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An identification tool for the Australian weedy *Sporobolus* species based on random amplified polymorphic DNA (RAPD) profiles

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Abstract. Based on morphological features alone, there is considerable difficulty in identifying the 5 most economically damaging weed species of *Sporobolus* [*viz*. *S. pyramidalis* P. Beauv., *S. natalensis* (Steud.) Dur and Schinz, *S. fertilis* (Steud.) Clayton, *S. africanus* (Poir.) Robyns and Tourney, and *S. jacquemontii* Kunth.] found in Australia. A polymerase chain reaction (PCR)-based random amplified polymorphic DNA (RAPD) technique was used to create a series of genetic markers that could positively identify the 5 major weeds from the other less damaging weedy and native *Sporobolus* species. In the initial RAPD profiling experiment, using arbitrarily selected primers and involving 12 species of *Sporobolus*, 12 genetic markers were found that, when used in combination, could consistently identify the 5 weedy species from all others. Of these 12 markers, the most diagnostic were $UBC51_{490}$ for *S. pyramidalis* and *S. natalensis*; $UBC43_{310,2000,2100}$ for *S. fertilis* and *S. africanus*; and $OPA20_{850}$ and $UBC43_{470}$ for *S. jacquemontii*. Species-specific markers could be found only for *S. jacquemontii*. In an effort to understand why there was difficulty in obtaining species-specific markers for some of the weedy species, a RAPD data matrix was created using 40 RAPD products. These 40 products amplified by 6 random primers from 45 individuals belonging to 12 species, were then subjected to numerical taxonomy and multivariate system (NTSYS pc version 1.70) analysis. The RAPD similarity matrix generated from the analysis indicated that *S. pyramidalis* was genetically more similar to *S. natalensis* than to other species of the '*S. indicus* complex'. Similarly, *S. jacquemontii* was more similar to *S. pyramidalis*, and *S. fertilis* was more similar to *S. africanus* than to other species of the complex. *Sporobolus pyramidalis*, *S. jacquemontii*, *S. africanus*, and *S. creber* exhibited a low within-species genetic diversity, whereas high genetic diversity was observed within *S. natalensis*, *S. fertilis*, *S. sessilis*, *S. elongates*, and *S. laxus*. Cluster analysis placed all of the introduced species (major and minor weedy species) into one major cluster, with *S. pyramidalis* and *S. natalensis* in one distinct subcluster and *S. fertilis* and *S. africanus* in another. The native species formed separate clusters in the phenograms. The close genetic similarity of *S. pyramidalis* to *S. natalensis*, and *S. fertilis* to *S. africanus* may explain the difficulty in obtaining RAPD species-specific markers. The importance of these results will be within the Australian dairy and beef industries and will aid in the development of integrated management strategy for these weeds.

Additional keywords: diagnostics, fingerprinting, genetic diversity, PCR, rat's tail grasses, species identification.

Introduction

Of the 24 species of the genus *Sporobolus* that have been reported to be present in Australia, 11 have been included in the weedy '*Sporobolus indicus* complex' (Simon 1999). Five of these [*viz*. giant rat's tail grasses *S. pyramidalis* P. Beauv and *S. natalensis* (Steud.) Dur. and Schinz, giant Parramatta grass *S. fertilis* (Steud) Clayton, Parramatta grass *S. africanus* (Poir.) Robyns and Tourney, and American rat's tail grass *S. jacquemontii* Kunth.] are now serious weeds (Laffan 1986; Betts *et al*. 1990; Anon. 1997) and 4 (*viz*. *S. elongatus* R. Br., *S. creber* De Nardi, *S. laxus* B. K. Simon, *S. sessilis* B. K. Simon) are less economically damaging. Of the 2 remaining members of the *S. indicus* complex [*S. indicus* (L.) R. Br. and *S. diandrus* P. Beauv],

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S. diandrus has not yet been reported in Australia, whereas a phylogenetic study (Shrestha *et al*. 2003) involving one suspected *S. indicus* seed sample from Victoria, has raised the possibility that this species is now present in Australia. This has yet to be confirmed.

The two giant rat's tail grasses and giant Parramatta grass constrain Australian pasture production, with their effects mainly on the dairy and beef industries. The distribution of these 3 weeds is still increasing and therefore it is estimated that they will have an even greater impact on these industries. Their main effect is on animal production as they are unpalatable and reduce the carrying capacity of the pasture. Eco-climatic modelling has suggested that the giant rat's tail grasses have the potential to establish in most areas receiving as little as 500 mm annual rainfall. Thus, 108 million ha of Queensland (60% of the total land area) and 233 million ha of Australia (30% of the total land area; Anon. 1999) may be under threat.

The past taxonomic identification of weedy (from native) *Sporobolus* species has been based on morphological characters alone (Simon 1993; Simon and Jacobs 1999) and has resulted in many misidentifications. This inability to clearly identify native and introduced minor weeds from the seriously weedy species has created many difficulties for the development of effective weed management strategies. Identification based on electrophoretic analysis of seed proteins, using SDS-PAGE, has been attempted (Vieritz 1993); however, due to certain limitations of this technique, it could not be developed into a reliable diagnostic protocol. In this context, nucleic-acid-based molecular marker techniques could provide reliable identification options. The polymerase chain reaction (PCR)-based random amplified polymorphic DNA (RAPD) technique (Welsh and McClelland 1990; Williams *et al*. 1990) has been widely used for plant species and varietal identification (Graham *et al*. 1994; Joel *et al*. 1996; Golembiewski *et al*. 1997). The method can also be used for inferring the nature of taxonomic relationships (Graham *et al*. 1996; Moodie *et al*. 1997; Lopez-Martinez *et al*. 1999) among closely related species. In the RAPD technique, molecular markers are produced following the PCR amplification of DNA fragments using randomly selected short oligonucleotide primers (usually 10 bases long) of arbitrary sequence. The polymorphic DNA fragments produced can be compared among a range of species, and suitable fragments selected to act as species identification markers. The qualitative data on presence or absence of the RAPD fragments in each species can be used to generate similarity indices (Dice 1945; Sokal and Michener 1958; Sokal and Sneath 1963) that in turn are used to reveal genetic relationships among the species.

In the present investigation, a RAPD-PCR technique was used to develop molecular markers that are useful for the identification of the 5 major Australian weedy *Sporobolus* species. The approach was also used to study the inter- and intra-specific variation among various species of the *S. indicus* complex with a view to understanding the genetic diversity and the interrelationship of the members of the *S. indicus* complex and their relationship to the Australian native species. This information can in turn be used in weed management strategy development.

Materials and methods

Plant materials

Fifty-five seed samples, belonging to 14 species of *Sporobolus* (11 species of the *S. indicus* complex and 3 Australian native species), were examined in this study (Table 1). Thirteen of these seed samples were collected from sites around Australia with the help of the Queensland Department of Primary Industries (QDPI) and the Queensland Department of Natural Resources and Mines (QNRM); 29 were provided by B. K. Simon, Queensland Herbarium (QH, Brisbane); 5 came from the University of New England; 5 came from unknown sources; 1 from the Maitland City Council; and 2 from landholder sites in New South Wales (NSW) and Queensland (Qld). Species identification was carried out by morphological identification undertaken on mature, reproductive plants (B. K. Simon, QH).

