

RESEARCH ARTICLE

Within variety flowering time variation leads to yield variation in Sri Lankan traditional rice “*Sudu wee*”

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Abstract: Sri Lankan traditional rice is an important breeding resource for development of rice for ecological adaptation during the era of climate change. There are around 2000 accessions belonging to more than 500 varieties comprised of one to several accessions in each with wider morphological differences, flowering times and yield variations. Genetic identity of Sri Lankan traditional rice accessions belonging to each rice variety is not established at molecular level. Variety *Sudu wee* comprises of around 30 accessions at PGRC. *Sudu wee* could be useful in re-introduction programmes of traditional rice due to relatively shorter crop duration. The objective of the present study was to evaluate the diversity of 29 *Sudu wee* accessions from PGRC, based on days to flowering, (DF), 12 morphological and 2 physiological characters during non-inductive photoperiod season. DF varied from 62 to 106 days while accession 4193 did not flower during the experimental period of nearly 7 months. Leaf temperature at heading (LeT) and Leaf temperature difference at heading (LeTD), vegetative morphological characters and yield components varied among *Sudu wee* accessions. Seventy nine percent of total observed variation among accessions was explained through Principal Component Analysis. In the dendrogram of accessions, 10 clusters were formed at the rescale distance of 5. Seed morphology indicated the genetic similarity among accessions while significantly different DF could be an indication of flowering time gene variation among similar genotypes. Increased DF reduced the grain yield increasing the vegetative growth among accessions of *Sudu wee*. Our results would be useful in future breeding for manipulating flowering time and yield within similar genetic backgrounds.

Keywords: days to flowering variation, morphology, Sri Lankan rice, *Sudu wee*, yield.

INTRODUCTION

Sri Lanka is an island in the tropics consisting of 46 agro-ecological sub regions based on the natural altitude, latitude, elevation, temperature and rainfall which may contribute to the diversity of approximately 2000 accessions of Sri Lankan traditional rice (*Oryza sativa indica*) collection in terms of days to flowering (DF), plant architecture, yield and photoperiod responsiveness (Irangani and Shiratake, 2013). The farmer varieties from different agro-ecological sub regions of Sri Lanka had been explored in 1960's and conserved at IRRI gene bank (Personal communication). In 1980s, Plant Genetic Resources Centre (PGRC), Sri Lanka, received a subset of the above collection. There are around 500 varieties in the collection while most of the varieties contain more than one accession. Our research group had characterized 755 accessions belonging to several varieties to be used as a breeding resource for manipulation of photoperiod responsiveness, nutrient quality and abiotic stress tolerance in the future: Using 384 accessions belonging to 55 varieties from above, a mini-core collection was developed for flowering time variation (Rathnathunga *et al.*, 2016b). Among the several Sri Lankan rice varieties incorporated in breeding programmes at IRRI, submergence tolerance had been achieved through *sub1A-2* gene of Sri Lankan rice *James wee* (Singh *et al.*, 2010). With the introduction of new improved varieties from IRRI in 1960's, Sri Lankan traditional rice had been withdrawn from regular farmer fields. Desirable characters including short plant height, photoperiod irresponsiveness, non-logging and higher yields led the shift of farmer choice towards new improved rice varieties. However, undesirable

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agronomic characters are not always a unique feature in traditional rice (Team of NRC 12-129, 2014; Team of NRC 12-129, 2015a; Team of NRC 12-129, 2015b).

Current Sri Lankan traditional rice cultivation is around 0.1% from total cultivated land area, which covers about 1,000 ha annually (Wang *et al.*, 2012). Attempting the re-introduction of traditional rice to farmer fields as a component in organic agriculture mainly, several traditional rice farming and introductory projects were conducted in Sri Lanka through the Department of Agriculture and other organizations (Berger, 2009; Dharmasena, 2010; Fisher and Lucy, 2012; Vanlanka Community Foundation, 2014).

