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Short Communication

Screening of Sri Lankan Rice (*Oryza sativa* L.) Landraces for Drought Tolerance

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ABSTRACT. *Twenty four (24) traditional Sri Lankan rice landraces were screened for drought tolerance at seedling stage in the greenhouse at the Open University of Sri Lanka, Nawala. Four week old seedlings grown in pots filled with soil were exposed to drought condition by disconnecting water supply for two weeks. Drought tolerance was evaluated by measuring plant height and leaf width after two weeks of recovery. Agro-morphological characterization of rice landraces was made on vegetative characters and yield attributed characters. The Simple Sequence Repeat (SSR) marker analysis was employed to determine the association between allelic diversity and drought tolerance of traditional rice landraces. Comparatively, the landraces Suwandal, Kirimurunga and Suduru samba showed tolerance to drought. Meanwhile, the SSR marker RM252 have shown the potential candidacy for tracing drought tolerance compared to other markers used in the study.*

Keywords: *Drought tolerance, SSR markers, Traditional rice landraces*

INTRODUCTION

Rice is the staple food in Sri Lanka and the total extent of cultivated rice is 34% of the arable land. Rice is cultivated during two seasons *Yala* (dry) and *Maha* (wet). *Yala* season experience longer dry conditions while *Maha* has comparatively shorter periods of drought. The extent of land cultivated under the *Yala* season is less than that of *Maha*. Further, climate condition of Sri Lanka is experiencing unexpected fluctuations of extended dry spells all over the island. Most of cultivated area (70%) lies in North, North Central, Eastern and Uva Provinces which are highly vulnerable to climate change (Somaratne and Dhanapala, 1996).These unexpected changes and the present trends of changing climate over the island threaten national food security.

The drought affects crop yield by interrupting floret initiation and grain filling in reproductive stage of the plant (Mostajeran and Rahimi-Eichi, 2009). The response of rice to drought depends on the developmental stage of canopy and alters water use efficacy by changing the leaf water potential leading to a high demand of transpiration water (Boonjung and Fukai, 1996). In addition, drought reduce nutrient uptake by lowering the absorption of inorganic nutrients (Farooq *et al*., 2009). Further, decreased availability of CO_2 limits the stomatal diffusion of CO_2, and as consequence, mesophyll cells lead to an alteration of photosynthesis due to drought stress (Chaves *et al*., 2009). The drought induce formation of

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the reactive oxygen species (ROS) including superoxide radical, hydroxyl free radical (.OH), hydrogen peroxide (H_2O_2) and atomic oxygen which cause peroxidation of lipids, denaturation of proteins, mutation of DNA causing various types of cellular oxidative damage bringing burnt leaves in the plant (Sharma and Dubey, 2005).

Studies show that Traditional agriculture practices coupled with native paddy varieties have proven to be more successful in facing climate change events such as droughts (Sharma and Rai, 2010). It has been reported that Sri Lankan rice cultivars consists of many abiotic and biotic stress tolerant traits with diverse agronomical characters (Ranawaka and Dahanayake, 2012). Cultivars showing these traits are of importance in breeding programs to produce drought tolerance rice cultivars in Sri Lanka.

The natural genetic variation of rice germplasm is often explored by subjecting the rice plant to stressed environments or by identification of quantitative trait loci (QTLs) which can subsequently be used with marker-assisted selection (De Costa *et al*., 2012). The markers such as SSR are used in breeding for the identification and/or confirmations of traits of importance. These technologies minimize the difficulties in preparation of sample, time consuming processes and derived information are deliberately informative (Matin *et al*, 2012). Therefore, the objectives of the present study were to screen the drought tolerance traditional rice landraces by means of morphological characters and SSR markers, and selecting appropriate markers for occurrence of drought tolerance trait.

METHODOLOGY

Twenty four rice varieties obtained from Rice Research and Development Institute, Batalagoda, Sri Lanka, were used for the study. Among them, the rice cultivar *Dular* was indicated as a drought resistant variety (Hoque and Kobata, 1998). Others were traditional rice varieties in Sri Lanka. The experiments were carried out in a green house in The Open University of Sri Lanka, Nawala, Sri lanka.