DNA extraction

Extraction of DNA was carried out from seed tissue (*c.* 10–50 mg of caryopses) using the technique of Edwards *et al*. (1991). In this procedure, caryopses were placed in Eppendorf tubes (1.5 mL), surface-sterilised (2% v/v sodium hypochlorite solution for 10 min), rinsed (3 or 4 times with sterile distilled water), and ground in an extraction buffer (800 µL; 200 mM Tris-HCl pH 7.5, 250 mM NaCl, 25 mM EDTA, 0.5% SDS). Samples were well shaken and then further centrifuged (18 890*g* for 2 min). Supernatant aliquots (600 µL) were transferred to fresh Eppendorf tubes and combined with isopropanol (600 µL). Following further centrifugation (18 890*g* for 5 min) the supernatants were carefully discarded and pellets dried overnight at 4°C in the fridge. The pellets were then dissolved in TE buffer (Tris EDTA buffer, 50–300 µL, depending upon the size of the pellet; 10 mM Tris HCl pH 8.0 and 1 mM EDTA pH 8.0) and stored at 4 ± 1°C. Estimation of the amount of DNA extracted from the various seed samples was carried out using a spectrophotometric method (GeneQuant II, RNA/DNA calculator, Amersham Pharmacia, Biotech, Australia).

Generation of RAPD markers for species identification

In preliminary trials, an optimised RAPD-PCR reaction and cycling condition was identified. In a final reaction volume (25 µL), the optimised RAPD-PCR reaction mixture consisted of genomic DNA (25 ng), $MgCl_2$ (2.5 mM), reaction buffer (2.5 µL; 670 mM Tris-HCl pH 8.8 at 25°C, 166 mM $(NH_4)_2SO_4$, 4.5% Triton X-100, 2 mg/mL Gelatin), dNTPs (0.2 mM), *Taq* polymerase (1.1 units), and a decanucleotide primer (0.4 µM). The primers selected were obtained from 2 sources (OPA primer set, Operon Technologies, Alameda, USA, and a UBC primer set, University of British Columbia, Oligonucleotide Synthesis Laboratory, Vancouver, British Columbia, Canada). The optimised RAPD-PCR program consisted of 1 cycle of 1 min at 94°C, 35 cycles of 10 s at 94°C, 30 s at 38°C, and 60 s at 72°C, followed by 5 min extension at 72°C. Initially, 120 decamer primers (20 from the OPA set, and 100 from the UBC set) were screened against genomic DNA of *S. pyramidalis*. From this, 49 primers giving banding profiles with *Sporobolus* DNA were chosen and used in a RAPD-PCR profiling experiment involving 9 species (6 from the *S. indicus* complex and 3 natives). Deoxyribonucleic acid was extracted from seed of the 9 species and tested with each of the 49 primers in an attempt to

Table 1. *Sporobolus* seed samples used in the RAPD investigation showing status, place and date of collection, and source

QDPI, Queensland Department of Primary Industries; QNRM, Queensland Department of Natural Resources and Mines; QH, Queensland Herbarium; UNE, University of New England, Armidale; MCC, Maitland City Council, NSW; –, unknown

Sporobolus species, status	Sample ID/ abbreviation	Place of collection	Date of collection	Source
S. pyramidalis	1/sp1	Gympie, Qld	1995	D. S. Loch, QDPI
(introduced serious	22/sp22	Mackay, Qld	–	–
weed)	48/sp48	Fox Tail Flats, Qld	22/4/1999	W. Vogler, QNRM
	49/sp49	Gympie, Qld	6/4/1999	W. Vogler, QNRM
	55/sp55	Kilcoy, Qld	15/1/1997	W. Vogler, QNRM
S. jacquemontii	87/sj87	Mareeba, Qld	1991 AQ381528	B. K. Simon, QH
(introduced minor	114/sj114	Fox Tail Flats, Qld	4/1999	M.Yee, QDPI
weed)	116/sj116	Gyndah, Qld	–	S. Bray, QDPI
	135/sj135	North Kennedy, Qld	8/6/1995 AQ638698	B. K. Simon, QH
S. fertilis	25/sf25	Toolara, Qld	25/11/1998	–
(introduced serious	140/sf140	Wide Bay, Qld	9/5/1994 AQ627161	B. K. Simon, QH
weed)	36/sf36	Grafton, NSW	4/3/1999	R. D. B. Whally, UNE
	45/sf45	Wollombi, NSW	11/3/1999	B. Warby, MCC
	84/sf84	Morton, Qld	21/1/1997 AQ660362	B. K. Simon, QH
	92/sf92	Nambucca, NSW	21/1/1997	B. K. Simon, QH
	17/sf17	Nambucca, NSW	17/2/1999	–
S. africanus	12/sa12	Gympie, Qld	2/11/1998	–
(introduced minor	5/sa5	Unknown, NSW	–	R. D. B. Whally, UNE
weed)	125/sa125	Taree, NSW	–	–
	143/sa143	Perth, WA	5/11/1995	B. K. Simon, QH
S. indicus (supposed	138/si138	Horsham, Vic.	3/1994 AQ626781	B. K. Simon, QH
not in Australia)	160/si160	Unknown, Mexico	8/1991 AQ520220	B. K. Simon, QH
S. natalensis	151/sn151	Chatsworth, Qld	11/5/1995 AQ636832	B. K. Simon, QH
(introduced serious	152/sn152	Wide Bay, Qld	3/1/1990 AQ459339	B. K. Simon, QH
weed)	153/sn153	Wide Bay, Qld	4/1995 AQ635642	B. K. Simon, QH
	83/sn83	Port Curtis, Qld	11/1993 AQ622016	B. K. Simon, QH
	110/sn110	Gympie, Qld	3/1999	P. Jones, QDPI
	157/sn157	Blouberg, South Africa	1991 AQ517699	B. K. Simon, QH
	159/sn159	Pretoria, South Africa	20/2/1992 AQ532340	B. K. Simon, QH
S. sessilis	126/ss126	Wide Bay, Qld	1/11/1995 AQ488451	B. K. Simon, QH
(native)	128/ss128	Unknown, PNG	24/4/1991	B. K. Simon, QH
	129/ss129	Yogyakarta, Indonesia	11/9/1992 AQ547041	B. K. Simon, QH
	130/ss130	Port Curtis, Qld	1/2/1993 AQ564757	B. K. Simon, QH
S. diandrus	154/sd154	Java, Indonesia	4/9/1992 AQ547035	B. K. Simon, QH
(supposed not	155/sd155	Java, Indonesia	9/9/1992 AQ 547019	B. K. Simon, QH
in Australia)	156/sd156	Kuala Lumpur, Malaysia	11/1992 AQ563368	B. K. Simon, QH
S. elongatus	91/se91	Wide Bay, Qld	5/7/1996 AQ489955	B. K. Simon, QH
(endemic)	41/se41	Talarm, NSW	2/4/1992	R. D. B. Whally, UNE
	73/se73	Gyndah, Qld	4/1999	S. Bray, QDPI
S. creber	10/scr10	Chatsworth, NSW	11/1998	D. Flower –
(endemic)	11/scr11	Bular, Kikivan, Qld	11/1998	J. Clery –
	37/scr37	Armidale, NSW	7/3/1999	R. D. B. Whally, UNE
S. laxus	88/sl88	Bundaberg, Qld	15/11/1996 AQ572692	B. K. Simon, QH
(endemic)	90/sl90	Mt Bartle Frere, Qld	17/11/1996 AQ657711	B. K. Simon, QH
S. carolii	42/sca42	Mallawa area, NSW	8/2/1999	R. D. B. Whally, UNE
(endemic)	76/sca76	Ban ban Station, Qld	4/1999	S. Bray, QDPI
	104/sca104	Glentulloch, Qld	31/3/1999	D. S. Loch, QDPI
	105/sca105	Roma, Qld	30/3/1999	D. S. Loch, QDPI
S. australasicus	95/sau95	Gregory North, Qld	30/4/1994 AQ632322	B. K. Simon, QH
(endemic)	107/sau107	Keilambiti, Qld	9/4/1999	P. Jones, QDPI
	146/sau146	South Kennedy, Qld	12/8/1997 AQ661416	B. K. Simon, QH
	147sau147	Camooweal, Qld	28/4/1995 AQ587010	B. K. Simon, QH
S. actinocladus	94/sac94	Derby, WA	29/3/1996 AQ532951	B. K. Simon, QH
(endemic)	106/sac106	Roma, Qld	30/3/1999	D. S. Loch, QDPI
	148/sac148	Perth, WA	4/4/1995 AQ598102	B. K. Simon, QH

