FR 1708

Final Report: Grant No. RG/2011/BT/06

Section 1

1. Information regarding Project/Project Personnel:

i) Contract Number : **RG/2011/BT/06**

ii) Title of the Project:

Assessment of Genetic diversity and Tracing the origin of weedy rice populations found in rice fields in Sri Lanka

iii) Principal Investigator: **Prof. (Mrs) S. R. Weerakoon**

iv) Co- Investigators: **Dr. O.V.D.S.J. Weerasena** and **Mrs. A.S. K. Abeysekara**

v) Institute (s) where research was being carried out :

1. **The Open University of Sri Lanka, Nawala**
2. **Institute of Biochemistry, Molecular Biology and Biotechnology, University of Colombo, Colombo 03.**

vi) Date of award /date Project was initiated: 01.01.2012
Date of funds were released- 10.04.2012

vii) Date of completion of the project: 30. 06. 2015
(an extension was granted from 01.01.2015 to 30.06.2015)

viii) Total allocation of funds (Rs) : **2,671,492.00**

ix) Total spent (Rs): **2,650,767.86**

x) Number of Research Students employed : **One**

xi) Post graduate degree completed with dates :

M. Phil. thesis of Ms K.D.K. Karunarathna is at the writing up stage.

xii) Number of Technical Assistants and/or laborers employed and period of service: **None**

xiii) Publications/Communications arising from the project during the reporting period

a. Abeysekera, A. S. K., Weerakoon, S. R., Karunaratna,K. D. K. and Johnson, D. E. (2012). Morphological variation in offspring derived from single panicle of weedy rice. *Annals of Sri Lanka Department of Agriculture (ASDA)*.**14**: 1-9.

b. A.S. K. Abeysekera, S. R. Weerakoon, K.D.K. Karunarathnaand D.E Johnson (2012) Variation in themorphology of offspring derived from single panicle of weedy rice, The 6th International Weed Science Congress Proceeding: Pp. 48.

c. K.D.K. Karunarathna, S.R. Weerakoon, O.V.D.S.J. Weerasena and S. Somarathna (2013). Assessment of morphological diversity of Weedy rice (Oryza sativa f. spontanea) bio-types found in rice fields in Kurunagala District, Sri Lanka, The sixth Annual Scientific sessions, IBMBB, University of Colombo: Pp30.

d. K. D. K. Karunarathna, S. R. Weerakoon, S. Somaratne, O.V.D.S.J. Weerasena, A. S. K. Abeysekera. Phenotypic and genotypic variationin different weedy rice (*oryza sativa* f. *spontanea*) bio-type populations in matara and kurunegala districts, srilanka,The24th APWSS Conference Proceeding: Pp. 77.

e. S. Somaratne,K. D. K. Karunarathna, S. R. Weerakoon,A. S. K. Abeysekera, O.V.D.S.J Weeresena. Salient characters of Weedy rice (O*ryza sativa* f.*spontanea)* populations in highly infested areas in Sri Lanka, 1stRuhuna International Science and Technology Conference, University of Ruhuna, Matara (RITSCON 2014): Pp 20.

f. K. D. K. Karunarathna, S. R. Weerakoon, S. Somaratne, O.V.D.S.J. Weerasena (2014).Molecular and Agro-morphological affinities among weedy, wild and cultivated rice varieties in different climatic zones of Sri Lanka. OUSL Annual Academic Sessions, 2014: Pp 319-323.

g. K. D. K. Karunarathna, S. R. Weerakoon, S.Somaratne, ,O.V.D.S.J.Weeresena, A.S.K.Abeysekera (2014). Affinities among traditional rice variety "*Pachchaperumal*" and closely related weedy rice eco-types in Sri Lanka: a combine approach of molecular and agro-morphological characters.(Submitted to NSF Journal and accepted with revisions).

h. K. D. K. Karunarathna, S. Somaratne, S. R. Weerakoon, ,O.V.D.S.J.Weeresena,and A.S.K.Abeysekera. (2015). Eco-climatic dependency of weedy rice (*Oryza sativa* f. *spontaneae*) distribution in Sri Lanka. 25th APWSS 2015 Conference. Pp. 11-12.

i. K. D. K. Karunarathna, S. Somaratne, S. R. Weerakoon and O.V.D.S.J. Weerasena (2015). Diversity of weedy rice *(Oryza sativa* f. *spontanea)* populations in Sri Lanka: An application of Self Organizing Map (SOM)-Manuscript submitted to Journal of Agriculture (Cambridge) – Under reviewing

Section 2

Executive summary of the project-

Weedy rice of the family Poaceae is a weed accompanying rice and is widely distributed in rice-planting areas all over the world, particularly in South and South-east Asia, South and North America and Southern Europe (Ferreero *et al.*, 1999).Weedy rice is taxonomically classified as the same species as cultivated rice (*Oryza sativa)* but is strongly characterized by seed shattering and dormancy, which apparently increase the distribution of this species. As a notorious weed occurring commonly in rice fields, weedy rice causes significant yield reduction (Labrada, 2002) and affects the quality of rice grain (Hougland and Paul, 1978). Most weedy rice strains possess seeds with red pericarps, thus it is also referred to as red rice, although some strains have white pericarps (Gealy *et al.*, 2003). The incidence of weedy rice, a problem, has aggravated with the increase of direct-seeded rice in several countries. Morphologically weedy rice is highly variable and appears to be an intermediate between wild and cultivated rice. Long-term sympatric distribution has led to similarities between weedy and cultivated rice through natural hybridization and introgression, making the control of weedy rice very difficult when compared with other weeds (Qianjin *et al.*, 2006). Weedy rice was reported first in the Eastern Provinces of Sri Lanka in early 1990s, has now spread into many rice growing areas irrespective of the agro-ecological zones in the country (Abeysekera, 2010). Although, currently Department of Agriculture has already developed the effective controlling package and giving awareness program to the farmers, they are reluctant to remove weedy rice, because it is identical to cultivated rice in first few generations. Therefore, weedy rice problem has aggravated and reduced the cultivated rice yield. Selective herbicides cannot be used to control weedy rice during the crop growth stages due to its physiological and morphological similarity (Abeysekera *et al.*, 2010). Thus, farmers are unable to take a clean paddy harvest. Although rice is a self pollinated plant, cross pollination in rice takes place to a certain extent, Previous data reported that natural out-crossing between different rice varieties in Sri Lanka ranges between 0.34 and 0.67 %, resulted in considerable increases of weedy rice in rice fields by out-crossing wild rice (*O. nivara* and *O. rufipogan*) and cultivated rice (Chen *et al.*, 2004). Recent results (RRDI Annual reports, 2010) report the out-crossing rate ranged between 0-20 percent. Present research focuses on the level of genetic diversity of weedy rice population found in different agro-ecological zones where weedy rice problem is considerably high. The present research carried out on the agro-morphological and molecular studies in order to explore the possible origin of weedy rice by comparing the genetic relationships of weedy rice populations with a wide range of recommended-cultivated rice (*O. sativa*) varieties and wild rice (*O. rufipogon* and *O. nivara*) populations. Once the potential out crossing cultivated rice varieties are identified, it is possible to avoid the cultivation of such rice varieties in the areas of high weedy rice infestation and to lessen the weedy rice threat in Sri Lanka.

Objectives of the project

- Assessment of the level and distribution of genetic diversity of weedy rice population in Sri Lanka.
- Tracing the possible genetic origin of weedy rice found in the paddy fields in Sri Lanka.

Specific Objective/s

- Agro-morphological and molecular characterization of weedy rice genotypes and their potential parents (cultivated rice and/or wild rice) in rice fields of different agro-ecological zones/ Districts (Kurunegala, Matara, Hambantota, Putlam, Mannar, Polonnaruwa, Anuradhapura, Ampara, Kandy, Matale, Jaffna and Batticalo) in Sri Lanka.
- Identification of recommended/cultivated rice varieties (*O. sativa*) and/or wild rice varieties (*O. nivara* and *O. rufipogan* etc.) with high percentage of out crossing to be able produce weedy rice eco-types.

Methodology:

Agro-Morphological characterization

The seeds of presumed different weedy rice eco-types were collected from five different locations in each twelve different districts (Kurunegala, Matara, Hambantota, Puttalam, Mannar, Pollonnaruwa, Anuradhapura, Ampara, Kandy, Matale, Jaffna and Batticallo), cultivated rice varieties and wild rice varieties were also collected from respective rice fields in Sri Lanka. Collected seeds 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. Five replicates each with a single plant was planted in plastic pots with paddy soils. Replicates were arranged in a Complete Randomized Design (CRD). Morphological characterization using thirty (30) characters of different rice varieties were made using the Standard Characterization Catalogue (PGRC, 1999).

Molecular Characterization

Total genomic DNA was extracted from 7-day old seedlings of WR eco-types, cultivated rice varieties and wild rice varieties using Ceygen Plant total DNA purification kit. Ten SSR primer pairs were used. The primer sequences and amplification conditions for primers were obtained from http://www.gramene.org/. A four-primer system (Schuelke, 2000) was used, which included a universal M13 oligonucleotide (TGTAAAACGACGGCCAGT), labeled with one of four fluorescent dyes (6-FAM, NED, PET or VIC) (Table 1). The fluorescent dyes allowed the products to be perplexed 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 30 µl volumes of which containing 1 x PCR buffer, 1mM dNTPs, 2µM SSR primers, 2mM $MgCl_2$, 50 ng of genomic DNA and 0.5 Units of Taq polymerase. The reaction conditions were: 95 °C for 1min, followed by 30 cycles of 95 °C (30 sec), 55 °C (1 min), and 72 °C (1 min), with 10 subsequent cycles of 95 °C (30 sec), 53 °C (45 sec), and 72 °C (1 min), and a final extension at 72 °C for 10 min. The SSR alleles were resolved on an ABI Prism 3100 DNA sequencer using GeneScan 4.1 software, and sized precisely using GeneScan600LIZladder. Fragment analysis using capillary electrophoresis was performed using GENE MAPPER software and identified different peaks among weedy rice, cultivated rice varieties and wild rice varieties. Collected data were normalized (z-score) to have a zero mean and standard deviation of one (1).

Major findings:

The major findings of the study include the following.

1. There was a close affinity of weedy rice eco-types of Wet zones with wild rice variety *O. rufipogan* implying that the origin of the WR eco-types in the wet zone is centred on *O. rufipogan.*
2. Weedt rice eco-types in the Dry zone was associated with the wild rice variety *O. nivara* suggesting that *O. nivara* is the ancestor of the Dry zone weedy rice eco-types.
3. The out-crossing potential of cultivated rice is significantly vary and the cultivated rice variety Bg379-2 possesses higher potential of out-crossing with weedy rice eco-types.
4. The SSR marker RM280 is the most reliable maker (at 0.92 PIC value) out of ten SSR markers to identify and differentiate the weedy rice eco-type in Sri Lanka.

