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RESEARCH ARTICLE

DISTRIBUTION PATTERNS AND GENETIC RELATIONSHIPS OF WEEDY RICE (*ORYZA SATIVA* F. *SPONTANEA*) POPULATIONS IN DIFFERENT CLIMATIC ZONES IN SRI LANKA

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ABSTRACT

Weedy rice (*Oryza sativa* f. *spontanea*) (WR) is widely distributed in rice growing areas in all climatic zones in Sri Lanka and shows spatial distribution and genetic affinities to cultivated rice. The present study attempts to relate the agro-morphological and molecular data to the distribution pattern/s of WR populations in different climatic zones. WR eco-types were collected from five different locations in twelve districts representing Wet, Dry and Intermediate zones. Five replicates of each eco-type planted in plastic pots and arranged in CRD. Agro-morphological characterization of WR, wild rice and cultivated rice was based on the Standard Characterization Catalogue**.** Ten SSR primer pairs were used for molecular study. Capillary electrophoresis was performed using GENE MAPPER software and identified different peaks among samples. The principle component analyses (PCA) were carried out for data. PCA of morphological variables of WR populations results nine components explaining 73.87% and 10 labeled SSR primer pairs resulted six components explaining 80.34% of total variation. Occurrence of agro-morphological characters of WR, wild and cultivated rice showed a weak trend with climatic zones. The pattern of genetic diversity and differentiation of WR populations suggest the common origin centered on the species; *O. nivara* for dry zone WR eco-types and *O. rufipogon* for wet zone eco-types.

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INTRODUCTION

Weedy rice (*Oryza sativa* f. *spontanea*) is one of the most nuisance weeds possessing higher morphological plasticity and mimics the wild and cultivated rice. The term "weedy rice" generally includes all the species of the genus *Oryza* which conspecific with rice and is in rotation with rice weeds. Weedy rice (WR) reported particularly in South and South-East Asia, South and North America, and Southern Europe (Mortimer *et al*., 2000; Chauhan and Johnson, 2010; Chauhan, 2012). Emergence of WR leads to high production costs and reduction of yield (Azmi and Karim, 2008) and lowered commodity value by staining the grains with undesirable pericarp color (Mortimer *et al*., 2000). Presently, WR has reached a considerable competitive level of infestation threatening the sustainability of rice cultivation especially in Asian countries (Londo and Schaal, 2007). WR was first reported in 1992 from Ampara District, Sri Lanka and spreading into many areas of the country. WR occurs in all agro-ecological zones of the country with varying population densities (Abeysekara *et al*., 2010). WR was formed from selection and adaptation of wild rice (De Wet and Harlan, 1975; Harlan, 1992). It was suggested that de-domestication of crop species to a wild or feral form would probably occur when domesticated rice was abandoned (Bres-Patry *et al*., 2001) and WR originated from hybridization between cultivated rice and its progenitor type (Tang and Morishima, 1996). WR may also originate from ongoing and multi directional hybridization between weedy rice and cultivated types as well as hybridization among weedy types (Londo and Schaal, 2007). Recently it was noted that conspecific weedy rice could also unexpectedly evolve from hybridization of its cultivated relatives (Ishikawa *et al*., 2005; Reagon *et al*., 2010; Xiong *et al*., 2012). It has been demonstrated that weedy type offspring could emerge after inter-subspecies and inter- varietal hybridization in rice (Xiong *et al*., 2012). For WR in USA it was believed that they have evolved directly from within domesticated lineages (Reagon *et al*., 2010). After the formation of weedy rice, the continual gene flow from the cultivated crop into neighboring weedy populations in combination with natural selection has been proposed to play a critical role in the adaptive evolution of WR (Jiang *et al*., 2012; Sun *et al*., 2013). Previous data

