



Eco-geographic Variation of Common Wild Rice- *Oryza rufipogon* Griff. in Sri Lanka

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Manuscript Details

Manuscript Submitted : 25/06/2018

Manuscript Revised : 01/07/2018

Manuscript Accepted : 02/07/2018

Manuscript Published : 15/07/2018

Available On

<https://plantaescientia.website/ojs>

ISSN 2581-589X

Cite This Article As

S Sandmal, A Tennakoon, DABN Amrasekera, B Marambe and D Ratnasekera (2018) Eco-geographic variation of common wild rice-*Oryza rufipogon* Griff., *Pla. Sci.* 2018; Vol. 01 Iss. 02: 36-43.

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ABSTRACT

Wild species of rice (*Oryza*) have superior agronomic characteristics to be incorporated in rice breeding programs worldwide. Population studies of wild relatives of rice in Sri Lanka have not been well documented despite a few attempts. In the present study, phenotypic diversity of *Oryza rufipogon* populations exist in Sri Lanka were characterized based on nine quantitative morphological traits. Populations (P1, P2, P3, P4 and P5) were established in a common-garden and were characterized. The results revealed moderate phenotypic diversity among *O. rufipogon* populations studied. However, flag leaf length and awn length were the most variable traits while plant height, flag leaf angle, flag leaf panicle neck length and spikelet angle were the least variable traits. *O. rufipogon* can be simply distinguished using flag leaf length and width, panicle branching type and distance from panicle base to lowest spikelet insertion. The dendrogram results indicated that four main clusters are at a similarity level of 98.73, showing the diversely related populations with a high identity based on higher similarity values. P1 and P2 populations grouped together by forming the first cluster. The second, third and fourth clusters consisted of P3, P5 and P4 populations, respectively. One population from the first cluster and P3, P5 and P4 populations can be used for conservation. This study highlights the phenotypic diversity of *O. rufipogon* populations existing in Sri Lanka across the geographic locations and Knowledge on such morphological diversity provides opportunities to design conservation strategies and the potentials of using particular population based on breeding objectives.

Keywords: Genetic differentiation, Morphological variation, *O. rufipogon*, Population divergence, Wild rice

INTRODUCTION

The rice genus *Oryza* contains approximately 23 species distributed in Asia, Africa, Australia and America. Of the species, only two are cultivated and the remaining 21 species are wild relatives of rice (Vaughan, 1989; Vaughan et al., 2003). Species in the *Oryza* genus are highly diverse morphologically (Clayton and Renvoize, 1986; Vaughan, 1994), and are represented genetically by different genome types, i.e. AA, BB, CC, BBCC, CCDD, EE, FF, GG, JJHH and JJKK genomes (Ge et al., 1997), providing valuable opportunities to explore variations for rice breeding programs. Of the wild relatives, species containing AA-genome are the most accessible genetic resources because of their high genetic compatibility with cultivated rice and therefore, transfer of alien genes from those wild relatives can be easily achieved through sexual hybridization (Ren et al., 2003).

Oryza rufipogon Griff. is known as the putative ancestor of Asian cultivated rice (*Oryza sativa* L.), is the most significant germplasm for rice genetic improvement (Oka, 1988). As an example, for the development of high yielding hybrid rice varieties, a male sterility (MS) gene found in *O. rufipogon* was first introduced to the cultivated rice. Other agronomically favourable characters, such as genes resistant to tungro virus and bacterial leaf blight in rice (Xa23 gene) and tolerant to acid sulfate soil found in common wild rice, are of great importance for rice genetic improvement (Song et al., 2005). *O. rufipogon* is broadly distributed in the regions of tropics and subtropics in Asia (Vaughan, 1994). This species is recorded to occur in 113 countries in the world. *O. rufipogon* has species-specific features compared with other *Oryza* species.