Though rice is the major crop in Sri Lanka in terms of the extent of land and consumption, due to unfavourable climatic and environmental conditions and reduction of land use, the rice production has been adversely affected (Bambaradeniya and Amerasinghe, 2004). Therefore, it is a necessity to increase the rice production by enhancing the genetic diversity through breeding new varieties to meet future challenges including climate change (Yoshida, 1983). Photoperiod is a major environmental factor which affects the growth and architecture of rice plants. This could be a major determinant for date fixed (Chandrarathna, 1964); period-fixed (DF not affected by sowing date) and date fixed (DF affected by sowing date) varieties widely contributed to the flowering time variations of Sri Lankan rice germplasm. Chandrarathna (1954) concluded that *Heenati*, *Vellaiilankalaya* and *Devaraddari* as period-fixed varieties and *Kohu ma wee* as a date-fixed variety. *Sudu wee* had been considered as short aged variety in farmer fields. According to our unpublished data, *Sudu wee* accessions are date fixed. We observed photoperiod and temperature sensitivity of most *Sudu wee* accessions at early vegetative stage and at flower initiation (unpublished data). Hence, characterization of *Sudu wee* accessions would be useful to assess the diversity among accessions on DF and morphological variations in order to determine the effect of DF variations on yield.

MATERIALS AND METHODS

Rice Accessions

In 1960's Sri Lankan traditional rice germplasm had been explored, collected and were deposited in IRRI gene bank. Later, a replica of the whole collection was brought back to Sri Lanka. However, as passport data are not completely available at the Plant Genetic Resource Centre (PGRC), the exact exploration sites are not known. During the collection, a farmer variety with very similar characters from different geographical locations of Sri Lanka was collected and preserved at PGRC under same variety name with different accession numbers. Twenty nine accessions of Sri Lankan traditional rice variety *Sudu wee* (Accession numbers: 3227, 3462, 3469, 3510, 3512, 3790, 3857, 3858, 3860, 3915, 3927, 3999, 4193, 4194, 4195, 4594, 4801, 4963, 5675, 5676, 6338, 6340, 6341, 6342, 6343, 6344, 6345, 6680 and 6705) were obtained from the PGRC, Sri Lanka.

Field experiment

Sudu wee accessions were grown at the Rice Research and Development Institute, Batalagoda, Sri Lanka (located in agro-ecological zone, IL1 of latitude 7° 29' 12" N and longitude 80° 21' 53" E with a height of 137 m above mean sea level). Average temperature during the cropping season was around 32°C and the soil was dark brown earth (DBE). Each replicate consisted of a plot of 9 plants in 3 rows: 20 cm × 20 cm within and between rows and 40 cm between plots in a Complete Randomized Design (CRD) with 4 replicates. The experiment was carried out in late short day (SD) season (*Maha*), from December, 2012 to long day (LD) season (*Yala*) till July, 2013. The seeds were sown in a nursery bed in December, 2012 with 15 cm spacing in between each accession. Seedlings were transplanted after 21-days. Fertilizer application, pest and disease management and weed control were carried out according to the recommendation by the Department of Agriculture, Sri Lanka. The basal dressing of urea, TSP and MOP (of 50, 62.5 and 50 kg/ ha) was applied during the land preparation. Top dressings of 61.75, 123.5 and 123.5 kg/ ha of urea were applied at 2 weeks, 5 weeks and 7 weeks of planting, respectively. Manual weeding was done at regular intervals and the competition from weeds was kept minimal. Approximately permanent standing

water level of 5 cm was maintained throughout the experiment.

Evaluation of morphological traits

Following characters were measured from each replicate from each accession: Days to Flowering (DF), Plant height at maturity (PH), Culm length at maturity (CL), Culm number at maturity (CN), Culm diameter at maturity (CD), Panicle length at maturity (PL) Grain length at maturity (GL), Grain width at maturity (GW), Leaf number at maturity (LN), Leaf length at maturity (LL), Leaf width at maturity (LW), Leaf temperature at heading (LeT), Leaf temperature difference at heading (LeTD), Shoot weight at maturity (SW) and Panicle weight at maturity (PW). LeT was measured using a Leaf Temperature meter (Specturm technologies. Inc., Australia) and LeTD was calculated by subtracting LeT from the ambient temperature. Dry weights of SW and PW were measured at 14% moisture level. With respective to each character in a given accession, average value of replicates was considered for analysis. Measurement techniques were according to modified descriptors of rice published by the Team of NRC research project 12-129 (2014) which was based on IBPGR-IRRI Rice Advisory Committee (1980), Bioversity International, IRRI and WARDA (2007) and PGRC (1999).