find weedy species-specific polymorphic markers. Twelve primers, revealing high degrees of polymorphisms between weedy and native species, were chosen for further study in a marker confirmation experiment. In this experiment, 52 (out of the original 55) seed samples from 14 species (11 from the *S. indicus* complex and 3 Australian natives) were used. In all cases, the RAPD-PCR products were analysed on a 1.5% (w/v) agarose gel in TBE buffer (Tris borate EDTA buffer, 1×) and visualised after staining in ethidium bromide for 45 min and de-staining for 10 min in water and photographed using a Polaroid camera system.

Study of genetic diversity and relationship

Gel photographs (taken from the marker confirmation experiment described above) for 6 randomly selected primers were scored for the presence or absence of RAPD fragments across 45 selected native and weedy species samples and a data matrix created. The matrix was analysed using a numerical taxonomy and multivariate system (NTSYS-pc, Version 1.70; Exeter Software, Setauket, New York). Similarity indices were calculated using a similarity for qualitative data (SIMQUAL) computer algorithm. From these similarity indices, sequential, agglomerative, hierarchical, and nested (SAHN) clustering was performed using the unweighted pair group method of arithmetic averages algorithm (UPGMA; Sneath and Sokal 1973). From this, phenograms were developed to show the relationships among members of different species. Estimates of similarity were based on the following 3 different measures.

(*1*) Simple matching coefficient (SM) (Sokal and Michener 1958):

$$Sij = a + d/a + b + c + d$$

(*2*) Dice's coefficient of similarity (Dice 1945; Nei and Li 1979):

$$Sij = 2a/2a + b + c$$

(*3*) Jaccard's coefficient (Jaccard 1908):

$$Sij = a/a + b + c$$

where S*ij* is the similarity between 2 individuals, *i* and *j*; *a* is the number of bands present in both *i* and *j*; *b* is the number of bands present in *i* and absent in *j*; *c* is the number of bands present in *j* and absent in *i*; and *d* is the number of bands absent from both *i* and *j*.

Results

Identification of weedy species-specific RAPD markers

Of the 120 decamer primers used in the initial primer screening using genomic DNA of *S. pyramidalis*, only 49 primers were selected to be used in the RAPD profiling experiment and, of these 49 primers, only 24 produced scorable bands. From these 24 primers, 174 bands were amplified in the 3 major weedy species (*S. pyramidalis*, *S. jacquemontii*, and *S. fertilis*) included in the experiment. Of these, 116 (67%) were polymorphic and 58 (33%) were monomorphic (Table not shown; Shrestha 2001).

From the RAPD profiling experiment (Table 2), 26 polymorphic RAPD bands were revealed that could be useful in identifying the various weedy *Sporobolus* species. When these markers were re-examined in a marker

Table 2. The 26 weedy species-specific RAPD markers observed after using various primers in a RAPD profiling experiment

The size of the various markers involved is also indicated

Primer code	Primer sequence 5′ to 3′	Weedy *Sporobolus* spp.	Marker size (base pairs)
OPA05	AGGGGTCTTG	*S. pyramidalis*	320
OPA20	GTTGCGATTC	*S. pyramidalis*, *S. jacquemontii*, *S. fertilis*	900
OPA20	GTTGCGATTC	*S. jacquemontii*	850
UBC05	CCTGGGTTCC	*S. pyramidalis*, *S. jacquemontii*	1210
UBC12	CCTGGGTCCA	*S. fertilis*	1600
UBC12	CCTGGGTCCA	*S. pyramidalis*, *S. jacquemontii*	1200
UBC13	CCTGGGTGGA	*S. pyramidalis*, *S. jacquemontii*, *S. fertilis*	520
UBC13	CCTGGGTGGA	*S. pyramidalis*, *S. jacquemontii*, *S. fertilis*	1000
UBC13	CCTGGGTGGA	*S. pyramidalis*, *S. jacquemontii*, *S. fertilis*	2000
UBC43	AAAACCGGGC	*S. fertilis*	310
UBC43	AAAACCGGGC	*S. pyramidalis*	600
UBC43	AAAACCGGGC	*S. pyramidalis*, *S. jacquemontii*, *S. fertilis*	650
UBC43	AAAACCGGGC	*S. fertilis*	2100
UBC43	AAAACCGGGC	*S. fertilis*	2200
UBC51	CTACCCGTGC	*S. pyramidalis*, *S. jacquemontii*	490
UBC51	CTACCCGTGC	*S. pyramidalis*, *S. jacquemontii*, *S. fertilis*	950
UBC52	TTCCCGGAGC	*S. fertilis*	660
UBC58	TTCCCGGAGC	*S. fertilis*	650
UBC59	TTCCGGGTGC	*S. pyramidalis*, *S. jacquemontii*, *S. fertilis*	590
UBC59	TTCCGGGTGC	*S. pyramidalis*, *S. jacquemontii*	1400
UBC61	TTCCCCGACC	*S. pyramidalis*, *S. jacquemontii*	850
UBC61	TTCCCCGACC	*S. fertilis*	240
UBC75	GAGGTCCAGA	*S. pyramidalis*, *S. jacquemontii*	510
UBC75	GAGGTCCAGA	*S. pyramidalis*, *S. jacquemontii*, *S. fertilis*	950
UBC92	CCTGGGCTTT	*S. fertilis*	950
UBC92	CCTGGGCTTT	*S. pyramidalis*, *S. jacquemontii*	1030

Table 3. Potential use of various markers in the identification of 5 major weedy species
+, Markers always useful for identification; +/−, markers sometimes useful, seen with some individuals but not in all