Section 3

Report in detail

Agro-Morphological characterization

The seeds of presumed different weedy rice eco-types were collected from five different locations in each twelve different districts (Kurunegala, Matara, Hambantota, Puttalam, Mannar, Pollonnaruwa, Anuradhapura, Ampara, Kandy, Matale, Jaffna and Batticallo) (Table 1), cultivated rice varieties and wild rice varieties were also collected from respective rice fields in Sri Lanka. Collected seeds 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. Five replicates each with a single plant was planted in plastic pots with paddy soils. Replicates were arranged in a Complete Randomized Design (CRD). Morphological characterization using thirty (30) characters of different weedy rice/wild rice and cultivated rice varieties were made using the Standard Characterization Catalogue (PGRC, 1999) (Table 2).

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

trict	Location	Weedy rice eco-type	Cultivated type
runagala	Kurunagala BulunahalaYaya	KBW1, KBW2	Bg 358 (KC1)
	Kumbukwawa DahampalaYaya	KDW1, KDW2, KDW3	Bg 379-2 (KC2)
	Kuliyapitiya HambalawaYaya	KHW1, KHW2	Bg 358 (KC3)
	Kurunagala Ibbagamuwa BulunwawaYaya	KIW1, KIW2	Bg 359 (KC4)
	Kurunagala Kuliyapitiya AhalaYaya	KAW1	Bg 379-2 (KC5)
tara	Matara Weligama Mudugamuwa	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)
uradhapura	Anuradhapura Kunchikulama	AKW1,AKW2	Bg 352 (AKC)
	Anuradhapura Thambuththegama	ABW1	Bg 352 (ABC)
	Anuradhapura Puliyankulama	APW1,APW2	Bg 352 (APC)
	Anuradhapura Shrawasthipura	ASW1	Bg 352 (ASC)
	Anuradhapura Thalawa	ATW1,ATW2	Bg 352 (ATC)
mbantota	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)
tticalo	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)
llonaruwa	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)
ndy	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)
uniya	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. Agro-morphological characters used for the characterization of WR eco-types, cultivated rice varieties and wild rice varieties in Sri Lanka (PGRC, 1999).

Character code	Character	Description
1	Seedling height (cm)	Recorded at the five leaf stage
2	Leaf blade length (cm)	Measured from top most leaf below the flag leaf on the main culm at late vegetative stage.
3	Leaf blade width (mm)	Measured at the widest portion of the leaf blade
4	Leaf blade pubescent	1. Glabrous 2. Intermediate 3. Pubescent
5	Leaf blade color	1. Pale green 2. Green 3. Dark green 4. Purple tips 5. Purple margins 6. Purple blotch 7. Purple
6	Basal leaf sheath color	1. Green 2. Purple lines 3. Light purple 4. Purple
7	Leaf angle	1. Erect 2. Intermediate 3. Horizontal 4. Descending
8	Flag leaf angle	1. Erect 2. Intermediate 3.Horizontal 4.Descending
9	Ligule length (mm)	Measured at late vegetative stage
10	Ligule color	0.Absent 1. White 2. Purple lines 3. Purple
11	Collar colour	1.Pale green 2. Green 3. Purple
12	Auricle colour	0.Abscent 1. Pale green 2. Purple
13	Days of heading	No. Of days from effective seeding to 50% heading
14	Culm length (cm)	From ground level to the base of the panicle
15	Culm number	Total no. Of grain bearing and non bearing tillers
16	Culm angle	1.Erect 3.Intermediate 5.Open 7.Spreading 9.Procumbent
17	Inter node color After Full Heading	1.Green 2.Light gold 3.Purple lines 4.Purple
18	Culm strength	1.Strong 3.Moderately strong 5.Intermediate 7.Weak 9.Very week
19	Panicle length (cm)	From the base to the tip of the panicle
20	Panicle type	1. Compact 5.Intermediate 9.Open
21	Secondary branching	0.Abscent 1.Light 2.Heavy 3.Clustering
22	Panicle exsertion	0.Well exsertion 3.Moderately 5.Justexterted 7.Partlyexserted 9. Enclosed
23	Awning after full heading	0. Absent 1.Short and partly awned 5.Short and fully awned 7.Long and partly awned 9. Long and fully awned
24	Apicus color	1.White 2.Straw 3. Brown 4.Red 5.Red apex 6.Purple 7.Purple apex
25	Lemma and palea color	0.Straw 2.Gold 3.Brown spot on straw 4.Brown 5.Reddish to light purple 6.Purple spots on straw 7.Purple 8.Black 9.White
26	Lemma and palea pubescence	1.Glabrous 2.Hairs on lemma keel 3. Hairs on upper portion 4.Short hairs 5.Long hairs
27	Sterile lemma color	1.Straw 2.Gold 3.Red 4.Purple
28	Sterile lemma length	1.Short 3. Medium 5. Long 7. Extra long 9. Asymmetrical
29	100 grain weight	A random sample of 100 well developed grains dried 13% moisture content
30	Seed coat color	1.White 2. Light brown 3. Speckled brown 4. Brown 5. Red 6. Variable purple 7. Purple

Molecular Characterization

Total genomic DNA was extracted from 7-day old seedlings of WR eco-types, cultivated rice varieties and wild rice varieties using Ceygen Plant total DNA purification kit.

DNA extraction from WR/Wild and Cultivated rice leaves

The DNA extraction was carried out by DNA extraction kit from the CEYGEN BIOTECH (PhytoSpin™ Plant Genomic DNA extraction kit) and followed their extraction protocol;

Extraction Protocol

1. Added 400µl grind and lysis buffer for each sample (50-250 mg) of plant material.
2. Disrupted the plant material.
3. Incubate at 65°C for 10 min, mixing the tube by inversion every 2-3 min.
4. Added 130 µl of precipitation buffer (PB), invert several times and incubate on ice for 5 min.
5. Centrifuge at maximum speed for 10min in a Microcentrifuge.
6. Transfer the supernatent to sterile 1.5ml centrifuge tube. Avoid transfer of any precipitate.
7. Added 1.5 volumes binding buffer (BB) (If 400 µl is recovered from previous steps, added 600µl binding buffer.)
8. Applied 650µl of mixture to a phytospin column and centrifuge for 1-10 mins. Until all solution has passed through the column.
9. Repeated above step with the remaining volume.
10. Washed the Phytospin column by adding 500µl of Wash buffer (WB) to the column and centrifuge (2-8 mins.) until all liquid has passed to the collection tube and discard the flow through.
11. Wash the Phytospin column by adding 500µl of 70% Ethanol to the column and centrifuge (2-8 mins.) until all liquid has passed to the collection tube and discard the flow through.
12. Place the Phytospin column in sterile 1.5ml Centrifuge tube.
13. Added 50-100µl preheated (65°C) Elution buffer (EB) or sterile water to each tube. Let stand for 1-5 mins. and then centrifuge for 1-2 min at maximum speed to elute the DNA.

Quantification of DNA

The agarose gel electrophoresis was done for the DNA samples and concentration markers. The concentrations of the DNA were determined by comparing band intensities with known concentration markers. (Figure 1)

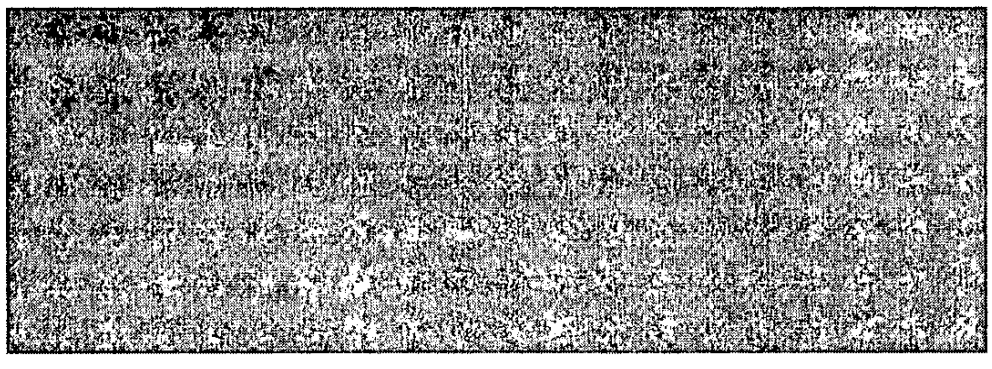

Figure 1. Genomic DNA from the rice leaves

Designing of PCR primers

Ten SSR primer pairs (M13 labeled) were design for the molecular characterization (Table 3). The primer sequences and amplification conditions for primers were obtained from http://www.gramene.org/. A four-primer system (Schuelke, 2000) was used, which included a universal M13 oligonucleotide (TGTAAAACGACGGCCAGT), labeled with one of four fluorescent dyes (6-FAM, NED, PET or VIC) (Table 4). The fluorescent dyes allowed the products to be perplexed during electrophoresis; a special forward primer composed of a concentration of the M13 oligonucleotide; and the pig tail reverse primer for SSR PCR amplification.

Table 3. 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
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 4. Four labeled primers used for the Capillary electrophoresis

Oligo name	Oligo sequence (5'-3')	Color of the Label Primer
5'- FAM- M13 (-21)	5'(FAM) TGT AAA ACG ACG GCC AGT 3'	Blue
5'- NED- M13 (-21)	5'(NED) TGT AAA ACG ACG GCC AGT 3'	Yellow
5'- PET- M13 (-21)	5'(PET) TGT AAA ACG ACG GCC AGT 3'	Red
5'- VIC- M13 (-21)	5'(VIC) TGT AAA ACG ACG GCC AGT 3'	Green

Primer reconstitution

Lyophilized primers were reconstituted in PCR grade water to 500 µM concentration and appropriate dilutions were made.

Precence nmoles of tube = x nmol
Needed concentration of Primer stock = 500 µM
Needed volume of TE buffer/ water to dissolve 25 nmoles to prepare 500 µM Primer stock is V_0;

$$\frac{\text{x nmol}}{V_0 \mu l} = \frac{500\ \mu\text{moles}}{1\text{L}}$$

$$\frac{\text{x nmol}}{V_0\ \mu l} = \frac{500 \times 10^3 \text{nmoles}}{1 \times 10^6\ \mu l}$$

$$V_0 = 2x\ \mu l$$

PCR reaction with Genomic DNA

The PCR was carried out using genomic DNA which extracted from weedy rice, wild rice and cultivated rice leaves. The components of the PCR reaction are shown in Table 5.