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reported that natural out-crossing between different rice varieties in Sri Lanka ranges between 0.34 and 0.67 % resulted in considerable increases of WR in rice fields by out-crossing wild rice (*O. nivara* and *O. rufipogon*) and cultivated rice (Chen *et al*., 2004). Recent results reports the out-crossing rate ranged between 0-20%. Therefore, several eco-types can be identified. Genetic variations are expected but, precise reports from Sri Lanka in this context are very rare. As phenotypes are expression of underlying genetic variations, genetic variation amongst WR eco-types in Sri Lanka is expected. Molecular markers are useful and informative tool for estimating the genetic diversity and genetic relationships in closely related genotypes to date, SSR markers have been used as allele-specific and co-dominant markers in population genetic and evolutionary studies of many plants (Mckhann *et al*., 2004; Upadhyaya *et al*., 2006). The close morphological similarity makes it difficult to distinguish between WR eco-types and cultivated rice varieties in the field. The studies related to the genetic diversity of WR populations and eco-climatic trend in distribution of WR are limited. Further, lack of such studies precludes the WR control and management in the country. In general, the distribution of weeds is facilitated by the changing climate. In the present study, it was attempted to employ agro-morphological and molecular data to recognize the distribution pattern/s of WR populations in different eco-climatic zones in Sri Lanka.

MATERIALS AND METHODS

Agro-morphological characterization

Seeds of presumed different WR eco-types were collected from five different locations in each twelve different districts representing Wet, Dry and Intermediate zones in Sri Lanka. (Wet Zone: Matara, Matale, Kandy Intermediate Zone: Kurunegala, Dry Zone: Hambantota, Puttalam, Mannar, Pollonnaruwa, Anuradhapura, Ampara, Jaffna and Batticallo) (Table 1), representing Wet, Dry and Intermediate zones in Sri Lanka. The cultivated rice varieties included breeding lines developed by Batalagoda (Bg series), Bombuwala (Bw series) and Ambalantota (At series). Though the description and nomenclature of wild rice species *O. nivara* and *O. rufipogon* is controversial, the present study followed the Sharma and Shanty's treatment (Sharma and Shastry, 1965). The cultivated rice variety and the wild rice variety or varieties were also collected from the same locations. The collected seeds of WR eco-types, cultivated and wild rice varieties (*O. nivara* and *O. rufipogon*) were subjected to dormancy breaking treatments and sown in plastic trays in a plant house at the Open University of Sri Lanka, Nawala, Sri Lanka. A total of five replicates of each eco-type and wild rice varieties were planted in plastic pots with representative paddy soils from each location. Replicates were arranged in Complete Randomized Design (CRD). Thirty six (36) Agro-morphological characters of WR eco-types, cultivated rice varieties and wild rice varieties were measured using the Standard Characterization Catalogue (PGRC 1999).

Molecular characterization

Total genomic DNA was extracted from 7-day old seedlings of respective WR eco-types, wild rice and cultivated types using Ceygen Plant total DNA purification kit. A total of ten SSR primer pairs were used (Table 2) for molecular study. SSR markers were obtained from Gramene (http://www.gramene.org/). A four-primer system was used, which included a universal M13 oligonucleotide (TGTAAAACGACGGC CAGT), labeled with one of four fluorescent dyes (6-FAM, NED, PET or VIC). Fluorescent dyes allow the products to be four plexed during electrophoresis; a special forward primer composed of a concentration of the M13 oligonucleotide; and the pig tail reverse primer for SSR PCR amplification. All amplification reactions were carried out in a total volume 30µl of which consist 1 x PCR buffer, 1mM dNTPs, 2µM SSR primers, 2mM $MgCl_2$, 50ng of genomic DNA and 0.5 Units of Taq polymerase. SSR alleles were resolved on an ABI Prism 3100 DNA sequencer using Gene Scan 4.1 software, and sized precisely using Gene Scan 600 LIZ ladder. Fragment analysis using capillary electrophoresis was performed using GENE MAPPER software and identified different peaks among WR eco-types and wild rice varieties.

Analysis of data

The collected data sets were subjected to data preprocessing to transform into homogenous variables. The principle component analysis (PCA) was carried out on the agro-morphological and molecular data of WR eco-types, cultivated rice varieties and wild rice varieties in twelve districts using SPSS PC Ver. 20.

RESULTS

Agro-morphological Characterization

The Principle Component Analysis of the 36 morphological variables of the WR populations has resulted nine components which explain 73.87% of total variation in the data set (Table 3). The biplot of the PCA1 and PCA2 scores reveled that there were four groups A, B, C and D (Figure 1). Wild rice varieties *O. nivara* and *O. rufipogon* were fallen into one group (Figure 1. Group A). The rest of the cultivated rice varieties and WR eco-types formed into separate clusters (Figure 1. Group B and D). The cluster C consists most of the cultivated rice varieties grown in Anuradhapura and Polonnaruwa Districts. Most of the WR eco-types in Sri Lanka belong to one cluster (Figure 1. Group D). Distribution of agro-morphological characters of WR, wild and cultivated rice showed a weak trend with climatic zones indicating the plasticity of morphological features of WR enabling them to grow in any climatic zone.

