International Research Symposium
Rajarata University of Sri Lanka

IRSyRUSl 2015

Genetic Diversity of Selected Sri Lankan Rice Accessions Based on Agro-Morphological Characters

Weerasinghe P. A.[3], Wijayawardhana H. C. D. [1]*, Herath H. M. V. G.[2], Herath H.M.D.A.K.[4]

Department of Plant Sciences, Faculty of Agriculture, Rajarata University of Sri Lanka[3,4], Department of Agricultural Biology, Faculty of Agriculture, University of Peradeniya, Sri Lanka[1,2]

ABSTRACT

The agro-morphological characterization is fundamental to provide information for plant breeding programmes. **.** *The aim of the present study was to characterize 20 Sri Lankan rice (Oryza sativa L.) accessions based on qualitative and quantitative agro-morphological characters. An experiment was conducted in the plant house of Faculty of Agriculture, Rajarata University of Sri Lanka, using a complete randomized design with three replicates. The IRRI standard evaluation system for rice was employed to study 17 different agro-morphological traits such as: vegetative traits (leaf length, leaf width, leaf blade pubescent, leaf blade color, basal leaf sheath color, leaf angle, ligule length, ligule color, culm length, culm number, culm angle, intermodal color, ligule shape, auricle color) and reproductive traits (flag leaf angle, panicle length, panicle exertion). Further, shoot and root dry weights were recorded. Correlation matrix of principle component (PC) analysis and single linkage cluster analysis were used to study the pattern of variation. Rice accessions of 4595 (Suwandel), 4759 (Kuruluthuda), 5650 (Rathal) and 3671 (Suduru Samba) could not grow well under the experimental condition, thus these accessions were omitted from the analysis. Polymorphism was observed among 17 of 19 traits evaluated. The first five PCs explained over 85% of the total variation of the all traits studied. Traditional varieties representing rice accessions showed the highest PC1 scores and new improved varieties representing rice accessions showed the highest PC2 scores. Based on both vegetative and reproductive characters, all the accessions were classified in to four clusters and the existence of these clusters was proved by Wilks' lambda statistics under multivariate analysis of variance (MANOVA). There was a high variability among the traditional rice accessions for their vegetative and reproductive parameters studied. These results can be used to identify the phenotypically divergent sources for traits of interest in breeding programs.*

KEYWORDS: *Agro-morphological characters, Breeding programmes, Genetic diversity, Rice*

[1] Corresponding Author: Wijayawardhana HCD: dwdarshani@yahoo.com

1 Introduction

Assessment of diversity is an essential component in germplasm characterization and conservation. Due to importance of rice as a second food crop in the world, its diversity has a great interest. Several tools are now in hand to study the variability and relationships between cultivars including isozyme analysis, storage protein study and molecular marker based studies. Among them, morphological evaluation is considered as a preliminary step for classification and estimation of germplasms due to its easiness and cost effectiveness.

Several breeding tools such as introduction, selection, recombination breeding and heterosis breeding etc. are employed to increase yield levels of rice after the green revolution (Roy and Sharma, 2014). Although rice yield has improved through these breeding strategies, subsequent yield stagnation has been observed in many breeding programmes. Therefore, breaking of rice yield stagnation through genetic improvement has prioritized objective in rice breeding programmes (Khush, 2005). Since the green revolution, the diversity of rice crop has been used in solving today's food problems (Khush and Virk 2000).

Agro-morphological traits play a crucial importance in identifying varieties and their characterization. This ultimately serves breeders for crop improvement (Laxuman *et al*., 2011). Sri Lankan rice germplasm exhibit a wide diversity in agronomicaly important traits, grain qualities and medicinal values. Therefore its diversity has a great interest towards plant breeders to use them in crop improvement programs.

1.1 Statement of the Problem

Frequent introduction of modern high yielding varieties to farmer fields has resulted the loss of gene pool of rice varieties in the farmer fields.

1.2 Objectives of the Study

This study was conducted to identify whether there is a significant genetic diversity exists in frequently cultivating local rice varieties for the possible exploitation in rice breeding programmes.