Table 5. Used PCR reagents/ Concentrations/ Volumes for the PCR optimization

Reagents	Required concentration	Stock concentration	Stock volume (µl)
PCR buffer	1x	10x	2.5
$Mgcl_2$	1.5mM	25mM	1.5
Ultra-Pure water	-	-	3.3
dNTP mix	1mM	10mM	2.5
Forward primer	10µM	50 µM	5.0
Reverse Primer	10µM	50 µM	5.0
Taq DNA polymerase	1U	5U	0.5
DNA	50ng	10ng	5.0

The reaction conditions were: 95 °C for 1min, followed by 30 cycles of 95 °C (30 sec), 55 °C (1 min), and 72 °C (1 min), with 10 subsequent cycles of 95 °C (30 sec), 53 °C (45 sec), and 72 °C (1 min), and a final extension at 72 °C for 10 min (Table 6,7).

Table 6. Thermocyclerprogramme for 1st PCR Cycles

Initial denaturation temperature	95°C	1min
Denaturation temperature	95°C	30S
Annealing temperature	55°C	1min
Extension temperature	72°C	1min
Cool	4°C	

Number of cycles 30

Table 7. Thermocyclerprogramme for 2nd PCR Cycles with Labeled Primers

Initial denaturation temperature	95°C	1min
Denaturation temperature	95°C	30S
Annealing temperature	53°C	45S
Extension temperature	72°C	1min
Final extention	72°C	10 min
Cool	15°C	

Number of cycles 10

A fluorescent labeled DNA size strand LIZ 600 with known fragments size was mixed with each 2.5µl amplified PCR products and 5µl of deionized formamide loading solution and 2.5µl of ultra-pure water. To get a good signal the PCR product with formamide and the size standard were mixed properly by quick vortexing. Then the samples were loaded in 96 well plates (Axygen Biosciences, USA). Samples were denatured 95°C for 2 min. chilled immediately by well plate on ice. The denatured amplified products were separated through ABI Prism 3100 DNA sequencer using GeneScan 4.1 software, and sized precisely using GeneScan600LIZladder. Electrophoresis parameters were used as 3 kv of sample injection voltage for 45 sec., 10kv of running voltage for 75 min. at 44°C. The method was repeated again for the reliability. Different peaks among weedy rice, cultivated rice varieties and wild rice varieties were identified. An electrophorogram resulted is given in Figure 2. Collected data were normalized (z-score) to have a zero mean and standard deviation of one (1).

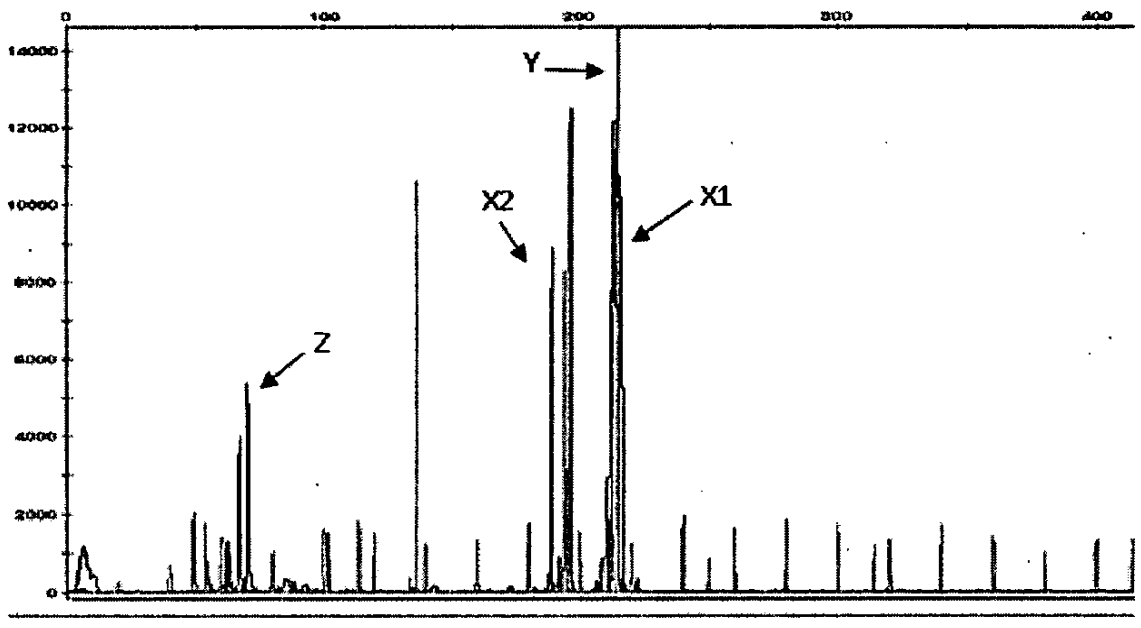

Figure 2. Electrophorogram of Wild (Z), Weedy (X1, X2) and Cultivated rice variety (Y) in Intermediate Zone (IL1) of Sri Lanka

Statistical analyses

The calculation of mean and standard deviation and the multiple range tests were carried out on the agro-morphological characters of rice varieties. Reduction of variables (scalar) was performed to identify the most important variables. A total of eight variables were chosen from the dataset (8 out of 30 variables).

The principle component analysis was carried out on the agro-morphological molecular data using SPSS PC Ver. 20 (2010). The phylogenic analysis of molecular data was performed using GenesScan 4.0.

Results/outputs:

Agro-Morphological characterization:

The summary of the variation in the measurable agro-morphological characters across different weedy rice (WR) eco-types indicated that the variation of these characters is of limited importance in separation of weedy rice eco-types in different eco-climatic zones of the country (Table 8).

Table 8. The variation of measurable agro-morphological characters across different WR eco-types in different eco-climatic zones in Sri Lanka.(Sdh - seedling height; LBL-leaf blade length; LBW-leaf blade width; CulmL- culm Length ;PanicleL-panicle Length; DaysofH- Days of heading; Gw100- 100 grain weigth; LiguleL- Ligule length)

Colloection Sites	Ecotype	Sdlh	LBL	LBW	PanicleL	Gw100	LiguleL	DaysofH	CulmL
Ampara	AAC	31.16(0.97)	58.8(0.98)	9.70(0.37)	26.40(0.76)	2.0(0.09)	18.00(0.71)	78.40(1.40)	115.8(0.86)
	AAW	23.30(0.77)	37.6(0.75)	11.0(0.35)	26.90(0.58)	2.76(0.03)	10.10(0.71)	99.00(1.23)	97.10(0.78)
	ABC	26.46(0.74)	49.20(1.16)	12.70(0.89)	21.06(1.23)	1.80(0.03)	8.70(0.58)	78.60(0.75)	103.20(2.42)
	ABW	31.52(1.07)	52.60(2.82)	8.30(0.44)	18.20(0.81)	2.17(0.08)	5.40(0.68)	81.20(3.43)	105.70(2.26)
	ACC	32.40(0.86)	58.30(0.54)	9.30(0.34)	25.50(1.17)	2.68(0.06)	17.40(0.93)	79.80(1.72)	116.20(0.20)
	ACW	26.98(0.69)	40.00(0.71)	10.80(0.34)	27.40(0.97)	2.69(0.05)	15.70(1.37)	79.20(1.66)	96.30(1.04)
	ADC	26.20(0.86)	49.72(0.86)	11.30(0.37)	21.40(1.13)	1.89(0.04)	13.60(1.18)	79.80(2.15)	96.80(0.86)
	ADW	33.26(1.18)	51.20(1.77)	7.60(0.51)	24.12(2.35)	2.16(0.09)	9.70(0.49)	77.60(1.54)	108.50(3.06)
	AEC	30.10(0.40)	58.60(0.80)	11.70(0.37)	26.90(0.75)	2.70(0.07)	15.80(1.39)	83.40(2.80)	116.60(0.66)
	AEW	25.66(0.94)	32.90(1.23)	10.70(0.25)	15.06(0.78)	1.89(0.04)	0.00(0.00)	93.20(1.66)	93.76(3.24)
Anuradhapura	ABC	17.50(0.22)	35.40(1.03)	8.26(0.12)	12.62(1.66)	2.17(0.09)	9.70(0.49)	77.60(1.54)	102.44(2.46)
	ABW1	21.50(0.35)	39.20(3.15)	10.38(0.26)	16.64(0.74)	1.89(0.04)	0.00(0.00)	93.20(1.66)	93.96(3.33)
	AKC	17.94(0.12)	36.50(1.24)	8.30(0.09)	13.72(1.15)	2.68(0.05)	10.10(0.71)	80.60(2.29)	114.80(0.85)
	AKW1	28.10(7.23)	46.50(1.20)	9.10(0.19)	18.80(1.08)	2.17(0.08)	5.40(0.68)	78.00(1.23)	106.10(2.26)
	AKW2	22.70(0.54)	51.90(1.29)	10.24(0.11)	16.54(1.19)	2.68(0.03)	15.70(1.37)	79.20(1.66)	118.90(0.80)
	APC	18.30(0.20)	37.80(0.58)	8.10(0.10)	18.82(0.45)	2.68(0.08)	18.00(0.71)	78.40(1.40)	115.80(0.86)
	APW1	21.40(0.37)	41.20(2.89)	9.40(0.19)	16.24(1.91)	1.79(0.03)	8.70(0.58)	78.60(0.75)	103.20(2.42)
	APW2	22.00(0.35)	53.80(1.98)	10.62(0.19)	15.00(2.02)	2.67(0.06)	17.40(0.93)	79.80(1.72)	116.20(0.20)
	ASC	18.20(0.20)	36.00(0.84)	8.20(0.12)	16.50(2.42)	1.89(0.04)	13.60(1.18)	79.80(2.15)	112.20(4.07)
	ASW1	21.20(0.34)	49.20(1.96)	10.70(0.25)	12.76(1.04)	2.71(0.07)	15.80(1.39)	83.40(2.80)	116.60(0.66)
	ATC	18.70(0.20)	37.20(0.86)	8.20(0.12)	13.46(1.35)	2.53(0.11)	11.80(0.46)	96.00(4.05)	100.64(0.87)
	ATW1	21.90(0.29)	44.00(1.14)	10.10(0.19)	14.80(1.36)	2.28(0.05)	14.50(0.55)	93.60(1.50)	97.60(1.24)
	ATW2	22.70(0.20)	48.20(2.35)	10.90(0.19)	10.12(0.79)	2.67(0.07)	14.50(2.52)	100.00(0.71)	101.58(0.57)
Batticalo	BCC	26.20(0.86)	49.72(0.86)	11.30(0.37)	21.40(1.13)	1.89(0.04)	13.60(1.18)	79.80(2.15)	96.80(0.86)