Statistical analysis

Data were analyzed using PCA with correlation matrix through SPSS software (version 20) to define the patterns of variation between all explanatory variables. Grouping of variables into PCs was noted and thereby the dimension of the data set reduced. The 14 accessions which flowered during the experimental period of 200 days were clustered using Hierarchical Cluster Analysis through SPSS software. The closely related accessions were grouped and sorted into clusters, using the first four PC scores. Measure of dissimilarity was given by the Euclidean distance and the clustering method was Ward's linkage. The number of clusters was determined at the rescaled distance of 5. A regression analysis was carried out to evaluate the significant correlation between DF and other quantitative morphological traits using Minitab software (version 14)

RESULTS AND DISCUSSION

Flowering time and morphological variation among accessions of Sri Lankan traditional rice variety *Sudu wee*

This experiment was carried out at the onset of non-inductive photoperiod season to exploit the maximum flowering variation due to photoperiod sensitivity. Sri Lankan traditional rice is known to be photoperiod sensitive where highly short-day sensitive varieties such as *Ma wee* are grown only during short -day season (*Maha*) (Chandraratne, 1964; Irangani and Shiratake, 2013; Team of NRC 12-129, 2015b). Days to flowering (DF) among 29 *Sudu wee* accessions varied from 62 - 106 days except for accession 4193 which did not flower during the experimental period (Table 1). Flowering time range in *Sudu wee* was comparatively narrow in contrast to 56 to 200+ days among 16 accessions of *Hondarawala* variety and 57 - 179 days among 12 accessions of *Sulai* variety (Rathnathunga and Geekiyanage, 2015 and Rathnathunga *et al.*, 2016a). Flowering time variations during late short day season towards day neutral to long day season could be due to the presence of date fixed forms explained by Chandrarathna (1964). Variations in physiological characters, morphological characters and yield components among *Sudu wee* accessions, narrowed within clusters of dendrogram at rescale distance of 5 (Table 1 and Table 2).

Relationship between days to flowering on vegetative growth and yield components

There were positive correlations between DF and quantitative morphological characters of PH, LL and CD and a negative correlation between PW (Table 3). Similar observations were reported for *Sulai* variety where positive correlations were present between DF and vegetative growth characteristics (PH, CN, LN, LL and LW) (Rathnathunga and Geekiyanage, 2015). In a photoperiod experiment with *Sulai*, *Kohu mawee* and *Deveraddili*, the yield was reduced under LD conditions where flowering was delayed in contrast to SD conditions (Geekiyanage *et al.*, 2012).

Table 1: Descriptive statistical explanation of the variation of quantitative characters among the accessions of *Sudu wee*.

Character	Unit	Range	Average	Standard deviation
DF	days	62 – 106 *	78.8	10.6
LN	number	15 – 94	47.2	18.4
LL	cm	38.9 - 70.3	53.0	8.8
LW	cm	9.6 - 15.2	12.0	1.6
LiL	cm	0.5 - 5.4	1.6	0.9
PH	cm	83.8 - 167.0	139.4	21.1
CL	cm	50.8 - 143.7	109.3	21.6
CN	number	8 - 25	13.9	4.3
CD	cm	0.4 - 0.8	0.5	0.1
PL	cm	13.8 - 30.6	24.4	4.2
GL	cm	0.6 - 0.9	0.8	0.1
GW	cm	0.3 - 0.4	0.3	0.0
SW	g	38.9 - 229.9	131.4	53.0
PW	g	3.9 - 59.9	30.9	15.3

* Except accession number 4193 which did not flowered during the experimental period of 200 days.