1.3 Review of Literature

Agro-morphological characterization is used extensively as a fundamental tool in evaluation and characterization of germplasm. Several researchers have used agro-morphological characterization in characterization studies of rice germplasm. do Nascimento et al. (2011) obtained a considerable polymorphism in accessions of upland rice from Brazil. Sarawgi et al. (2013) obtained a significant variation in rice accessions in Hyderabad by using agro-morphological characters. In India, Roy and Sharma (2014) analyzed the genetic diversity of Indian rice landraces by using agro-morphological traits. Further, Bajracharya et al. (2006) investigated the genetic relationship among high altitude land races of rice in Nepal by using agro-morphological characters associated with SSR markers.

Ogunbayo et al. (2005) investigated the phylogenic diversity and relationship among the accessions of rice from Genetic Resource Unit from the Africa Rice Center by using morphological and RAPD techniques. Here, one of the evaluation techniques was based on PC analysis. Tehrim et al. (2012) also investigated the genetic diversity of commercial and primitive cultivars of Pakistan rice by using multivariate techniques on the basis of agronomic characters. Further, Gana et al. (2013) have used PC analysis effectively to explore the pattern of variation among Nigerian rice accessions with the aim of identifying the traits that contribute to create the variability and use them in rice breeding programmes. Moreover, Ali et al. (2000) had used cluster analysis to group rice cultivars together with greater similarity.

2 Methodology

A pot experiment was conducted to evaluate the genetic diversity existing in selected Sri Lankan rice accessions based on agro-morphological characters exist in rice plant. The experiment was conducted in the plant house of Faculty of Agriculture, Rajarata University of Sri Lanka. Mid-day temperature inside the plant house during the experiment period was 32 – 36 oC.

Table 1: Agro Morphological Traits of Rice and Measurement Details

Serial No.	Agro morphological trait	Plant growth stage	Sample size	Unit	Method of measurement	Scale used
1	Leaf length	Late vegetative, Penultimate leaf	5	cm	Measuring ruler	-
2	Leaf width	Late vegetative, Penultimate leaf	5	cm	Measuring ruler	-
3	Leaf blade pubescence	Late vegetative, Penultimate leaf	-	-	Observation	1-glabrous including ciliated margins, 2-intermediate, 3-pubescent
4	Leaf blade color	Late vegetative, penultimate leaf	-	-	Observing and comparing with photographs	1-pale green, 2-Green, 3-dark green, 4-Purple tips, 5-purple margins, 6-purple blotch, 7-purple (full)
5	Basal leaf sheath color	Early to late vegetative, penultimate leaf	-	-	Observing and comparing with photographs	1-green, 2-purple lines, 3-light purple, 4-purple
6	Leaf angle	Prior to heading, penultimate leaf	-	-	Observing and comparing with diagrams	1-erect, 5-horizontal, 9-drooping
7	Ligule length	Late vegetative	5	mm	Measuring ruler	-
8	Ligule color	Late vegetative	-	-	-	1-white, 2-purple lines, 3-purple
9	Culm length	After flowering	5	cm	Meter ruler	-
10	Culm number	After flowering	5	-	Counting-total number of culms	-
11	Culm angle	After flowering	-	-	Observing and comparing with diagrams	1-erect, 3-intermediate, 5-open, 7-spreading, 9-procumbent
12	Internode color	After flowering	3	-	Observing	1-green, 2-light gold, 3-purple lines, 4-purple
13	Ligule shape	Late vegetative	-	-	Observing and comparing with diagrams	1-acute to acuminate, 2-2-cleft, 3-truncate
14	Auricle color	Late vegetative	-	-	Observing and comparing with photographs	1-pale green, 2-purple
15	Flag leaf angle	After heading	5	-	Observing and comparing with diagrams	1-erect,3-intermediate, 5-horizontal, 7-descending
16	Panicle length	Near maturity	5	cm	Measuring ruler	-
17	Panicle exertion	Near maturity	-	-	Observing	1-wellexerted, 3-moderately well exerted, 5-just exerted, 7-partly exerted, 9-enclosed