Molecular characterization

The scatter plot of the first and second principle components showed a clear genetic variation and differentiation pattern of weedy rice populations in Sri Lanka. The Principle Component Analysis of the 10 labeled SSR primer pairs of the WR population has resulted six components which explain 80.34% of total variation in the dataset (Table 4). The biplot of the PCA1 and PCA2 scores revealed that there were three groups (Figure 2). Wild rice varieties *O. nivara* and dry zone (Anuradhapura and Puttalam Districts) WR eco-types belong to one group (Figure 2. Group A) suggesting a possibly origin of dry zone WR eco-types from *O. nivara*. Wet zone (Matara, Matale and Kandy Districts) WR eco-types and *O. rufipogon* were fallen into one group (Figure 2. Group B) suggesting, *O. rufipogon* as a contributive wild rice for origin of WR eco-types in Wet zone. The rest of the cultivated rice varieties and WR eco-types found in the intermediate zone (Kurunegala Districts) formed a separate cluster (Figure 2. Group C).

Table 1. Population samples of weedy rice (*Oryza sativa* f. *spontanea*) collected from different locations in Sri Lanka

District	Location	Weedy rice eco-type	Cultivated type
Kurunagala	KurunagalaBulunahalaYaya	KBW1, KBW2	Bg 358 (KC1)
	KumbukwawaDahampalaYaya	KDW1, KDW2, KDW3	Bg 379-2 (KC2)
	KuliyapitiyaHambalawaYaya	KHW1, KHW2	Bg 358 (KC3)
	KurunagalaIbbagamuwaBulunwawaYaya	KIW1, KIW2	Bg 359 (KC4)
	KurunagalaKuliyapitiyaAhalaYaya	KAW1	Bg 379-2 (KC5)
Matara	MataraWeligamamudugamuwa	MWW1,MWW2,MWW3,MWW4	Bg 379-2 (MWC)
	MataraMapalanaKamburupitiya	MKW1,MKW2,MKW3,MKW4	Bg 307 (MKC)
	MataraPalolpitiyaAkurugoda	MPW1, MPW2, MPW3, MPW4	Bg 352 (MPC)
	MataraHakmanaKomangoda	MHW1, MHW2, MHW3, MHW4	At 362 (MHC)
	MataraMorawaka	MMW1, MMW2,MMW3	Bg 379-2 (MMC)
Anuradhapura	Kunchikulama;	AKW1,AKW2	Bg 352 (AKC)
	Thambuththegama	ABW1	Bg 352 (ABC)
	Puliyankulama	APW1,APW2	Bg 352 (APC)
	Shrawasthipura	ASW1	Bg 352 (ASC)
	Thalawa	ATW1,ATW2	Bg 352 (ATC)
Hambantota	Hambantota Ranna	HRW	At 362 (HRC)
	HambantotaAngunukola	HAW	At 362 (HAC)
	HambantotaKatuwawa	HKW1,HKW2	At 362 (HKC)
	HambntotaBallagaswawa	HBW1, HBW2	At 362 (HBC)
	HambantotaAmbalantota	HMW	At 362 (HMC)
Matale	MataleNagahathannaMaiwela	MMW	Bg 1/94 (MMC)
	MatalePahalaYatawara	MYW	Bg 358 (MYC)
	MataleGaloya	MGW	Bg 352 (MGC)
	MataleNawaragoda	MNW	Bg 358 (MNC)
	MataleGolahanWaththa	MWW	Bg 1/94 (MWC)
Puttalam	Puttalam Madampe	PMW	At 362 (PMC)
	PuttalamMarawila Dankotuwa	PDW	Bg 358 (PDC)
	PuttalamAnamaduwa	PAW1, PAW2	Bg 11 (PAC)
	PuttalamRajakatuwa	PRW	Bg 11 (PRC)
	PuttalamKaruwalagaswawe	PKW	Bg 358 (PKC)
Batticalo	Batticalo Kalawanchikudy	BKW	At 362 (BKC)
	BatticaloKattankudy	BKaW	Bg 358 (BKaC)
	BatticaloPunnakuda	BPW	Bg 11 (BPC)
	BatticaloThavapuram	BTW	Bg 11 (BTC)
	BatticaloChenkaladi	BCW	Bg 358 (BCC)
Pollonaruwa	Pollonaruwa Kaduruwela	PKW	At 362 (PKC)
	Pollonnaruwa Hingurakgoda	PHW	Bg 358 (PHC)
	PollonaruwaBakamuna	PBW	At 362 (PBC)
	PollonaruwaJayanthipura	PJW	At 362 (PJC)
	PuttalamKaruwalagaswawe	PGW	Bg 358 (PGC)
Mannar	Mannar 1	MAW	At 362 (MAC)
	Mannar 2	MBW	Bg 358 (MBC)
	Mannar 3	MCW	At 362 (MCC)
	Mannar 4	MDW	At 362 (MDC)
	Mannar 5	MEW	Bg 358 (MEC)
Ampara	Ampara 1	AAW	At 362 (AAC)
	Ampara 2	ABW	Bg 358 (ABC)
	Ampara 3	ACW	At 362 (ACC)
	Ampara 4	ADW	At 362 (ADC)
	Ampara 5	AEW	Bg 358 (AEC)
Kandy	Kandy 1	KAW	At 362 (KAC)
	Kandy 2	KBW	Bg 358 (KBC)
	Kandy 3	KCW	At 362 (KCC)
	Kandy 4	KDW	At 362 (KDC)
	Kandy 5	KEW	Bg 358 (KEC)
Vauniya	Vauniya 1	VAW	At 362 (VAC)
	Vauniya 2	VBW	Bg 358 (VBC)
	Vauniya 3	VCW	At 362 (VCC)
	Vauniya4	VDW	At 362 (VDC)
	Vauniya5	VEW	Bg 358 (VEC)