Besides its distribution in Sri Lanka (normally occurred in wet zone) *O. rufipogon* is found in Australia, south and southeast Asia, and Papua New Guinea. It is well grown in moist clay, loam and black soil. Generally, *Oryza rufipogon* is a perennial, cross-pollinator, photoperiod sensitive, found in permanently wet regions in terms of swampy areas like marshes, open ditches, swamps, grasslands, ponds, billabongs, pools along river banks, at the edges of lakes, and in or at the margins of rice fields. Some populations grow under submergence and saline conditions. The water depth preferences are between 0.2 - 4m (Duistermaat, 1987). Flowering times vary from place to place (Field observation) with peak flowering time from December to January (Liyanage and Senanayake, 2010).

In Sri Lanka, our recent field observations showed that the current geographic range of this species has significantly reduced, and the extant of populations have gradually decreased in size owing to habitat destruction and degradation as a result of anthropogenic activities such as agriculture, industry and urbanization (Liyanage and Senanayake, 2010). Above drastic human activities have destroyed natural habitats, leading to habitat fragmentation that affects to reduced population sizes and isolation among populations, leading to 'isolation by distance' (IBD) (Zhao et al., 2013).

Most studies related to wild relatives of rice so far have been based on field surveys and collections of conservation neglecting population studies that could contribute to knowledge about genetic diversity and eco-geographic variation among natural wild rice populations. Effective conservation of these species is urgently needed to preserve the remaining populations. Our knowledge of the genetic diversity of the few natural populations on *Oryza rufipogon* species in Sri Lanka is rather limited, and a better understanding of its population genetics will be of great significance in the *in-situ* germplasm collection.

Understanding genetic diversity and eco-geographic variation of wild rice (*Oryza rufipogon*) in Sri Lanka is a timely requirement to implicate conservation & utilization of such endangered species. Comprehensive understanding of eco-genetic diversification and eco-geographic variation (in terms of morphologically) of wild rice (*Oryza rufipogon*) was focused in this study enabling their effective use in rice improvement.

MATERIALS AND METHODS

Five *O. rufipogon* populations were sampled representing a wide distribution of natural habitats in the country based on GPS coordinates obtained from previously reported information (Liyanage and Senanayake, 2010), with each population of 25 individuals that were randomly collected at least 5 m distance between two individuals (Figure 1, Table 1). Plants were established in cement pots at the size of 16 cm × 16 cm × 18 cm in a common garden at the Faculty of Agriculture, University of Ruhuna (Latitude 06.060337°N and Longitude 80.5681455°E) during January to December 2017 to characterize plant morphology. Phenotyping was conducted for nine quantitative parameters and was measured as described in the Bioversity International, International Rice Research Institute (IRRI), and the Africa Rice Center (Center, 2007). All measurements were taken after the time of flowering from three randomly selected tillers per plant, as flowering time is considered to be involved in the creation of various different characters of the *Oryza* species (Grillo et al., 2009).

DATA ANALYSIS

All the analyses were conducted using SAS 9.1 (SAS, 2002) and Minitab 17 (Minitab, 2014) statistical software packages. Analysis of variance was performed for each parameter separately to identify specific characters that are significantly different among populations. Descriptive statistics were used to obtain the coefficient of variance. Quantitative variables were subjected to Pearson's correlation analysis. A hierarchical cluster analysis was performed using the cluster variable function.

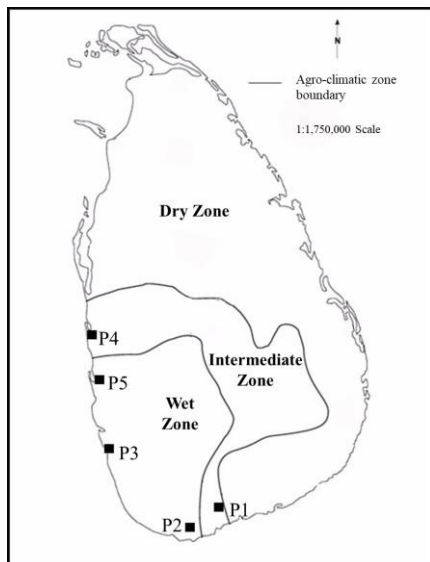


Figure 1. Geographical locations of the five *O. rufipogon* populations collected from natural habitats in Sri Lanka. The population codes at each collection site are shown in Table 1. Divisions of the Dry, Intermediate and Wet zones are represented by the solid lines.

