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Short Communication

Clustering of Selected Sri Lankan Rice (*Oryza sativa* L.) Cultivars Based on Plant Architecture and Evaluation of Their Association to Relative Water Content under Drought

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ABSTRACT: *Certain rice cultivars when get exposed to drought, develop morphology that enables them to acquire drought tolerance. In addition, general morphology of a plant before its exposure to drought may also be a determinant of its final response to drought. Ten Sri Lankan rice cultivars were clustered according to ten morphological parameters under well-watered conditions and their associations to relative water content (RWC) under drought was assessed. The cluster analysis, and the component 1 of principal component (PC) analysis (mainly contributed by plant height (PH), leaf length (LL) and culm length (CL)), grouped the cultivars as traditional and newly-improved (exception Kalu Heenati). No significant ($p<0.05$) difference in RWC under drought was detected on 5^{th}, 12^{th} and 18^{th} day after imposing drought (DAID). When PC2 (mainly contributed by leaf width (LW) and leaf angle (LA)) and PC1 are considered together, the groups were significantly ($p<0.05$) different in RWC on the 12^{th} DAID. Hence, PH, leaf length (LL), CL, LW and LA before exposure to drought has an effect on the RWC of a plant under drought.*

Keywords: *Drought, plant architecture, relative water content, Sri Lankan rice cultivars*

INTRODUCTION

Rice (*Oryza sativa* L.) is the staple food of many Sri Lankans. In the dry-zone low lands, drought is a major limitation for the cultivation of rice (Peter, 2011; Chithranayana and Punyawardena, 2014). With increasing trends of occurrence, drought can fuel a food crisis in Sri Lanka in years to come. To produce rice amid drought prone conditions, rice plants that are tolerant to drought stress must be identified and be used as parents in plant breeding programs. Rice respond to drought in many ways such as avoidance, tolerance, escape, and recovery (Fang and Xiong, 2015), mostly by altering its morphology. Changes in morphology that are commonly associated with drought stress responses include, leaf rolling (Wopereis *et al*., 1996), leaf drying (Courtois *et al*., 2000), root elongation (Ekanayake *et al*., 1985), delay or complete absence of flowering (Saini and Westgate, 1999), and reduced panicle exertion (O'Toole and Namuco, 1983). The general leaf, root and flowering morphology of a rice cultivar may give it an added advantage in acquiring tolerance against

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drought over another. The cumulative effect of its general morphology and the morphology acquired as a result of drought responses, ultimately decide the overall response of the rice cultivar to drought stress. In the current study, selection of Sri Lankan rice cultivars was clustered based on ten morphological parameters taken at the vegetative stage under well-watered conditions. Further, the associations of these clusters to relative water content under drought were assessed.

METHODOLOGY

Planting Materials and Morphological Screening

Seeds of the rice cultivars At307, Bg300, At354, Bg359, Bg360, *Gonabaru* (accession no. 2056), *Yakada Wee* (accession no. 3445), *Kalu Heenati* (accession no. 2197), *Kuru wee* (accession no. 3254), and *Norukkan* (accession no. 3241) were collected from the Rice Research and Development Institute, Bathalagoda and Plant Genetic Resource Center, Gannoruwa, Sri Lanka. Five seedlings per each variety were transplanted in to a pot and plants were maintained under saturated water levels in a plant house at the University of Peradeniya. The morphological evaluation was carried out using the standard evaluation system (SES) developed by the International Rice Research Institution, Philippines (IRRI, 2002) for the traits basal leaf sheath color (BLSC), culm angle (CA), culm length (CL), culm number (CN), leaf angle (LA), leaf blade color (LBC), leaf blade pubescence (LBP), leaf length (LL), leaf width (LW) and plant height (PH).

Evaluation of Drought Stress

For the drought experiment four seedlings per each cultivar were transplanted and a cultivar was replicated twice. Plants were maintained under saturated water level until drought was imposed by draining the existing water and withholding watering when > 85% of the plants reached a four-leaf stage. Drought responses were assessed on the 5^{th}, 12^{th} and 18^{th} days after imposing drought (DAID) by measuring relative water content (RWC) and leaf dryness. The leaf dryness was measured according to the SES scale (IRRI, 2002), RWC was measured as described in Dasgupta (2015) and the RWC% was calculated according to Slayter (1967).