The regression analysis of *Sudu wee* accessions indicated significantly positive correlations between DF with that of PH, LL and CD ($p = 0.022$, $p = 0.004$, $p = 0.033$) and a significantly negative correlation between DF and PW ($p = 0.015$) (Table 4 and Figure 2). A similar trend had been observed among *Hondarawala* accessions in a previous study (Rathnathunga *et al.*, 2013). Increased DF reduces the grain yield and increases the vegetative growth among accessions of *Sudu wee*, *Sulai*, *Kohu ma wee* and *Deveraddili* emphasizing the positive relationship between flowering time and vegetative growth and negative relationship between flowering time and yield in Sri Lankan traditional rice *Oryza sativa indica* in general.

Principal Component Analysis and Cluster Analysis revealed the variation in DF among *Sudu wee* accessions

First four Principal components (PC) explained 78.9% of total observed variation. PH, CL, LL and SW included in PC1. DF, LL, PL and PW were included in PC2 while LeT, LeTD, LW and GW were included in PC3. CN, LN LW and SW were included in PC4. The PC1, PC2, PC3 and

PC4 explained 21.3%, 20.5%, 20.1% and 17.0% of variance, respectively.

According to the hierarchical cluster analysis, 3 major clusters were identified at the rescale distance of 15, which were further separated into 10 clusters at rescaled distance of 5 indicating similar DF groups in most clusters (Figure 1 and Table 2). Meanwhile, within cluster variations in DF in clusters 1, 2, 3, 8 and 9 may represent the variations in flowering time gene within potentially similar genetic backgrounds. DF variation was evident among accessions of different clusters. According to the seed morphology, which is a common feature in determination of varietal purity, similar genetic backgrounds of accessions are indicated (Figure 3). Cluster 4 consists of accessions 5676, 5675 and 3790 that may have similar genetic background for flowering time genes while their other genes may be different as evident in variation in morphology. Molecular characterization on genetic basis for flowering time variation in *Sudu wee* would provide more precise information on pleiotropic effect of flowering time on vegetative growth and yield.

Table 2: Variation of quantitative morphological characters of *Suduwee* within clusters which were derived through Ward's linkage method.

Cluster	PGRC	DF	LeT	LN	LL	LW	PH	CL	CN	CD	GL	GW	PL	SW	PW
1	4195	76 b	26.67	40.5	52.0	15.2	138.5	115.5	8.5	0.65	0.70	0.35	17.00	123.80	36.58
	3510	83 a	27.33	40.3	49.0	13.8	125.8	107.0	8.8	0.50	0.88	0.38	16.75	76.70	31.00
	4194	79 b	30.00	50.8	56.5	14.8	154.3	143.7	8.3	0.55	0.70	0.35	15.50	112.85	40.13
	3512	84 a	27.67	34.8	63.4	12.8	166.3	140.5	9.0	0.65	0.83	0.40	17.00	133.05	39.80
2	6705	91 a	30.33	22.3	61.0	12	162.8	140.8	12.5	0.65	0.80	0.30	20.50	186.38	41.80
	6345	82 b	31.33	40.8	62.0	11.2	162.5	131.2	8.8	0.78	0.80	0.33	20.15	194.33	42.68
	3858	80 b	32.67	23.5	69.4	13.4	151.0	112.5	13.5	0.50	0.83	0.30	16.00	142.55	42.45
	3999	75 c	31.67	37.3	58.1	12.8	154.8	127.5	9.3	0.35	0.80	0.30	14.75	123.63	24.03
	6343	79 b	32.33	40.3	45.8	12.6	148.3	121.8	8.3	0.80	0.68	0.38	13.25	104.73	27.43
3	3227	90 a	29.33	52.0	53.7	10.4	157.3	113.3	18.3	0.50	0.70	0.40	20.50	166.15	3.93
	3462	106 b	30.67	61.8	59.2	11.6	167.0	114.5	16.0	0.60	0.88	0.30	28.50	228.33	13.03
4	5676	90 a	29.33	22.8	58.9	11.6	156.0	121.5	16.3	0.70	0.90	0.30	22.95	54.93	12.30
	5675	88 a	28.00	63.8	59.1	13.6	146.0	96.5	14.5	0.40	0.68	0.33	20.73	48.90	5.03
	3790	90 a	30.00	48.0	58.4	10	144.4	81.4	20.5	0.60	0.78	0.30	21.38	74.50	4.65
5	6344	79	30.67	19.0	70.3	12.8	132.0	87.5	11.5	0.65	0.75	0.30	19.25	38.85	8.90