	BCW	30.10(0.40)	58.60(0.80)	11.70(0.37)	26.90(0.75)	2.70(0.07)	15.80(1.39)	83.40(2.80)	116.60(0.66)
	BKaC	27.23(0.84)	40.00(0.91)	10.50(0.20)	28.13(0.83)	2.66(0.05)	15.13(1.61)	78.00(1.47)	96.38(1.34)
	BKAC	26.00(.)	40.00(.)	12.00(.)	24.50(.)	2.80(.)	18.00(.)	84.00(.)	96.00(.)
	BKaW	3.26(1.18)	51.20(1.77)	7.60(0.51)	24.12(2.35)	2.16(0.09)	9.70(0.49)	77.60(1.54)	108.50(3.06)
	BKC	23.30(0.77)	37.60(0.75)	11.00(0.35)	26.90(0.58)	2.76(0.03)	10.10(0.71)	99.00(1.23)	97.10(0.78)
	BKW	31.52(1.07)	52.60(2.82)	8.30(0.44)	18.20(0.81)	2.17(0.08)	5.40(0.68)	81.20(3.43)	105.70(2.26)
	BPC	25.66(0.94)	32.90(1.23)	10.70(0.25)	15.06(0.78)	1.89(0.04)	0.00(0.00)	93.20(1.66)	93.76(3.24)
	BPW	31.16(0.97)	58.80(0.98)	9.70(0.37)	26.40(0.76)	2.76(0.09)	18.00(0.71)	78.40(1.40)	115.80(0.86)
	BTC	26.46(0.74)	49.20(1.16)	12.70(0.89)	21.06(1.23)	1.80(0.03)	8.70(0.58)	78.60(0.75)	103.20(2.42)
	BTW	32.40(0.86)	58.30(0.54)	9.30(0.34)	25.50(1.17)	2.68(0.06)	17.40(0.93)	79.80(1.72)	116.20(0.20)
Hambantota	HAC	24.36(1.17)	58.60(0.80)	7.60(1.36)	13.72(1.15)	2.80(0.06)	15.60(1.33)	74.40(1.36)	116.20(0.44)
	HAW	31.52(1.07)	46.80(1.46)	7.20(1.39)	13.00(1.14)	2.89(0.22)	12.00(1.05)	81.00(1.14)	118.20(1.28)
	HBC	23.90(0.43)	44.70(2.31)	10.20(1.24)	17.72(1.56)	2.37(0.25)	14.10(1.82)	78.40(1.44)	117.80(0.87)
	HBW1	24.66(0.85)	48.72(1.75)	4.00(0.32)	17.20(1.40)	2.63(0.04)	13.40(2.50)	74.40(1.36)	117.20(0.58)
	HBW2	30.40(0.93)	52.86(1.59)	3.00(0.32)	18.80(1.08)	2.39(0.13)	15.40(1.21)	75.20(0.86)	115.40(0.98)
	HKC	24.80(1.03)	40.00(0.71)	7.00(1.22)	18.20(0.85)	2.51(0.12)	18.00(0.71)	74.40(1.17)	116.30(0.66)
	HKW1	27.30(1.43)	45.60(1.94)	5.00(0.63)	10.22(0.79)	2.72(0.06)	9.80(1.24)	77.00(1.05)	115.60(1.21)
	HKW2	32.96(1.43)	45.70(1.27)	3.80(0.20)	13.12(1.38)	2.69(0.03)	12.00(1.05)	81.00(1.14)	118.20(1.28)
	HMC	19.00(0.65)	40.86(1.12)	8.80(0.97)	14.64(1.55)	2.13(0.21)	12.20(2.63)	78.60(0.68)	116.50(0.32)
	HMW	25.40(1.12)	53.80(4.55)	2.80(0.58)	16.82(1.39)	2.61(0.06)	14.80(1.16)	78.20(1.16)	117.20(0.58)
	HRW	23.25(0.43)	50.87(0.86)	4.00(0.39)	16.54(1.74)	2.66(0.05)	11.90(1.27)	80.30(1.69)	115.65(0.54)
Kandy	KAC	30.60(2.46)	46.42(3.32)	12.60(1.21)	11.76(0.99)	2.77(0.06)	13.60(0.81)	79.40(2.40)	114.70(1.93)
	KAW	24.90(2.60)	48.72(1.75)	4.80(0.58)	12.56(1.43)	2.67(0.09)	10.20(1.98)	77.20(0.74)	117.20(0.64)
	KBC	29.40(2.73)	50.86(0.42)	5.80(0.20)	10.26(0.79)	2.86(0.22)	8.80(2.48)	77.00(1.05)	117.40(1.30)
	KBW	24.60(3.94)	54.32(4.05)	4.20(0.97)	14.62(1.34)	2.79(0.06)	14.20(2.35)	71.60(1.21)	116.70(0.77)
	KCC	22.30(0.37)	34.74(1.41)	3.80(0.20)	16.02(1.19)	2.57(0.15)	5.40(0.40)	77.00(1.58)	115.10(1.35)
	KCW	27.80(3.30)	34.90(1.83)	4.60(0.51)	19.02(0.72)	2.50(0.12)	12.20(1.39)	77.80(2.40)	115.50(0.42)
	KDC	24.12(1.90)	44.34(2.43)	4.00(0.45)	20.02(0.58)	2.28(0.22)	7.00(0.32)	74.60(1.33)	116.00(0.42)
	KDW	28.60(1.63)	44.00(3.81)	10.40(1.69)	14.96(2.15)	1.94(0.03)	12.20(2.11)	81.00(1.95)	116.30(0.58)
	KEC	22.90(1.76)	48.50(4.54)	7.40(2.16)	14.40(1.29)	2.27(0.26)	12.00(1.87)	78.40(4.35)	115.90(0.19)
	KEW	29.80(0.58)	56.22(2.32)	8.60(0.93)	12.76(1.04)	2.71(0.07)	15.80(1.39)	83.40(2.80)	116.30(0.26)
Kurunegala	KAW1	28.60(0.51)	58.40(0.90)	11.70(0.37)	26.90(0.75)	2.72(0.08)	15.80(1.39)	83.40(2.80)	116.60(0.66)
	KBW1	29.20(0.46)	61.50(0.50)	10.80(0.46)	26.90(0.58)	2.69(0.06)	10.10(0.71)	80.60(2.29)	114.80(0.85)
	KBW2	23.66(0.79)	44.90(2.26)	7.80(0.51)	18.20(0.81)	2.35(0.23)	5.40(0.68)	78.00(1.23)	106.10(2.26)
	KC1	17.60(0.53)	39.44(0.83)	11.20(0.68)	22.24(0.86)	2.53(0.11)	11.80(0.46)	96.00(4.05)	100.64(0.87)
	KC2	17.20(0.66)	39.50(0.82)	10.60(0.70)	21.52(0.57)	2.28(0.05)	14.50(0.55)	93.60(1.50)	97.60(1.24)
	KC3	16.70(0.44)	34.58(1.37)	10.40(0.58)	24.88(0.48)	2.67(0.07)	14.50(2.52)	100.00(0.71)	101.58(0.57)
	KC4	18.96(0.38)	37.76(1.34)	10.26(0.24)	24.46(1.54)	2.51(0.12)	7.00(0.89)	97.00(1.23)	104.92(1.60)
	KC5	17.30(0.54)	36.20(0.98)	10.60(0.70)	22.24(0.89)	2.30(0.14)	12.10(0.68)	90.00(2.26)	97.36(1.42)
	KDW1	28.90(0.37)	59.20(0.82)	11.00(0.35)	27.40(0.97)	2.69(0.03)	15.70(1.37)	79.20(1.66)	118.90(0.80)
	KDW2	27.62(0.73)	45.82(3.67)	6.80(0.37)	24.12(2.35)	2.24(0.12)	9.70(0.49)	77.60(1.54)	102.44(2.46)
	KDW3	17.00(1.19)	32.90(1.23)	6.00(0.71)	15.06(0.78)	1.89(0.04)	0.00(0.00)	93.20(1.66)	93.96(3.33)
	KHW1	27.90(0.29)	59.10(1.11)	11.90(0.68)	26.40(0.76)	2.68(0.08)	18.00(0.71)	78.40(1.40)	115.80(0.86)
	KHW2	25.86(2.08)	53.40(2.84)	12.80(1.16)	21.06(1.23)	1.81(0.04)	8.70(0.58)	78.60(0.75)	103.20(2.42)
	KIW1	27.90(0.10)	58.20(0.51)	11.50(0.47)	25.50(1.17)	2.66(0.06)	17.40(0.93)	79.80(1.72)	116.20(0.20)
	KIW2	23.20(0.81)	49.72(0.86)	9.70(0.93)	21.40(1.13)	1.89(0.04)	13.60(1.18)	79.80(2.15)	112.20(4.07)
Mannar	MAC	23.20(0.86)	52.56(3.56)	3.60(0.40)	18.28(0.99)	2.62(0.16)	14.80(2.15)	68.60(1.03)	116.40(0.40)
	MAW	34.82(1.53)	48.72(1.75)	9.80(1.46)	11.76(0.99)	2.77(0.06)	12.60(1.03)	79.60(1.60)	114.60(0.75)
	MBC	26.00(0.71)	55.96(3.57)	5.20(0.86)	18.04(1.31)	1.80(0.07)	11.40(1.63)	76.20(2.85)	115.40(0.40)
	MBW	41.30(1.52)	58.92(1.74)	11.00(1.00)	10.26(0.79)	2.86(0.22)	9.20(0.86)	78.40(1.29)	119.20(0.86)