Twenty rice accessions were selected by considering the popularity among Sri Lankan farmers and the seeds were obtained from the Plant Genetic Resources Centre (PGRC), Gannoruwa, Sri Lanka. Selected rice varieties and their accessions numbers were, H4 (2851), Bg300 (2840), Bg305 (10617), Bg350 (2837), Bg352 (7182), Bg357 (8919), Bg359 (8923), Bg360 (8920), Bg450 (2835), Ld356 (9202), *Suwandel* (4595), *Kaluheenati* (4740), *Wedaheenati* (2340), *Pachchaperumal* (3136), *Murungakayan* (3809), *Rathkanda* (3223) *Raththawalu* (5566),*Kuruluthudu* (4759), *Rathal* (5650) *and Sudurusamba* (3671). Seedlings were prepared by using sand containing petrydishes. After 10 days, uniformly appearing healthy two seedlings were transplanted in each pot containing sand as the potting medium. Three replicate pots were made for each rice accession and the pots were arranged as complete randomized design inside the plant house. Plants were fertilized by using Urea, Tripple supper phosphate and Murate of potash, according to the recommendations by Department of Agriculture, Sri Lanka for 120 bushels per acre yield level (Fertilizer usage in paddy cultivation, 2010). Further, Yoshida nutrient media (Yoshida *et al*., 1976) was used to provide micronutrients.

Rice accessions were evaluated for 19 different agro-morphological characters including both vegetative and reproductive characters and dry weight measurements. Morphological trait measurement techniques were based on International Rice Research Institute (IRRI) standard evaluation system of rice (IRRI, 1980). The measurement details, images and pictorial guides used for data collection are present in table 1 and figure 1. Dry weight of shoots and roots were recorded after uprooting plants and drying at 60 °C until reaching in to a constant weight (Januškaitienė. 2010). All recorded data were analyzed using principle component (PC) analysis with correlation matrix, single linkage cluster analysis and multivariate analysis of variance (MANOVA) procedures in Statistical Analysis System software (SAS, Version 9).