Table 2. Ten SSR primer pairs used for the study

Oligo name	Oligo sequence (5'- 3')
M13RM11F	TGTAAAACGACGGCCAGT TCTCCTCTTCCCCCGATC
PigtRM11R	GTTTCTTATAGCGGGCGAGGCTTAG
M13RM14F	TGTAAAACGACGGCCAGTCCGAGGAGAGGAGTTCGAC
PigtRM14R	GTTTCTTGTGCCAATTTCCTCGAAAAA
M13RM21F	TGTAAAACGACGGCCAGTACAGTATTCCGTAGGCACGG
PigtRM21R	GTTTCTTGCTCCATGAGGGTGGTAGAG
M13RM 44F	TGTAAAACGACGGCCAGTACGGGCAATCCGAACAACC
PigtRM44R	GTTTCTTTCGGGAAAACCTACCCTACC

Continue……

M13RM84F	TGTAAAACGACGGCCAGTTAAGGGTCCATCCACAAGATG
PigtRM84R	GTTTCTTTTGCAAATGCAGCTAGAGTAC
M13RM167F	TGTAAAACGACGGCCAGTGATCCAGCGTGAGGAACACGT
PigtRM167R	GTTTCTTAGTCCGACCACAAGGTGCGTTGTC
M13RM205F	TGTAAAACGACGGCCAGTCTGGTTCTGTATGGGAGCAG
PigtRM205R	GTTTCTTCTGGCCCTTCACGTTTCAGTG
M13RM211F	TGTAAAACGACGGCCAGTCCGATCTCATCAACCAACTG
PigtRM211R	GTTTCTTCTTCACGAGGATCTCAAAGG
M13RM280F	TGTAAAACGACGGCCAGTACACGATCCACTTTGCGC
PigtRM280R	GTTTCTTTGTGTCTTGAGCAGCCAGG
M13RM332F	TGTAAAACGACGGCCAGTGCGAAGGCGAAGGTGAAG
PigtRM332R	GTTTCTTCATGAGTGATCTCACTCACCC

Table 3. Summary of the principle component analysis carried out on the 36 morphological characters of the different rice varieties across different climatic zones in Sri Lanka