Seeds were allowed to break dormancy at 50 ^{o}C for 5 days. Seed surface was sterilized by keeping them in 70% alcohol for 2 minutes. Seeds were washed thoroughly with distilled water and were dipped in 2% Clorox for about 30 minutes. Finally they were thoroughly washed with distilled water and kept in an incubator at 35 ^{o}C for seven days under dark conditions. Germinated seeds were planted in plastic buckets filled with soil collected from paddy fields, up to ¾ of the total depth according to Completely Randomized Design (CRD) with 3 replicates and 5 plants per replicate. From these plants, data of vegetative characters, yield and yield attributed characters were taken. These characters were shoot length (cm), number of tillers/plant, number of reproductive tillers/plant, panicle length (cm), number of spicklets/plant, number of fertile spicklets/plant, grain yield/plant (g) and hundred seed weight (g)/plant.

Dormancy broken seeds were sterilized and allowed to germinate. Experiment was conducted at the seeding stage. Plants were grown in two experiments under stress and non-stress conditions. Germinated seeds were planted in plastic buckets filled with homogenized soil up to ¾ of the total depth in a Completely Randomized Design with 3 replicates and 5 plants/replicate. Supplying of water was discontinued four weeks after planting. Two weeks after 80% of the plants were allowed to completely wither and plants were evaluated for drought resistance by measuring plant height and leaf-width of the recovered plants.

Seeds of rice landraces were grown in soil filled plastic trays for two weeks and leaves were harvested. Total genomic DNA was extracted using Quagen plant DNA extraction kit. Randomly selected four (4) drought-tolerances related SSR markers (Table 1) (*RM252*, *RM234*, *RM242* and *RM2125*) were used to screen rice varieties using extracted DNA from seedlings. PCR amplification reactions were done in 12.5 µl reaction volume. SSR markers were amplified by thermo cycler (Gradient Thermal Cycler-Life ECO TC-96/G/H(b)C. Bir/ferrotec) as follows: 35 cycles of 5 min initial denaturation at 95 ^oC, denaturation for 1 min at 95 ^oC, annealing for 1 min at 55 ^oC, extension for 2 min at 72 ^oC and final extension at 72 ^oC for 7 min. Amplified products were separated by 1% Agarose Gel Electrophoresis.

Table 1. Selected SSR markers for drought and salinity tolerance

Locus	Location	Sequence	Annealing Temperature (^0C)	Product size/range (bp)
RM252F	Chr.4	TTCGCTGACGTGATAGGTTG		
RM252R	Chr.4	ATGACTTGATCCCGAGAACG	55	216
RM234F	Chr.7	ACAGTATCCAAGGCCCTGG		156
RM234R	Chr.7	CACGTGAGACAAAGACGGAG	55	
RM242F	Chr.9	GGCCAACGTGTGTATGTCTC		225
RM22RR	Chr.9	TATATGCCAAGACGGATGGG	55	
RM 2125F	Chr.10	TACCTCCTAGCTTTACTTAT		72-123
RM2125R	Chr.10	ACTGATCTCTATCTCATTGT	55	

Source: Khowaja *et al*., (2009), Islam *et al*., (2012)

The summary of statistics such as mean and standard deviation were calculated for Agro-morphological measurements using SPSS PC Ver.23.

RESULTS AND DISCUSSION

The tested landraces showed significant variation with respect to agro morphological characters (Table 2). The shoot length ranged from 78.6 cm to 168.2 cm and among the tested cultivars five of them showed shoot length above 120cm while *Ma wee* was the tallest (168.2 cm) and *Kaluheenati* was the shortest (78.6cm). Highest number of tillers was observed in the cultivar *Halsudu wee* which is seven tillers but in majority of cultivars (62.5%) it was less than 5. The highest number of reproductive tillers was seen in cultivar *Rathuheenati*. The longest panicle, highest number of spicklets and fertile spicklets were seen in cultivar *Godaheenati*. However the highest 100 seed weight was observed in *Murungakayan.*

