Correlation analysis of drought, salinity and submergence tolerance in some traditional rice cultivars of Sri Lanka

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Abstract- Searching new materials for biotic and abiotic stress tolerance from diverse sources such as traditional rice gene pool is important as there is limited variability for the traits in the improved rice cultivars. Identification and introgression of favorable alleles into elite breeding lines from traditional rice cultivars to increase the biotic and abiotic stress tolerances in cultivated rice are still progressing. This will prevent genetic erosion of cultivated varieties in Sri Lanka hence many improved rice cultivars are derived from few parental lines. Identification of rice cultivars with multiple tolerances are very much needed to utilize in the regions where micro climate of the crop is changing time to time due to short term flooding in prolonged rainy seasons, unexpected drought seasons comes with delayed monsoon rain or increasing salinity in coastal belt with intrusion of tidal waves. For selecting rice cultivars with multiple tolerances and identification of mode of tolerance at different abiotic stresses, thirty three traditional rice cultivars were separately evaluated for drought salinity and submergence tolerance at seedling stage. Each experiment was carried out according to the randomized complete block design with four replicates and twenty plants were included in to each replicate. In drought tolerant screening water cut was done at four weeks after planting and five days after 80% of the plants were completely withered. Plants were watered once again for recovery before evaluation. In salinity tolerance screening, rice seedlings were grown in soil filled trays and equal volume (500 ml) of 5 dS/m saline solution was added to each and every tray at 2 weeks after planting (2 WAP), 3 WAP and 4 WAP to make the soil salinized. Plants were evaluated 10 days after the 3rd salinity treatment. In submergence tolerance screening, two week old rice seedlings were subjected to 14 day complete submergence stress and plants were evaluated after two week recovery period under de-submerged conditions. Control experiment was carried out parallel to each stress condition. Plant survival percentages were calculated after each abiotic stress treatment. Among tested 34 traditional rice cultivars 53%, 72%, and 25% of rice cultivars scored more than 50% of survival rates at drought, salinity and submergence stress respectively. Dik wee and Gonabaru scored more than 50% survival rates at each drought, salinity and submergence stress while Kahata wee, Maddaikaruppan, Manamalaya, Mas Samba and Rath kara wee scored in-between 40%-49% survival rates at each stress condition. Pearson's correlation analysis showed 0.9256 of positive correlation in survival rates of drought and submergence stressed traditional rice cultivars. This finding proves that the mode of tolerance at drought and submergence stress is somewhat similar in these rice cultivars while the mode of tolerance at salinity and submergence or salinity and drought are differed

Index Terms- Drought, salinity and submergence tolerance, traditional rice, seedling stage

I. Introduction

Rice is grown in varied environmental conditions where it shows different levels of reactions to abiotic stresses, depending on the environmental conditions of origin and, cultivation. Rice has been adapted to tropical, sub tropical and temperate climates (Lafitte, 2004). It is grown in uplands where very little water is required in seedling establishment stage like Chena cultivation in Sri Lanka or direct sawing in southern Russia, and in low land, waterlogged soils as usually the case in lower river estuaries of south Asia. Rice cultivars affected by flooding in these areas have developed submergence tolerance. Further it is moderately tolerant to salinity where it is grown under seepage in coastal areas and moderately tolerant to soil acidity where it has been adapted to upper catchment areas of rivers with acidic soils developed due to excess run off, but rice is sensitive to chilling and does not acclimate to freezing (Reyes et al. 2003).

Drought stress

Drought and high salinity are the most important environmental factors that cause osmotic stress and dramatically limit plant growth and crop productivity (Boyer, 1982). Drought is a major cause of yield loss in rain-fed rice, grown on over 40 million ha in Asia. (Venupeasad et al. 2007).

However, rice consumes about 90% of the freshwater resources in Asia used for agriculture (Bhuiyan, 1992). About 80% of the world's rice is grown under irrigated (55%) and rain fed lowland (25%) ecosystems. Development of rice cultivars with less water requirement indirectly protects natural water resources.