Component	PCA1	PCA2	PCA 3	PCA 4	PCA 5	PCA 6	PCA 7	PCA 8	PCA 9
Eigen value	4.739	3.839	3.801	3.467	2.461	2.25	2.222	2.019	1.796
Percentage of variance explain	13.165	10.663	10.557	9.632	6.837	6.251	6.171	5.609	4.988
Cumulative Percentage of variance explain	13.165	23.828	34.385	44.017	50.854	57.105	63.277	68.886	73.874
Leaf Senescence	0.864	0.274	0.08	-0.029	-0.194	0.011	0.076	-0.087	-0.007
Panicle Shattering	0.823	0.157	0.015	0.053	-0.274	0.012	-0.016	0.133	0.175
Apicus color	0.806	0.091	-0.05	0.265	0.052	-0.251	-0.077	0.001	-0.217
Panicle type	0.803	0.115	-0.107	0.275	0.186	-0.228	0.014	-0.074	-0.121
Lemma and palea pubescence	0.797	0.301	-0.007	0.048	-0.372	-0.046	-0.077	0.036	-0.117
100 grain weight	-0.431	0.128	0.175	-0.007	0.338	-0.051	-0.089	0.1	0.122
Culm number	0.057	0.845	0.107	0.037	0.102	-0.029	0.019	-0.002	-0.047
Culm angle	0.328	0.768	-0.039	0.092	-0.019	-0.223	0.055	0.103	-0.005
Flag leaf angle	0.237	0.749	0.072	0.103	-0.185	0.209	0.089	0.072	-0.001
Collar colour	0.416	0.696	-0.178	0.186	0.057	-0.003	-0.165	0.099	-0.081
Ligule length (mm)	-0.144	0.634	0.186	0.056	0.214	0.223	0.138	-0.034	0.258
Days of heading	-0.018	0.1	-0.903	-0.051	-0.02	-0.016	0.02	-0.001	0.069
Sterile lemma color	0.055	-0.185	-0.771	-0.114	-0.124	0.003	-0.102	-0.084	0.061
Culm length (cm)	-0.212	0.104	0.744	0.095	0.197	0.095	0.344	0.138	-0.009
Leaf angle	0.162	0.091	0.516	0.22	0.201	0.047	-0.141	0.078	0.43
Leaf blade width (mm)	0.115	0.408	-0.459	0.22	-0.069	0.137	-0.209	-0.397	0.234
Leaf blade length (cm)	-0.159	0.107	0.42	0.414	0.294	0.248	-0.357	0.137	0.01
Culm strength	0.17	0.215	-0.019	0.772	-0.043	0.047	-0.013	0.139	0.084
Awn color at maturity	0.235	0.017	0.09	0.701	-0.167	0.053	0.282	-0.023	-0.253
Panicle threshability	0.166	-0.018	0.062	0.649	0.05	-0.062	-0.009	0.303	0.356
Seed coat color	0.048	0.233	0.324	0.599	0.116	-0.103	0.096	0.042	0.341
Awning after full heading	0.088	0.111	0.034	0.594	-0.084	0.218	0.537	0.111	-0.229
Seedling height (cm)	-0.043	-0.299	0.445	0.529	0.333	0.262	-0.131	-0.088	0.082
Auricle colour	-0.356	0.001	0.1	0.031	0.815	-0.05	0.156	-0.005	-0.04
Ligule color	-0.118	0.104	0.279	-0.117	0.725	0.228	0.141	0.04	0.055
Sterile lemma length	-0.07	0.005	0.266	0.136	0.059	0.817	0.079	-0.054	-0.14
Panicle axis at maturity	0.124	-0.089	0.519	-0.096	-0.051	-0.668	0.127	-0.098	0.159
Lemma and palea color	0.243	0.017	0.275	0.303	-0.038	-0.49	0.262	-0.138	-0.257
Ligule shape at late vegetative stage	-0.296	0.347	0.242	0.064	0.382	0.47	0.299	0.05	-0.072
Secondary branching	-0.178	0.127	0.121	0.089	0.239	-0.044	0.785	-0.188	-0.106
Basal leaf sheath color	0.154	-0.018	0.128	0.082	0.117	-0.019	0.709	0.432	0.106
Leaf blade color	0.05	-0.022	0.068	0.106	0.042	0.18	0.137	0.804	0.109
Leaf blade pubescent	-0.084	0.241	0.143	0.28	0.075	-0.216	-0.191	0.644	-0.259
Inter node color After Full Heading	-0.169	0.163	-0.006	0.457	-0.327	-0.006	-0.039	0.498	-0.01
Panicle exsertion	-0.33	0.039	-0.226	0.098	0.014	-0.22	-0.038	-0.106	0.754
Panicle length (cm)	0.309	0.416	-0.251	-0.041	0.293	0.01	0.149	-0.173	-0.432