Table 2. Summary of the traits of rice landraces

Cultivar	Shoot length (cm)L	Number of tillers	Number of reproducti ve tillers	Panicle length (cm)	Number of spikelets	Number of fertile spikelets	Weight of 100 (g)
Kuruluthuda	129.7 (10.9)^a	5 (0.57)^b					
Ma wee	168.2 (1.2)^a	4 (0.33)^c					
Dular	121.8 (3.1)^b	3 (0.25)^c	1 (0.25)^b	15.8 (0.1)^b	39 (3.42)^b	31 (3.83)^b	1.835 (0.03)^b
Moddikuruppan	127.2 (10.0)^b	5 (0.33)^b					
Murungakayan	110.7 (4.9)^c	4 (0.57)^c	1.00	17.3 (0.1)^a	53 (2)^a	26 (4.5)^b	2.76 (0.006)^a
Suduheenati	112.5 (7.96)^c	5 (0.25)^b	1.00	14.6 (0.6)^b	32 (1.5)^b	15.00 (0.5)^c	1.496 (0.06)^b
Suwandal	115.6 (8.8)^c	3 (0.28)^c					
Dahanala	95.6 (2.7)^d	5 (0.37)^b	1.00	15.5 (0.7)^b	23 (0.91)^c	12.00 (0.23)^c	1.65 (0.02)^b
Halsudu wee	92.0 (3.9)^d	7 (0.50)^a	2 (0.37)^b	9.5 (0.4)^d	18 (1.43)^c	12 (0.4)^c	1.622 (0.09)^b
Handiran	108.6 (5.3)^c	3 (0.33)^c	1.00	13.8 (0.3)^b	33 (1.33)^b	21 (0.88)^c	2.087 (0.059)^a
Herathbanda	104.5 (4.5)^c	3 (0.28)^c	1.00	16.3 (0.2)^b	45 (2.4)^b	18 (1.20)^c	2.043 (0.063)^a
Kaluheenati	78.6 (4.1)^e	3 (0.51)^c	1.00	11.2 (0.7)^c	28 (5.97)^c	14 (2.29)^c	1.36 (0.13)^b
Rathuheenati	110 (6.4)^c	4 (0.88)^c	3 (0.5)^a	14.5 (0.8)^b	45(7.5)^b	18 (4)^c	1.635 (0.04)^b
Kirimurunga	112.3 (14.2)^c	5 (0.88)^b	1.00	14.4 (0.2)^b	20 (0.88)^c	10.00 (0.43)^d	2.19 (0.008)^a
Madael	89.9 (7.0)^d	3 (0.58)^c	1 (0.4)^b	15.3 (1.5)^b	30 (3.94)^c	17 (1.96)^c	1.722 (0.13)^b
Masura	110.9 (5.2)^c	3 (0.37)^c					
Pachchaperumal	98.2 (0.6)^d	4 (0.28)^c	2 (0.33)^b	13.6 (0.7)^b	29 (7.81)^c	14 (3)^c	1.403 (0.02)^b
Pokkali	115.1 (12.5)^c	5 (0.66)^b	2 (0.33)^b	13.1 (0.8)^b	30 (0.66)^c	18 (2.51)^c	1.951 (0.09)^b
Pokkaliyan	115.3 (14.3)^c	3 (0.33)^c	2 (0.5)^b	18.9 (0.2)^a	70 (6.5)^a	45 (5)^a	1.92 (0.03)^b
Suduru samba	95.3 (2.1)^d	4 (0.40)^c					
Godaheenati	130.8 (4.0)^a	5(1)^b	2.00 (0.6)^b	17.3 (0.6)^a	68 (18.9)^a	30 (3.17)^b	2.327 (0.127)^a
Madathawalu	92.9 (4.2)^d	3 (0.33)^c	1.00	11.5 (0.5)^c	44 (4)^b	16 (2.5)^c	1.705 (0129)^b
Behethheenati	98.7 (4.4)^d	7 (0.86)^a	1.00	12.9 (.09)^c	61 (21.5)^a	43 (17)^a	0.977 (0.022)^c
Rathsuwandal	97 (9.5)^d	4 (0.28)^c	2 (0.25)^b	15.4 (1.5)^b	66 (20.6)^a	38 (7.30)^a	0.910 (0.014)^c

The mean values are followed standard deviation within parenthesis. Means with different superscripts in the same column are significantly different ($p< 0.05$)

Means of shoot length and leaf width are shown graphically in Fig.1 and 2.Varieties such as *Murungakayan, Suwandal, Rathuheenati, Kirimurunga, Suduru samba* and *RathSuwandal* were able to regain shoot length compared to the control after a drought period (Fig.1). Certain varieties such as *Murungakayan, Suwandal* and *Kirimurunga* showed tolerance by surviving in the induced drought duration. Thus, the results indicate that *Murungakayan, Suwandal* and *Kirimurunga* possess the ability for drought tolerance and recovery after a drought period.

