

Morphological variability pattern of Sri Lankan weedy rice - an ecological appraisal

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ABSTRACT Weedy rice (*Oryza sativa* L. f. *spontanea*) is one of the most widespread and problematic weeds in rice ecosystems with diverse characteristics. The study was carried out to determine the morphological variation pattern of the weedy rice populations in relation to agro-ecology of Sri Lanka. Twelve weedy rice populations collected from infested locations in Ampara, Matara and Kurunegala districts representing dry, wet and intermediate zones were evaluated in a common garden for ten quantitative traits to estimate the phenotypic diversity. The diversity level of weedy rice populations was high as revealed by Shannon-Weaver Index. Dry zone of Sri Lanka has more diversity hotspots of weedy rice. Analysis of variance revealed significant differences ($p < 0.05$) among populations than within populations implying the presence of substantial amount of genetic variability. Seed shattering percentage exhibited the highest variation while thousand seed weight showed the lowest variation explained by coefficient variation (CV). Principal component analysis indicated that the first two components accounted for 72.3% of the total variation and number of tillers, plant height (cm) at both seedling and heading stages, panicle length (cm), seed shattering % and the thousand seed weight (g) were the major determinants of genetic diversity in the weedy rice collection. Clustering identified two clusters and they were not associated with the geographical distribution of the populations. All the analysis based on plant morphology

suggested that weedy rice in Sri Lanka has great variability but no association with ecology of the country.

KEYWORDS *Oryza sativa* f. *spontanea*, diversity, genetic resources, coefficient variation, PCA

INTRODUCTION

Rice (*Oryza sativa* L.) is one of the major staple crops in the world and is particularly important in Asia, where approximately 90% of world's rice is produced and consumed (Khush 2004, Zeigler and Barclay 2008.). It is the staple food of Sri Lankans, providing 45% of total calorie requirement and 40% of total protein requirement of an average Sri Lankan diet (Census and Statistics 2007). It occupies 17.6% (0.7 million ha) of the total agricultural land area in the island (Agstat 2008), contributing 14.2% to total agricultural GDP of the island (Census and Statistics 2009, Central Bank 2009). Rice cultivation is distributed in almost all agro-ecological zones except for elevations above 2000m (Gunatilaka and Somasiri 1995). According to the spatial distribution of rainfall, Sri Lanka has traditionally been generalized into three climatic zones in terms of Wet Zone (rainfall >2500mm), Dry zone (rainfall <1750mm) and Intermediate zone (between 1750 to 2500 mm) (Punyawardena et al. 2003). The year is divided into two seasons coinciding with the monsoon rain as "Maha" (northeast monsoon falls during December to February) and "Yala" (southwest monsoon falls during May to September) and rice lands are cultivated in these two distinct seasons. Cultivars used in ancient time, were entirely traditional and most cultivars were tall with droopy leaves which may have facilitated the direct-seeded rice crop to overcome heavy weed infestation with trivial yield (Senadhira et al. 1980). The current rice production model with a few high-yielding modern varieties over a massive area has significantly improved the food security in the country, but has

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concurrently generated many troubles in rice ecosystems. Among these, the emerging of weedy rice with wide-ranging variation in morphological characteristics has significantly affected rice production in the country.

Weedy rice (*Oryza sativa* L. f. *spontanea*) is one of the most widespread and problematic weeds found in rice ecosystems worldwide (Ferrero et al. 1999, Mortimer et al. 2000). The control of this weed is problematic because of the close affinity with cultivated rice, in terms of morphological and physiological characteristics. This similarity has even led weedy rice to be classified as the same species as cultivated rice (*Oryza sativa* L.) (Vaughan et al. 2001). Weedy rice commonly causes a considerable reduction of rice yield because of its competition for resources with the cultivated rice and has a negative effect on the grain quality of cultivated rice (Kwon et al. 1991, Pantone and Baker 1991).

Weedy rice was first identified as a threat from Vavunia, Ampara, and Batticaloa districts of Sri Lanka in the mid 1990's (Marambe and Amarasinghe 2000). Yet, the origin of weedy rice populations in Sri Lanka is not clear. The yield losses have been estimated to vary from 10-100%, depending on the shattering ability and the level of infestation of weedy rice populations (Marambe 2005). Except for a few preliminary studies (Marambe and Amarasinghe 2000, Marambe 2005, Perera et al. 2010, 2012), no well-organized research has been carried out on morphological variability of weedy rice in relation to their ecology after it was first reported in Sri Lanka. Survey studies conducted by the Department of Agriculture, Sri Lanka in 1997-98 indicated that approximately 200 ha of cultivated land in the Ampara district (southeastern part of the country) has been seriously infested by weedy rice. In addition, weedy rice has been identified throughout the eastern province of Sri Lanka (Marambe, Amarasinghe 2000).