Table 4. Summary of the PCA analysis of ten molecular data of weedy rice and cultivated rice varieties of different eco-climatic zones of Sri Lanka

Component	PCA1	PCA2	PCA3	PCA4	PCA5	PCA6
Eigen value	5.13	3.805	2.67	1.988	1.392	1.084
Percentage variance explain	25.648	19.025	13.348	9.938	6.962	5.418
Cumulative Percentage variance explain	25.648	44.674	58.022	67.96	74.921	80.339
RM211	0.883	-0.066	-0.064	0.237	0.125	-0.125
RM167	0.789	-0.334	0.062	0.328	-0.279	-0.017
RM332	0.683	-0.356	-0.092	0.272	0.306	-0.053
RM44	0.493	0.480	-0.051	-0.354	-0.349	0.077
RM280	-0.122	0.681	-0.488	0.204	0.045	-0.368
RM14	0.517	0.600	-0.055	-0.072	-0.099	0.316
RM205	0.298	0.237	0.806	-0.225	0.221	-0.023
RM21	-0.427	0.433	0.408	0.623	0.089	0.031
RM11	0.412	0.347	-0.310	-0.448	0.436	-0.167
RM84	-0.268	-0.107	-0.376	0.439	0.219	0.274

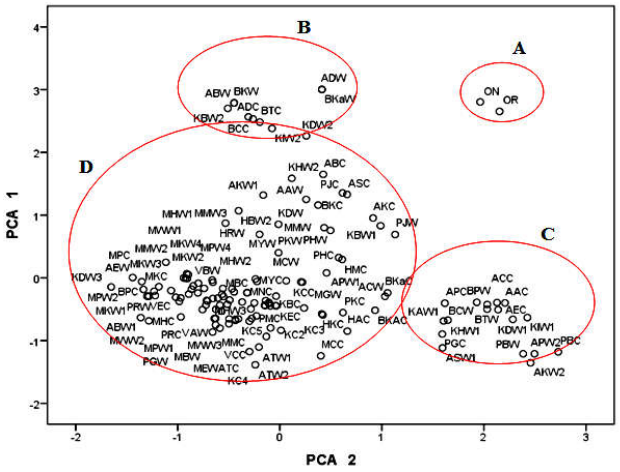

Figure 1. Biplot resulted from plotting of Principle Component scores of axis 1 and 2 from the analysis of agro-morphological data using PCA1 and PCA2. (Percent of variance explained from the PCA1 = 13.17%, Percent of variance explained from the PCA2 = 23.83%, Total Percent of variance explained = 37%)

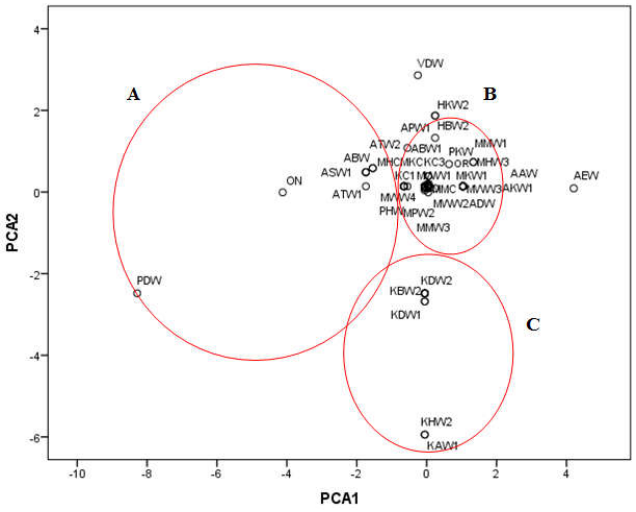

Figure 2. Biplot resulted from plotting of Principle Component scores of axis 1 and 2 from the analysis of Molecular data using PCA1 and PCA2. (Percent of variance explained from the PCA1 = 25.68%, Percent of variance explained from the PCA2 = 44.67%, Total Percent of variance explained = 70.35%)