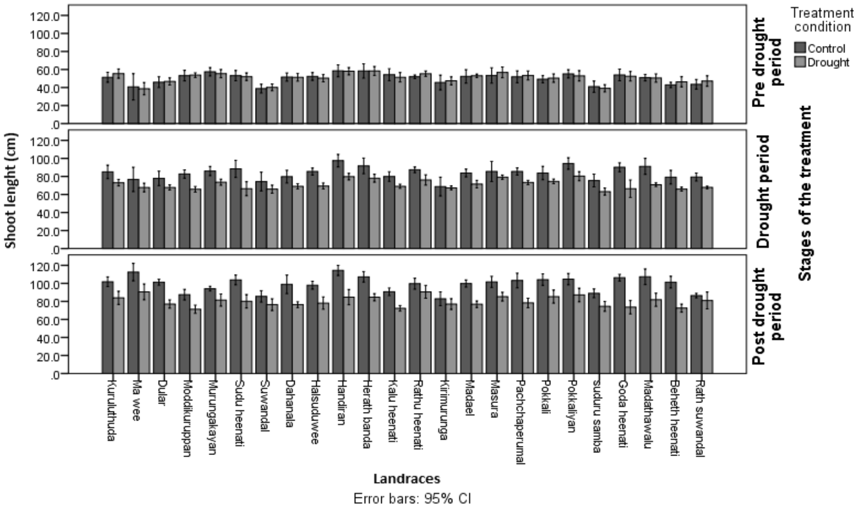

Fig. 1. The influence of drought on the shoot length of rice landraces under different water regimes

Similar approach can be used to describe the patterns in Fig.2. The cultivars such as *Ma wee, Suwandal, Kalu heenati, Kirimurunga, Pachchaperumal, Suduru samba* and *Beheth heenati*r egained to leaf width after the drought period compared to the control. Cultivars *Mawee* and *Kirimurunga* were able to regain the leaf width as in the control indicating tolerance to drought. This implies that *Mawee* and *Kirimurunga* both have tolerance to drought. However, the drought reference cultivar *Dular* showed different results in this study. Comparatively, certain local cultivars have showed better resistance and recovery potential than *Dular*.

Leaf width is reduced due to leaf rolling which reduce the transpiration during a drought stress. According to Rauf *et al*. (2016), this dehydration avoidance mechanism was brought by two possible ways by altering the leaf tissue structure. They are by bulliform cells which are adaxial cells in the epidermis of the leaf and large hypodermal cells shrinking due to loss of water or to a differential distribution of schlerenchyma cells. Stunting is apparently a temperature response to drought rather than an avoidance mechanism. Drought causes reduction of cell by losing cell water content and ultimately the growth of the plant (Hasanuzzaman*et al*., 2013). In the present study, regaining of shoot height and leaf width was observed. This indicates that the plant is readjusting to initial metabolic state after a drought period.

