like that of prickly paddy melon seed. All melons exhibited similarities in plant growth habit (several branches with a main trailing stem), light green stem, cordate leaf shape, and monoecious flowering, i.e. male and female flowers on the same plant. All plants showed protandry, (clear male flowering period before the onset of female flower production), after which, both male and female flowers were produced simultaneously on the plants.

In conclusion, this research showed that there were some key morphological and molecular differences between colocynth (*Citrullus colocynthis*), camel melon (*Citrullus lanatus* var. *citroides*) and prickly paddy melon (*Cucumis myriocarpus*). Our study results have obvious implications for the management of these species, as proper identification is the key to successful weed management. This work provides further evidence that DNA sequence analysis can be a useful tool in assessing the taxonomic identity of invasive weed species.

ACKNOWLEDGMENTS

We thank the EH Graham Centre for Agricultural Innovation for the funding of a PhD scholarship and a New Research Initiative Grant for this project, the WWAI for providing access to the DNA barcoding research laboratory as well as all the people who assisted in seed procurement.

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