Although weedy rice has imposed negative impacts on cultivated rice, the genetic diversity of weedy rice might be a driving force in domestication and represents a potential source for cultivated rice improvement (Chen et al. 2001). Wide phenotypic variations are found in weedy rice populations all over the world, such as plant height, tillers, panicle morphology, seed size, seed weight and phenology (Delouche 2007).

Therefore, it is essential to evaluate the magnitude of variation pattern of the weedy rice from diverse environmental conditions to observe association with the agro-ecology of Sri Lanka, to explore the accessibility of different genotypes and possibility to incorporate favorable

traits of weedy rice into cultivated rice. With the above background information, the present investigation was undertaken to study the morphological variation pattern of the weedy rice populations and identification of major traits contributing to the diversity. This knowledge will be useful for genetic improvement of cultivated rice.

MATERIALS AND METHODS

Seed collection An extensive field survey was carried out in rice growing areas of Ampara, Matara and Kurunegala districts at seed maturity stage and seeds were collected from weedy rice infested locations (Fig. 1, Table 1) representing dry, wet and intermediate zones. Thirty individuals were collected with about 10 m interval for each weedy rice population. Each population is apart from the other for at least 5 km. The details of locality, preferably the longitude and latitude of each site were recorded using a Global Positioning System (GPS). The distribution of weedy rice was mapped using ArcView GIS software.

Field experiment Seeds of the weedy rice samples were placed in an oven (-55°C) for 5-7 days to break the seed dormancy, presoaked in tap water for 24 hours before they were sown directly to the nursery fields. For the field layout, complete randomized design was used with 4 replicates (plots) for each weedy rice population. For each replicate (plot), 25 weedy rice seedlings (14-day old) from the same population were planted in a 5m x 5m plot, with 25cm between hills and rows. All the management practices including fertilizer application, pest and disease management etc. were performed according to the recommendation of the Department of Agriculture, Sri Lanka. Weeds occurring in the plots were hand-removed periodically.

Morphological evaluation A total of ten (10) quantitative characteristics were measured at various growth stages using the standard evaluation system for rice developed by the International Rice Research Institute (IRRI) as indicated in table 2. The panicles of the weedy rice were enclosed by nylon mesh bags to avoid the loss of seed due to shattering before the seeds matured. To avoid an edge effect, only the nine plants in the middle of the plot were characterized.

Statistical analyses For the description of 10 quantitative traits, the following parameters were calculated: mean, standard deviation (SD), maximum and minimum values, coefficient of variation (CV, %). For the estimation of morphological diversity, data of each trait in every

population was divided into ten grades (from $\leq X-2*SD$ to $\geq X+2*SD$ with $1/2SD$ difference between two adjacent grades. Shannon-Weaver Index was calculated according to the formula: $H^1 = -\sum P_i \ln P_i$ (P_i : the proportion of the number i of entries in each grade) (Shannon and Weaver 1949). The averages of Shannon diversity index were estimated for each population based on the equal contribution of 10 traits. Statistical analyses were furnished using Excel. One-way analysis of variance (ANOVA) was conducted to estimate the morphological variation within and among populations using MINITAB ver14. Principal component analysis (PCA) was also used to determine the extent and pattern of variation of the major traits contributing to the diversity. The analyses were conducted using the software Minitab 14.

Cluster analysis by unweighted pair group method with arithmetic mean (UPGMA) (Sneath and Sokal 1973), and the dendrogram was constructed by Multivariate Statistical Package MSVP 3.1 (Kovach 1998).

Table 1 Sampled locations of 12 weedy rice populations from Matara, Kurunegala and Ampara in Sri Lanka