A proper understanding of the physiological mechanisms for drought stress tolerance must be needed for achieving drought tolerance in rice. Though conventional plant breeding techniques are time-consuming, it has been immensely helpful in releasing drought-tolerant varieties. However, this is not adequate to cope up with the future demand for rice, as drought seems to spread to more regions and seasons across the world. Identification of favorable alleles for introgression into rice varieties will give a chance to utilize natural gene pool for the development of drought stress tolerance in rice. Drought stress most severely impacts yield when applied during the reproductive stage of the rice plant. In other growth stages drought stress limits yield causing poor seedling establishment and poor tiller number.

Salinity stress

Soil salinity has been identified as caused by three different reasons; natural, clearing of native vegetation, and irrigation (Manneh et al. 2007). Soil salinity constraints rice production and over 30% of the irrigated rice area in the world is affected by saline conditions due to irrational management and defective irrigation practices (Yeo and Flowers, 1984). Current guidelines (Maas and Grattan, 1999; Hanson et al. 1999) indicate that rice yields decrease 12% for every unit (dS/m) increase in above 3.0 dS/m. According to the classification of classes of soil salinity, low salinity; EC 2-4 dS/m can be caused by natural salinity and irrigation salinity. Species with low-moderate salt tolerance can be grown successfully under this salt stress. Moderate to high salt tolerant plant species are needed when the electrical conductivity falls between 4-8 dS/m. This salinity is caused by irrigation water logged. Under high-salinity condition with EC value more than 9 dS/m only halopytes can be grown in the areas like seeping. Utilization of unexploited genetic variation in sub species of rice cultivars with distinguishable level of tolerance for biotic and abiotic stresses avoids genetic engineering techniques such as gene transformation to develop stress tolerant cultivars with less chance for acquiring considerable tolerance in the case of traits, which are controlled by many genes. Introgression of Japonica rice cultivars with Indica rice cultivars to overcome the narrow gene pool is well practiced in broad areas of development of stress tolerant rice cultivars. Further Indica rice cultivars named Pokkali and Nona-Bokara are well-known salt tolerant rice cultivars with high heritability values (Gregorio and Senadhira, 1993) but it is said that salt tolerance is co-inherited with other undesirable agronomical characters (Heu and Koh, 1991).

The growth under saline condition depends on the reducing ability of sodium and chloride uptake while maintaining potassium uptake in to the plant (Koyama et al. 2001). The development of appropriate technique for management of salinity is critical for optimizing rice performance under saline or potentially saline conditions. Mass and Grattan (1999) reported that yields cannot be improved under salt-stressed conditions by increasing the seeding rate. And they found that, high field-water levels are more growth limiting than shallow water levels. Therefore, the solution for rice growers who are facing salinity problems is, irrigation management strategies that maintain low levels of salinity stress while minimizing high field water levels (Grattan et al. 1999). Various methods such as soil reclamation, excessive irrigation, and soil drainage are used to minimize soil salinity; they are always laborious and expensive. Other strategies such as varietal improvement have to be done for constant and profitable rice production.

Submergence stress

An increase in the frequency and magnitude of hydrological fluctuations is seen in modern agriculture as a result of global climate change as well as poor management of agricultural lands. Almost one-third of world's rice lands are at a low elevation and rainfed, and a large proportion of it is prone to both drought and flash flooding. Submergence affects 15 million ha of low land rice growing areas in South and South East Asia. In Sri Lanka alone, rice lands with flash flooding exceed 25,000 - 40,000 ha.

Ismail and Mackill (2009, www.knowledgebank.IRRI) suggest that there would be a different mechanism in Goda Heenati which is a submergence tolerant Sri Lankan traditional rice cultivar (www.knowledgebank.IRRI). Studies conducted in Sri Lanka (Ranawake et al. 2010 a; Ranawake et al. 2010b; Ranawake et al. 2011a, Ranawake et al. 2011b) showed that some other traditional rice cultivars have also shown different tolerance mechanisms to submergence at seedling stage indicating the need of further systematic study of tolerant levels and mechanisms of traditional rice cultivars.