Cluster	PGRC	DF	LeT	LN	LL	LW	PH	CL	CN	CD	GL	GW	PL	SW	PW
6	6341	68 a	30.33	32.0	38.9	12.6	101.0	77.8	10.5	0.35	0.70	0.38	16.75	120.38	33.80
	4801	68 a	26.67	59.5	40.4	12.6	100.3	78.1	13.0	0.45	0.78	0.35	13.83	83.18	31.13
7	4594	91	26.33	44.3	42.5	13.8	83.8	50.8	13.3	0.45	0.80	0.30	20.00	49.83	9.95
8	6338	64 b	31.00	52.5	47.2	10.4	134.5	113.9	14.3	0.35	0.70	0.33	17.43	123.03	33.05
	6340	62 b	31.00	51.3	42.0	9.8	130.5	107.0	16.0	0.55	0.60	0.30	16.50	124.38	28.40
	3927	69 a	33.00	75.3	44.1	9.8	122.3	96.6	12.5	0.45	0.7	0.3	15.03	125.93	49.58
	6342	65 b	32.33	15	48.1	9.6	127.3	99.3	20.5	0.45	0.80	0.30	16.05	152.70	40.13
9	6680	77 b	30.00	53.3	55.0	13	129.3	107.3	13.8	0.50	0.85	0.30	16.00	185.98	46.50
	3469	70 c	28.67	55.0	51.5	10.2	153.5	124.5	16.3	0.60	0.70	0.30	15.25	193.38	31.70
	3857	80 b	29.00	56.0	60.7	12.4	163.0	135.6	19.8	0.65	0.78	0.30	17.00	151.60	44.88
	4963	85 a	30.00	61.3	48.8	12.6	137.4	114.7	18.0	0.75	0.75	0.40	18.20	183.48	59.88
	3915	71 c	30.33	75.0	41.2	10.2	128.0	103.8	13.8	0.50	0.78	0.35	17.68	145.20	38.65
10	3860	63	31.00	93.5	45.7	10	125.3	97.5	24.8	0.45	0.60	0.30	18.85	229.93	44.50

Note: Different letters indicate the significant differences among DF within each cluster at rescale distance of 5, which are not for comparison of DF among clusters.

Table 3: Correlation among the quantitative characters within accessions of *Sudu wee*.

DF	LN	LL	LW	LiL	PH	CL	CN	CD	PL	GL	GW	SW	PW	LeT	
LN	-0.185 0.347														
LL	0.525 0.004*	-0.437 0.020*													
LW	0.320 0.097	-0.280 0.149	0.266 0.171												
LiL	-0.148 0.451	-0.096 0.627	0.046 0.818	0.412 0.029*											
PH	0.431 0.022*	-0.156 0.429	0.713 0.000*	-0.034 0.862	0.129 0.514										
CL	0.127 0.520	-0.133 0.501	0.464 0.013*	0.080 0.684	0.306 0.113	0.855 0.000*									
CN	-0.062 0.753	0.363 0.058	-0.095 0.632	-0.613 0.001*	-0.261 0.180	-0.020 0.919	-0.219 0.262								
CD	0.403 0.033*	-0.210 0.282	0.376 0.048*	0.093 0.637	0.038 0.849	0.512 0.005*	0.460 0.014*	-0.108 0.583							
PL	-0.262 0.179	-0.018 0.928	0.192 0.329	0.017 0.930	0.308 0.111	0.338 0.078	0.412 0.029	0.052 0.793	0.384 0.044*						
GL	0.307 0.112	-0.134 0.495	0.096 0.625	-0.136 0.491	-0.165 0.402	0.070 0.725	0.012 0.950	0.067 0.736	0.086 0.665	-0.051 0.796					
GW	0.203 0.300	0.088 0.654	-0.162 0.411	0.340 0.077	0.146 0.458	0.021 0.917	0.123 0.532	-0.420 0.026*	0.211 0.282	0.008 0.969	-0.161 0.412				
SW	-0.056 0.776	0.334 0.082	0.003 0.986	-0.356 0.063	0.116 0.558	0.360 0.060	0.434 0.021*	0.318 0.099	0.142 0.472	0.295 0.127	-0.109 0.582	0.054 0.786			
PW	-0.456 0.015*	0.174 0.376	-0.173 0.380	0.001 0.996	0.233 0.234	-0.007 0.973	0.374 0.050*	-0.062 0.756	0.118 0.551	0.367 0.054	-0.184 0.348	0.214 0.275	0.536 0.003*		
LeT	-0.261 0.179	-0.056 0.776	0.104 0.600	-0.481 0.010*	-0.259 0.184	0.230 0.239	0.200 0.307	0.128 0.518	-0.005 0.979	0.115 0.560	-0.293 0.130	-0.145 0.461	0.341 0.076	0.266 0.171	
LeTD	0.261 0.179	0.056 0.776	-0.104 0.600	0.481 0.010*	0.259 0.184	-0.230 0.239	-0.200 0.307	-0.128 0.518	0.005 0.979	-0.115 0.560	0.293 0.130	0.145 0.461	-0.341 0.076	-0.266 0.171	-1.000 -