Population code	Climatic zone	Location (District/locality)	Latitude (N)	Longitude (E)
P1	wet	Matara/Pasgoda	N6°13'15.1"	E80°36'00.1"
P2	wet	Matara/Pitabeddara	N6°10'20.5"	E80°27'58"
P3	wet	Matara/Kotapola	N6°15'42.0"	E80°36'16.80"
P4	wet	Matara/Thihagoda	N6°04'18.5"	E80°33'50"
P5	dry	Ampara/Akkareipattu	N7°13'08.41"	E81°48'52.98"
P6	dry	Ampara/Thottama	N7°8'36.1"	E81°40'60"
P7	dry	Ampara/Lahugala	N6°52'03.40"	E81°43'05.85"
P8	dry	Ampara/Malwatta	N7°19'17.30"	E81°43'56.19"
P9	Inter	Kurunegala/Mawathagama	N7°25'45.00"	E80°26'36.60"
P10	Inter	Kurunegala/Bingiriya	N7°39'49.50"	E80°11'37.20"
P11	Inter	Kurunegala/Kurunegala	N7°28'30.50"	E80°22'14.00"
P12	Inter	Kurunegala/kuliyapitiya	N7°27'42.90"	E80°04'48.40"

Inter = Intermediate

RESULTS AND DISCUSSION

The weedy rice coming from different locations of the country has great diversity in morphological characteristics. But there was no significant correlation of morphological variation with geographic distances.

Fig 1 Representation of collected geographical locations of weedy rice in Sri Lanka

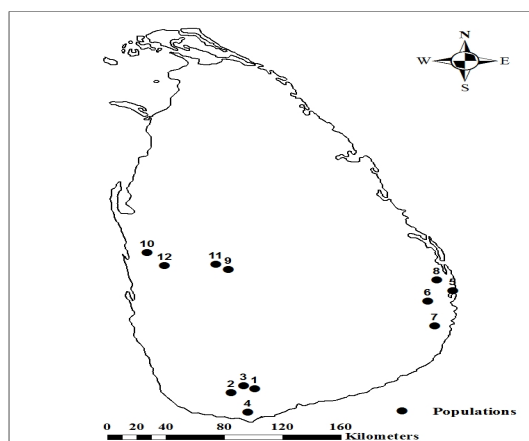


Table 2 Selected morphological traits and their methods of measurement for the characterization

Trait	Code	Method of measurement
Plant height at seedling stage	PH-S	Height from ground level to the tip of the leaf of the tallest tiller at three weeks after transplanting
Plant height at heading stage	PH-H	Height from the ground level to the base of the panicle of the tallest tiller after heading
No of tillers at seedling stage	T-S	Total number of tillers of each plant at three weeks after transplanting
No of tillers at heading stage	T-H	Total number of tillers of each plant after heading
1000-Seed Weight (g)	S-WT	Weight of 100 filled seeds/100)*1000
Panicle Length (cm)	PANL	Length was measured from the base to the tip of the panicle. Average length of 05 panicles were recorded
Number of panicles per plant	PAN/P	All number of panicles of each plant at maturity
Seed Shattering per panicle	SS/P	Number of seeds shattered/Number of panicles per plant
Number of filled seeds per panicle	FS/PA	Number of filled seeds from 03 panicle/03)+Number of filled seeds shattered/panicle
Total Number of seeds per panicle	TS/PA	Total number of seeds from 03 panicles/03)+Total number of seeds shattered/panicle

Table 3 Eigen value and cumulative percentage variance explained by first three principal components (PCs)

PC	Eigen value	Cumulative % variance explained
1	3.8036	38%
2	3.4308	72.3%
3	1.3456	85.8%

Extent and distribution pattern of morphological

variation: The first three PCs explained over 85% of the total variation of the 10 quantitative traits (Table 3). The PC1 and PC2 accounted for 38% and 34.3% of the total variation, respectively.

The first two components account for about 72.3% of total variation (Table 4). The first principal component accounted for about 38% of total variance was positively correlated mainly with number of tillers at both seedling and heading stage and number of panicles per plant. A negative correlation was observed with plant height (cm) at heading stage, panicle length (cm). The second component accounted for 34.3% of total variance positively correlated with thousand seed weight (g) and plant height at seedling stage (cm). It was also correlated, albeit to a lower degree, with total number of seeds per panicle. A negative correlation was found with seed shattering % per panicle. PCA analysis indicated that number of tillers, plant height (cm) at both seedling and heading stages, panicle length (cm), seed shattering % and the thousand seed weight (g) were the major determinants of the diversity and the most important traits among the 10 traits measured and accounted for all phenotypic variation (Table 4).

Representing all the populations in a bi-dimensional space of the first two components, it was not possible to identify well-separated groups of populations and most populations were fairly spread between positive and negative values of component 01 and more concentrated around positive values of component 02.