Data Analysis

The ten rice cultivars were grouped based on selected morphological parameters by carrying out a cluster analysis (CA) and a principal component analysis (PCA). The CA was performed using a complete linkage method with Euclidean distance in Minitab 17 (Minitab Inc, USA). The PCA was performed with a correlation matrix and visualized based on a score plot of the first and second order principal components (PC). The cultivars grouped based on the PC1 and PC2 and groups created considering both the first and second order PCs as positively and negatively, were tested for equal variance in RWC% measured on the 5^{th}, 12^{th} and 18^{th} DAID using SAS v9.1.4 (SAS Institute Inc, USA) and was subjected to an one-way analysis of variance (ANOVA) evaluated at a significance cutoff of $p < 0.05$. Mean separation was performed using Duncans' multiple range test. Further, an ANOVA was performed to evaluate the significance ($p < 0.05$) of RWC% within the cultivars irrespective of the PCA grouping and mean separation was carried out using the Duncans' multiple range test.

RESULTS AND DISCUSSION

Based on the principal component analysis, the morphological characters (BLSC, CL, LA, LBP, CA, CN, LBC, LL, LW and PH) clustered the cultivars in to four groups, with the first two components capturing nearly 65% of the total variation seen among the cultivars (Table 1). The remaining 35% of the variation was accounted by the remaining eight PCs. The morphological parameters PH, LL and CL are the major contributors to PC1 and LW and LA are the major contributors to PC2 (Table 1).

Table 1. Contribution of morphological parameters of ten rice cultivars for the estimation of the first four principal components under well-watered conditions

Variable	PC1	PC2	PC3
Plant height	0.452	0.221	-0.035
Leaf width	-0.257	0.448	-0.158
Culm length	0.431	0.07	-0.227
Leaf length	0.413	0.33	-0.124
Basal leaf sheath color	0.217	-0.188	0.637
Culm number	-0.014	0.364	0.385
Leaf angle	0.208	0.497	0.023
Leaf blade color	0.369	-0.151	-0.1
Leaf blade pubescence	-0.165	0.349	0.498
Culm angle	0.344	-0.278	0.307
Eigen value	***3.965***	***2.572***	***1.451***
Proportion	***0.396***	***0.257***	***0.145***
Cumulative proportion	***0.396***	***0.654***	***0.799***

Considering all morphological traits taken under well-watered conditions, the ten cultivars were clustered in to two groups based on 33.3% similarity (Figure 1). The grouping included the improved cultivars and the traditional landrace *Kalu Heenati* in one group, and the remaining traditional rice cultivars in the other. The PC1 divided the cultivars in to the same groups (A and C; B and D in Figure 2). The grouping based on PC2 was not reflective of whether or not the cultivars were traditional or newly improved. Thus, the parameters PH, LL and CL, the major contributors of PC1 were the most influential in grouping the cultivars as traditional and newly improved (including the exception *Kalu Heenati*). With the green revolution the breeding targets was to increase the yield of cultivars by altering the plant architecture to produce shorter, lodge resistant plants with multiple short culms (Yoshida, 1981). Given that PH, LL and CL, the major contributors of PC1 were key targets during the development of newly improved cultivars the observed grouping is expected.

Under drought conditions, the cultivars within these two groups (traditional cultivars and newly improved along with *Kalu Heenati*) showed no significant difference ($p < 0.05$) in RWC % on 5^{th}, 12^{th} and 18^{th} DAID. Hence, when considered exclusively, the parameters PH, LL and CL that loaded to PC1 did not create a significant variation in RWC % under drought.

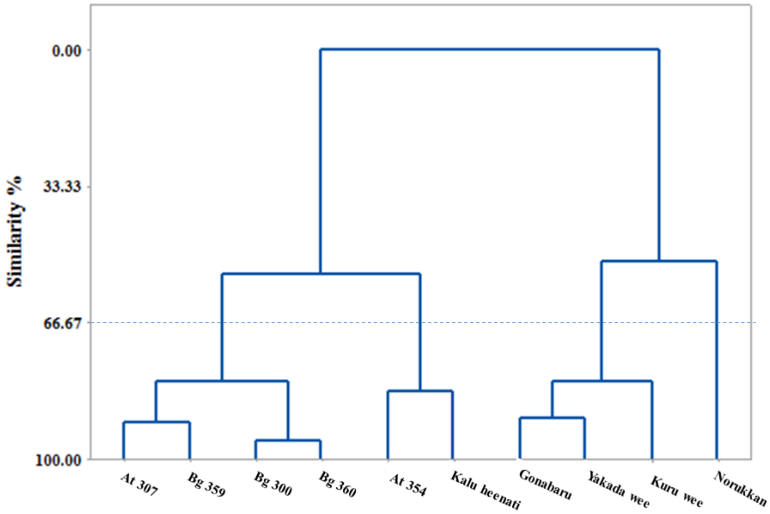

Figure1.Cluster analysis of ten Sri Lankan rice cultivars based on morphological parameters observed under well-watered conditions