RESULTS AND DISCUSSION

A considerable variation of characteristics was found among populations for most of the traits measured. Morphological diversity was measured by 9 quantitative traits are presented in Table 2. ANOVA results indicated that evaluated quantitative traits showed moderate variations among populations of *O. rufipogon*. Such morphological variation shows significance in crop improvement on the basis of incorporate desirable traits in different aspects. The highest plant height observed in P4 and P5 populations belonged to the intermediate and wet zones respectively. Population P2 showed the significantly highest culm girth and P1 recorded the lowest ($p < 0.05$). The highest length from flag leaf base to panicle neck (FLPNL) was recorded from plants in the P2 population.

In general morphological differences among *O. rufipogon* populations are directly correlated to their natural habitats, life cycle and breeding system (Banaticla-Hilario et al., 2013). Previous studies indicated that, culm related traits with wider culm diameter and less number of tillers were significantly associated with crop physiology and yield production by increasing plant lodging resistance (Chuanren et al., 2004). *O. rufipogon* showed markedly longer culm length and wider culm diameter, greater plant height and longer panicle length implying that higher lodging resistance had developed in *O. rufipogon*. The presence of such characteristics may help to survive in permanently inundated habitats (Banaticla-Hilario et al., 2013). In addition, longer plant height, longer flag leaf length and greater flag leaf width of *O. rufipogon* probably more appropriate for waterlogging environmental conditions. As reported by Maduakor and Lal (1989), morphological traits are vital as a tool for assessing the extent of genetic diversity of plant genetic resources. Therefore, the morphological variability observed among populations is indicative of the

genetic divergence (Richards, 2000). Wild species of rice may have morphological, biochemical, anatomical adaptations for their survival in the particular environmental conditions.

Oryza rufipogon plants are found under different agro-ecological conditions of the island. This wild rice species have adapted to climatic conditions prevailing in the Hambantota, Matara, Kalutara, Puttalam and Colombo districts of Sri Lanka (Liyanage and Senanayake, 2010). For example, the P2 population from the wet zone in the Matara district showed vigorous plant growth with the highest CG and FLPNL. Moreover this population is grown on the river. The higher annual rainfall and favourable environmental conditions of this population compared to those in the other population would have led to the higher growth performance of *O. rufipogon* in the wet zone. In addition to that, plants in P3 may have a higher ability to pest attacks due to its AWL. But no significant difference with population P4. Highest FLL was observed in both P3 and P5, which are belonged to the wet zone. Hence, plants were morphologically adapted to wet environments together with high temperature and water availability to grow and survive by increasing plant height and culm girth when increasing water level (Banaticla-Hilario et al., 2013). Sri Lankan researchers have found several morphological differences among populations of *O. rufipogon* species collected from different locations of Sri Lanka. There are no documentary evidence to support these eco-morphological differences.

Table 3 shows the within and among population variations of each parameter. Some traits showed higher variability among populations resulting in a high coefficient of variation (CV%) in PNSPL (44.65%) while some traits such as PH, CG and FLW showed a comparatively lower CV% (9-13%). Though, some traits (PH, CG, FLA and SPA) showed higher within-population variance than among population variance. Trait-specific variation patterns were observed within populations. For example, PH of P5, CG of P2, FLL and FLW in P5, AWL in P1, and PNSPL of P3 and P5, SPA of P4 showed high variations within the populations.

Quantitative traits are important for plant description (Kurlovich, 1998) and largely influenced by the socio-economic scenario and natural selection (Hien et al., 2007). The coefficient of variation is a valuable tool for gaining comparisons of variability in different traits. A reasonable amount of genetic variation was exhibited for the traits evaluated. Plant height was the only character with a coefficient of variation (CV) value less than 10%. However most traits have CV values above 10% and as high as 44.65%. For the grain yield per plant representing that selection based on these characters is expected to be effective (Zafar et al., 2004).