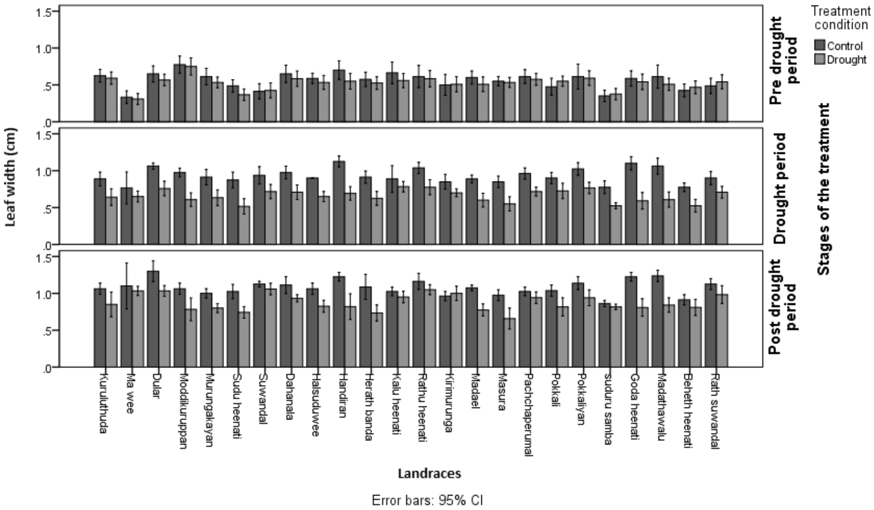

Fig. 2. The influence of drought on the leaf width of rice landraces under different water regimes

Certain cultivars were not represented in Fig. 1 and 2, which gave an indication of how cultivars responded to drought and how they recovered after a drought period. Despite the fact that, the cultivars *Murungakayan*, *Rathuheenati* and *Rathsuwandal* were able to regain the shoot length, they showed poor recovery in leaf width. However, the cultivars *Mawee, Kaluheenati*, *Pachchaperumal* and *Behethheenati* were poor in recovering shoot length. A cultivar which possesses both tolerance and recovery can be considered as an resistant variety to drought (Zulkarnain *et al*., 2009). Thus, according to the results of this study, *Suwandal*, *Kirimurunga* and *Suduru samba* could be identified as better varieties compared to *Dular*.

With reference to RM product size (216bp), only the *RM252* indicated bands in the agarose gel and the rest of the markers used in the study did not produce bands. In this study, it was observed that *RM252* could be considered as a potential marker for tracing the drought tolerance in rice landraces. However, the results of agro-morphological characterization and molecular analysis have indicated divisive findings. Even though, the land races *Halsudu wee, Handiran, Herath Banda, Madeal, Pokkali* and *Pokkaliyan* produced bands in the gel, they did not show morphological characteristics of drought tolerance (Fig. 3). However, the land races *Suwandal, Kirimurunga* and *Suduru samba* indicated a potential drought resistance while lacking any band patterns in the gel. The land races *Rathuheenati* and *Behethheenati* demonstrated drought tolerance, implying that these land races possibly possess drought resistance at the gene level. In addition to molecular and morphological evidences, anatomical and physiological evidences should seek the establishment of drought tolerance of rice landraces.

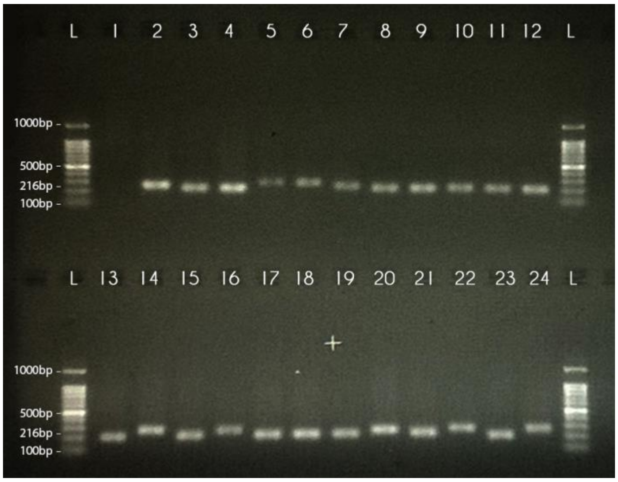

Fig. 3. DNA profile of the 24 rice land races with SSR marker RM 252

Legend: 1-Kuruluthuda, 2- Mawee, 3- Dular, 4- Moddikuruppan, 5- Murungakayan, 6- Suduheenati, 7- Suwandal, 8- Dahanala, 9- Halsuduwee, 10- Handiran, 11- Herathbanda, 12- Kaluheenati, 13- Rathuheenati, 14- Kirimurunga, 15- Madeal, 16- Masura, 17- Pachchaperumal, 18- Pokkali, 19-Pokkaliyan, 20- Suduru samba, 21-Godaheenati, 22- Madathawalu, 23- Behethheenati, 24- Rathsuwandal, L- Ladder DNA

CONCLUSIONS

The results of this study revealed that drought tolerance is directly associated with phenotypic variation and indirectly related to gene level manifestation. The landraces *Suwandal*, *Kirimurunga* and *Suduru samba* were found to be drought tolerant. The SSR marker *RM252* is a potential candidate as marker for tracing drought tolerance in rice landraces.

