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A COMPARATIVE PHENOLOGICAL AND GENETIC DIVERSITY ANALYSIS OF TWO INVASIVE WEEDS, CAMEL MELON (*Citrullus lanatus* (Thunb.) Matsum. & Nakai var. *lanatus*) AND PRICKLY PADDY MELON (*Cucumis myriocarpus* L.), IN INLAND AUSTRALIA

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ABSTRACT

The biological attributes of two invasive weed species, prickly paddy melon and camel melon, were studied in different disturbed habitats of the Riverina region, NSW during 2010-2011. Seedlings first germinated in early to mid November 2010, once optimal soil temperatures were achieved. Flowering began in both species, generally 35 to 45 days following seedling establishment. Both species exhibited monoecious tendencies, with production of male flowers rapidly followed by production of both male and female flowers on the same vine. Both species exhibited prolific fruit production at all sites, until senescence occurred, at 150-180 days following establishment. Date of senescence varied among sites and species. Molecular genetic sequences analysis of chloroplast (MatK) and nuclear (G3pdh) genes was used to assay population genetic diversity and to verify species identity of melon species sampled from geographically diverse locations in Australia. Genetic variation within the species was not observed among the Australian populations at either of the assayed genes. This lack of genetic diversity may have resulted from a limited entry by each of the species into Australia and or sustained population bottlenecks following their entry. The absence of genetic diversity among Australian populations in both species provides some indication that future bio-control measures may be applicable across the invasive range of these species. Comparisons with sequences from overseas vouchers indentified the Australian specimens of prickly paddy and camel melon as Cucumis myriocarpus and Citrullus lanatus var. citroides respectively. The latter result is discordant with contemporary herbaria identifications which describe Australian camel melon as Citrullus lanatus var. lanatus.

Keywords: Cucurbitaceae, genetic variation, invasive weeds, molecular identification, non-native, Phenology, sequence analysis,

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INTRODUCTION

Globally, invasive plants are a significant threat to biodiversity conservation (McNeely, 1997). Invasive plants are defined as non-native species that have successfully spread outside their native range and are associated with significant environmental and long-term sustainability issues (Richardson *et al.* 2000). Their impacts include loss of biodiversity and crop yield. Australia is currently dealing with impacts associated with invasion of over 200 significant weed invaders (www.weeds.org.au/WONS/).

There are five cucurbit species which were introduced to Australia as potential feed crops or as seed contaminants. Camel melon and prickly paddy melon are the most common (Martin *et al.,* 2006), introduced into Australia in the mid 1800s, and are invasive weeds of crop plants and natural habitats (Cunningham *et al*., 1992; Parsons and Cuthbertson, 2001). Although these melons are native to Africa, camel melon was most likely imported from its secondary centre of origin in India, Pakistan or Afghanistan, to use as camel fodder (Barker, 1964; Parsons and Cuthbertson, 2001). However, semi-arid regions of Australia have experienced serious invasive problems (Barker, 1964; PlantNET website, 2011). Today, camel melon is widely distributed throughout Australia (Australian Virtual Herbarium, 2010, www.avh.rbg.vic.gov.au). Globally, it is problematic in S. Europe and is a declared noxious weed in state of California, USA (Grubben, 2004; USDA web site, 2011).

After introduction to Australia, both species were subsequently reported as weeds as early as 1900. In recent years they have become problem weeds in dryland cotton, sorghum, and wheat, fallow croplands, and naturalized settings (Borger and Madin, 2005; Leys *et al.,* 1990; Johnson *et al.,* 2006). Both species are also considered to be potentially toxic to sheep and livestock (Cunningham *et al*., 1992, Parsons and Cuthbertson, 2001, Mc Kenzie *et al.,* 1988). In feeding trials, *Cucumis myriocarpus* fruit ingestion was reported to have caused death of cattle (Mc Kenzie *et al.,* 1988). Cost of chemical control of these weeds can be high, with repeated herbicide application required for management (Rosskopf *et al.,* 1999).