Cell Contents: Pearson correlation between the individual variables,* Significant at $p < 0.05$ (+) positive, (-) negative

Table 4: Regression analysis for quantitative characters of *Sudu wee* accessions.

	Character	The regression equation	R- sq	P
1	PH	PH = 71.96 + 0.8564 DF	18.60%	0.022
2	LN	LN = 72.41 - 0.3199 DF	3.40%	0.347
3	LL	LL = 18.79 + 0.4339 DF	27.60%	0.004
4	LW	LW = 8.167 + 0.04849 DF	10.20%	0.097
5	CL	CL = 89.01 + 0.2584 DF	1.60%	0.52
6	CN	CN = 15.97 - 0.02556 DF	0.40%	0.753
7	CD	CD = 0.1662 + 0.004864 DF	16.30%	0.033
8	PL	PL = 32.45 - 0.1028 DF	6.80%	0.179
9	GL	GL = 0.5816 + 0.002333 DF	9.40%	0.112
10	GW	GW = 0.2736 + 0.000712 DF	4.10%	0.3
11	SW	SW = 153.6 - 0.2820 DF	0.30%	0.776
12	PW	PW = 82.76 - 0.6582 DF	20.80%	0.015
13	LeT	LeT = 33.49 - 0.04536 DF	6.80%	0.179
14	LeTD	LeTD = 0.614 + 0.04536 DF	6.80%	0.179