The boxplots (Figure 2) clearly illustrated overall patterns of entire distribution of populations in relation to parameters, providing a convenient way to visualize the variation of nine morphological traits across populations.

Table 1 : Habitat information and location details of the seven *O. rufipogon* populations used in the present study.

Location	Accession Code	GPS Value		Habitat
		Latitude	Longitude	
Weerakatiya, Hambantota	P1	06°09'18.64"	80°46'02.83	Lake
Thihagoda, Matara	P2	06°00'01.60"	80°33'43.90"	River
Nugagoda Junction, Waskaduwa, Kalutara	P3	06°38'07.08"	79°57'03.24"	River
Madampe, Puttalam	P4	07°28'02.93"	79°49'40.97"	Stream
Colombo-Negombo Rd, Seeduwa	P5	07°06'51.4"	79°53'15.3"	Adjacent to paddy field

Table 2: Estimates of morphological trait differentiation among five *Oryza rufipogon* populations

Character*	Population				
	P1	P2	P3	P4	P5
PH (cm)	121.9 ^b	131.6 ^{ab}	126.5 ^b	140.1 ^a	141.33 ^a
CG (cm)	1.45 ^c	1.82 ^a	1.66 ^b	1.61 ^b	1.63 ^b
FLL (cm)	19.27 ^{bc}	15.65 ^d	21.49 ^{ab}	17.58 ^{cd}	22.78 ^a
FLW (mm)	0.87 ^{ab}	0.83 ^b	0.71 ^c	0.96 ^a	0.86 ^b
FLA	58.87 ^b	69.97 ^{ab}	73.13 ^{ab}	80.7 ^a	74.2 ^{ab}
FLPNL (cm)	10.33 ^b	17.7 ^a	10.34 ^b	9.31 ^b	11.45 ^b
AWL (cm)	3.58 ^d	4.27 ^c	6.23 ^a	5.85 ^{ab}	5.54 ^b
PNSPL (cm)	1.917 ^c	2.354 ^c	4.327 ^a	4.67 ^a	3.34 ^b
SPA	43 ^b	65.39 ^a	59.5 ^a	52 ^{ab}	56.83 ^a

*Trait descriptions in the study. PH: plant height, CG: culm girth, FLL: flag leaf length, FLW: flag leaf width, FLA: flag leaf angle, FLPNL: flag leaf panicle neck length, AWL: awn length, PNSPL: panicle neck spikelet length, SPA: spikelet angle. Within a row values on correspondent trait, means followed by the same letters are not significantly different at $p=0.05$.

Population P4, which belongs to the intermediate zone is morphologically distinct from each other as shown by a distinguishable cluster in the dendrogram (Figure 3). Hence, the results showed that *O. rufipogon* plants are adapted to survive in the intermediate zone. Because the current geographic range of *O. rufipogon* has significantly reduced, and the extant populations have gradually decreased in size owing to habitat destruction and degradation resulting from agriculture, industry and urbanization (Liyanage and Senanayake, 2010). Above drastic human activities have destroyed natural habitats, leading to habitat fragmentation

that affects to reduced population sizes and isolation among populations, leading to 'isolation by distance' (IBD). Gradually decrease of population size experience bottlenecks, genetic drift and inbreeding which lead to the loss of alleles and to an increased homozygosity that affect to population extinction (Young et al. 1996). As reported by Banaticla-Hilario & Almazan (2010) natural populations of *O. rufipogon* can be simply distinguished using flag leaf length and width, panicle branching type and distance from panicle base to lowest spikelet insertion. The presence of stolons and long, strong and spreading culms may help to make

O. rufipogon suitable for permanently inundated habitats
(Banaticla-Hilario and Almazan, 2010).

Variation of Growth Characteristics of Within and Among Populations
Table 3. Standard deviation of different traits within and among populations.