Importance of genetic variability

Extent of genetic variability present in a gene pool is an important factor for genetic improvement in rice. Sri Lanka traditional rice gene pool consists of many abiotic and biotic stress tolerant traits with divers agronomical characters (Ranawake et al. 2010a; Ranawake et al. 2010b; Ranawake et al. 2011a; Ranawake et al. 2011b; Ranawake et al. 2012) The selected abiotic stress tolerant rice cultivars have the potential of direct introduction in to farmer fields (Djilianov et al. 2005) or utilization of them in the breeding programs implement to develop abiotic stress tolerance rice cultivars (Djilianov et al. 2005; Ashfaq et al. 2012). Evaluation of salinity, drought and submergence tolerance in 33 traditional rice cultivars was done at Faculty of Agriculture, University of Ruhuna, Sri Lanka with the aim of identifying stress tolerant traditional rice cultivars.

II. MATERIALS AND METHODS

Thirty three traditional rice cultivars were collected from Plant Genetic and Resource Center (PGRC) Gannoruwa, Peradeniya, Sri Lanka for the study.

Screening for level of drought tolerance

Seed dormancy was broken by keeping seeds at 50o C for 5 days. Seed surface sterilization was done by dipping seeds in 70% ethyl alcohol for 2 minutes and dipping seeds in 5% Chlorex solution for 30 minutes. Seeds were washed out thoroughly by distilled water. Dormancy broken seeds were germinated at 35o C and planted in plastic boxes (15 cm X 7.5 cm X 15 cm) filled with homogenized soil up to ¾ of the total depth according to the randomized complete block design with 20 plants per replicate and four replicates for each cultivar. Water cut was done at four weeks after planting. Five days after 80% of the plants were completely withered plants were watered once again for recovery. Ten days after watering, plants were evaluated according to survival percentage.

Screening for level of Salinity tolerance

Germinated seeds were planted in trays (45 cm X 30 cm X 5 cm) according to the randomized complete block design (RCBD) with 3 replicates and with 20 plants for each replicate. Trays were filled with homogenized soil up to 3 cm depth. Equal volume (500 ml) of 5 dS/m saline solution was added to each and every tray at 2 Weeks after planting (2 WAP), 3 WAP and 4 WAP as 1st, 2nd & 3rd salinity treatments. Electrical conductivity (EC) of soil solution was measured at each time.

Equal volume of water was added to all the trays every other day. Plant survival percentage was recorded on the 10th day after 3rd salinity treatment.

Screening for level of submergence tolerance

Experiment was carried out according to the randomized complete block design (RCBD) with 4 replicates. Each replicate contained 20 plants. Uniformly germinated seeds were planted in trays (60 cm X 90 cm) and maintained them at control growth conditions for 2 weeks. Two week old seedlings were subjected to 14 day complete submergence conditions separately and control experiment was carried out all along the experiment period. After complete submergence period plants were allowed two week period for recovery at control growth conditions. Plant survival percentage was recorded at the end of the experiment.

III. Results and Discussion

Among tested traditional rice cultivars 53%, 72% and 25% of rice cultivars scored more than 50% survival rates at drought, salinity and submergence stress respectively (Table 1). In fact Sri Lankan traditional rice cultivars show exceptional levels of tolerance. For example in a study of 76 rice cultivars, nine Sri Lankan indigenous cultivars were among the top thirteen for survival under submergence for 2 weeks (Singh et al. 2010). However this is a great figure comparing that only 6% of 3156 rice cultivars tested for tolerance at the Huntra Rice experiment research station Thailand survived at 10 day submergence (Setter et al. 1997) and only 2% of 18,115 lines were submergence tolerance at 10 days at IRRI (Setter and Laureles, 1996).

Heeneti-309 was the best submergence tolerant rice cultivar with the survival rates more than 95% at the seedling submergence stress. Rathel and Matholuwa also scored more than 85% survival rates at two week submergence stress. Interestingly these three cultivars scored more than 60 % of survival rates at the drought stress. This type of rice cultivars are very much suitable for the areas where rice cultivation totally depends on the monsoon rains. Due to climate change, sometimes monsoon rain prevails more weeks than usual and sometimes it onset later than the expected duration causing drought seasons in the middle of the crop. These areas are frequently affected by floods as well as by drought.