Primer	Fragment size (base pairs)	*Sporobolus* spp. (and sample no.)				
		pyramidalis (5)	*jacquemontii* (4)	*natalensis* (5)	*fertilis* (4)	*africanus* (4)
OPA20	850	−	+[A]	−	−	−
OPA20	900	+	+	+	+	−
UBC13	2000	+	−	+/−	+/−	+/−
UBC43	310	−	−	−	+[A]	+[A]
UBC43	470	−	+[A]	−	−	−
UBC43	650	+	−	+	+/−	+/−
UBC43	2100	−	−	−	+[A]	+[A]
UBC43	2000	−	−	−	+[A]	+[A]
UBC51	490	+[A]	−	+[A]	−	−
UBC75	950	+	+	+	+/−	−
UBC92	930	−	−	+/−	+	+/−
UBC92	1000	+	−	+/−	+	+/−

[A]Various weedy species-specific markers.

confirmation experiment involving 49 seed samples coming from all 12 *Sporobolus* species, 12 bands were revealed to be the most useful in separating the various weedy species (Table 3). The important RAPD-PCR markers observed were $UBC51_{490}$ for *S. pyramidalis* and *S. natalensis*, $UBC43_{310}$, $UBC43_{2100}$, and $UBC43_{2000}$ for *S. fertilis* and *S. africanus*, and $UBC43_{470}$ and $OPA20_{850}$ for *S. jacquemontii* (Figs 1, 2, 3, 4, and 5). More importantly, the DNA fingerprint profile generated by a single primer UBC43 can positively identify 5 major weedy *Sporobolus* species of Australia (Figs 1 and 2).

Genetic diversity and relationship based on RAPD data

The RAPD similarity matrix generated by the NTSYS pc program revealed the degree of inter- and intra-specific genetic diversity among various *Sporobolus* species under study. Inter-species genetic diversity ranged from 10 to 76% in pair wise comparisons between the various species of the weedy *S. indicus* complex. The highest genetic diversity (76%) was revealed between the *S. sessilis* sample obtained from Papua New Guinea (ss 128) and a Queensland sample of *S. fertilis* (sf140). The least genetic diversity (10%) was revealed between the *S. creber* sample of NSW (scr 37) and the *S. elongatus* sample of Qld (se73). Individuals of American rat's tail grass (*S. jacquemontii*) and giant rat's tail grass (*S. pyramidalis*) were 65–83% similar, whereas *S. natalensis* and *S. pyramidalis* individuals were 65–85% similar. Similarity value between *S. fertilis* and *S. pyramidalis* individuals ranged from 45 to 65%, whereas between *S. fertilis* and *S. natalensis* they ranged from 48 to 65% (Table 4).

Giant rat's tail grass *S. pyramidalis* was found to be genetically most similar to another giant rat's tail grass, *S. natalensis* (65–85% similar), whereas it was most genetically dissimilar to *S. elongatus* (40–56% similar).

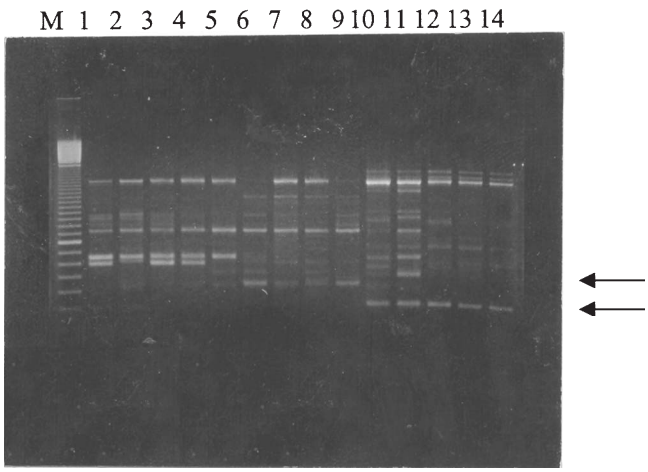

Fig. 1. RAPD profile generated by primer UBC43 with 14 samples of *Sporobolus* belonging to 3 species (sp, *S. pyramidalis*; sj, *S. jacquemontii*; sf, *S. fertilis*). Lanes marked with M are 100 bp molecular weight markers and lanes 1–14 are sp1, sp22, sp48, sp49, sp55, sj87, sj114, sj116, sj135, sf25, sf140, sf36, sf45, sf120. Arrows indicate *S. jacquemontii* (470 bp) and *S. fertilis* (310 bp)-specific markers.

Giant rat's tail grass *S. natalensis* was most dissimilar to *S. sessilis* (47–68% similar). American rat's tail grass *S. jacquemontii* was most similar to giant rat's tail grass *S. pyramidalis* (65–83% similar) and most dissimilar to *S. africanus* (43–55% similar). Giant Parramatta grass *S. fertilis* was shown to be most similar to *S. africanus* (70–83% similar) and most dissimilar to *S. sessilis* (24–68% similar) (Table 4). Inter-specific genetic similarity between the species of the *S. indicus* complex and Australian natives considered in the present study (*viz*. *S. carolii*, *S. actinocladus*, and *S. australasicus*) ranged from 37% (between *S. carolii* and *S. fertilis*) to 90% (between

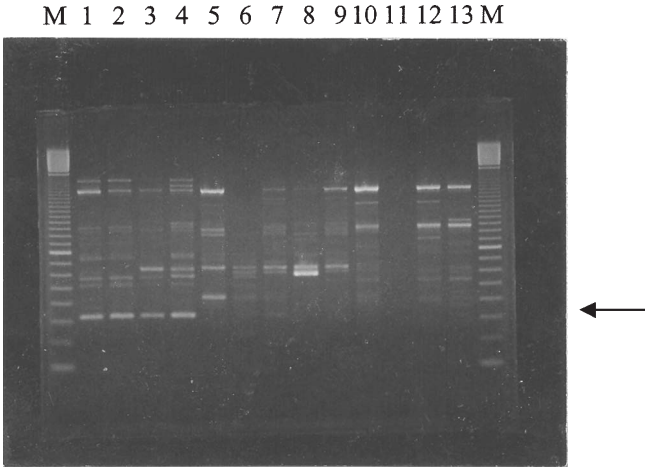

Fig. 2. RAPD profile generated by primer UBC43 with 13 samples of *Sporobolus* belonging to 3 species (sa, *S. africanus*; sn, *S. natalensis*; and ss, *S. sessilis*). Lanes marked with M are 100 bp molecular weight markers and lanes 1–13 are sa12, sa5, sa125, sa143, sn151, sn152, sn153, sn83, sn110, ss126, ss128, ss129, ss130. Arrow indicates *S. africanus* (310 bp)-specific marker (310 bp, lanes 1–4).

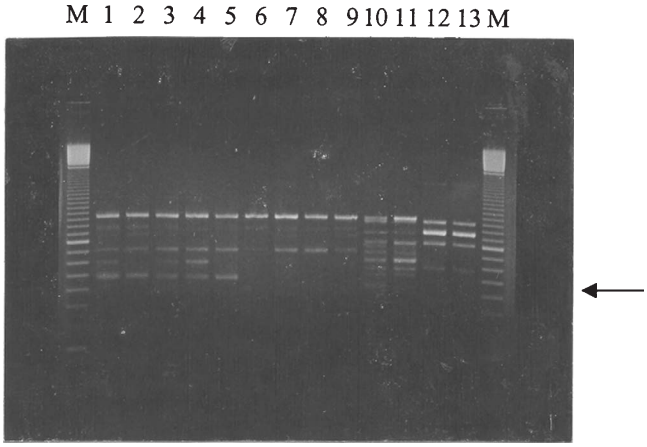

Fig. 3. RAPD profile generated by primer UBC51 with 13 samples of *Sporobolus* belonging to 3 species (sp, *Sporobolus pyramidalis*; sj, *S. jacquemontii*; and sf, *S. fertilis*). Lanes marked with M are 100 bp molecular weight markers and lanes 1–13 are sp1, sp22, sp48, sp49, sp55, sj87, sj114, sj116, sj135, sf25, sf140, sf36, sf45. Arrow indicates *S. pyramidalis* (490 bp)-specific marker (490 bp, lanes 1–5).