Traits	Within Population					Among Population
	P1	P2	P3	P4	P5	
PH	5.36 (4.39)	8.8 (6.69)	8.2 (6.48)	10.38 (7.41)	15.43 (10.92)	12.39 (9.36)
CG	0.1306 (8.96)	0.2829 (15.55)	0.1181 (7.1)	0.1449 (9)	0.1089 (6.67)	0.2005 (12.25)
FLL	2.438 (12.65)	1.66 (10.6)	2.12 (9.86)	2.012 (11.44)	4.27 (18.75)	3.645 (18.83)
FLW	0.0734 (8.4)	0.0728 (8.77)	0.0502 (7.04)	0.0516 (5.38)	0.1042 (12.03)	0.1069 (12.6)
FLA	11.42 (19.4)	13.53 (19.34)	24.11 (32.97)	10.84 (13.44)	18.69 (25.18)	17.39 (24.36)
FLPNL	2.251 (21.79)	5.32 (30.03)	1.527 (14.77)	1.542 (16.56)	3.025 (26.4)	4.235 (35.8)
AWL	0.795 (22.18)	0.2865 (6.71)	0.467 (7.48)	0.655 (11.2)	0.9 (16.24)	1.195 (23.44)
PNSPL	0.402 (20.95)	0.379 (16.12)	1.727 (39.93)	0.827 (17.72)	1.275 (38.18)	1.483 (44.65)
SPA	9.84 (22.89)	9.39 (14.36)	8.02 (13.48)	21.52 (41.38)	16.36 (28.79)	15.43 (27.88)

Values presented within parenthesis are CV%

Table 4. Pearson correlation matrix among nine morphological parameters of Sri Lankan wild rice populations.

Trait	PH	CG	FLL	FLW	FLA	FLPNL	AWL	PNSPL	SPA
PH	1.0000								
CG	0.0320	1.0000							
FLL	0.0933	-0.1625	1.0000						
FLW	0.2535	-0.0644	-0.1284	1.0000					
FLA	0.4609*	0.1797	0.1338	0.1552	1.0000				
FLPNL	-0.0027	0.6072*	-0.3365*	-0.2186	-0.3207*	1.0000			
AWL	0.2919*	0.1263	0.1797	-0.3186*	0.3334*	-0.2530	1.0000		
PNSPL	0.3197*	0.0577	0.2626	0.0816	0.3689*	-0.2974*	0.5155*	1.0000	
SPA	0.0659	0.2023	-0.0348	-0.1160	0.1131	0.1991	0.1100	0.0513	1.0000