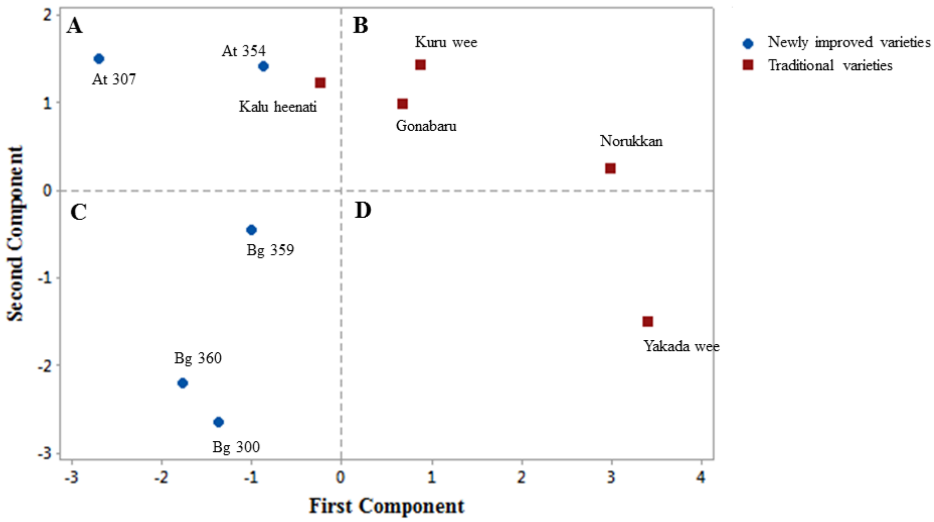

Figure 2. The score plot of principal component 1 and principal component 2. Four groups are indicated as A, B, C and D.

In the study, the drought conditions were imposed by withholding water. With time the soil loose water due to evapotranspiration. Based on the of soil moisture content measurements, even though the watering was withheld, the actual soil moisture content dropped to a low level (< 20 %) only beyond 8th DAID (data not shown). Hence, even though the plants where under drought treatment at 5th DAID, the plants were not physiologically experiencing drought as they had access to soil moisture (leaf drying observations based on SES 0 to 20%).