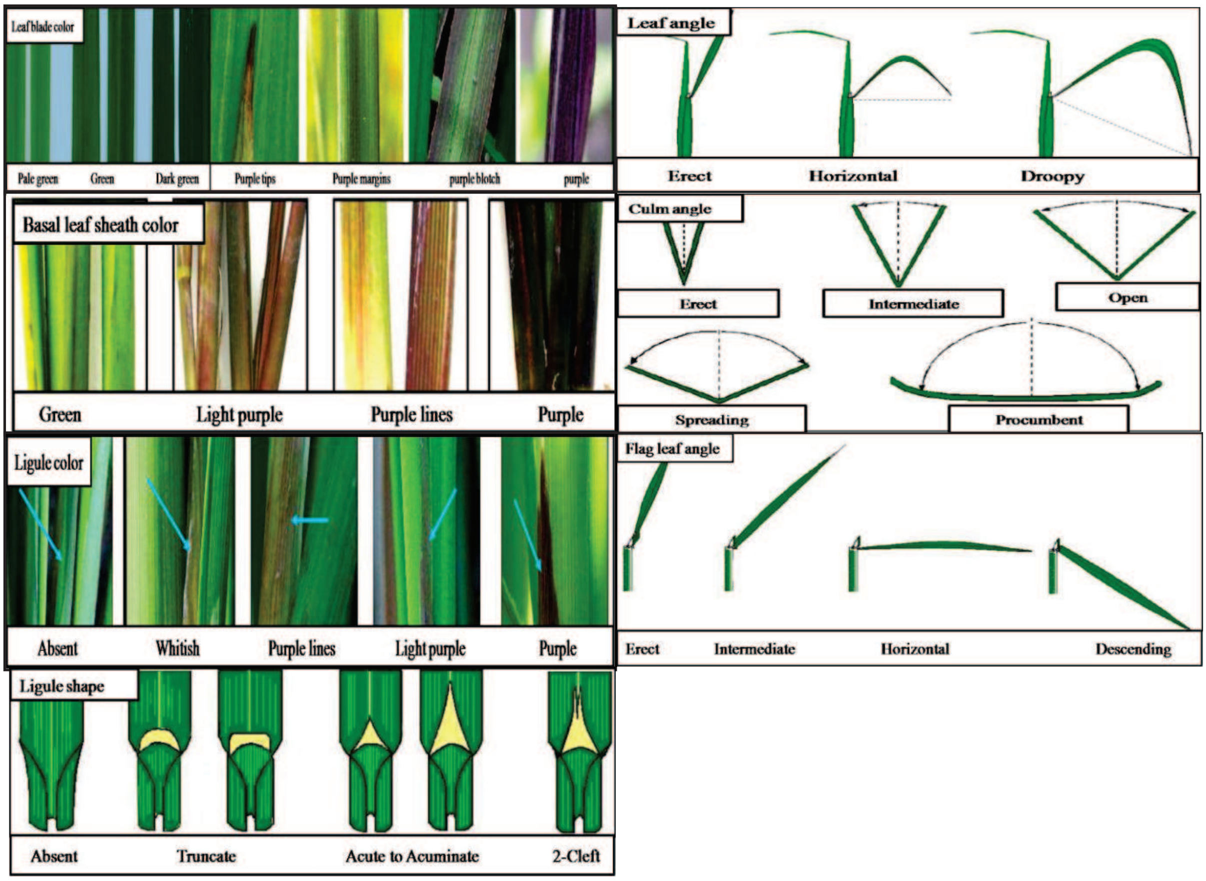

Figure 1: Used images and pictorial guides to observe some agro- morphological characters.

3 Results and Discussion

3.1 Growth of rice accessions during experimental period

Four rice accessions namely, 4595 (Suwandel), 4759 (Kuruluthudu), 5650 (Rathal) and 3671 (Suduru Samba) showed late and improper reproductive development during experiment period. Therefore, in the analysis, data from subjected four rice accessions were removed. Even though they were kept in pots while providing extra micro nutrients up to six months, proper panicle development and panicle exersion could not be observed. Further, plenty of undeveloped, white color, shrinked spikelets were observed in panicles and furthermore, milking and grain development was not observed in such spikelets. Instead of this, such unexerted panicles were consisted with unfilled grains.

Table 2: Eigen value, factor scores and contribution of the first five principle component axes to variation in rice accessions

Parameter	PC 1	PC 2	PC 3	PC 4	PC 5
Leaf length	0.3628	-.1985	-.1734	0.1518	0.0901
Leaf width	-. 2182	0.2357	0.2844	-.2978	0.1558
Leaf blade pubescent	0.2440	0.0674	-.1293	-.3348	0.3497
Leaf blade color	0.1748	0.3567	0.2598	0.2952	-.0822
Basal leaf sheath color	0.1694	0.3182	-.3104	0.1875	0.2603
Leaf angle	-.0691	0.1341	-.0623	0.6500	0.1642
Ligule length	0.2464	-.3208	0.0140	-.1554	0.4109
Ligule color	0.2539	0.3828	0.0224	-.2107	0.1505
Culm length	0.0872	-.4356	0.0686	0.1554	0.3545
Culm number	0.0589	0.0044	-.5162	-. 0525	-.3397
Culm angle	0.3008	0.0629	0.1066	0.2086	0.1666
Internode color	0.2878	0.3397	0.0287	-.1682	0.0757
Flag leaf angle	0.2089	-.0222	0.3059	0.2228	-.1229
Panicle length	0.1231	-.1999	0.4887	-.0644	-.0851
Panicle exertion	0.2965	0.1072	0.2144	-.0532	-.2813
Shoot dry weight	0.3459	-.1730	0.0463	-.0100	-.3182
Root dry weight	0.3477	-.1144	-.1994	-.0837	-.2731
Eigenvalue	5.0511	3.2227	2.8376	1.9164	1.5048
Proportion of the variance %	29.71	18.96	16.69	11.27	8.85
Cumulative variance %	29.71	48.67	65.36	76.64	85.49

3.2 Principle component analysis

The first five PCs explained over 85% of the total variation of nineteen qualitative and quantitative traits studied. The PC1 and PC2 were accounted for 29.71% and 18.96% of the total variation, respectively. PC1 and PC3 explained the 46.46% of the total variation covered by all rice accessions (Table 2). Traditional varieties showed the highest PC1 scores and new improved varieties obtained the comparatively highest PC2 scores (figure 2). Further, rice accessions of 2835 (Bg450) and 8920 (Bg360) showed the extremely high scores for both PC1 and PC2, which reflect the dark green leaf blade color, purplish color basal leaf sheaths, ligules and internodes (Table 2).