All the Intermediate zone populations comprising Mawathagama-P9, Bingiriya- P10, Kurunegala- P11, and Kuluyapitiya-P12 obtained positive PC1 Scores, with Kotapola-P3 at Wet zone and Thottama-P6 at Dry zone which reflects the difference in number of tillers at both seedling and heading stage and number of panicles per plant at mature stage (Table 4). Apparently, the PC2 differentiated populations with respect to the plant height at seedling stage (cm), thousand seed weight (g) and seed shattering% per panicle (Table 4).

Grouping of weedy rice populations

The 12 weedy rice populations coming from different geographic regions were grouped into 02 clusters at a Euclidean distance of 204.03 (Fig 3). The number of populations per cluster varied from 06 populations (Pasgoda-P1, Pitabeddara-P2, and Thihagoda-P4 at wet zone, Akkareipattu-P5 and Lahugala-P7 at dry zone and Mawathagama-P9 at intermediate zone) associated with cluster 01 and other 06 populations (Kotapola-P3 at wet zone, Thottama-P6, Malwatta-P8 at dry zone and Bingiriya-P10, Kurunegala-P11 and Kuliyaipitiya-P12 at intermediate zone) were found in cluster 02. The numbers of populations within a cluster were not similar and all populations within a geographical zone were not grouped in to one cluster. Most wet zone populations except Kotapola-P3, were grouped within cluster 01 at Euclidean distance 68.01 and intermediate zone populations except Mawathagama-P9, were grouped within cluster 02 at Euclidean distance between 0 and 68.01.

Table 4 The eigen values of the correlation matrix for the 10 characteristics of 12 populations of weedy rice (values in each parenthesis correspond to the eigen value and proportion of total variation documented by each component).

Trait	1 st principle component (38%)*	2 nd principle component (34.3%)*
Number of tillers at heading stage	0.469	-0.102
Number of tillers at seedling stage	0.467	0.111
Plant height at heading stage (cm)	-0.455	-0.103
Panicle length (cm)	-0.417	-0.107
Number of panicles per plant	0.345	-0.311
1000 Seed weight (g)	-0.145	0.446
Total number of seeds per panicle	0.145	0.192
Plant height at seedling stage (cm)	-0.044	0.491
Seed shattering (%)	-0.035	-0.449

*Values in brackets represent the percentage of the variance explained by each component

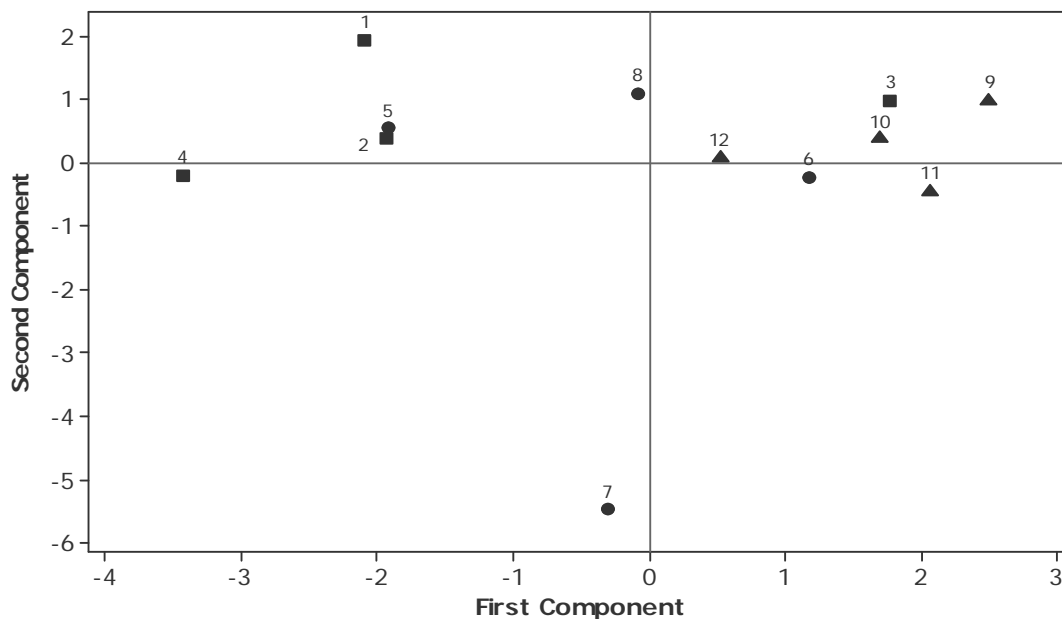


Fig 2 Distribution of wet zone (), dry zone () and intermediate zone () weedy rice populations on the first and second principal component