Rice cultivar Dik Wee I scored more than 60 % survival rates at all the stress conditions. This is an exceptional rice cultivar in terms of abiotic stress tolerance.

Table 1 Survival rates of traditional rice cultivars after drought, salinity and submergence stress

Accession Number	Name	Survival rate (%) Submergence	Salinity	Drought
3550	Bathkiri el	0.0^{r}	19.8^{q}	86.7^{a}
2203	Dikwee I	60.0^{h}	64.4^{d}	60.0^{k}
3543	Gonabaru	55.56^{j}	57.9^{g}	69.4^{g}
3692	Handiran	8.3^{q}	29.1^{l}	73.3^{e}
3707	Heenati	8.3^{q}	0.0^{y}	60.0^{k}
3132	Heenati-309	96.74^{a}	0.0^{y}	50.0^{m}
3641	Heen dik Wee	0.0^{r}	91.2^{a}	20.0^{q}
3644	Herath	75.0^{e}	27.7^{n}	53.3^{l}
3642	Kahata Samba	33.3^{n}	25.4^{o}	80.0^{b}
3387	Kahata wee	70.0^{f}	48.5^{h}	73.3^{e}
3713	Kalukanda	20.8^{p}	59.0^{f}	46.67^{n}
3162	Kiri naran	36.84^{m}	0.0^{y}	60.0^{k}
3388	Maddai karuppan	53.33^{k}	74.2^{c}	40.0^{o}
3721	Manamalaya	79.2^{d}	79.3^{b}	46.67^{n}
2349	Mas samba	60.0^{h}	45.1^{j}	86.7^{a}
3472	Masuran	0.0^{r}	16.1^{t}	20.0^{q}
3214	Matholuwa	86.2^{b}	14.9^{u}	75.0^{c}
3388	Maddai karuppan	53.33^{k}	74.2^{c}	40.0^{o}
3142	Molaga samba	0.0^{r}	16.1^{t}	72.2^{f}
3672	Mudaliwi	75.0^{e}	22.1^{p}	62.5^{i}
3663	Murunga	25.0^{o}	19.7^{r}	60.0^{k}
3639	Polayal	66.7^{g}	8.3^{w}	26.7^{p}
3592	Ranhiriyal	58.3^{i}	5.8^{x}	80.0^{b}
2196	Rathel	86.4^{b}	0.0^{y}	60.0^{k}
3684	Rathkara	75^{e}	48.5^{h}	66.6^{h}
3390	Rathu heenati	80.0^{c}	12.5^{v}	55.56^{k}
3473	Ratu wee	41.7^{l}	35.8^{k}	12.5^{r}
3629	Ruwan raththaran	0.0^{r}	0.0^{y}	73.3^{e}
3605	Seedevi	8.3^{q}	63.2^{e}	50.0^{m}
3725	Sivuru wee	0.0^{r}	28.2^{m}	46.67^{n}
3171	Sudu hetada	0.0^{r}	0.0^{y}	61.7^{j}
3160	Valihundiran	80.0^{c}	16.4^{s}	20.0^{q}
3445	Yakada wee	0.0^{r}	47.2^{i}	73.9^{d}

Superscript letters indicate DMRT groups. The same letters in the same raw are not significantly differed.

According to the applied bio assay conditions, twenty three rice cultivars scored more than 50% survival rates at drought stress. Mas Samba and Bathkiriel were the best among all the other cultivars those scored 86.7% survival rates.

At each stress condition Dik wee and Gonabaru scored more than 50% survival rates (Table1). The genetic constituents of this type of cultivars must be dissected to understand the alleles responsible for tolerant levels. Kahata wee, Maddaikaruppan, Manamalaya, Mas Samba and Rath kara wee scored in-between 40%-49% survival rates at each stress condition (Table 2). Out of 33 traditional rice cultivars Heeneti-309 scored the highest survival rate at both submergence (96.74%) and drought (86.67%) stresses but at salinity stress it scored only 19.81% survival rate.

Table 2 Rice cultivars scored given survival rates at each salinity, drought and submergence stress.