S. actinocladus and *S. elongatus*) (similarity matrix not shown).

RAPD similarity matrix showed a low genetic diversity within giant rat's tail grass (*S. pyramidalis*), American rat's tail grass (*S. jacquemontii*), Parramatta grass (*S. africanus*), and *S. creber* (similarity values of 90–98%, 93–100%, 90–95%, and 92–97%, respectively) as compared with the other 5 species under study (*viz*. giant rat's tail grass *S. natalensis* and giant Parramatta grass *S. fertilis*, *S. sessilis*, *S. elongates*, and *S. laxus*; similarity values were 54–93%, 65–100%, 70–98%, 75–84%, and 90%, respectively) (Table 4).

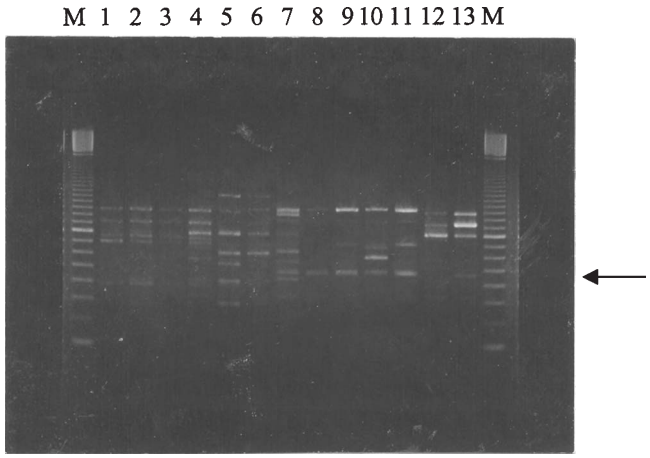

Fig. 4. RAPD profile generated by primer UBC51 with 13 samples of *Sporobolus* belonging to 3 species (sa, *S. africanus*; si, *S. indicus*; and sn, *S. natalensis*). Lanes marked with M are 100 bp molecular weight markers and lanes 1–13 are sa12, sa5, sa125, sa143, si138, si160, sn151, sn152, sn153, sn83, sn110, sn157, sn157. Arrow indicates *S. natalensis* (490 bp)-specific marker (490 bp, lanes 7–11).

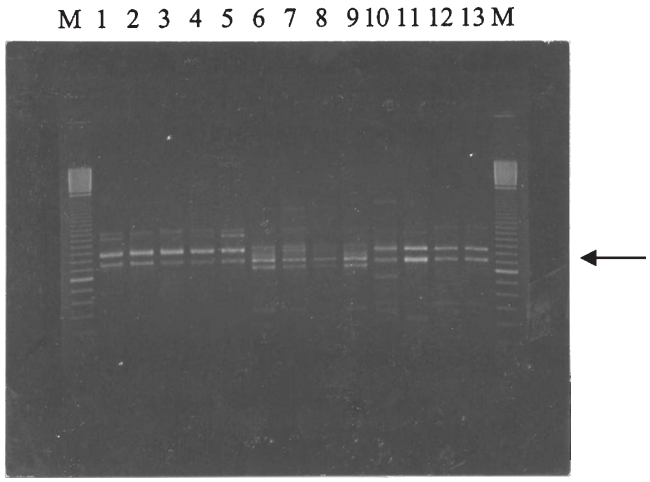

Fig. 5. RAPD profile generated by primer OPA20 with 13 samples of *Sporobolus* belonging to 3 species (sp, *S. pyramidalis*; sj, *S. jacquemontii*; and sf, *S. fertilis*). Lanes marked with M are 100 bp molecular weight markers and lanes 1–13 are sp1, sp22, sp48, sp49, sp55, sj87, sj114, sj116, sj135, sf25, sf140, sf36, sf45. Arrow indicates *S. jacquemontii* (850 bp)-specific marker (850 bp, lanes 6–9).

Three similarity coefficients were used to generate phenograms to aid in the visualisation of the genetic relationships among the 9 species of the *S. indicus* complex studied and the 3 Australian native species studied. The topology of the phenogram generated from the simple matching coefficient was found to be slightly different from that generated from the Dice (Fig. 6) and Jaccard's coefficients (Fig. 7). In the phenograms generated from the Dice and Jaccard's coefficients, all the species of the *S. indicus* complex were grouped into one major cluster and

Table 4. Part of RAPD similarity matrix showing percentage genetic similarity and diversity among various species of the *S. indicus* complex
Data matrix being too large could not be presented

Comparison of one *Sporobolus* spp. with another	Genetic similarity (%)	Genetic diversity (%)
S. pyramidalis v. S. pyramidalis	90–98	2–10
S. jacquemontii v. S. jacquemontii	93–100	0–7
S. jacquemontii v. S. pyramidalis	65–83	17–35
S. fertilis v. S. fertilis	65–100	0–35
S. fertilis v. S. pyramidalis	45–65	35–55
S. fertilis v. S. jacquemontii	45–50	50–55
S. africanus v. S. africanus	90–95	5–10
S. africanus v. S. pyramidalis	43–58	42–57
S. africanus v. S. jacquemontii	43–55	45–57
S. africanus v. S. fertilis	70–83	17–30
S. natalensis v. S. natalensis	54–93	7–46
S. natalensis v. S. pyramidalis	65–85	15–35
S. natalensis v. S. jacquemontii	53–68	32–47
S. natalensis v. S. fertilis	48–65	35–52
S. natalensis v. S. africanus	51–65	35–49
S. sessilis v. S. sessilis	70–98	2–30
S. sessilis v. S. pyramidalis	45–59	41–55
S. sessilis v. S. jacquemontii	63–71	29–37
S. sessilis v. S. fertilis	24–68	32–76
S. sessilis v. S. africanus	29–73	27–71
S. sessilis v. S. natalensis	47–68	32–53
S. elongatus v. S. elongatus	75–84	16–25
S. elongatus v. S. pyramidalis	40–56	44–60
S. elongatus v. S. jacquemontii	50–69	31–50
S. elongatus v. S. fertilis	41–65	35–59
S. elongatus v. S. africanus	55–66	34–45
S. elongatus v. S. natalensis	48–68	32–52
S. elongatus v. S. sessilis	66–73	27–34
S. creber v. S. creber	92–97	3–8
S. creber v. S. pyramidalis	50–55	45–50
S. creber v. S. jacquemontii	53–70	30–47
S. creber v. S. fertilis	55–65	35–45
S. creber v. S. africanus	60–68	32–40
S. creber v. S. natalensis	48–73	27–52
S. creber v. S. sessilis	65–77	23–35
S. creber v. S. elongatus	73–90	10–27
S. laxus v. S. laxus	90	10
S. laxus v. S. pyramidalis	45–50	50–55
S. laxus v. S. jacquemontii	58–65	35–42
S. laxus v. S. fertilis	50–60	40–50
S. laxus v. S. africanus	58–68	32–42
S. laxus v. S. natalensis	48–65	35–52
S. laxus v. S. sessilis	68–80	20–32
S. laxus v. S. elongatus	73–84	16–27
S. laxus v. S. creber	75–80	20–25

the 3 Australian native species formed a second, distinct cluster. Species of the *S. indicus* complex were further divided into 3 subclusters (Figs 6 and 7).