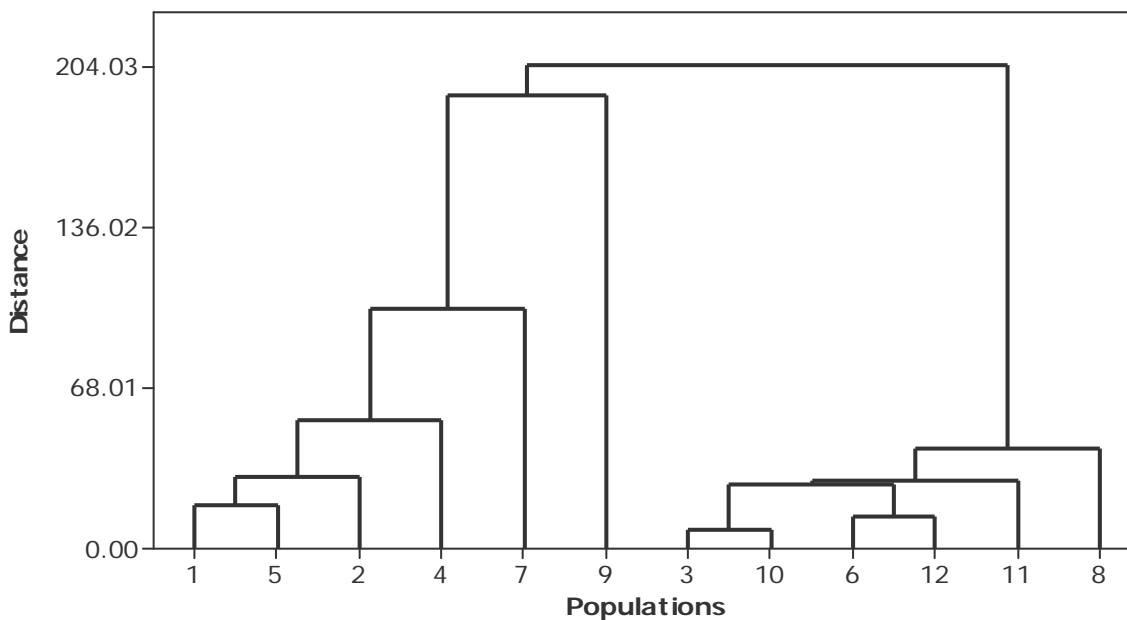


Fig 3 Dendrogram of weedy rice populations obtained through Ward Linkage and Euclidean distance based on 10 vegetative and reproductive characteristics

Estimate and analysis of diversity

Table 5 reflects the estimate of Shannon diversity index (H^1), characterized by geographical distance comprising of dry, wet and intermediate zones. H^1 was estimated for each of the 10 vegetative and reproductive characters in three geographical zones and twelve weedy rice populations. The average value of Shannon-Weaver Index (H^1) from the 12 populations was 1.06132 with variation of 0.047603 (Table 5). The highest value of H^1 was found in the Population-7 Lahugala/Ampara from Dry zone. Comparatively, dry zone of Sri Lanka has more diversity hotspots of weedy rice.

Table 5 Diversity index of 12 populations calculated by Shannon-Wiener Index

Population	Shannon-Wiener Index	
	Mean	Sd
1	0.775144	0.223215
2	1.02664	0.196157
3	1.143693	0.233919
4	1.109035	0.146128
Wet Zone Average	1.013628	0.199855
5	1.17835	0.17897
6	1.095954	0.248689
7	1.247665	0.17897
8	1.039721	0.163376
Dry Zone Average	1.140423	0.192501
9	1.061297	0.279622
10	1.005063	0.255724
11	0.978901	0.244703
12	1.074378	0.196731
Intermediate Zone Average	1.02991	0.034838
Total Average	1.06132	0.047603

Variation of morphological traits

The results of analysis of variance (ANOVA) in (Table 6) showed variation of measured traits among and within populations and reflects the higher (86.24%) variation in among populations and comparatively lower (13.75%) variation within populations for selected traits. Plant height (cm) at heading stage was the most variable character among populations while total number of seeds per panicle was the least variable character.

The mean, standard deviation, maximum value, minimum value and coefficient of variation (CV %) for each trait were shown in Table 7 to describe the level of traits variation based on all the 12 weedy rice populations. Seed shattering % per panicle is the highest variable character (CV-89.72%) and ranged from 3.33% to 73.89%. While thousand seed weight (g) is the least variable trait, ranged from 20.99% to 29.19 % (Table 7).