*p < 0.05

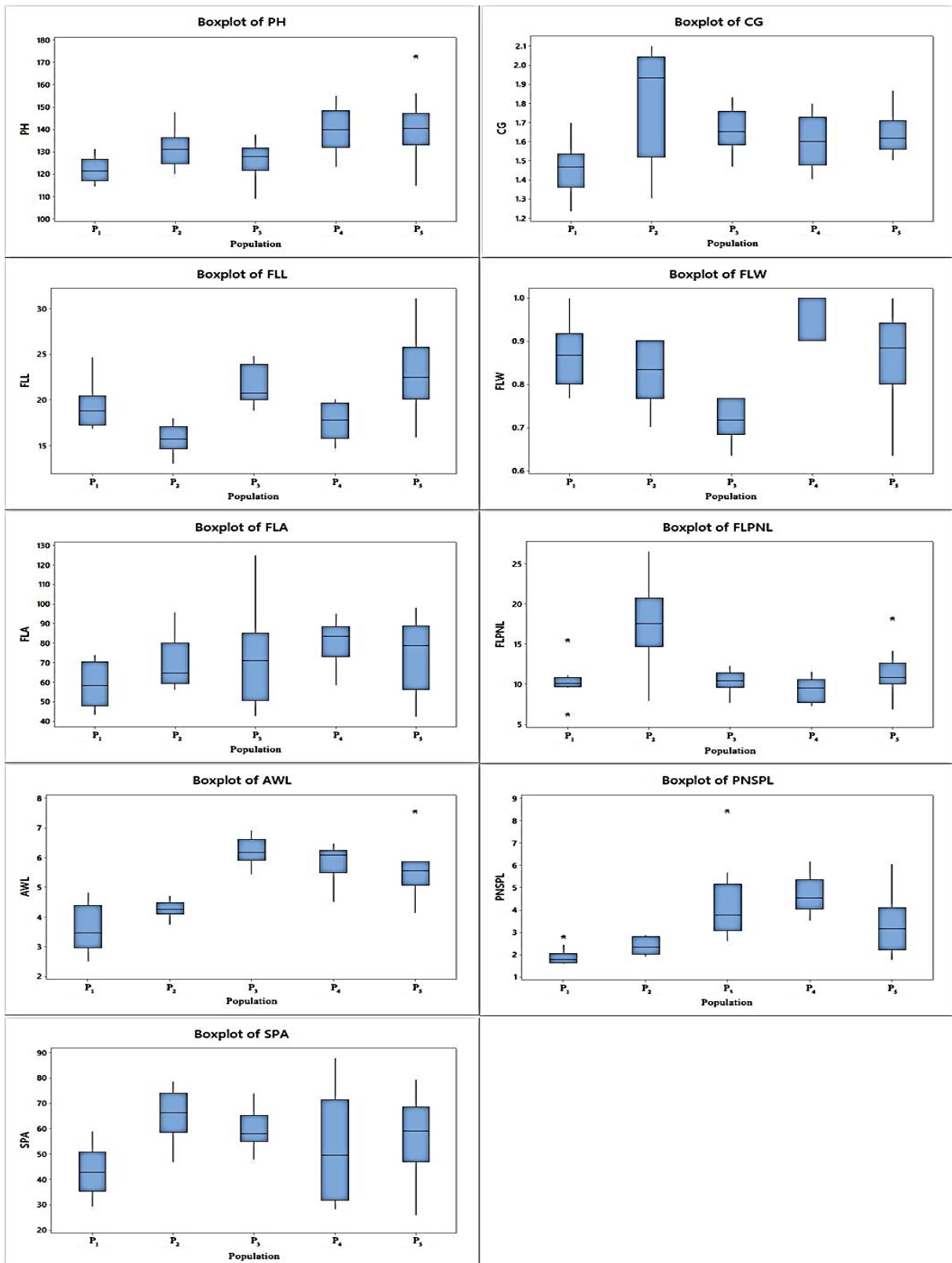


Figure 2: Variation of each morphological trait in different populations. Outliers are marked by stars (*). The size of each boxplot represents degree of variation in each population with respect to each trait. Upper and lower horizontal lines around median line shows IQR range. Vertical lines represent the whiskers which shows the minimum and maximum data distribution without be an outlier.

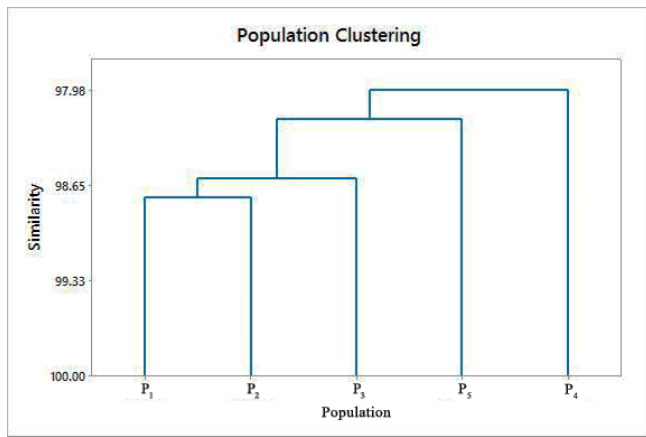


Figure 3. A dendrogram shows the morphological relationship among the five populations of *O. rufipogon* in Sri Lanka.