Genetic diversity and evolution of diversity can be important factors contributing to the success of any invasive species (Sexton *et al.,* 2002; Prentis *et al*., 2008). Novel populations of introduced species often go through population bottle-necks (reductions in effective population size) during and after the introduction phase. The intensity, duration and frequency of these bottle-neck events can increase the effects of genetic drift in these populations and subsequently lead to loss of their genetic diversity. In some instances

this may promote adaptations which facilitate establishment in a new environment (Prentis *et al.,* 2008).

Currently, little is known about the genetic diversity and identity of wild melons in Australia. A review of existing Australian references shows that authors have often confused identification and description of these two species. Globally, a detailed botanical description of *Cucumis myriocarpus* was given by Jeffrey (1975) and Kirkbride (1993). However they have been little studied in Australia, except in management trials (Felton *et al*., 1994, Leys *et al*., 1990).

Taxonomically, Jeffrey (1975) classified *Cucumis myriocarpus* into two subspecies, including *Cucumis myriocarpus* subsp. *myriocarpus* and *Cucumis myriocarpus* subsp. *leptodermis*. The subspecies are cross-compatible (Jeffrey 1975; Van Raamsdonk *et al.* 1989). These subspecies designations are used varyingly among Australian herbaria, with Queensland refering to specimens as subsp. *myriocarpus,* and NSW, ACT and VIC refering to them as subsp. *leptodermis* (ANHSIR extracts, 2011). Other states do not use any sub-specific classifications. Our germplasm collection to date suggests this likely represents a single subspecies. Camel melon in Australia was described by Jacobs and Pickard (1981) as *C. lanatus* var. *lanatus*, however this sub-specific nomenclature is only used by herbaria in NSW and QLD. *C. l*. var. *lanatus* has cross compatibility with its relative, *Citrullus colocynthis* (Singh, 1978), another invasive melon naturalised across north Australia (Australian Virtual Herbarium).

By use of genetic sequence analysis, one can carefully assess variation and potential hybridisation among species and evaluate phylogeographic relationships (Dane *et al.* 2007; Dane and Lang 2004). This study uses sequence analysis to identify species and population genetic diversity in two invasive melon species, and examines their phenology in inland NSW. A sound understanding of the biology and taxonomy of camel melon and prickly paddy melon is required to assist managers in correctly identifying and managing these species.

MATERIALS AND METHODS

Naturally occurring populations of camel and prickly paddy melon were identified in four locations within 50 km of Wagga Wagga NSW with red earth soils. Locations included: Charles Sturt University, equine paddock, Wagga campus; (ES), perennial pasture, Lake Albert, (LA); CSU horticulture irrigation site, Wagga (HA) and mixed farming site, Galore (GA). Table 1 presents information on individual experimental sites. The existing vegetation at all study sites included a variety of common broadleaf weeds and annual grasses, both native and introduced.

Table-1. Experimental location details.

Location	GPS coordinates	Soil type	Paddock history
ES	35 04 015 S, 147 21 17 E	Clay loam	Permanent grazed pasture
LA	35 09 031 S, 147 22 33 E	Red loam	Pasture followed by fallow
HA	35 41 193 S, 139 20 35 E	Red loam	Cropping followed by fallow
GA	34 59 45 S, 146 48 36 E	Sandy loam	Grazed pasture

Phenological characteristics of camel melon and prickly paddy melon seedlings and plants were studied in these locations during the summer of 2011. In some sites, species range overlapped. Specifically, prickly paddy melon was studied at HA and LA sites and camel melon studied at ES and GA sites. At each site, species in question were monitored by tagging, with a minimum of 12 plants tagged per site per species.