	MCC	23.50(2.01)	45.44(2.53)	3.60(0.51)	16.84(2.06)	5.71(3.32)	12.40(2.04)	78.40(1.29)	117.60(0.93)
	MCW	32.66(0.79)	54.80(2.31)	8.60(0.93)	13.72(1.15)	2.80(0.06)	15.60(1.33)	74.40(1.36)	116.80(0.49)
	MDC	27.20(3.81)	41.32(1.12)	5.00(0.89)	20.82(0.47)	1.54(0.10)	11.60(1.81)	74.40(1.36)	117.00(0.63)
	MDW	31.40(1.78)	47.46(2.79)	13.00(0.95)	18.80(1.08)	2.48(0.11)	14.60(1.54)	74.00(0.95)	114.40(1.47)
	MEC	24.90(1.62)	41.90(1.84)	6.00(0.84)	20.42(1.91)	1.81(0.29)	10.00(0.71)	74.00(0.95)	116.60(0.40)
	MEW	37.80(3.49)	51.76(1.08)	6.60(0.60)	16.54(1.19)	2.56(0.14)	5.60(1.17)	74.00(1.14)	116.20(0.49)
Matale	MGC	22.30(0.37)	34.74(1.41)	3.80(0.20)	16.02(1.19)	2.57(0.15)	5.40(0.40)	77.00(1.58)	115.10(1.35)
	MGW	29.40(2.06)	55.80(3.94)	10.80(1.93)	13.60(1.03)	2.70(0.06)	15.80(1.39)	84.40(3.03)	114.20(1.81)
	MMC	24.60(3.94)	54.32(4.05)	4.20(0.97)	14.62(1.34)	2.79(0.06)	14.20(2.35)	71.60(1.21)	116.70(0.77)
	MMW	34.40(1.12)	52.02(1.38)	10.80(0.97)	18.30(2.01)	2.35(0.22)	15.80(1.16)	79.80(1.74)	116.30(0.60)
	MNC	24.12(1.90)	44.34(2.43)	4.00(0.45)	20.02(0.58)	2.28(0.22)	7.00(0.32)	74.60(1.33)	116.00(0.42)
	MNW	32.26(1.40)	46.74(1.60)	9.00(1.38)	9.86(0.64)	2.93(0.20)	12.80(2.35)	76.80(0.80)	116.60(1.20)
	MWC	21.30(0.89)	41.32(1.12)	4.00(0.32)	16.60(1.08)	2.10(0.19)	9.60(0.51)	71.80(0.58)	116.20(0.20)
	MWW	24.90(2.60)	48.72(1.75)	4.80(0.58)	12.56(1.43)	2.67(0.09)	10.20(1.98)	77.20(0.74)	117.20(0.64)
	MYC	27.80(3.30)	34.90(1.83)	4.60(0.51)	19.02(0.72)	2.50(0.12)	12.20(1.39)	77.80(2.40)	115.50(0.42)
	MYW	29.60(0.68)	48.10(1.63)	11.20(0.86)	12.96(1.74)	2.29(0.22)	12.80(0.82)	81.20(2.13)	116.70(0.37)
Matara	MHC	26.76(1.70)	33.42(1.00)	9.40(0.81)	21.70(2.07)	2.57(0.09)	3.00(0.32)	84.00(1.70)	116.00(0.35)
	MHW1	40.00(1.88)	52.32(3.34)	10.80(0.97)	10.12(0.79)	2.89(0.22)	11.40(2.84)	76.40(0.87)	115.50(2.21)
	MHW2	35.06(1.99)	59.62(1.24)	11.20(0.86)	12.42(1.44)	2.74(0.03)	12.80(1.98)	77.00(0.89)	116.60(1.13)
	MHW3	26.66(3.69)	46.70(1.51)	10.80(1.93)	15.90(1.23)	2.66(0.16)	12.00(2.49)	71.20(1.07)	116.00(0.52)
	MHW4	36.80(4.06)	51.96(2.29)	9.00(1.38)	18.12(1.34)	2.48(0.13)	11.80(1.77)	79.80(1.99)	116.20(0.82)
	MKC	28.60(2.83)	42.70(1.96)	6.80(0.37)	19.42(1.43)	1.55(0.24)	3.60(0.81)	81.60(0.93)	115.80(0.46)
	MKW1	46.26(3.16)	60.44(0.48)	4.00(0.32)	16.64(0.74)	2.58(0.09)	5.60(1.17)	74.00(1.14)	116.00(0.42)
	MKW2	40.18(5.52)	43.20(2.39)	3.00(0.32)	18.82(0.45)	2.50(0.15)	14.80(2.15)	68.60(1.03)	116.50(0.32)
	MKW3	23.44(4.55)	50.42(1.03)	2.80(0.58)	16.24(1.91)	2.55(0.11)	11.40(1.63)	76.20(2.85)	115.60(0.40)
	MKW4	27.70(1.02)	49.26(0.91)	3.00(0.32)	15.00(2.02)	2.73(0.09)	12.40(2.04)	78.40(1.29)	117.70(0.85)
	MMC	23.76(2.03)	40.08(0.35)	8.60(0.51)	20.12(1.83)	1.91(0.12)	2.60(0.60)	84.20(1.16)	116.60(0.81)
	MMW1	31.10(1.31)	48.56(1.33)	4.80(0.58)	16.88(1.03)	2.77(0.08)	5.80(0.49)	76.20(1.07)	114.70(1.22)
	MMW2	36.76(1.72)	61.36(0.53)	4.20(0.97)	19.26(1.32)	2.02(0.15)	7.20(0.37)	73.60(1.03)	116.40(0.48)
	MMW3	29.10(2.53)	44.40(2.16)	4.60(0.51)	17.54(1.67)	2.11(0.19)	10.60(0.40)	75.20(1.07)	116.00(0.22)
	MPC	23.56(2.74)	36.30(1.87)	4.00(0.32)	22.88(1.03)	2.31(0.23)	3.20(0.49)	82.60(0.75)	116.30(0.83)
	MPW1	37.18(2.24)	51.66(1.72)	7.60(1.36)	16.50(2.42)	2.75(0.05)	11.60(1.81)	74.40(1.36)	117.20(0.49)
	MPW2	35.84(3.49)	49.10(0.64)	7.00(1.22)	12.76(1.04)	2.69(0.09)	10.00(0.71)	74.00(0.95)	116.50(0.47)
	MPW3	36.94(2.88)	42.38(1.22)	10.20(1.24)	13.46(1.35)	2.52(0.11)	10.60(1.03)	77.60(1.69)	116.20(0.56)
	MPW4	37.42(2.58)	50.86(0.42)	8.80(0.97)	14.80(1.36)	2.57(0.11)	12.40(0.51)	84.40(3.03)	116.20(0.26)
	MWC	20.80(2.13)	44.70(2.31)	3.80(0.92)	18.30(1.61)	1.86(0.19)	2.40(0.24)	81.80(2.58)	116.20(0.44)
	MWW1	35.00(2.16)	55.32(3.44)	5.00(0.32)	15.20(1.93)	2.69(0.06)	12.60(1.03)	79.60(1.60)	114.80(0.85)
	MWW2	38.90(3.77)	52.02(1.38)	7.20(1.39)	18.30(1.71)	2.69(0.03)	9.20(0.86)	78.40(1.29)	118.90(0.80)
	MWW3	40.50(0.89)	48.10(1.63)	5.00(0.63)	13.42(1.52)	2.56(0.07)	15.60(1.33)	74.40(1.36)	116.90(0.37)
	MWW4	26.30(4.41)	59.76(0.59)	3.80(0.20)	12.62(1.66)	2.50(0.06)	14.60(1.54)	74.00(0.95)	114.40(1.37)
Polonnaruwa	PBC	22.00(0.35)	53.80(1.98)	10.62(0.19)	15.00(2.02)	2.67(0.06)	17.40(0.93)	79.80(1.72)	116.20(0.20)
	PBW	22.70(0.54)	48.20(2.35)	10.24(0.11)	12.18(2.13)	2.67(0.07)	14.50(2.52)	100.00(0.71)	101.58(0.57)
	PGC	21.20(0.34)	49.20(1.96)	10.70(0.25)	12.76(1.04)	2.71(0.07)	15.80(1.39)	83.40(2.80)	116.60(0.66)
	PGW	21.50(0.35)	39.20(3.15)	10.38(0.26)	16.64(0.74)	1.89(0.04)	0.00(0.00)	93.20(1.66)	93.96(3.33)
	PHC	21.40(0.37)	41.20(2.89)	9.40(0.19)	16.24(1.91)	1.79(0.03)	8.70(0.58)	78.60(0.75)	103.20(2.42)
	PHW	22.10(1.27)	44.00(1.14)	10.10(0.19)	14.80(1.36)	2.2(0.05)	14.50(0.55)	93.60(1.50)	97.60(1.24)
	PJC	18.20(0.20)	36.00(0.84)	8.20(0.12)	16.50(2.42)	1.89(0.04)	13.60(1.18)	79.80(2.15)	112.20(4.07)
	PJW	17.50(0.22)	35.40(1.03)	8.26(0.12)	12.62(1.66)	2.17(0.09)	9.70(0.49)	77.60(1.54)	102.44(2.46)
	PKC	18.30(0.20)	37.80(0.58)	8.10(0.10)	18.82(0.45)	2.68(0.08)	18.00(0.71)	78.40(1.40)	115.80(0.86)
	PKW	17.94(0.12)	37.20(0.86)	8.20(0.12)	13.46(1.35)	2.53(0.11)	11.80(0.46)	96.00(4.05)	100.64(0.87)
Puttalam	PAC	37.18(2.24)	51.66(1.72)	7.60(1.36)	16.50(2.42)	2.75(0.05)	11.60(1.81)	74.40(1.36)	117.00(0.63)
	PAW1	40.50(0.89)	48.10(1.63)	5.00(0.63)	13.42(1.52)	2.56(0.07)	15.60(1.33)	74.40(1.36)	116.80(0.49)

	PAW2	26.30(4.41)	59.76(0.59)	3.80(0.20)	12.62(1.66)	2.50(0.06)	14.60(1.54)	74.00(0.95)	114.40(1.47)
	PDC	23.44(4.55)	50.42(1.03)	2.80(0.58)	16.24(1.91)	2.55(0.11)	11.40(1.63)	76.20(2.85)	115.40(0.40)
	PDW	35.00(2.16)	55.32(3.44)	5.00(0.32)	15.20(1.93)	2.69(0.06)	12.60(1.03)	79.60(1.60)	114.60(0.75)
	PKC	36.94(2.88)	42.38(1.22)	10.20(1.24)	13.46(1.35)	2.52(0.11)	10.60(1.03)	77.60(1.69)	116.00(0.63)
	PKW	40.18(5.52)	43.20(2.39)	3.00(0.32)	18.82(0.45)	2.50(0.15)	14.80(2.15)	68.60(1.03)	116.40(0.40)
	PMC	27.70(1.02)	49.26(0.91)	3.00(0.32)	15.00(2.02)	2.73(0.09)	12.40(2.04)	78.40(1.29)	117.60(0.93)
	PMW	38.90(3.77)	52.02(1.38)	7.20(1.39)	18.30(1.71)	2.69(0.03)	9.20(0.86)	78.40(1.29)	119.20(0.86)
	PRC	35.84(3.49)	49.10(0.64)	7.00(1.22)	12.76(1.04)	2.69(0.09)	10.00(0.71)	74.00(0.95)	116.60(0.40)
	PRW	46.26(3.16)	60.44(0.48)	4.00(0.32)	16.64(0.74)	2.58(0.09)	5.60(1.17)	74.00(1.14)	116.20(0.49)
Vauniya	VAC	23.20(0.86)	52.56(3.56)	3.60(0.40)	18.28(0.99)	2.62(0.16)	6.60(0.40)	74.60(1.40)	115.00(1.34)
	VAW	34.82(1.53)	48.72(1.75)	9.80(1.46)	11.76(0.99)	2.77(0.06)	13.60(0.81)	79.40(2.40)	114.40(1.86)
	VBC	26.00(0.71)	55.96(3.57)	5.20(0.86)	18.04(1.31)	1.80(0.07)	8.40(0.75)	73.80(0.86)	116.20(0.49)
	VBW	41.30(1.52)	58.92(1.74)	11.00(1.00)	10.26(0.79)	2.86(0.22)	8.80(2.48)	77.00(1.05)	116.80(1.39)
	VCC	23.50(2.01)	45.44(2.53)	3.60(0.51)	16.84(2.06)	5.71(3.32)	7.40(2.23)	78.00(2.30)	116.40(0.40)
	VCW	32.66(0.79)	54.80(2.31)	8.60(0.93)	13.72(1.15)	2.80(0.06)	16.40(1.08)	74.40(1.50)	116.60(0.60)
	VDC	27.20(3.81)	41.32(1.12)	5.00(0.89)	20.82(0.47)	1.54(0.10)	3.80(0.73)	82.60(1.66)	115.80(0.20)
	VDW	31.40(1.78)	47.46(2.79)	13.00(0.95)	18.80(1.08)	2.48(0.11)	10.20(1.46)	72.80(1.66)	116.60(0.93)
	VEC	24.90(1.62)	41.90(1.84)	6.00(0.84)	20.42(1.91)	1.81(0.29)	2.60(0.24)	81.40(0.75)	116.80(0.80)
	VEW	37.80(3.49)	51.76(1.08)	6.60(0.60)	16.54(1.19)	2.56(0.14)	8.40(2.09)	80.20(1.66)	116.20(0.20)
Wild	*O.nivara*	20.90(0.33)	17.90(0.93)	6.80(0.41)	30.50(0.45)	1.78(0.05)	17.30(0.89)	82.60(2.02)	124.36(2.15)
	O.rufipogan	21.30(0.58)	31.00(0.47)	10.60(0.40)	29.80(0.58)	1.46(0.12)	12.40(0.81)	89.60(1.21)	129.60(1.03)