Relationship Among Morphological Traits

The correlation between parameters that was estimated using the Pearson correlation matrix is presented in Table 4. The majority of the correlations were statistically significant ($p < 0.05$) according to the degree and direction (positive or negative) of the correlation values. A strong positive correlation (>0.3) was observed in PH with FLA and PNSPL, and CG with FLPNL and PNSPL with FLA and AWL while a strong negative correlation (>0.3) was observed in FLPNL with FLL and FLA.

Correlation is a measure of the degree to which variables differ together or a measure of the intensity of the relationship. The efficiency of selection for yield mostly depends on the direction and magnitude of the relationship between yield and its components and among themselves. In the present investigation, the correlation analysis indicated that the FLPNL was positively correlated with culm girth. Plant height showed positive and significant correlation with FLA, AWL and PNSPL. The FLL had a significant and negative association with FLPNL. This information can be used to recognize phenotypically different sources for traits of interest in breeding programs.

Identification of Closely Related Populations

The results indicated that the five main clusters are at a similarity level of 99.22, showing the diversely related populations with a high identity based on higher similarity values (Figure 3). In addition, P1 and P2 populations grouped together (similarity level = 98.73) forming the first cluster. Most interestingly P4 population was clustered separately with minimum similarity level. The results indicated that the four main clusters at a similarity level of 98.73, showing the most closely related populations with a high identity based on higher similarity values (Figure 3). The second, third and fourth clusters consisted of P3, P5 and P4 populations, respectively.

Populations from the different natural habitats (P1 and P2) clustered together (Table 1). Though P1 belongs to the

intermediate zone, morphological variations of these two populations were closely related to each other. The P4 population separated from all populations having the high FLA and the FLW as a result of adapting to its natural habitat. In addition P3 and P5 populations were clearly separated from others (Figure 3).

Habitat specificity-driven morphological adaptation of different *O. rufipogon* populations plays a critical role in conservation and sustainable utilization of gene pool. In view of conservation of *O. rufipogon*, results of this study indicated that four out of five populations should be subjected for attentive while P3, P5 and P4 and one population from P1 and P2 can be used for conservation. This result suggests a larger influence of ecological factors (compared to geographical) on the morphology of species. *O. rufipogon* has possibly undergone and are still undergoing ecological speciation (Banaticla-Hilario et al., 2013). In general, *O. rufipogon* is found in relatively stable habitats even though *O. nivara* occupies disturbed habitats (Kariali et al., 2008). Fluctuating environmental conditions may have influenced *O. rufipogon* to adapt to different geographic regions in Sri Lanka even in closely related populations.

CONCLUSION

The present study focused on the genotypic diversity of *Oryza rufipogon* in natural wild rice populations in Sri Lanka. Flag leaf length and awn length were the most variable traits while plant height, flag leaf angle, flag leaf panicle neck length and spikelet angle were the least variable traits. Increasing desirable traits are also possible using the trait from appropriate wild species. Likewise, some of the identified wild rice (*O. rufipogon*) populations with beneficial characters could be of direct interest for desirable traits. Some *O. rufipogon* populations grouped in the same cluster along with indicating close genetic relatedness. According to efficient conservation strategies, this study indicated that one population can be conserved instead of two populations that are representing in the same cluster. Information concerning the morphological variation of *O. rufipogon* that has been generated from this study reflects a baseline study in understanding its diversity in Sri Lanka. This result suggests a larger influence of ecological factors on the morphology of populations. All these results clearly indicate that the existing variations amongst the morphological traits of Sri Lankan wild rice (*O. rufipogon*) suggest that they can be used to distinguish it from weedy rice and cultivated rice. Moreover morphological variations are a reflection of highlighting genetic diversity which fulfils the knowledge gap regarding overall morphological variations of *O. rufipogon* populations in Sri Lanka with proper sampling strategies. Therefore, the genetic diversity information obtained will be useful in the efficient use of Sri Lankan wild rice germplasm collection. Moreover, this information will be useful in the management of *O. rufipogon* populations to design conservation strategies and the potentials of using particular population based on breeding objectives.

ACKNOWLEDGEMENTS

This research was financially supported by the National Research Council (Grant No. NRC 15-108) and National Science Foundation of Sri Lanka.

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