In December-April 2011, seedlings less than 21 days of age were tagged and studied for growth and development. To prevent initial mortality, plants of both species were kept relatively weed free during their initial establishment up to 60 days after emergence (DAE) and growth analyses taken approximately every 15 days until plant senescence was noted. At each visit, data on seedling establishment, time to first flowering, and time to fruit set were noted, along with notable events including infestation by pests and pollinators. At maturity, in late March through April, six actively flowering and fruiting vines of prickly paddy melon were collected from LA and HA sites, and camel melon from ES and GA sites. Above ground plant dry weight was recorded after drying in a dehydrator at 85° C for 7 days. Leaf area was estimated with a LA meter using 5 leaves each from the terminal, middle and lower portion of each vine. Length of longest vine per sample was measured. Fruit number/vine and fruit weight were recorded. In April, 10 mature fruit were collected per each of five plants in the case of prickly melon at LA and HA, and one fruit from 10 plants, in the case of camel melon, from ES and GA locations. Sampled plants were separated by 10 m or more at each site. Seed number/fruit was obtained after fruit dissection.

Additional germination data was collected in November 2010 on species establishment at each site. Date of first seedling emergence for each species was recorded using 1 m^2 grids placed randomly at locations at least 10 m apart and recording the number and approximate age of plants falling within 12 grids in each site. Data was

collected on at least 5 dates in November and throughout the season as germination events occurred in association with rainfall patterns.

Genetic variation within Australian melon samples was assayed by sequencing two informative nuclear and chloroplast gene regions, previously shown as informative for detection of population genetic variation and species identification in curcurbits (Demesure *et al*., 1995; Dane *et al.,* 2007). For these assays, fruit from 22 geographically distinct sites for camel melon and 16 sites for prickly paddy melon were collected across inland Australia including representative sites from NSW, VIC, SA and NT. Seed (1 seed/sample) was ground and prepared for DNA extraction, using two replicate samples per population. DNA was extracted using a Corbett 1820 extractor robot with associated extraction reagents (QIAGEN). Polymerase chain reaction (PCR) was used to amplify chloroplast *MatK* using primers 1R KIM (5′ ACC CAG TCC ATC TGG AAA TCT TGG TTC) and 3F_KIM (5′ CGT ACA GTA CTT TTG TGT TTA CGA G) (Ki-Joong Kim unpublished). An Intronic region within the nuclear *G3pdh* gene was targeted using primers *G3pdh* (5′ CAG GCT AAT GGA AAG GGT TT) and *G3pdh*_R (5′ TTG TAT CCT CCG CTT CC) (Demesure *et al*., 1995). Sample PCR's (15 µl) contained, 1.5 µl of 10X PCR Buffer, 0.9 µl of 50 µM $MgCl_2$, 0.3 µl of 10 mM dNTP, 0.3 µl each of 5 µM forward and reverse primers and 0.075 µl of Platinum® Taq DNA polymerase (Invitrogen Pty.), 9.675 µl of water and 2 µl genomic DNA. The PCR thermal profile used for both genes was: 94°C denaturing for 2 minutes, followed by 40 cycles of (94°C denaturing for 30 seconds, 52°C annealing for 30 seconds, 72°C extension for 60 seconds), 72°C extension for 7 minutes, 10°C hold. PCR's were checked for quality and size using a UV visualiser (Gel Doc Pty.) after electrophoresis through a 1.5% TAE agarose gel at 170V for 7 minutes. Quality PCR products were bi-directionally sequenced at the Australian Genomic Research Foundation (AGRF), Brisbane Australia. Sequences were aligned using SeqMan DNA star software and edited using Bioedit (Hall, 1999). Reference sequences of taxonomically identified voucher accessions were downloaded from the National Barcode of Life website (www.ncbi.nlm.nih.gov/genbank/barcode.html). Sample sequences were compared against references using neighbour-joining distance analysis and tree reconstruction as implemented in MEGA 4 (Tamura *et al.,* 2007).

RESULTS

As presented in Table-2 and -3, seedling emergence of both melon species was noted from early November 2010 onwards. Time of emergence varied with species and location of study, from November 1 to 15, 2010. For both species, length of mature vines extended 2 to 3

m in length. Leaves of prickly paddy melon were smaller than those of camel melon. However, an average prickly melon produced greater leaf numbers/plant than camel melon of similar maturity. First fruit formation was observed in both species between 35 and 49 days after emergence, depending on site. Emergence was later at the Lake Albert (LA) site for prickly paddy melon. Camel melons matured several weeks earlier at the Galore site (GA). Prickly melon exhibited variation in growth and development depending on location. A comparison of both melon species with respect to total biomass production suggested that species produced similar total biomass under favourable growth conditions.