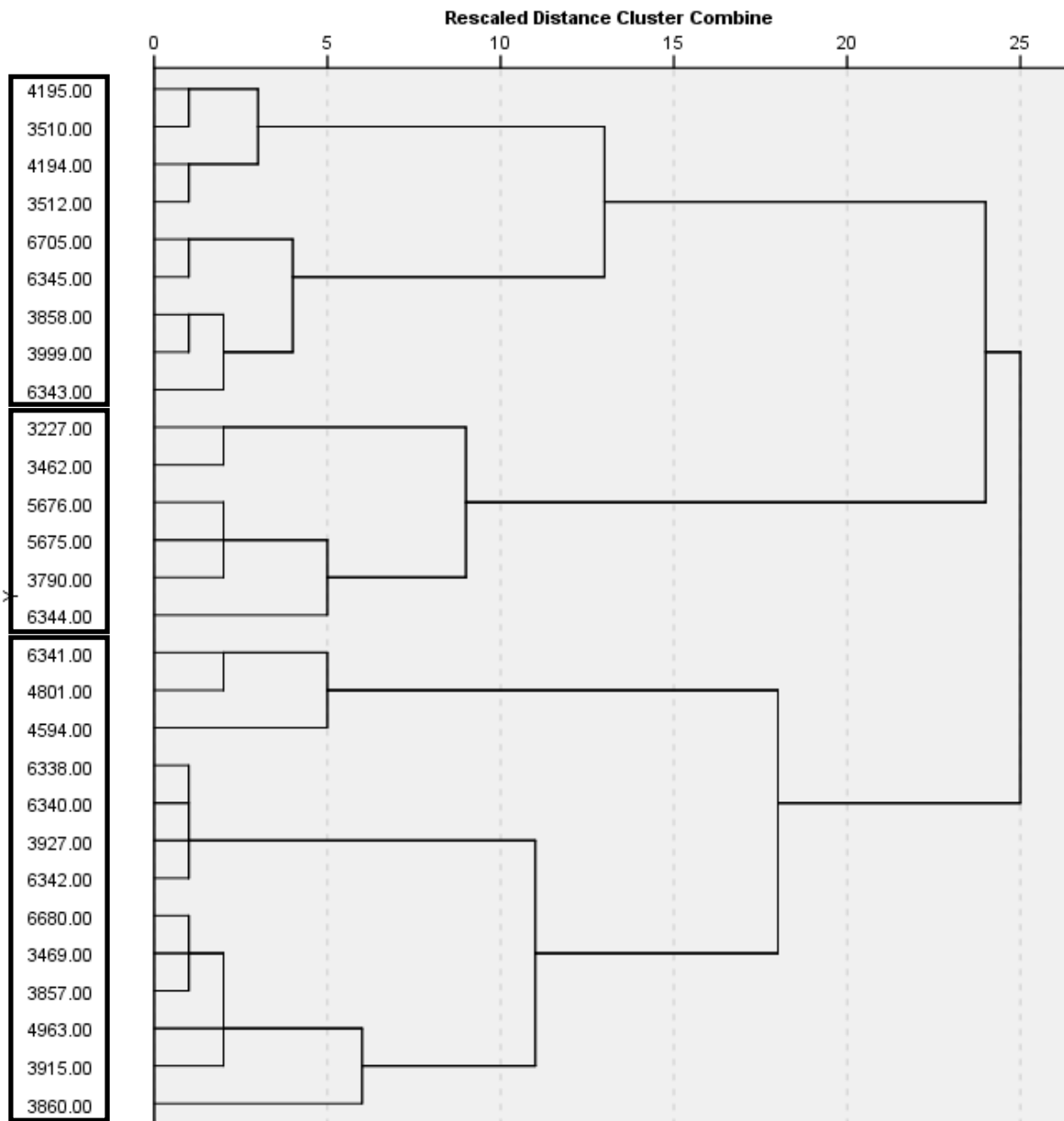


Figure 1: Dendrogram of *Sodu wee* accessions derived through Ward's linkage method of Cluster Analysis based on DF, 12 morphological and 2 physiological characters.