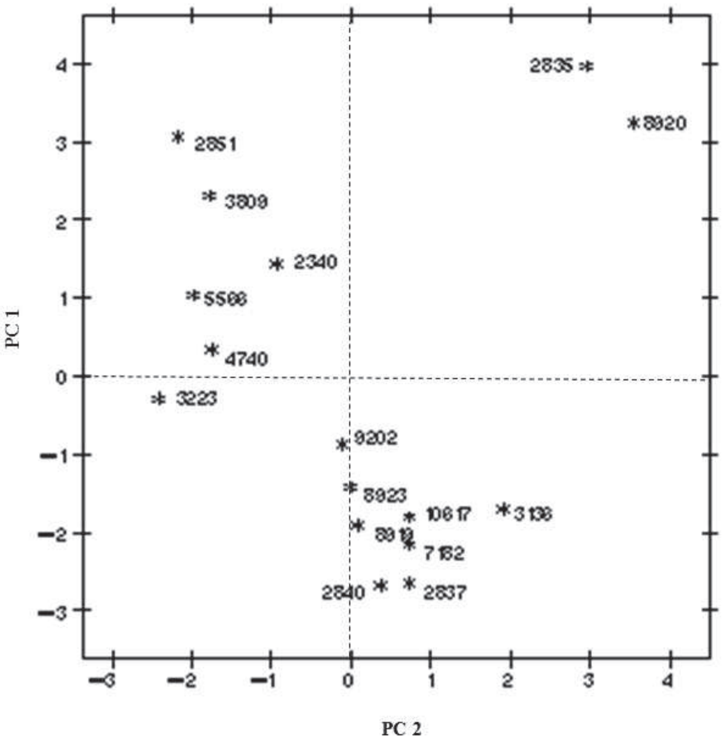

Figure 2: The score plot of PC1 and PC2 based on measured parameters.

3.3 Grouping of rice accessions

Sixteen rice accessions were grouped in to 4 clusters at 0.5 minimum distance between clusters (figure 3). Number of accessions per cluster varied from 1 in cluster II and 10 in cluster IV. Cluster I, II and III represented the accessions of traditional varieties and cluster IV represented the accessions of improved varieties. Thus, considerably highest variation was observed with traditional varieties representing rice accessions. Accessions of Bg300 and Bg305 were first formed in to a group at 0.1163 minimum distance between clusters. Therefore, these two accessions were the morphologically closest accessions among the tested ones. Accession of *Wedaheenati* was formed in to cluster at quite lengthy distance (0.5396), thus this was the distant accession. Further, the old improved rice variety H4 representing rice accession was grouped with traditional varieties containing group under the cluster I at a minimum distance of 0.4312. Furthermore, Traditional variety related accession of *Pachchaperumal* was grouped with new improved varieties (NIVs) related accessions containing group in cluster IV at 0.3303 minimum distance. Moreover, cluster IV contained NIVs representing rice accessions which were bred in Batalagoda (Bg-Batalagoda) and Labuduwa (Ld-Labuduwa).

3.4 Significance of observed clusters

The MANOVA analysis of the four rice accession clusters obtained reflected significant Wilks' lambda statistics (< 0.0001). Therefore, four groups of rice accessions resulted from cluster analysis are truly different clusters. Thus tested both vegetative and reproductive agro-morphological traits can be utilized effectively to group selected Sri Lankan rice accessions.