Both prickly melon and camel melon were highly plastic with respect to their growth and potential to produce biomass at various study sites; this is reflected in terms of their capacity to produce viable fruit. We observed that camel melons produced a maximum of 14 fruits /plant, but an average plant typically produced 2-3 fruit /plant. Similarly, an average prickly melon produced upwards of 80 melons under favourable conditions, and up to 120 under optimal conditions. Camel melons typically produced between 300 to 400 seed /fruit, depending on location of production, while smaller prickly paddy melons produced between 40 to 45 seed /fruit. Total seed production/plant was greater for prickly paddy melon in comparison to camel melon at all study sites.

Table-2. Phenology and growth parameters assessed for camel melon in two locations (ES) and (GA) in 2010-2011. ([1]SD = Standard Deviation).

S.N	Growth parameter	(ES)	SD[1]	(GA)	SD[1]
1	Time of first germination	Nov 15	-	Nov 1	-
2	Longest vine length (mm)	1700	700	2850	900
3	Leaf area /5 leaves (cm^2)	-	-	394	61
4	Duration to male flower(DAE)	41.9	11.4	35	1.8
5	Duration to female flower (DAE)	56.7	14.6	41.7	1.9
6	Duration to fruit (DAE)	63.8	15.1	47.3	1.7
7	Plant dry weight (g)	89.1	18.3	207	167
8	Fruit/plant	2.5	1.4	2.58	2.19
9	Seed /fruit	281	101	390	87
10	Dry wt. per seed (g)	0.07	0.004	0.06	0.0006
11	Male : female flower ratio	3.1	3.5	2.6	2.0

We observed that *C. myriocarpus* exhibited a greater male/female flower ratio than camel melon. *C. myriocarpus* flowered more profusely and produced larger numbers of fruit as well. Prickly

paddy melon produced an average of 80 fruits and 40 seed/fruit, while camel melon produced 3 fruit per plant, with seed number/fruit varying with location.

Table-3. Phenology and growth parameters assessed for prickly paddy melon in two locations (HA) and (LA) in 2010-2011.

S.N	Growth parameter	(HA)	SD[1]	(LA)	SD[1]
1	Time of first germination	Nov 1	-	Nov 15	-
2	Longest vine length(mm)	2400	700	1900	540
3	Leaf area /10 leaves (cm^2)	200	311	79	52
4	Duration to male flower (DAE)	33.7	5.2	39.4	4.3
5	Duration to female flower (DAE)	44.7	8.4	48.8	3.6
6	Duration to fruit (DAE)	51.6	9.1	52.3	2.2
7	Plant dry weight (g)	176	139	32	17
8	Fruit /plant	85	67	22	12
9	Seed/fruit	46	5	39	7
10	Dry wt. per seed (g)	0.020	0.001	0.022	0.001
11	Male : female flower ratio	5.9	3.3	4	2

[1] SD refers to standard deviation. SD was calculated generally for 12 plants per location.

Molecular studies revealed that the PCR products at *MatK* and *G3pdh* were successfully amplified from both melon species; PCR success at *MatK* was 77% and 100% at *G3pdh*. Sequence analysis at the assayed genes indicated an absence of intraspecific genetic variation in both species. *MatK* and *G3pdh* gene sequences obtained for prickly paddy melon samples showed 100% similarity to sequence accessions from *Cucumis myriocarpus* vouchers present overseas. *MatK* sequences obtained from Australian camel melons were 100% identical to those reported for overseas *C. colocynthis* accessions. *G3pdh* gene sequences for Australian camel melons were identical to a widespread *C. lanatus var citroides* genotype and differed from overseas *C. lanatus var lanatus* (cv. Crimson Sweet) and *C. colocynthis* sequence (PI- 432337) accessions by 1.34 and 1.84 %, respectively. Phylogenetic relationships at *G3pdh* reconstructed using neighbour-joining analysis indicated the Australian camel melon genotype was synonymous with *C. lanatus var citroide* accessions from Botswana, India, South Africa and the USA (Fig. 1).