Discussion

RAPD markers for species identification

Individual weedy species-specific markers were difficult to find for the 5 most serious weed species. For *S. jacquemontii*, 2 species-specific markers were found; however, only combined markers could be identified to separate the other 4 into 2 groups (*viz*. Group 1, *S. pyramidalis* and *S. natalensis*; Group 2, *S. fertilis* and *S. africanus*; Table 3). A possible reason for the lack of some species-specific markers was apparent in the results of the second study, which looked at the degree of genetic similarity among the species. It was found that only small genetic differences existed between *S. pyramidalis* and *S. natalensis*, and between *S. fertilis* and *S. africanus* (Figs 6 and 7, Table 4).

The molecular markers generated from this investigation can now be used to identify the 5 major weedy *Sporobolus* species of Australia provided all the reaction and cycling conditions of the RAPD technique are strictly maintained. Several factors (viz. template DNA concentration, magnesium concentration, primer annealing temperature, and primer base composition) affect the PCR reaction (Tingey *et al*. 1992). Furthermore, RAPD being a dominant marker system, several markers show a dominance/recessive inheritance pattern in diploid organisms. This results in a partial amplification of particular fragments and this makes identification of heterozygotes for the dominant allele difficult (Aman 1997). The resulting fragments may either be homozygous (AA) or heterozygous (Aa), and the absence of the fragment may indicate the underlying genotype (aa) (Weising *et al*. 1995). Therefore, a modified marker system, called the sequence characterised amplified regions (SCAR) markers (Paran and Michelmore 1993), has to be developed in order to convert the dominant weedy species-specific RAPD markers to codominant SCAR markers. The resultant specific PCR would create a robust diagnostic protocol for these weeds.

Analysis of genetic diversity and relationships

Genetic diversity studies at the molecular level have great implications for formulating weed management strategies, especially when there is taxonomic dispute regarding differentiating various species on a morphological basis (Lopez-Martinez *et al*. 1999). The present investigation has put forth the inter- and intra-specific genetic diversity and relationship among 9 member species of the *S. indicus* complex based on RAPD data. Among all 9 species, *S. natalensis* was found to be most genetically diverse (7–46% genetic diversity) followed by *S. fertilis* (0–35% genetic diversity) and *S. sessilis* (2–30% genetic diversity). Although all the representative samples of the 3 introduced weedy species, giant rat's tail grasses (*S. pyramidalis*, *S. natalensis*) and American rat's tail grass (*S. jacquemontii*), were from various places within Queensland, *S. natalensis* was found to be genetically more diverse (polymorphic) in comparison with the other 2 species (Table 4). In the case of Paramatta grasses, giant Parramatta grass *S. fertilis* was shown to be more diverse (0–35% genetic diversity) in comparison with Parramatta grass *S. africanus*. One previous RAPD-based genetic diversity study on giant rat's

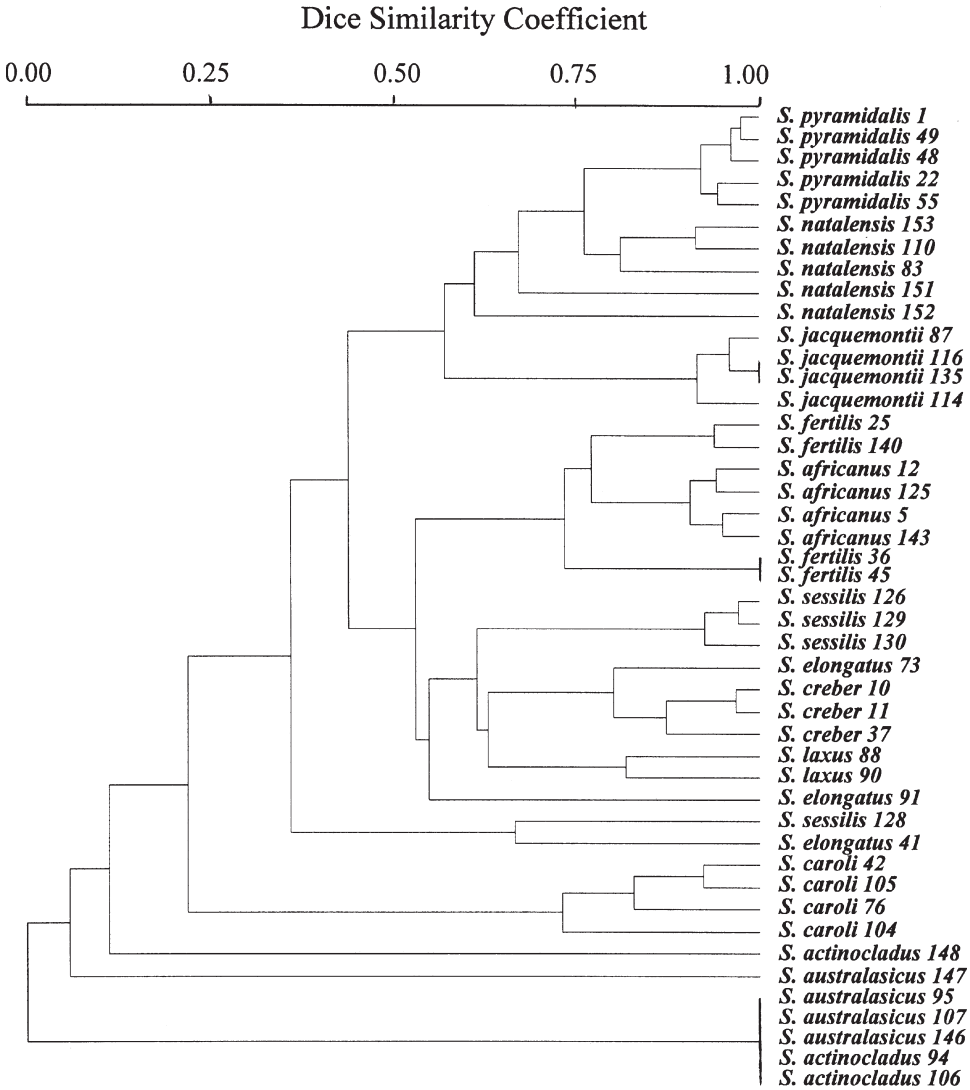

Fig. 6. A phenogram constructed for 45 samples taken from 12 *Sporobolus* species and created from the UPGMA cluster analysis using Dice's coefficient. All the species of the *S. indicus* complex formed one big cluster.

tail grasses had shown *S. natalensis* to be 16% dissimilar to *S. pyramidalis*, whereas there was only 8% diversity within *S. pyramidalis* (Hetherington 1997). This result is quite consistent with the present investigation where similarity between 2 giant rat's tail grasses ranged from 65 to 85% (i.e. 15–35% dissimilar), whereas similarity within *S. pyramidalis* individuals of this investigation was found to be 90–98% (2–10% dissimilar) (Table 4).