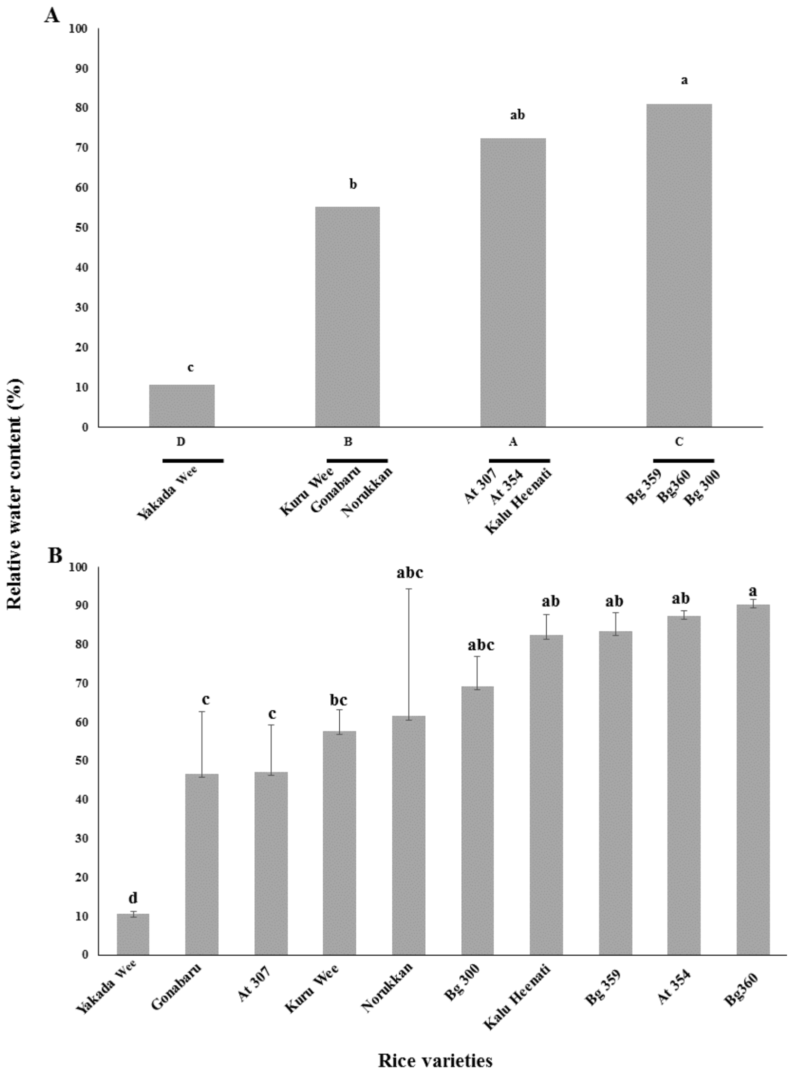

Figure 3. (A) Relative water content of groups A, B, C and D identified in principal component analysis at 12th day after imposing drought. (B) Variation of the relative water content in ten Sri Lankan rice cultivars under drought. The means with the same letters (a, b, and c) are not significantly different (p> 0.05).

As a result, no significant difference ($p > 0.05$) was revealed in the RWC % between the groups A, B, C and D on the 5th DAID. Similarly, no significant difference ($p > 0.05$) was seen between the groups at 18th DAID even though the plants were under severe water deficit. This could well be because at 18th DAID the plants belonging to all groups were very dry (leaf drying 80 to 100%) and thus, was beyond the stage that could show actual variations in RWC. However, at 12th DAID a significant difference ($p < 0.05$) was observed in the RWC % between the groups A, B, C and D as the plants were physiologically experiencing drought at a soil moisture level of approximately 15%. According to the mean separation (Figure 3A) the landrace *Yakada wee* (group D) was having a lower RWC compared to the cultivars in group C (Bg 359, Bg 300 and Bg 360), B (*Kuru wee*, *Gonabaru* and *Norukkan*) and group A (At 307, At 354 and *Kalu Heenati*).

While the RWC of group A was not significantly ($p < 0.05$) different from groups B and C, groups B and C was significantly ($p < 0.05$) different from each other (Figure 3A). Therefore, interestingly the traditional cultivars with the exception of *Kalu Heenati* was represented by the groups with the lowest RWC and the improved "Bg" rice cultivars were the best performers in terms of maintaining the RWC under drought conditions. However, the cultivats *Kalu Heenati*, At 354 and At 307 could not be resolved as clearly, as individually both *Kalu Heenati* and At 354 was not significantly ($p < 0.05$) different from the Bg cultivars. Further, At 307 was not significantly ($p < 0.05$) different from the remaining traditional cultivars (except *Kalu Heenati*) and Bg 300 (Figure 3B). Thus, it is possible that morphological parameters loaded to the PC1 and PC2 (PH, CL, LL, LW and LA) could create a significant variation in RWC% under drought around 12th DAID in the high RWC retaining groups (A, B and C; RWC >50%) and low RWC retaining group (D; RWC < 50%). However, among the high RWC retaining group groups B and C can be clearly separated group A cannot be separated with a clear-cut (Figure 3A). Thus, parameters such as PH, CL, LL, LW and LA that affect the plant architecture at vegetative stage under well-watered conditions is associated with the variations in RWC under drought around the 12th DAID.

CONCLUSIONS

The morphological parameters PH, LL and CL were the major contributors of clustering the selected cultivars as improved and traditional. In general, the traditional cultivars (except for *Kalu Heenati*) had low RWC compared to the improved "Bg" cultivars. The RWC% at 12th DAID reflected a significant difference among cultivar groups clustered based on plant morphological parameters PH, CL, LL, LW and LA. Vegetative parameters PH, CL, LL, LW and LA taken under well-watered conditions together can be used to identify the high-RWC retaining and low-RWC retaining cultivars under drought.

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