Survival%	Rice cultivar
> 50%	Dik Wee
	Gonabaru
40%-49%	Kahata Wee
	Maddaikaruppan
	Manamalaya
	Mas Samba
	Rathkara
30%-39%	Herath
20%-29%	Kahata Samba
	Kalu Kanda
	Mudali Wee

Murunga

The pair wise correlation analysis showed 0.9256 of positive correlation in survival rates of drought and submergence stressed traditional rice cultivars but the correlations between salinity and drought or submergence and salinity stress were negative (Table 3). On the other hand the highest survival percentage at salinity stress was scored by Herath (91.16%). Its survival percentage at salinity stress was only 20% while its submergence tolerance was around 75% (Table 1).

Table 3 Correlation coefficients for trait pairs

Trait pair	Correlation coefficient	P value
Submergence -drought	0.9256	0.001
Submergence- salinity	-0.0825	0.6532
Drought-salinity	-0.2663	0.406

Currently Marker Assisted Back-Crossing is being practiced to develop cultivars with tolerance to submergence (Neeraja et al. 2007). Mega varieties with SUB1A introgression have been developed and proved that SUB1A enhanced the level of submergence tolerance in introgression cultivars (Endang et al. 2009). It has been found that SUB1A mediates ABA responsiveness, thereby activating a cascade of stress responsive gene expression, thus mediating both submergence and drought tolerance through prevention of water loss from leaves and suppression of leaf elongation conserving carbohydrate reserves (Fukao et al. 2007). This result is in agreement with our bio assay data where many cultivars those submergence tolerance were drought tolerance at the seedling stage. The strong correlation (r=0.9256) in between submergence tolerance and drought tolerance emphasized the common features of mode of tolerance in submergence tolerance and drought tolerance.

It is worth studying traditional rice cultivars in systematic way for each tolerance to find new breeding materials and finding new abiotic stress tolerant genes within traditional rice cultivars.

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References

[1]. A. L. Ranawake S. A. P. Madhurangi and N. Dahanayake "Evaluation of level of drought tolerance in traditional rice cultivars in Sri Lanka at the seedling stage". *International Symposium on Agriculture and Environment (ISAE)*, 2012b, pp. 325 – 327.

[2]. A. L. Ranawake, N. Dahanayake and D.D. Senadhipathi, "Evaluation of level of submergence tolerance in traditional rice cultivars at post-germination stage", *8th Academic sessions, University of Ruhuna*, 2010b, p. 203.

[3]. A. L. Ranawake, N. Dahanayake and R. D. G. Kumari, "Assessment of submergence tolerance of some traditional and modern rice cultivars in Sri Lanka", *8th Academic sessions, University of Ruhuna*, 2010a, p. 187.

[4]. A. L. Ranawake, N. Dahanayake and U.T.D. Rodrigo, "Salinity tolerance in traditional rice cultivars" *International Sympoisum on Agriculture and Environment (ISAE)*, 2011b, p.136.

[5]. A. M. Ismail, E. S. Ella, G.V. Vergara and D. J. Mackill, "Mechanisms associated with tolerance to flooding during germination and early seedling growth in rice (*Oryza sativa*)" *Annals of Botany*, 2009, 103: 197-209.

[6]. A.L. Ranawake, U. G. S. Amarasingha and N.Dahanayaka, " Study on the submergence tolerance of some traditional rice cultivars in Sri Lanka under two different stress periods at seedling stage," *International Sympoisum on Agriculture and Environment (ISAE)*, 2011a, p. 28.

[7]. A.R. Yeo and T.J. Flowers, "Salinity Tolerance in Plants–Strategies for Crop Improvement" (eds.)Staples R.C. and Toenniessen G.H., Wiley-Inter-science Publication, Canada, 1984, pp.151-170.

[8]. B. Hanson, S. R. Grattan and A. Fulton, "Agricultural salinity and drainage", UC DANR Pub, (1999). 3375. p. 160

[9]. B. Manneh, P. Satm, P.C. Struik, S. Bruse-Oliver and F.A.V. Eeuwik, "QTL-based analysis of genotype by environment interaction for grain yield of rice in stress and non-stress environments", *Euphytica*, 2007, pp. 213-226.