The Principle Component Analysis (PCA) of the 30 morphological variables of the WR populations resulted nine components which explain73.87% of total variation in the data set (Table 9). The biplot of the PCA1 and PCA2 scores reveled that there were four groups (Figure 3).Wild rice varieties *O. nivara* and *O. rufipogan* were fallen into one group (Group A). The rest of the cultivated rice varieties and WR eco-types formed into separate clusters (Group B and D). The cluster C consists most of the cultivated rice varieties grown in Anuradhapura and Pollonnaruwa Districts. Most of the WR eco-types in Sri Lanka belong to one cluster (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 agro-ecological zone.

Table 9. Summary of the principle component analysis carried out on the 30 morphological characters of the different rice varieties across different eco-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 explain	13.165	10.663	10.557	9.632	6.837	6.251	6.171	5.609	4.988
Cumulative Perecentage explain	13.165	23.828	34.385	44.017	50.854	57.105	63.277	68.886	73.874
Leaf S	0.864	0.274	0.08	-0.029	-0.194	0.011	0.076	-0.087	-0.007
PanicleS	0.823	0.157	0.015	0.053	-0.274	0.012	-0.016	0.133	0.175
ApiculC	0.806	0.091	-0.05	0.265	0.052	-0.251	-0.077	0.001	-0.217
PanicleT	0.803	0.115	-0.107	0.275	0.186	-0.228	0.014	-0.074	-0.121
LPP	0.797	0.301	-0.007	0.048	-0.372	-0.046	-0.077	0.036	-0.117
Gw100	-0.431	0.128	0.175	-0.007	0.338	-0.051	-0.089	0.1	0.122
CulmNo	0.057	0.845	0.107	0.037	0.102	-0.029	0.019	-0.002	-0.047
CulmA	0.328	0.768	-0.039	0.092	-0.019	-0.223	0.055	0.103	-0.005
FLeafA	0.237	0.749	0.072	0.103	-0.185	0.209	0.089	0.072	-0.001
CollorC	0.416	0.696	-0.178	0.186	0.057	-0.003	-0.165	0.099	-0.081
LiguleL	-0.144	0.634	0.186	0.056	0.214	0.223	0.138	-0.034	0.258
DaysofH	-0.018	0.1	-0.903	-0.051	-0.02	-0.016	0.02	-0.001	0.069
SLC	0.055	-0.185	-0.771	-0.114	-0.124	0.003	-0.102	-0.084	0.061
CulmL	-0.212	0.104	0.744	0.095	0.197	0.095	0.344	0.138	-0.009
LeafA	0.162	0.091	0.516	0.22	0.201	0.047	-0.141	0.078	0.43
LBW	0.115	0.408	-0.459	0.22	-0.069	0.137	-0.209	-0.397	0.234
LBL	-0.159	0.107	0.42	0.414	0.294	0.248	-0.357	0.137	0.01
CulmS	0.17	0.215	-0.019	0.772	-0.043	0.047	-0.013	0.139	0.084
AwnC	0.235	0.017	0.09	0.701	-0.167	0.053	0.282	-0.023	-0.253
PanicleT	0.166	-0.018	0.062	0.649	0.05	-0.062	-0.009	0.303	0.356
SeedCC	0.048	0.233	0.324	0.599	0.116	-0.103	0.096	0.042	0.341
AwnAFH	0.088	0.111	0.034	0.594	-0.084	0.218	0.537	0.111	-0.229
Sdlh	-0.043	-0.299	0.445	0.529	0.333	0.262	-0.131	-0.088	0.082
AuricalC	-0.356	0.001	0.1	0.031	0.815	-0.05	0.156	-0.005	-0.04
LiguleC	-0.118	0.104	0.279	-0.117	0.725	0.228	0.141	0.04	0.055
SLL	-0.07	0.005	0.266	0.136	0.059	0.817	0.079	-0.054	-0.14
PanicleA	0.124	-0.089	0.519	-0.096	-0.051	-0.668	0.127	-0.098	0.159
LemmaPC	0.243	0.017	0.275	0.303	-0.038	-0.49	0.262	-0.138	-0.257
LiguleS	-0.296	0.347	0.242	0.064	0.382	0.47	0.299	0.05	-0.072
SecB	-0.178	0.127	0.121	0.089	0.239	-0.044	0.785	-0.188	-0.106
BLSC	0.154	-0.018	0.128	0.082	0.117	-0.019	0.709	0.432	0.106
LBC	0.05	-0.022	0.068	0.106	0.042	0.18	0.137	0.804	0.109
LBP	-0.084	0.241	0.143	0.28	0.075	-0.216	-0.191	0.644	-0.259
INCAF	-0.169	0.163	-0.006	0.457	-0.327	-0.006	-0.039	0.498	-0.01
PanicleE	-0.33	0.039	-0.226	0.098	0.014	-0.22	-0.038	-0.106	0.754
PanicleL	0.309	0.416	-0.251	-0.041	0.293	0.01	0.149	-0.173	-0.432

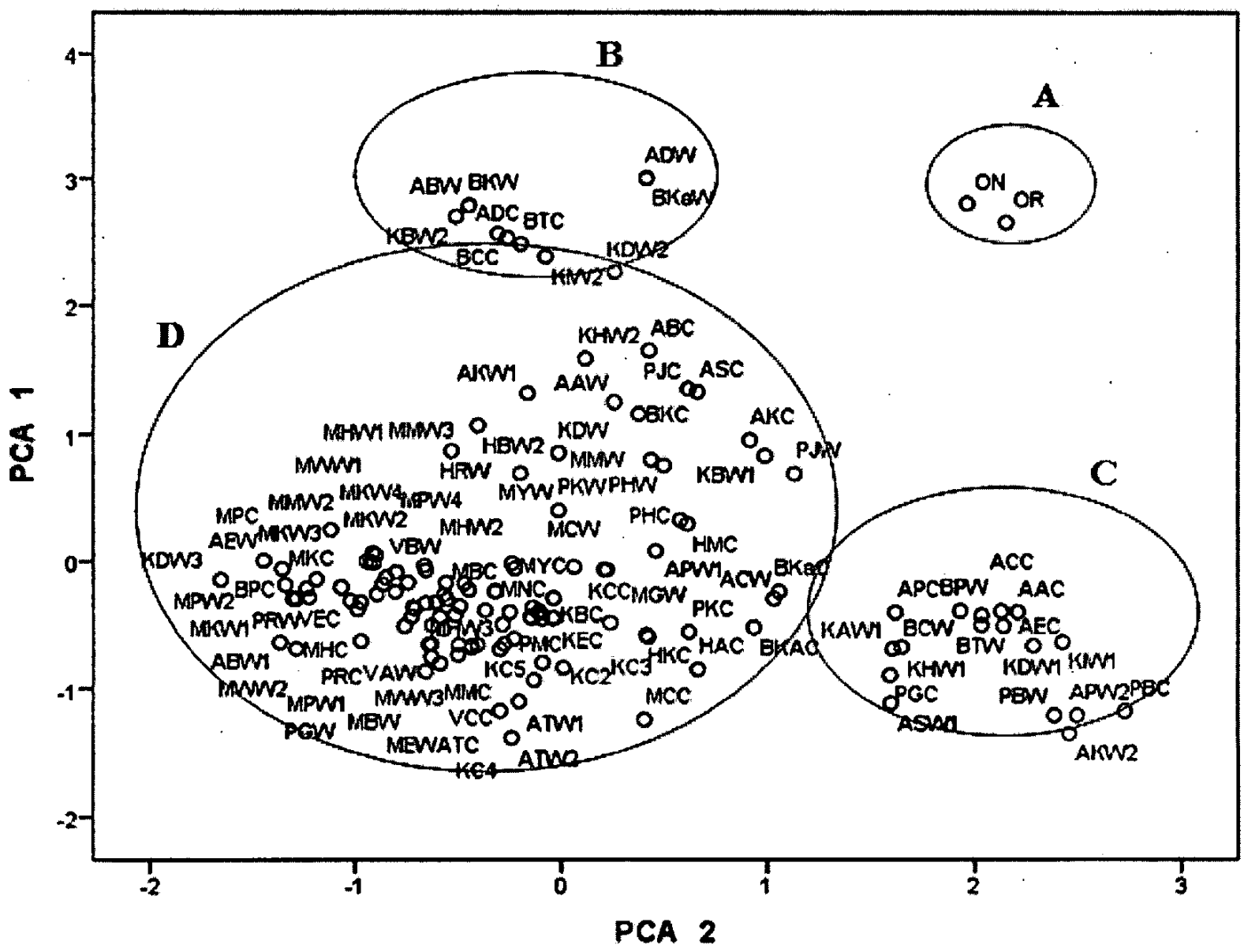

Figure 3: Biplot resulted from plotting of Principle Component scores of axis 1 and 2 from the analysis of agro-morphological data using PCA1 and PCA2. (Percent of variance explained from the PCA1 = 13.17%, Percent of variance explained from the PCA2 = 23.83%, Total Percent of variance explained = 37%).