DISCUSSION

Both prickly paddy and camel melon species share similarity in their growth and life history traits. They are annual vines with

branches ranging from 2-4 m in length with angled, tendrillar stems. Camel and prickly paddy melon growth was initiated with germination when the paddocks received first significant rainfall, which occurred from mid November – mid January continuing until onset of low temperatures in mid April, at which time the plants senesced.

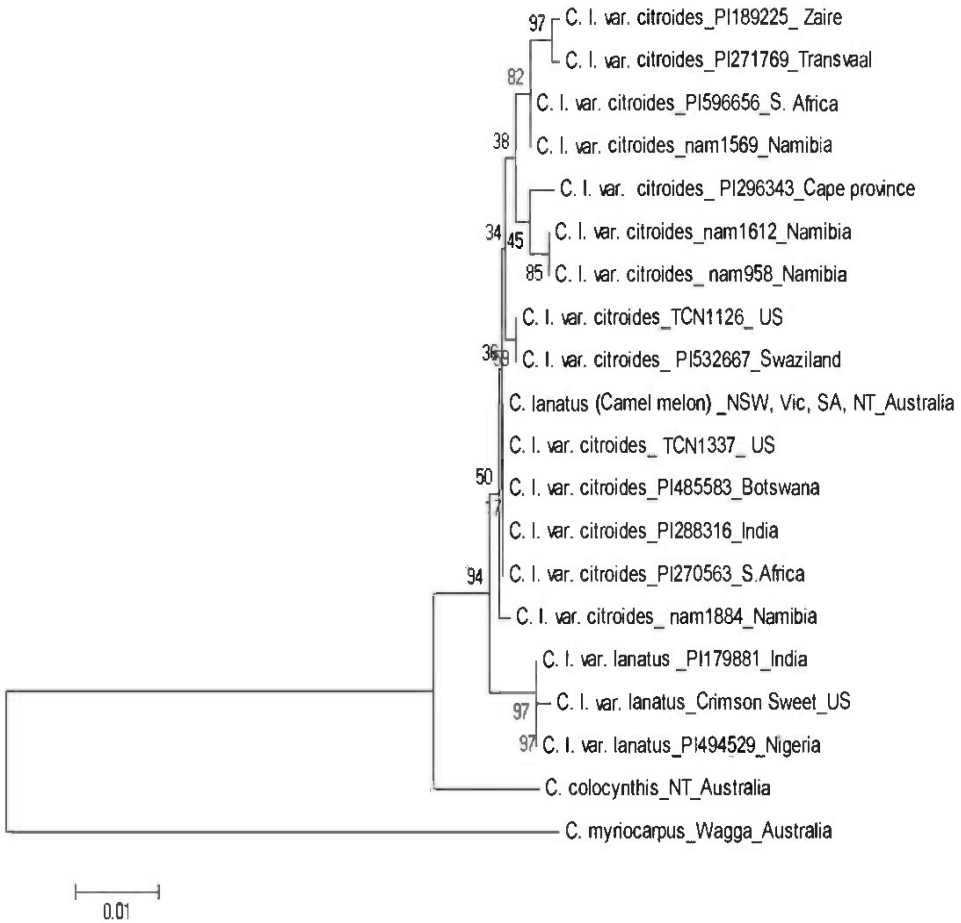

Figure-1. Neighbour joining tree showing genetic distance relationships among camel melon sampled from Australia and overseas melon accessions at the *G3pdh* intron -2 region. The values at nodes indicate the boot strap supports from 1000 replications. The scale bar equals one percent genetic difference.