DISCUSSION

The present results based on the PCA pattern of 10 selected SSR loci demonstrated that Sri Lankan WR populations possessed relatively high genetic diversity. In principle, a high level of genetic diversity of WR populations provides a broad genetic basis of potential adaptation to a wide range of climatic zones, which may increase the difficulty in controlling WR eco-types (Dekker, 1997; Holt and Hochberg, 1997). The results also showed that the genetic diversity of the wet zone weedy rice populations was inconsistent in distribution. Considerable variation was found across populations in dry and intermediate zones in Sri Lanka which could be attributed to different farming practices, seed sources and the number of rice varieties used in different climatic zones in Sri Lanka. WR is an autogamous species with an extremely low out-crossing rate and restricted pollen-mediated gene flow (Gealy *et al*., 2003; Chen *et al*., 2004). Low frequency of hybridization and introgression could play an important role in the long-term evolution of WR populations and in the maintenance of a certain amount of genetic diversity. Wild rice populations adjacent to rice fields had a higher genetic diversity than those at some distance from cultivated rice (Song *et al*., 2003; Cai *et al*., 2004). This indicates that introgression from cultivated rice can considerably shape genetic diversity of its wild relatives. Further, the differences in genetic diversity among WR populations might be associated with the weed management procedures. Farmers in the rice-planting regions usually remove weeds (including weedy rice) manually. Consequently, farmers pulled out the most obvious off-types of WR they encountered. This procedure might considerably reduce variation of WR if it infested rice fields for a longer period of time. After a certain period, WR individuals morphologically similar to cultivated rice were left in the fields. Selective removal by humans will tend to even up weedy rice within a population. The observed differentiation of WR populations is probably caused by limited exchange of genetic materials among WR populations because of the inbreeding nature of weedy rice with an extremely low out-crossing rate. In principle, considerable gene flow is an evolutionary force that tends to maintain genetic homogeneity among populations (Slatkin, 1987) and, in contrast, limited gene flow may promote substantial genetic differentiation among populations. WR is always surrounded by rice cultivars in fields, genetic introgression from different cultivated rice varieties through time may increase variation among WR populations. It has been shown the importance of introgression from crop species, which may have a substantial impact on differentiation and evolutionary processes in wild and weedy populations (Ellstrand *et al*., 1999; Song *et al*., 2006). The results of the present study showed that weedy rice populations in wet zone had a very close genetic relationship with *Oryza rufipogon,* a wild rice species. This is clearly reflected by the PCA of WR eco types and cultivated rice varieties in the wet zone of the country (Matara, Matale and Kandy Districts). Further, PCA showed a relatively close genetic relationship of dry zone (Anuradhapura and Puttalam Districts) WR populations with *Oryza nivara,* a wild rice species. The above finding same is supported by the findings of morphological analysis of a study carried out on weedy rice which weedy rice showed intermediate characteristics of cultivated and wild rice variety (*O. nivara)* and, combined analysis of molecular and morphological has indicated a position of two weedy rice accessions between the cultivated and wild rice (*O. nivara)* which in-tern implied the potential of the occurrence of a hybrid between wild and cultivated rice (Subasinghe *et al*., 2007).

Conclusion

Weedy rice populations in climatic zones of the Sri Lanka are considerably varied in genetic diversity which in turn indicated their potential differentiation in relation to the climatic conditions of the zone under consideration. The pattern of genetic diversity and genetic differentiation of WR populations suggest the common origin which is possibly centered around the species; *O. nivara* for dry zone WR eco-types and *O.rufipogon* for wet zone eco-types. There are a number of contributive factors affect the diversity and the distribution of WR eco-type populations in different climatic zones of the country. Among these factors the effectiveness of weed management in the particular climatic zones, limited gene flow among weedy rice eco-types populations and introgression with different rice varieties over the time. The recent changes

of farming practices and cultivation methods with application of direct seeding and seedling broadcasting technologies with less weed management may have promoted the re-emergence and genetic diversification of WR in Sri Lanka. Effective methodologies for weed control and management must be developed to prevent WR from extensive spreading and infestation across all rice planting areas in Sri Lanka.

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