The clustering pattern in the phenograms, using the 3 different similarity coefficients (Figs 6 and 7), was slightly different. *Sporobolus pyramidalis*, *S. natalensis*, and *S. jacquemontii* all clustered together in all 3 phenograms, forming a first subcluster. Similarly, giant Parramatta grasses and Parramatta grasses clustered together in all the phenograms. In phenograms generated from Dice and Jaccard's coefficients, these formed the second subcluster. However, the phenogram generated using a simple matching coefficient separated the giant Parramatta grass and Parramatta grass group from the rest of the species under study (phenogram not shown). It has been pointed out that inclusion of data for absence of bands (e.g. in the analysis using the simple matching coefficient) from both individuals being compared reduces the level of similarity (Mace *et al*. 1999). Therefore, in the present context, phenograms generated from Dice and Jaccard's coefficients (Figs 1 and 2) have been considered more reliable in revealing the taxonomic relationship of various species of the *S. indicus* complex. This has been further substantiated by the fact that species of the complex formed a monophyletic clade in one phylogenetic study involving worldwide *Sporobolus*

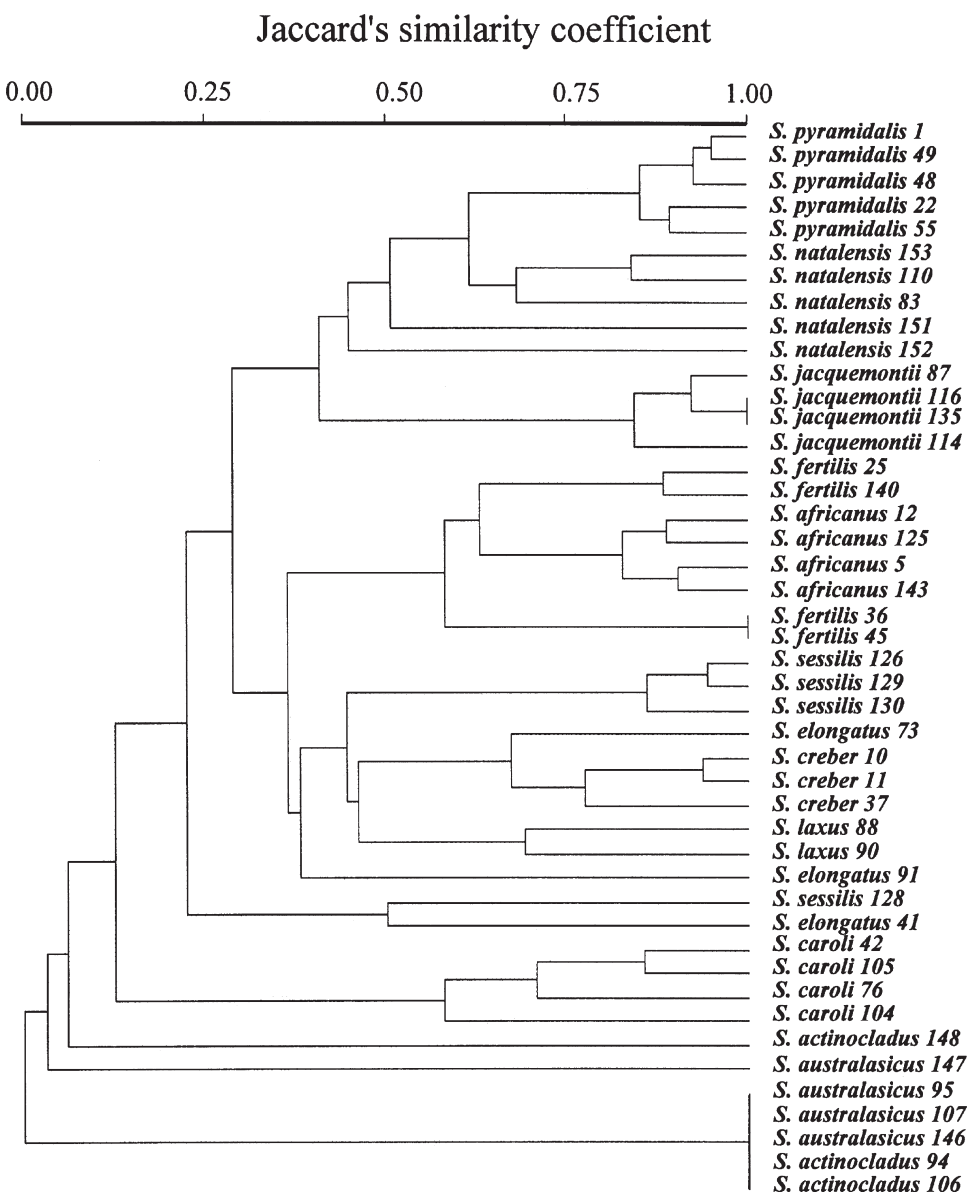

Fig. 7. A phenogram constructed for 45 samples taken from 12 *Sporobolus* species and created from the UPGMA cluster analysis using Jaccard's coefficient. All the species of the complex formed one big cluster.

species (Oritz-Diaz and Culham 2000) as well as in an earlier phylogenetic study based on internal transcribed spacers (ITS) sequences from our group (Shrestha *et al*. 2003). The 4 other, less problematic weedy species of the complex (*viz*. *S. sessilis*, *S. elongatus*, *S. creber*, and *S. laxus*), formed a third subcluster in various phenograms (Figs 6 and 7).

Clustering together of various species of the complex in the phenograms into 3 major clusters suggests the close relationship among the constituent species of each of these clusters (i.e. among *S. pyramidalis*, *S. natalensis*, and *S. jacquemontii*; *S. fertilis* and *S. africanus*; and *S. sessilis*, *S. elongatus*, *S. creber*, and *S. laxus*). The protein electrophoresis study of Vieritz (1993) also showed a close relationship among the *S. pyramidalis*, *S. natalensis*, and *S. jacquemontii* group and the *S. elongatus*, *S. creber*, *S. laxus*, and *S. sessilis* group.

A few differences in the way some individuals clustered were observed in the different phenograms. This may be attributed to one of a number of reasons. Firstly, as the RAPD-PCR searches the genome more widely, the observed variation then becomes greater due to the highly polymorphic DNA regions in those individuals. Secondly, as the RAPD-PCR itself is not a very robust technique, the bands thus generated are not of the same intensity. This then creates difficulties when scoring for presence or absence of bands. Thirdly, the scored bands generated by 6 primers may not

be sufficient for resolving various individuals clearly in the phenograms. Fourthly, the data matrix for analysis might have been very big. It has been mentioned that these clustering methods are sensitive to the number of operational taxonomic units (OTUs) in the various clusters, especially at high ranks (Sneath 1976).

The distinctness of the various species, and the taxonomic relationship among various weedy species of the complex, shown in this study, are similar to those previously generated using morphological and protein-based markers (Vieritz 1993; Simon 1996). As the present DNA-based studies are independent of environment and management practices (Morell *et al*. 1995), they offer a more reliable option for plant species–varieties–cultivars identification and genetic diversity analysis. The weedy species-specific RAPD fingerprints, as well as the genetic diversity and relationship analyses revealed in this present study, will be highly beneficial in formulating future weed-control strategies. This could either be achieved through positive identification (Joel *et al*. 1996) of these weeds in contaminated grass seed lots or aiding in the selection of appropriate herbicides for their control in pastures (Lopez-Martinez *et al*. 1999). Furthermore, this kind of knowledge may also be important to those who are attempting to introduce biological control agents to help to control these weeds (Nissen *et al*. 1995).