REFERENCES

Boonjung, B. and Fukai,S. (1996). Effects of soil water deficit at different growth stages on rice growth and yield under upland conditions. 1. Growth during drought. Field Crops Res. *48*, 37-45.

Chaves, M.M., Flexas, J. and Pinheiro, C. (2009). Photosynthesis under drought and salt stress: regulation mechanisms from whole plant to cell. Ann. Bot. *103*, 551 - 560.

De Costa, W.A.J.M., Wijeratne, M.A.D. and De Costa, D.M. (2012). Identification of Sri Lankan rice varieties having osmotic and ionic stress tolerance during the first phase of salinity stress. J.Nati. Sci. Found. Sri Lanka. *40,* 251-280.

Farooq, M., Wahid, A., Kobayashi, N., Fujita, D. and Basra, S.M.A. (2009). Plant drought stress: mechanisms and management. Agron.for Sustainable Development. *29,* 185-212.

Hasanuzzaman, M., Nahar, K., Alam, M., Roychowdhury, R. and Fujita, M. (2013). Physiological, Biochemical and molecular Mechanism of heat Stress Tolerance in Plant. Int. J. Molecular Sci. *14,* 9643-9684.

Hoque, M. and Kobata, T. (1998). Growth Responses of Drought Resistant Rice Cultivars to Soil Compaction under Irrigated and Succeeding Non-irrigated Conditions during the Vegetative Stage. Plant Prod. Sci. *1,* 183-190.

Islam, A.S.M.F., Ali, M.R., Gregorio, G.B. and Islam, M. R. (2012). Genetic diversity analysis of stress tolerant rice (*Oryza sativa* L.). African J. Biotech. *11,* 15123 - 15129.

Khowaja, F.S., Norton, G.J., Courtois, B. and Price, A.H. (2009). Improved resolution in the position of drought-related QTLs in a single mapping population of rice by meta-analysis http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2708188/

Matin, S., Ashrafuzzaman, M., Monirul Islam, M.D., Sikdar, S.U. and Zobayer, N. (2012). Molecular marker based (SSR) genetic diversity analysis in deep water rice germplasms of Bangladesh, Int. J.Biosci. *2,* 264 - 72.

Mostajeran, A. and Rahimi-Eichi, V. (2009). Effects of Drought Stress on Growth and Yield of Rice (*Oryza sativa* L.) Cultivars and Accumulation of Proline and Soluble Sugars in Sheath and Blades of Their Different Ages Leaves. American-Eurasian J. Agric. & Environ. Sci. *5,* 264 - 272.

Ranawaka, A.L. and Dahanayake, N. (2012). Understanding abiotic stress tolerant levels and mechanisms in some traditional rice cultivars in Sri Lanka. 9th Academic session, University of Ruhuna, p 23.

Rauf, S., Al-khayri, J.M., Zaharieva, M., Monneveux, P. and Khalil, F. (2016). Breeding strategies to Enhance Drought Tolerance in crops. pp. 365-370 In: Khayri, J.M. *et al*., (*Eds.*) Adv. Plant Breeding Strategies. Springer International Publications., Switzerland.

Sharma, P. and Dubey, R.S. (2005). Drought induces oxidative stress and enhances the activities of antioxidant enzymes in growing rice seedlings. Plant Growth Regulation. *46,* 209 - 221.

Sharma, G. and Rai, L.K. (2010). Climate Change and Sustainability of Agro diversity in Traditional Farming of the Sikvkim Himalaya, Mountain Institute of India. United Nations University, Tokyo and MacArthur Foundation.

Somaratne, S. and Dhanapala, A.H. (1996). Potential impact of global climate change on forest distribution in Sri Lanka. 129-135. In:Water Air Soil Pollutes. Springer International Publications., Switzerland.

Zulkarnain, W.M., Ismail, M.R., Ashrafuzzaman, M., Saud, H.M. and Haroun, I.C. (2009). Growth, Physiological and Biochemical Responses of Malaysia Rice Cultivars to Water Stress. Pertanika J.Tropi. Agric. *32,* 323-333.