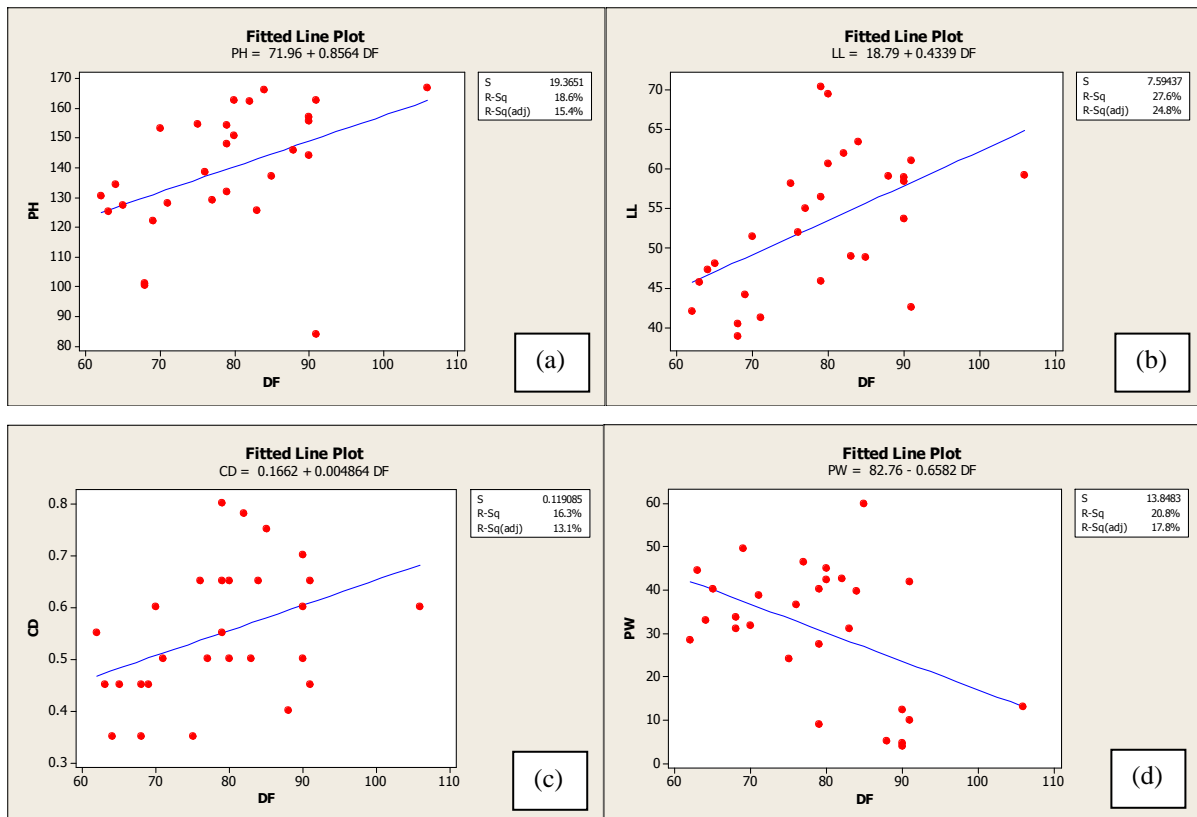


Figure 2: Regression analysis for relationships of (a) DF and PH, (b) DF and LL, (c) DF and CD and (d) DF and PW for the *Sudu wee* accessions.



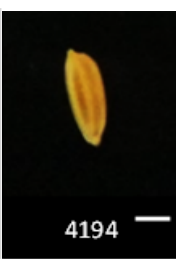




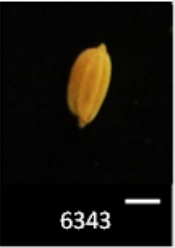







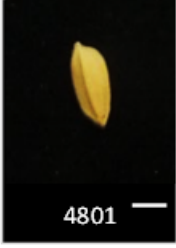
Cluster	Accession	DF	Seed morphology				
1	4195	76 b					
	3510	83 a					
	4194	79 b					
	3512	84 a					
2	6705	91 a					
	6345	82 b					
	3858	80 b					
	3999	75 c					
	6343	79 b					
3	3227	90 a					
	3462	106 b					
4	5676	90 a					
	5675	88 a					
	3790	90 a					
5	6344	79					
6	6341	68 a					
	4801	68 a					

Figure 3 (a): Seed morphology of *Sudu wee* accessions as shown in the dendrogram. Different letters indicate the significant differences among accessions within each cluster at rescale distance of 5 which are not for comparison of Days to Flowering (DF) among clusters 1 to 6.

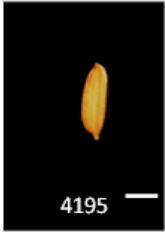




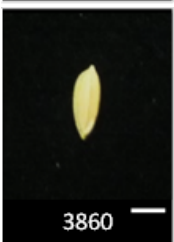
Cluster	Accession	DF	Seed morphology
7	4594	91	
8	6338	64 b	
	6340	62 b	
	3927	69 a	
	6342	65 b	
9	6680	77 b	
	3469	70 c	
	3857	80 b	
	4963	85 a	
	3915	71 c	
10	3860	63	

Figure 3 (b): Seed morphology of *Sudu wee* accessions as shown in the dendrogram. Different letters indicate the significant differences among accessions within each cluster at rescale distance of 5 which are not for comparison of Days to Flowering (DF) among clusters 7 to 10.

CONCLUSIONS

Accessions listed under *Sudu wee* at PGRC exhibited variations in morphological characters and flowering time during the experiment. Delayed flowering time increased the plant height and reduced the panicle weight.

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