Molecular characterization:

The scatter plot of the first and second principle components showed a clear genetic variation and differentiation pattern of WR populations in Sri Lanka. The Principle Component Analysis (PCA) 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 10). The biplot of the PCA1 and PCA2 scores revealed that there were three groups (Figure 4). Wild rice varieties *O. nivara* and dry zone (Anuradhapura and Puttalam Districts) WR eco-types belong to one group (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. rufipogan* were fallen into one group (Group B) suggesting, *O. rufipogan* 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 (Group C).

Table 10. Summary of the PCA analysis of ten molecular data of weedy rice, wild rice and cultivated rice varieties of different eco-climatic zoned 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 explain	25.648	19.025	13.348	9.938	6.962	5.418
Cumulative Percentage explain	25.648	44.674	58.022	67.96	74.921	80.339
RM211 A2	0.883	-0.066	-0.064	0.237	0.125	-0.125
RM211 A1	0.879	-0.090	-0.079	0.214	0.133	-0.09
: RM167 A1	0.789	-0.334	0.062	0.328	-0.279	-0.017
RM167 A2	0.786	-0.332	0.071	0.338	-0.268	-0.045
RM332 A2	0.683	-0.356	-0.092	0.272	0.306	-0.053
: RM332 A1	0.588	-0.08	-0.104	0.116	0.056	-0.015
RM44 A2	0.493	0.48	-0.051	-0.354	-0.349	0.077
RM14 A1	0.292	0.815	0.162	0.158	0.042	0.123
RM44 A1	0.411	0.75	0.034	-0.161	-0.271	0.018
RM280 A2	-0.108	0.689	-0.516	0.219	-0.027	-0.368
RM280 A1	-0.122	0.681	-0.488	0.204	0.045	-0.368
RM14 A2	0.517	0.6	-0.055	-0.072	-0.099	0.316
RM205 A2	0.298	0.237	0.806	-0.225	0.221	-0.023
RM205 A1	0.26	0.264	0.802	-0.133	0.242	0.014
RM21 A1	-0.345	0.417	0.428	0.658	0.074	-0.015
RM21 A2	-0.427	0.433	0.408	0.623	0.089	0.031
RM11 A1	0.412	0.347	-0.31	-0.448	0.436	-0.167
RM84 A2	-0.268	-0.107	-0.376	0.439	0.219	0.274
RM11 A2	0.135	0.028	-0.195	-0.099	0.738	0.135
: RM84 A1	0.121	0.251	-0.418	0.11	0.007	0.735

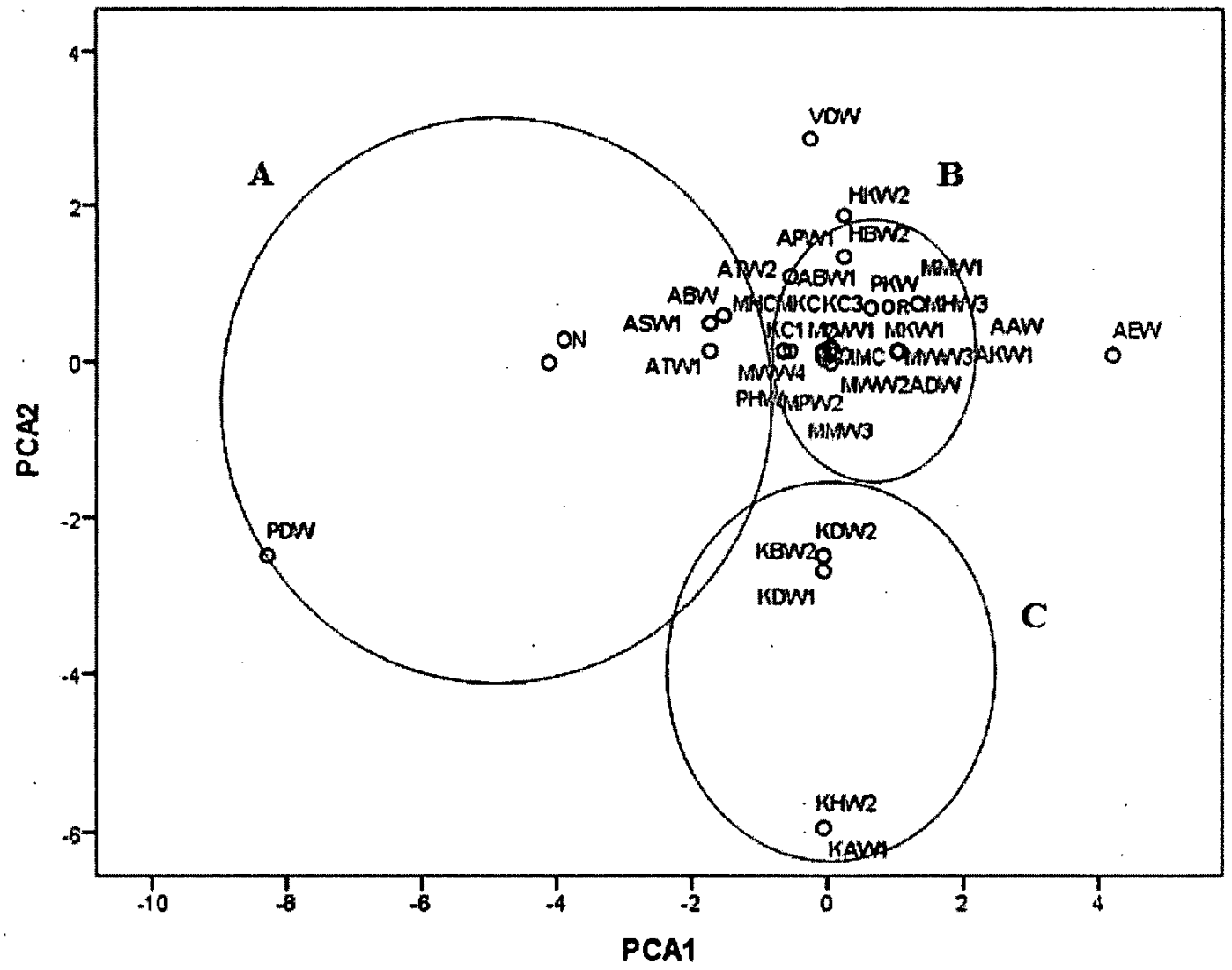

Figure 4: Biplot resulted from plotting of Principle Component scores of axis 1 and 2 from the analysis of Molecular data using PCA1 and PCA2. (Percent of variance explained from the PCA1 = 25.68%, Percent of variance explained from the PCA2 = 44.67%, Total Percent of variance explained = 70.35%).

Population structure analysis of weedy rice with cultivated and wild rice varieties using molecular data

The topology of the phylogenetic tree developed from the molecular data is shown in Figure 05. According to Figure 05 it is clear that there was a clustering tendency in which the association of dry zone weedy rice eco-types and *O. nivara;* weedy rice eco-types in wet zone closely associated with *O. rufipogan.* Based on the phylogenetic tree *O. rufipogan* (146) was closely related to Matara, Matale and Kandy district Weedy rice eco-types and also *O.naivara* (147) wild rice variety closely related to the Puttalam, Anuradhapura and Vauniya weedy rice eco-types. Most of the cultivated rice varieties in each climatic zone are grouped into large clusters with WR eco-types. The overall topology of phylogenetic tree was consistent with that of Huang *et al.* (2012), in which they suggested that the selected molecular datasets were effective for inferring the phylogeny of weedy rice.

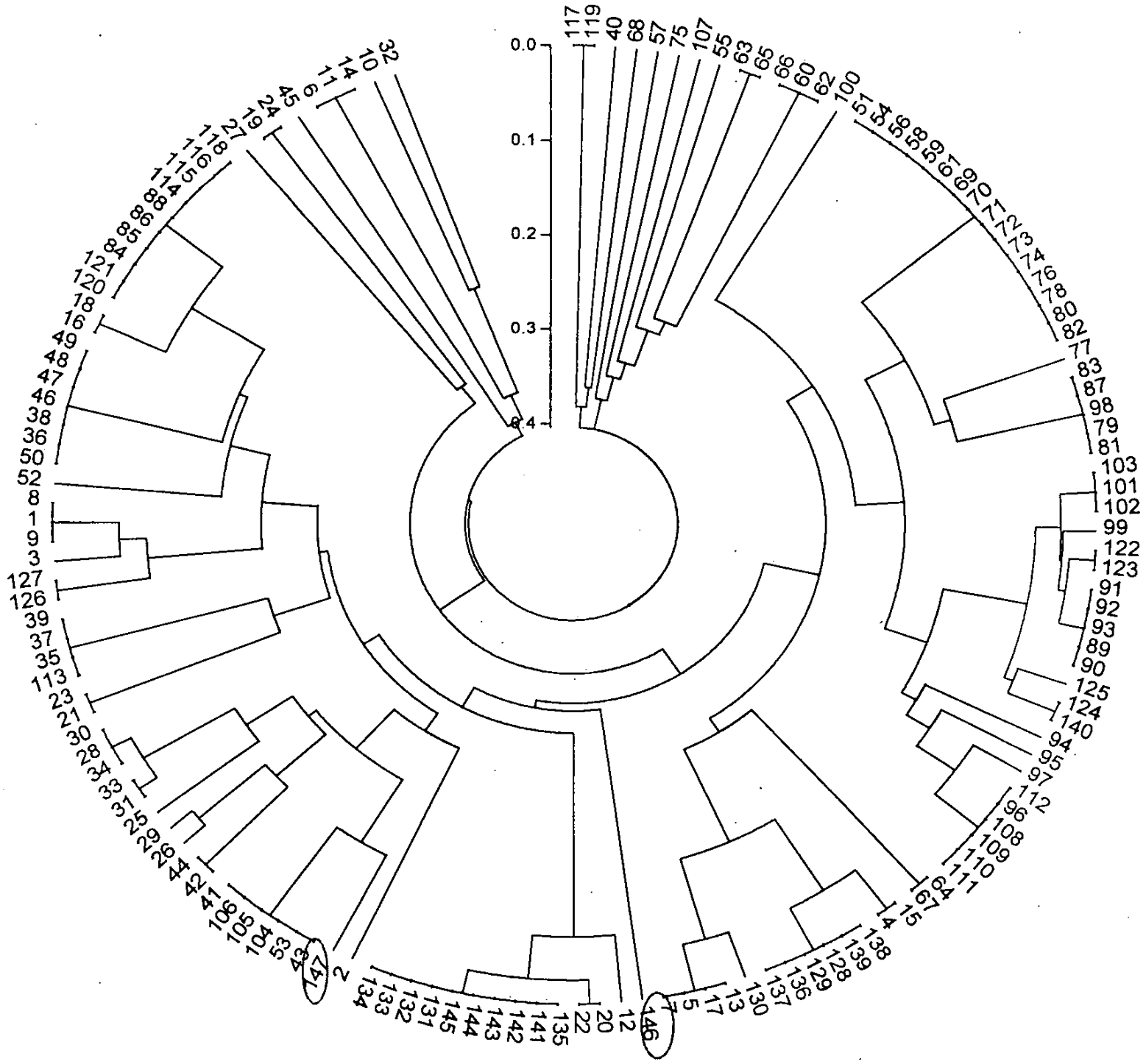

Figure 5. Population structure analysis of weedy rice with cultivated *Oryza* species and wild rice varieties. The circled numbers 146 and 147 indicate *O. rufipogan* and *O. nivara.*

Table 11. Summary of the parameters obtained from the electrophoresis analysis.