In general, there is a remarkably rich diversity in cultivated rice; however, a series of biotic and abiotic stresses continue to limit its productivity. Thus there is an urgent need to identify diverse sources of genes for tolerance to various stresses and broaden the rice gene pool. Because of their competitive nature, ecological adaptation and high diversity weedy rice is an important reservoir of useful genes and can be exploited both to broaden the existing narrow genetic base and enrich the existing varieties with desired agronomically important traits. In this study, variations were found in the 12 weedy rice populations across the three geographical zones with respect to the 10 quantitative morphological traits. High diversity was observed for the selected traits while there was no significant correlation among geographic distances. The morphological diversity observed, not only among the weedy rice populations but also within them, offers an array of traits that could be studied and incorporated to future rice-breeding programs. The variability in morphological characters have proved a useful tool in classification of plants and the information obtained could be of high interest to users of genetic diversity of plant genetic resources (Rogers and Appan 1973, Rogers and Fleming 1973, Maduakor and Lal 1989).

Table 6 Mean Squares (MS) and percentages of variation from ANOVA within and among populations

Traits	Mean Square of variation			Percentage of variation	
	Among population	Within population	Total	Among population	Within population
PH-S	77.4	10.6	88	87.9545	12.0454
PH-H	3227.1	45.2	3272.3	98.6187	1.3812
T-S	15.064	0.927	15.991	94.2029	5.7970
T-H	11.17	2.51	13.68	81.6520	18.3479
PAN/P	11.22	1.47	12.69	88.4160	11.5839
PANL	15.16	1.55	16.71	90.7241	9.2758
SS	1557.0	91.3	1648.3	94.4609	5.5390
FS/P	1037	270	1307	79.342	20.658
TS/P	5173	3861	9034	57.2614	42.7385
S-WT	15.48	1.76	17.24	89.7911	10.2088
Average	1113.959	428.6317	1542.59	86.2424	13.7575

Estimates of Shannon-Weaver diversity indices in this study showed that the highest diversity in dry zone weedy rice collections due to the ecological heterogeneity and climatic variations. This is also in consonance with Sanni et al (2008) who reported that Geographical and agro-ecological differences have their own influences as a factor for diversity variation of rice.

Table 7 Description of the variation of different traits of weedy rice populations in Sri Lanka.

Trait	Mean	Standard deviation (SD)	Max value	Min value	Coeff of variation %
Plant height at seedling stage (cm)	54.63	4.50	61.02	42.46	8.25
Plant height at heading stage (cm)	112.60	29.00	157.27	75.54	25.76
Number of tillers at seedling stage	3.793	1.969	7.208	1.595	51.92
Number of tillers at heading stage	10.987	1.676	12.592	7.342	15.25
Number of panicles per plant	9.226	1.685	12.432	6.650	18.26
Panicle length (cm)	22.884	1.950	26.103	6.650	18.26
Seed shattering (%)	22.22	19.94	73.89	3.33	89.72
Number of filled seeds per panicle	66.	16.10	90.20	29.87	24.40
Total number of seeds per panicle	111	36.0	221.3	80.6	32.40
1000 Seed weight (g)	25.27	2.056	29.19	20.99	8.14

Out of 10 traits, plant height is the most diverse trait among populations and between zones. Sanni et al. (2012) observed similar performance for phenological variables of landrace rice in Africa. This may be due to farmers' management practices to control weedy rice based on their height in the fields at early stage of growth, making huge variations of plant height. Plant height is one of the traits conferring competitive advantage to cultivated rice and taller weedy rice is able to capture more light and shade their neighboring rice plants (Kwon et al. 1992, Shivrain et al. 2010). At the same time, plants that mimic the crop, especially with the similar or shorter height, are difficult to locate and consequently have a better chance to reach maturity and then shatter on the soil surface.

Principal component analysis revealed that several characteristics played an important role in explaining part of the variability among weedy rice populations, particularly number of tillers and plant height (cm) at both seedling and heading stages, panicle length (cm), thousand seed weight (g) and seed shattering % per panicle most correlated with the two extracted components. Other weedy rice morphological studies agree partially with our results, indicating that diversity among populations is affected by plant height, 1000 seed weight and seed size (Arrieta-Espinoza et al. 2005, Zainudin et al. 2010).

The information obtained from the morphological characterization of Sri Lankan weedy rice reflects an initial step for understanding its diversity and complexity. Our results indicated a relatively high level of overall morphological variation in weedy rice populations collected covering three major zones in Sri Lanka.

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