[10]. C. N. Neeraja, R. Maghirang-Rodriguez A. Pamplona, S. Heuer, B.C.Y. Collard, E.M. Septiningsih, G. Vergara, D. Sanchez, K. Xu and A.M. Ismail, "A marker-assisted backcross approach for developing submergence-tolerant rice cultivars". *Theoretical and Applied Genetics*, 2007, pp. 767-776.

[11]. D. Djilianov, T. Georgieva D. Moyankova A.Atanassov K. Shinozaki S.C.M. Smeeken D.P.S. Verma and N. Murata, "Improved abiotic stress tolerance in plants by accumulation of osmoprotectants – gene transfer approach". *Biotechnol. & Biotechnol. Eq*., 2005, 19: 63-71.

[12]. D. Reyes, D. Rodriguez, M.P. O. Gonzales-Garcia Lorenzo G.Nicolas J.L. García-Martínez and C. Nicolás, "Overexpression of a Protein Phosphatase 2C from Beech Seeds in Arabidopsis Shows Phenotypes Related to Abscisic Acid Responses and Gibberellin Biosynthesis". *Plant Physiol*, 2006, pp. 1414-1424.

[13]. E.V. Maas and S. R. Grattan, "Crop yields as affected by salinity". In: Skaggs RW, Van Schilfgaarde J (eds.). Agricultural Drainage. Agron Monogr 38. ASA, CSSA, SSA, Madison, WI, 1999, pp. 55–108.

[14]. G. B. Gregorio and D. Senadhira,"Genetic analysis of salinity tolerance in rice (*Oryza sativa* L.)". *Theor. Appl. Genet* ,1993, pp. 333-338.

[15]. H. R. Lafitte, A. Ismail and J. Bennett, "Abiotic stress tolerance in rice for Asia: progress and the future "New directions for a diverse planet". *Proceedings of the 4th International Crop Science Congress*, Brisbane, Australia. Published on CDROM. 2004, www.cropscience.org.au

[16]. IRRI Annual Report 2009, www.knowledgebank.IRRI

[17]. J. S. Boyer, "Plant productivity and environment" *Science*, 1982, pp. 443–448

[18]. M. Ashfaq, A. S. Khan, S.H.U. Khan and R.V. Ahmad, "Association of various morphological traits with yield and genetic divergence in rice (*Oryza Sativa* L.)." *Int. J. Agric. Biol*, 2012, pp. 55–62.

[19]. M. Endang, A. M. Septiningsih, D. L. Pamplona Sanchez, *et al*., "Development of submergence-tolerant rice cultivars: the Sub1 locus and beyond" *Ann. Bot.*, 2009, pp. 151-160.

[20]. M. H. Heu and H. J. Koh, "Newly bred salt tolerance lines in rice". *Kor. J. Breed*, 1991, pp. 59-63.

[21]. M. Koyama, A. Levesley, R. Koebner, T. Flowers and A. Yeo Quantitative trait loci for component physiological traits determining salt tolerance in rice". *Plant Physiology*, 2001, pp. 406-422.

[22]. R.Venupeasad and H.R. Lafitte and G.N. Atlin "Response to Direct Selection for Grain Yield under Drought Stress in Rice," *Crop Sci*, 2007, pp. 285-293.

[23]. S. I. Bhuiyan, "Water management in relation to crop production: Case study on rice. Outlook Agric." 1992, pp. 293–299.

[24]. T. Fukao and J. B. Serres, "Ethylene—A key regulator of submergence responses in rice", *Plant Sci.*, 2007, pp. 43- 51.

[25]. T. L. Setter and E.V. Laureles, "The beneficial effect of reduced elongation growth on submergence tolerance of rice," *Journal of Experimental Botany*. 1996, pp. 1551-1559

[26]. T. L. Setter, M. Ellis, E. S. Laureles, D. Senadhira, S. B. Mishra, S. Sarkarung and S. Datta, "Physiology and genetics of submergence tolerance in rice," *Annals of Botany*, 1997, pp. 67-77.

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