Similar to Parsons and Cuthbertson (2001), we observed their monoecious growth habit, with both male and female flowers produced

on the same vine. Male flowers arise in leaf axils, and female flowers are solitary in camel melon and in prickly paddy melon arise in clusters. Flowering was first noted at 5 to 7 weeks following germination, continuing until senescence. In most occasions, the male flowers were the first to open, followed by the female flowers. Camel melon produced a larger plant with a much larger fruit than that of prickly melon, similar to Parsons and Cuthbertson (2001). We observed that prickly melon exhibited continuous staggered germination throughout the growing season. In contrast, camel melon germinated more sporadically with 3 to 4 seasonal flushes of germination observed. Camel melon evidently requires higher temperatures to flower and set fruit than prickly melon (Parsons and Cuthbertson, 2001). Both melons exhibited greater male fitness, resulting in more numerous male flowers than female flowers. Protandry is a common feature of most cucurbits, with male/female flower ratio >1.

DNA sequencing was used to examine population genetic diversity and species identity of wild melon present in Australia. Our results indicated an absence of genetic diversity within and among populations of both paddy melon and camel melon; in both cases, only a single fixed genotype was observed among widespread sample locations. This contrasts with evidence of some genetic variation present within overseas melon populations at the assayed genes (Liu, 2005; Dane *et al*., 2007). It is plausible the lack of observable genetic diversity among Australian melon populations resulted from a single Australian incidence of introduction in both species. This is potentially supported by historical records that indicate the brief period of introduction of camel melon seed into Australia to provide fodder for introduced camels used for land transport (Barker, 1964). Population bottlenecks following introduction to this country may also have been of sufficient size and duration to effectively eliminate all but the most common genotype available for both species. Regardless, the apparent absence of genetic diversity among Australian populations in both species provides some indication that future bio-remediation or bio-control measures may be applicable across the invasive range of these species.

Genetic analyses verified species identity of prickly paddy melon in Australia and provided evidence of a discrepancy in the nomenclature of Australian camel melon. Currently, camel melon is reported in Australia as *C. lanatus* var *lanatus* (ANHSIR extracts, 2011; Leys *et al.,* 1990, *plantnet.rbgsyd.nsw.gov.au, 2011*). However, sequence analysis at *G3pdh* indicated our Australian camel melon populations are synonymous with overseas citron melons identified by authorities as *C. lanatus* var *citroides* (Liu, 2005); we also observed

Australian camel melon sequences differed from the overseas accessions of *C. lanatus* var *lanatus*. Additional sequencing studies with other variable gene loci and overseas voucher specimens are now underway to investigate this important taxonomic finding.

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REFERENCES CITED

Anhsir extracts. 2011. Australian National Herbarium, Canberra. Personal communication.

Barker, H.M. 1964. *Camels and the Outback*. London: Angus & Robertson Ltd. Retrieved from the internet on 16- 6-2011. http://www.biology.iastate.edu/InternationalTrips/1Australia/05PAPERS/Camels.htm

Borger, J. and R. Madin. 2005. Paddy melon and Afghan melon. Farmnote No. 91/93 [Replaces 22/88]/2005, Department of Agriculture. Western Australia.

Cunningham, G., W.E. Mulham, P.L. Milthorpe and J.H. Leigh. 1992. Plants of Western New Southwales. Inkata Press, Melbourne.

Dane, F., J. Liu., and C. Zhang. 2007. Phylogeography of bitter apple, Citrullus colocynthis. Gen. Res. and Crop Evol. 54: 327- 336.

Dane, F. and P. Lang. 2004. Comparative analysis of *cp* DNA variability in wild and cultivated *Citrullus* species: implications for evolution of watermelon. Am. J. of Bot.91: 1922–1929.

Demesure, B., B.N. Sozi, and R.J. Petit. 1995. A set of universal primers for amplification of polymorphic non-coding regions of mitochondrial and chloroplast DNA in plants. Mol. Eco. 4: 129–131.

Felton, W. L., G. A. Wicks and S. M. Welsby. 1994. A survey of fallow practices and weed floras in wheat stubble and grain sorghum in northern New South Wales. Australian Journal of Experimental Agriculture 34(2), 229-236.

Grubben, G.J.H. 2004. Plant resources of Tropical Africa. *Vegetables*. PROTA Publication, Wageningen, Netherlands. pp. 252.