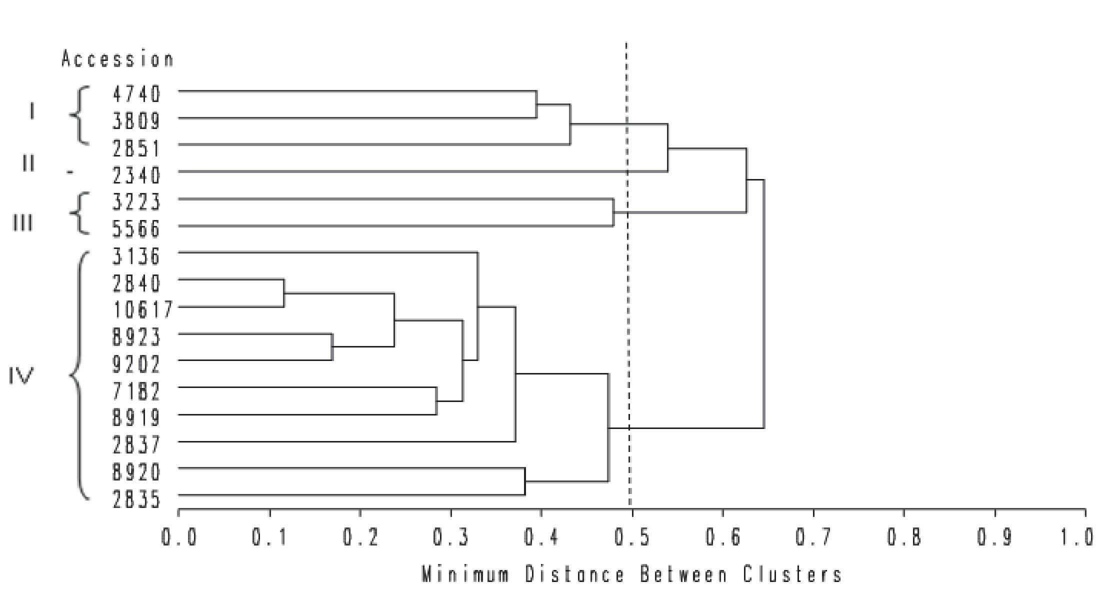

Figure 3: Dendrogram of rice accessions obtained through single linkage cluster analysis. Dashed line indicated the minimum distance between clusters. The clusters were identified as I, II, III and IV.

Results of the present study showed that selected vegetative and reproductive parameters can be used effectively to explore the genetic variability associated with the tested rice accessions. Though Sri Lankan rice varieties are broadly categorized in to two groups as traditional and improved, a wide variability among traditional variety group was observed. Present results were in conformity with Suriyagoda *et al*., (2011) and Wijayawardhana *et al.,* (2015) findings in terms of variability among traditional and newly improved rice varieties grown in Sri Lanka with few exceptions. This variability was most prominent among traditional variety containing rice accessions than NIVs containing accessions. Further, present study resulted a one cluster for NIVs and Suriyagoda *et al.,* (2011) obtained significantly different 2 clusters for NIVs. Here, Suriyagoda *et al*., (2011) had used further more rice varieties than the present study. Therefore, this difference is possible due to variations in genetic make-up of seed material, environment and field management practices (Singh and Rachie, 1985), soil type and soil fertility levels (Steel, 1972).

Three clusters obtained from traditional rice varieties containing accessions had high PC 1 scores than in NIVs. This represented the lengthy culms, ligules, leaves and highest dry weight in shoots and roots. The accession of old improved variety H4, which was clustered in to cluster I also showed similar agro-morphological characters as specified herewith. Further, varieties from cluster IV which was dominated by NIVs had highest PC 2 scores than PC1 scores. This was characterized by quite high leaf width, dark green leaf blades, presence of pubescence in leaf blades and quite narrow leaf angles.

Same authors have investigated the morphological diversity in same rice accessions only by using parameters existing in vegetative growth stage of rice from the same experiment (Wijayawardhana *et al*., 2015). The results obtained from the study showed a quite high variability than in previous study in terms of PC analysis and dendogram analysis. This variability can be resulted with added reproductive and dry weight parameters for the analysis. Further, the subsequent MANOVA analysis clearly indicates the greater importance of vegetative and reproductive parameters to differentiate selected Sri Lankan rice accessions.

Thus the identified agro-morphological characters to create genetic variability can be used by plant breeders to determine varieties for rice improvement programmes. Further, the present study gives detailed characterization of 16 local rice accessions with important agro-morphological characters, so that rice breeders can choose donor parents according to their genetic improvement programme as well as for conservation of the genetic resources.

4 Conclusions and recommendations

This study explored a significant level of genetic diversity existing in different accessions of rice studied, in relation to both vegetative and reproductive agro-morphological traits. Thus the measured traits can be used to evaluate the genetic diversity in selected Sri Lankan rice germplasm.

5 Acknowledgements

We are thankful to the Rajarata University of Sri Lanka for financial support (research grant No: RJT/RP & HDC/ 2013/Agric./R/04) and providing plant house to carry out this research work. Thanks are due to Mr. A.M.K.R. Banadara and Dr. W.C.P. Egodawatta for helping in statistical data analysis.