Marker	Major.Allele. Frquency	Genotype No	Sample Size	No. of obs.	Allele No	Availability	Gene Diversity	Hetero zygosity	PIC
RM11	0.45	13.00	147.00	147.00	10.00	1.00	0.63	1.00	0.56
RM21	0.47	19.00	147.00	147.00	19.00	1.00	0.71	1.00	0.68
RM14	0.25	21.00	147.00	143.00	23.00	0.97	0.85	0.50	0.83
RM44	0.31	19.00	147.00	145.00	24.00	0.99	0.79	0.99	0.76
RM84	0.38	19.00	147.00	147.00	22.00	1.00	0.75	0.96	0.71
RM167	0.27	17.00	147.00	132.00	21.00	0.90	0.84	0.85	0.82
RM205	0.23	16.00	147.00	147.00	19.00	1.00	0.85	0.91	0.84
RM211	0.60	15.00	147.00	123.00	17.00	0.84	0.62	0.43	0.61
RM280	0.14	23.00	147.00	145.00	30.00	0.99	0.92	1.00	0.92
RM332	0.33	25.00	147.00	130.00	28.00	0.88	0.81	1.00	0.79
Mean	0.34	18.70	147.00	140.60	21.30	0.96	0.78	0.86	0.75

The parameters obtained from electrophoresis analysis shown in Table 11, the comparison of values of Polymorphic Information Content (PIC) indicated that except RM11, RM21, RM211, the rest of the primers are of importance in assessing genetic diversity in the WR eco-types in different climatic zones in Sri Lanka. The PIC value of RM280 imply that it was the best primer for the separation (identify and differentiate) of WR eco-types.

Discussion:

The present results, especially based on the PCA pattern of 10 selected SSR loci demonstrated that Sri Lankan weedy rice populations possessed relatively high genetic diversity. In principle, a high level of genetic diversity of weedy rice populations provides a broad genetic basis of potential adaptation to a wide range of eco-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. Weedy rice 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 weedy rice 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. The differences in genetic diversity among weedy 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 weedy rice they encountered. This procedure might considerably reduce variation of weedy rice if it infested rice fields for a longer period of time. After a certain period, weedy rice 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 weedy rice populations is probably caused by limited exchange of genetic materials among weedy rice populations because of the inbreeding nature of weedy rice with an extremely low outcrossing 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. Weedy rice is always surrounded by rice cultivars in fields, genetic introgression from different cultivated rice varieties through time may increase variation among weedy rice populations. Ellstrand *et al.*, (1999) and Song *et al.*, (2006) have shown the importance of introgression from crop species, which may have a substantial impact on differentiation and evolutionary processes in wild and weedy populations.

The results showed that weedy rice populations from wet zone had a very close genetic relationship with *Oryza rufipogan* wild rice variety. This is clearly reflected by the PCA of weedy rice eco types and cultivated rice varieties in the wet zone of the country (Matara, Mathale and Kandy). PCA also showed a relatively close genetic relationship of dry zone (Anuradhapura and Puttalam) weedy rice populations with *Oryza nivara* wild rice variety. The analysis of molecular data provided valuable information for identification of different WR populations across different eco-climatic zones of Sri Lanka. The primer RM 280 could be considered as important marker in identification of weedy rice eco-types in Sri Lanka.

Conclusions:

Weedy rice populations in different climatic zones of the country 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 weedy rice populations suggest the common origin is centered around the species; *O. nivara* for dry zone WR eco-types and *O.rufipogan* for wet zone eco-types. WR eco-types in the intermediate zone shows close affinities with the cultivated rice variety (Bg379-2), suggesting a possible origin of WR outcrossing with cultivated rice. There are a number of 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 weedy rice in Sri Lanka. Effective methodologies for weed control and management must be developed to prevent weedy rice from extensive spreading and infestation across all rice planting areas in Sri Lanka.

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Gealy DR, Mitten DH, Rutger JN. 2003. Gene flow between red rice (Oryza sativa) and herbicide-resistant rice (O. sativa): implications for weed management. Weed Technology 17: 627–645.

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Problems if any, encountered during the implementation of the project:

The collection of WR from the Districts of Jaffna and Batticalo was not possible at the required time, due to the prevailing drought. Therefore, the samples were collected form the Districts of Jaffna and Batticalo during a latter period of a cultivation season.

Section 4

Impact of Research results:

i) Relevance of results achieved to scientific advancement

A primer was identified for the characterization of weedy rice eco-type which can be used in similar studies in future. The maker RM280 is a reliable maker in differentiation and identification of WR eco-types in Sri Lanka conserving time, money and labor.

ii) Relevance of results achieved to national/socio-economic development

The marker RM280 can be used to identify WR eco-types across different climatic zones with maximum cost-effectiveness.

The early identification of WR eco-type in infected areas and potential areas to be infected with WR eco-types could be promptly identified using the molecular marker chosen in the study which in turn prevents lowering the quality and quantity of rice yield. This can be led to reduce the famers' income.

iii) Dissemination/application of research output

A number of publications were made out of this research.
Agricultural biologist, Weed scientist, Agronomist and in general, paddy farmers will be benefited.

Section 5

Miscellaneous

i) List of major equipment acquired during the project period and their functionality

Major Equipment	*Functionality*
Plastic buckets	Planting rice plants
Garden gloves	For the plant house works
Glove box	For laboratary works
DNA extraction kit	Extract the DNA from rice leaves
Agarose	Agarose gel electrophoresis
Microcentrifuge tube	For the molecular works
Flat top reaction tube	For the molecular works
Centrifuge tube	For the molecular works

ii) List of publications/communications arising from the project and/or presentations made at seminars, workshops etc. (Please attach copies)

a. Abeysekera, A. S. K., Weerakoon, S. R., Karunaratna,K. D. K. and Johnson, D. E. (2012). Morphological variation in offspring derived from single panicle of weedy rice. *Annals of Sri Lanka Department of Agriculture (ASDA)*.**14**: 1-9.

b. A.S. K. Abeysekera, S. R. Weerakoon, K.D.K. Karunarathna and D.E Johnson (2012) Variation in the morphology of offspring derived from single panicle of weedy rice, The 6th International Weed Science Congress Proceeding: Pp. 48.

c. K.D.K. Karunarathna, S.R. Weerakoon, O.V.D.S.J. Weerasena and S. Somarathna (2013). Assessment of morphological diversity of Weedy rice (*Oryza sativa* f. *spontanea*) bio-types found in rice fields in Kurunagala District, Sri Lanka, The sixth Annual Scientific sessions, IBMBB, University of Colombo: Pp30.

d. K. D. K. Karunarathna, S. R. Weerakoon, S. Somaratne, O.V.D.S.J. Weerasena, A. S. K. Abeysekera. Phenotypic and genotypic variationin different weedy rice (*oryza sativa* f. *spontanea*) bio-type populations in Matara and Kurunegala districts, Sri Lanka,The 24th APWSS Conference Proceeding: Pp. 77.

e. S. Somaratne,K. D. K. Karunarathna, S. R. Weerakoon,A. S. K. Abeysekera, O.V.D.S.J Weeresena. Salient characters of Weedy rice (O*ryza sativa* f. *spontanea)* populations in highly infested areas in Sri Lanka, 1st Ruhuna International Science and Technology Conference, University of Ruhuna, Matara (RITSCON 2014): Pp 20.

f. K. D. K. Karunarathna, S. R. Weerakoon, S. Somaratne, O.V.D.S.J. Weerasena (2014).Molecular and Agro-morphological affinities among weedy, wild and cultivated rice varieties in different climatic zones of Sri Lanka. OUSL Annual Academic Sessions, 2014: Pp 319-323.

g. K. D. K. Karunarathna, S. R. Weerakoon, S.Somaratne, ,O.V.D.S.J.Weeresena, A.S.K.Abeysekera (2014). Affinities among traditional rice variety "*pachchaperumal*" and closely related weedy rice eco-types in Sri Lanka: a combine approach of molecular and agro-morphological characters.(Submitted NSF Journal).

h. K. D. K. Karunarathna, S. Somaratne, S. R. Weerakoon, ,O.V.D.S.J.Weeresena,A.S.K.Abeysekera. (2015).Eco climatic dependency of weedy rice (*Oryza sativa* f.*spontaneae*) distribution in Sri Lanka. 25th APWSS 2015 Conference. Pp. 11-12.

i. K. D. K. Karunarathna, S. Somaratne, S. R. Weerakoon and O.V.D.S.J. Weerasena (2015). Diversity of weedy rice *(Oryza sativa* f. *spontanea)* populations in Sri Lanka: An application of Self Organizing Map (SOM)- Manuscript submitted to Journal of Agriculture (Cambridge) – Under reviewing

Section 6

Financial Statement as at 22nd October, 2015

Category	**Total fund received**	**Total Expenditure**	**Balance** at 22.10.2015
Personnel RS	1485000.00	1485000.00	-
Personnel Others	109626.66	109626.66	-
Consumables	650715.34	640874.80	9840.54
Travel & Subsistence	350017.60	339134.00	10883.60
Miscellaneous	35132.40	35132.40	-
PG Registration	41000.00	41000.00	-
Total	2671492.00	2650767.86	20724.14

Summary Statement of Expenditure
(indicate under Personnel, Equipment, Consumables, Travel and Subsistence and Miscellaneous)

Personnel : **Rs.** 1485000.00 + 109626.66

Equipment and Consumables: **Rs.** 640874.80

Travel and Subsistences : **Rs.** 339134.00

Miscellaneus : **Rs.** 35132.40

Section 7

i) Grantees' signatures

Prof. (Mrs) S. R. Weerakoon

Dr O. V. D. S. J. Weerasena

Mrs. A.S.K. Abeysekara

ii) Comments of the Head of the Department/signature

The research is completed satisfactorily and important findings to the field of Botany and agriculture have been reported.
Several publications have been made which will be helpful in upgrading the research culture performance in the Department of Botany and the Open University. One M.Phil will be produced out of this project.

iii) Head of the Institution's signature

24/11/2015

Professor S. A. Ariadurai
Vice-Chancellor
The Open University of Sri Lanka
Nawala, Nugegoda.

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