Hall, T.A. 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis. http://www.mbio.ncsu.edu/BioEdit/bioedit.html

Jacobs, S. W. L and J. Pickard. (1981). Pl. New S. Wales 108.

Jeffrey, C. 1975. *Further notes on Cucurbitaceae: III*. Some southern African taxa. Kew Bull. 30: 475-493.

Johnson, A., J., Dellow and K. Pengilley. 2006. Central west weed survey, efficient weed control in central west NSW, Appendix one. DPI, NSW Pp 92-99. http//www.dpi.nsw.gov.au/agriculture /pestsweeds/weeds/publications/management/central-west

Kirkbride, J.H. 1993. Biosystematic monograph of the genus *Cucumis (Cucurbitaceae);* botanical identification of cucumbers. Parkway publishers. Pp1-35.

Leys, A. R., R.L., Amor, A. G. Barnett and B. Plater. 1990. Evaluation of herbicides for control of summer- growing weeds on fallows in south eastern Australia. Aus. J.of Exp.Agri. 30: 274-279.

Liu, J. 2005. Phylogeny and biogeography of water melon *(Citrullus lanatus (Thunb.)* Matsum. & Nakai) based on chloroplast, nuclear sequence and AFLP molecular marker data. M Sc. Thesis, Auburn University, Alabama. Retrived from the internet on 16-6-2011. (http://etd.auburn.edu/etd/bitstream/handle/10415/703/LIU_JIARONG_35.pdf?sequence=).

Martin,T. G., S. Campbell, and S. Ground. 2006. Weeds of Australian rangelands. The Rangeland J. 28: 3- 26.

McNeely, J. 1997. IUCN (International Union for conservation of Nature and Natural Resources) alien invasive species: a framework for action. In: Conserving Vitality and Diversity: Proceedings of the World Conservation Congress Workshop on Alien Invasive Species (eds. C.D.A. Rubec & G.O. Lee). IUCN and Environment Canada, Ottowa, pp. 3-9.

McKenzie, R.A., R.D. Newman, A.C., Rayner and P.J. Dunster. 1988. Prickly Paddy melon (*Cucumis myriocarpus*) poisoning of cattle. Aus. Vet. J. 65(6): 167-70.

Parsons, W. T. and W. G. Cuthbertson. 2001. Noxious weeds of Australia. CSIRO publishing Victoria, 405-411.

Prentis, P., E., Dormontt, A. Wilson, D. Richardson, And A. J. Lowe. 2008. Adaptive evolution in invasive species. Trends in Pl. Sci. 13:288-294.

Richardson D. M., P. Pysek, M. Rejmanek, M. G. Barbour, F. D. Panetta and C. J. West. 2000. Naturalisation and invasion of alien plants: Concepts and definitions. Div. and Distbs 6: 93-107.

Rosskopf, E. N., R. Charudattan, and J.B. Kadir. 1999. Use of plant pathogens in weed control. In Handbook of biological control: principles and applications of biological control, By T. S.

Bellows, T. W. Fisher, Elsevier Science & Technology publishers. Pg. 891.

Sexton, J. P., J. K. McKay and A. Sala. 2002. Plasticity and genetic diversity may allow salt cedar to invade cold climates in North America. Ecol App. 12: 1652–1660. [doi:10.1890/1051-0761(2002)012[1652:PAGDMA]2.0.CO;2]

Singh, A.K. 1978. Cytogenetics of semi-arid plants. III. A natural interspecific hybrid of Cucurbitaceae (*Citrullus colocynthis* Schrad x *C. vulgaris* Schrad). Cytologia 43: 569-576.

Tamura, K., J. Dudley, M. Nei and S. Kumar. 2007. MEGA4: Molecular Evolutionary Genetics Analysis (MEGA) software version 4.0. Mol. Bio. and Evol. 24: 1596-1599.

Van Raamsdonk, L.W. D., A. P. M. Den Nijs and M.C. Jongerius. 1989. Meiotic analyses of *Cucumis* hybrids and an evolutionary evaluation of the genus *Cucumis* (Cucurbitaceae). Pl. Sys. and Evol. 163: 133-146.