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References

Aman RA (1997) A comparative assessment of molecular techniques employed in genetic diversity studies (and their suitability in resource-limited settings). In 'Molecular genetic techniques in plant genetic resources. Report of an International Plant Genetic Resources Institute (IPGRI) Workshop'. 9–11 October 1995, Rome, Italy. (Eds WG Ayad, T Hodgkin, A Jaradat, VR Rao) pp. 119–127. (IPGRI)

Anon. (1997) Giant rats tail grass *Sporobolus pyramidalis* and *S. natalensis* declared. Queensland Department of Primary Industries, Pest Facts Sheet, Produced by Land Protection Sub Program, Queensland.

Anon. (1999) Giant rats tail grass. Best practice manual. Information series QI99034, Compiled by the Giant Rats Tail Grass Project Team, Department of Primary Industries.

Betts J, Fenton G, Moore K (1990) Giant Parramatta grass control. Agnote, NSW Agriculture and Fisheries, NSW.

Dice LR (1945) Measures of the amount of ecologic association between species. *Ecology* **26**, 297–302.

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.

Golembiewski RC, Danneberger TK, Sweeney PM (1997) Potential RAPD markers for use in the identification of creeping bent grass cultivars. *Crop Science* **37**, 212–214.

Graham GC, Henry RJ, Godwin ID, Nikles DG (1996) Phylogenetic position of Hoop pine (*Araucaria cunninghamii*). *Australian Systematic Botany* **9**, 893–902.

Graham GC, Henry RJ, Redden RJ (1994) Identification of navy bean varieties using random amplification of polymorphic DNA. *Australian Journal of Experimental Agriculture* **34**, 1173–1176.

Hetherington S (1997) Evaluation of endemic fungal pathogens as biological control agents of *Sporobolus* species. PhD thesis, The University of Queensland, Brisbane, Australia.

Jaccard P (1908) Nouvelles recherches sur la distribution florale. *Bulletin. Societe Vaudoise Sciences Naturelles* **44**, 223–270.

Joel DM, Portnoy V, Katzir N (1996) Identification of single tiny seeds of *Orobanche* using RAPD analysis. *Plant Molecular Biology Reporter* **14**, 243–248.

Laffan RA (1986) Parramatta grass (*Sporobolus africanus*) in the Coffs Harbour Agronomy district. In 'Parramatta grass and its control'. pp. 9–23. (NSW Department of Agriculture, Biological and Chemical Research Institute: Rydalmere, NSW)

Lopez-Martinez N, Salva AP, Finch RP, De Prado R (1999) Molecular markers indicate intraspecific variation in the control of *Echinochloa* spp. with quinclorac. *Weed Science* **47**, 310–315.

Mace ES, Lester RN, Gebhardt CG (1999) AFLP analysis of genetic relationships among the cultivated eggplant, *Solanum melongena* L., and wild relatives (Solanaceae). *Theoretical and Applied Genetics* **99**, 626–633. doi: 10.1007/S001220051277

Moodie M, Finch RP, Marshall G (1997) Analysis of genetic variation in wild mustard (*Sinapsis arvensis*) using molecular markers. *Weed Science* **45**, 102–107.

Morell MK, Peakall R, Appels R, Preston LR, Lloyd HL (1995) DNA profiling techniques for plant variety identification. *Australian Journal of Experimental Agriculture* **35**, 807–819.

Nei M, Li WH (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.

Nissen SJ, Masters RA, Lee DJ, Rowe ML (1995) DNA-based marker systems to determine genetic diversity of weedy species and their application to biocontrol. *Weed Science* **43**, 504–513.

Oritz-Diaz J, Culham A (2000) Phylogenetic relationships of the genus *Sporobolus* (Poaceae: Eragrostideae) based on nr DNA ITS sequences. In 'Grasses: systematics and evolution'. (Eds SWL Jacobs, J Everett, A Jaradat, VR Rao) (CSIRO Publishing: Melbourne, Vic.)

Paran I, Michelmore RW (1993) Development of reliable PCR based markers linked to downy mildew resistance genes in lettuce. *Theoretical and Applied Genetics* **85**, 985–993. doi: 10.1007/BF00215038

Shrestha S (2001) Molecular systematics of weedy *Sporobolus* species of Australia. PhD thesis, University of Queensland, Brisbane, Australia.

Shrestha S, Adkins SW, Graham GC, Loch DS (2003) Phylogeny of the *Sporobolus indicus* complex based on internal transcribed spacers (ITS) sequences. *Australian Systematic Botany* **16**, 165–176. doi: 10.1071/SB02009

Simon BK (1993) Key to Australian grasses. 2nd edn. Queensland Department of Primary Industries Information Series, Q18901.

Simon BK (1996) Taxonomy of weedy rats tail grasses (*Sporobolus* spp.). In 'Proceedings of the Workshop Weedy *Sporobolus* Grasses, Current Knowledge and Future Directions.' Gympie. pp. 22–27. (Queensland Department of Primary Industries: Brisbane)

Simon BK (1999) Taxonomy of the *Sporobolus indicus* complex. In 'Proceedings of the Workshop Weedy *Sporobolus* Grasses, Research Update'. Gympie. pp. 41–47. (Queensland Department of Primary Industries: Brisbane)

Simon BK, Jacobs SWL (1999) Revision of the genus *Sporobolus* (Poaceae, Chloridoideae) in Australia. *Australian Systematic Botany* **12**, 375–448.

Sneath PHA (1976) Phenetic taxonomy at the species level and above. *Taxon* **25**, 437–450.

Sneath PHA, Sokal RR (1973) 'Numerical taxonomy, the principles and practice of numerical classification.' (W. H. Freeman and Company: San Francisco, CA)

Sokal RR, Michener CD (1958) A statistical method for the evaluating systematic relationships. *University of Kansas Science Bulletin* **38**, 1409–1438.

Sokal RR, Sneath PHA (1963) 'Principles of numerical taxonomy.' (W. H. Freeman and Company: San Francisco, CA)

Tingey SV, Rafalski JA, Williams JGK (1992) Genetic analysis with RAPD markers. In 'Applications of RAPD technology to plant breeding'. Joint plant breeding symposia series. pp. 3–8. (Crop Science Society of America, American Society for Horticultural Science and American Genetic Association: Minneapolis, MN)

Vieritz AM (1993) Electrophoretic analysis of *Sporobolus* spp. by PAGE. Project Report 9.18 to Queensland Department of Primary Industries (QDPI).

Weising K, Nymbom H, Wolff K, Meyer W (1995) 'DNA fingerprinting in plants and fungi.' (CRC Press: Boca Raton, FL)

Welsh J, McClelland M (1990) Fingerprinting genomes using PCR with arbitrary primers. *Nucleic Acids Research* **18**, 7213–7218.

Williams JGK, Kubelic AR, Livak KJ, Rafalski JA, Tingey SV (1990) DNA polymorphism amplified by arbitrary primers are useful as genetic markers. *Nucleic Acids Research* **18**, 6531–6535.

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