6 References

Ali, SS, Jafri, SJH, Khan,TZ, Mahmood, A & Butt, MA 2000, 'Heritability of yield and yield components of rice' Pakistan Journal of Agricultural Research, vol. 16, pp.89 - 91.

Bajracharya J, Steele, KA, Jarvis, DI, Sthapit, BR & Witcombe, JR 2006, 'Rice landrace diversity in Nepal: variability of agro-morphological traits and SSR markers in landraces from a high altitude site'. Fields Crop Research, vol. 95, pp. 327-335.

do Nascimento, WF, da Silva, EF & Veasey, EA 2011, ' Agro-morphological characterization of upland rice accessions' , Sci. Agric., vol. 68, no. 6, pp. 652-660.

Fertilizer usage in paddy cultivation 2010, Extension and Training centre, Department of Agriculture, Gannoruwa, Peradeniya.

Gana AS, Shaba SZ & Tsado EK. 2013, 'Principal component analysis of morphological traits in thirty-nine accessions of rice (Oryza sativa L.) grown in a rainfed lowland ecology of Nigeria' Journal of Plant Breeding and Crop Science, vol. 5, pp. 120-126.

International Rice Research Institute and International Board for Plant Genetic Resources 1980. Descriptors for rice Oryza sativa L. The international Rice Research Institute, Manila, Philippines.

Januškaitienė, I 2010, 'Impact of low concentration of cadmium on photosynthesis and growth of pea and barley' Environmental research, engineering and management, vol 3, pp. 24-29.

Khush, GS 2005 'What it will take to feed five billion rice consumers by 2030', Plant Mol. Biol. Vol. 59, pp. 1-6.

Krush GS, Virk PS (2000), 'Rice breeding achievements and future strategies', Crop Improvement, vol 27, pp. 115-144.

Laxuman, C, Salimath, P & Varma, M 2011, Molecular mapping and tagging of quantitative trait loci in rice - Molecular breeding in rice, Lambert Academic publishing GmbH & Co, Germany.

Ogunbayo, SA, Ojo, DK, Guei, RG, Oyelakin, OO & Sanni, KA 2005, 'Phylogenetic diversity and relationships among 40 rice accessions using morphological and RAPDs techniques', African Journal of Biotechnoogy. Vol. 4, no. 11, pp. 1234-1244.

Roy, SC & Sharma, BD 2014, 'Assessment of genetic diversity in rice (Oryza sativa L.) germplasm based on agro-morphology traits and Zinc-iron content for crop improvement', Physiol. Mol. Biol. Plants. Vol. 20, no. 2, pp. 209-224.

Sarawgi, AK, Rao, LVS, Parikh, M, Sharma, B & Ojha, GC 2013, Assessment of variability of rice (Oryza sativa L.) germplasm using agro-morphological characterization', Journal of Rice Research, vol. 6, no. 1 available from <http:// www.drricar.org/assesment.pdf>. [12.09.2015]

Singh, SR & Rachie, KO 1985, Cowpea Research, Production and Utilization. John Wiley and Sons, New York.

Steel, WM 1972, Cowpeas in Nigeria, Ph.D thesis, University of Reading.

Suriyagoda, LDB, Thilakarathne, RMMS, Nissanka, S P & Samitha, S 2011, 'Morphological variation in selected rice (Oryza sativa L.) germplasm of Sri Lanka'. Journal of National Science Foundation Sri Lanka, vol. 39, pp. 129 - 137.

Tehrim, S, Pervaiz, ZH, Mirza MY, Rabbani, MA & Masood, S 2012, 'Assessment of phenotypic variability in rice (Oryza sativa L.) cultivars using multivariate analysis', Pak. J. Bot. vol. 44 , no. 3, pp. 999-1006.

Wijayawardhana, HCD, Herath, HMVG, Weerasinghe, PA, Herath, HMDAK 2015, 'Morphological variation in selected Sri Lankan rice (Oryza sativa L.) accessions in relation to the vegetative parameters', Tropical Agricultural Research, vol. 26, no. 2, pp. 380-389.

Yoshida, SI, Forno, DA, Cock, JH, Gomez, KA 1976, Laboratory manual for physiological studies of rice, International Rice Research Institute, Manila, Philippines.