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# Physiological and Molecular Characterization of Rice Varieties for Submergence Tolerance

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**ABSTRACT**

In rice crop, submergence is one of the limiting factors affecting productivity particularly in coastal irrigated ecosystem during wet season. Most of the widely cultivated rice varieties are vulnerable to flashfloods resulting in lowering the production under adverse climatic conditions. In present study, physiological traits like plant survival percentage, total shoot elongation, relative shoot elongation and SSR markers were used to characterize six widely cultivated rice varieties in comparison with swarna sub, a known submergence tolerant variety. None of the six widely cultivated varieties exhibited submergence tolerance at seedling stage reflecting absence of sub 1A, a submergence tolerant allele. Variety MTU 1064 expressed desirable traits like minimal shoot elongation under submergence and relative shoot elongation showing fast recovery after de-submergence at tillering stage for 10 days. Dendogram generated using 86 polymorphic SSR marker data grouped two varieties (MTU 1075 and MTU 1010) into cluster I, five varieties (MTU 1061, MTU 1064, MTU 7029, Swarna sub and BPT 3291) into cluster II. Characterization of widely cultivated rice varieties for submergence tolerance with molecular markers would help in introgression of sub 1A using marker assisted selection to enhance the rice productivity under flooded condition.

**Key words :** Diversity, Molecular characterization, SSR markers, Submergence.

Submergence of rice crop is one of the major constraints in coastal irrigated ecosystem limiting rice production during monsoon season. Rice crop is often inundated due to flash floods associated with ill drained conditions in low lands caused by unpredictable rainfall or heavy down pour in short period due to adverse climatic changes. Most of the widely cultivated rice varieties are vulnerable to submerged conditions. Ethylene response factor sub 1A was identified as major determinant of submergence tolerance on chromosome 9 (Xu *et al.,* 2006). Introgression of Sub 1A into mega varieties like Swarna, Samba mahsuri and IR 64 (Neeraja *et al.,* 2007, Septinguish *et al.,* 2009) using marker assisted breeding has resulted to improve mega rice varieties for submergence tolerance. The present study is aimed to characterize six widely cultivated rice varieties of coastal area of Andhra Pradesh using simple sequence repeats in comparison with swarna sub and to assess genetic diversity for further use in marker assisted back cross breeding for back ground selection.

## MATERIAL AND METHODS

Sowing of seven rice varieties (MTU 7029, MTU 1010, MTU 1075, MTU 1061, MTU 1064, BPT 3291 and Swarna sub) in 3 replications was taken up in plastic trays of size 36X 24X12 cm to assess submergence tolerance at seedling stage during 2010 wet season at Andhra Pradesh Rice Research Institute and Regional Agricultural Research Station, Maruteru. Seedlings in trays at 14 days after sowing were immersed in concrete tank for 14 days up to 1m depth and scoring of plant survival percentage was recorded at 10 days after de-submergence. For field level screening during wet season 2010, nursery was raised and thirty days old seedlings were transplanted with 4rows of 5m length in 3 replications in submergence pond and normal irrigated condition to assess submergence tolerance of rice varieties. Plants at 15 days after transplanting were completely submerged for 10 days. Submergence tolerance score scale (% plants survived 100% =1, 95-99%=3, 75-94% =5. 50-74%=7, 0-49=9%) was recorded as per the Standard Evaluation System

for rice, IRRI, 2002 at 10 days after de-submergence. Relative shoot elongation (RSE) under submergence was calculated as per Toojinda *et al.,* 2003.

$$\text{Relative shoot elongation} = \frac{\text{Elongation under submergence}}{\text{Elongation under non submergence}} \times 100$$

**DNA isolation and SSR analysis**

Healthy leaf samples were collected from 18 days old seedlings of nursery for isolation of DNA. Total genomic DNA was extracted using modified protocol of Zheng *et al* 1995. Fresh leaf samples were crushed in microcentrifuge tubes in Tris/SDS extraction buffer (50 mM Tris-HCl pH 8, 25 mM EDTA pH 8, 300 mM NaCl, 1% SDS (w/ v) per 40 ml of buffer) using Qiagen Tissue lyser. Tris saturated phenol (pH 8), chloroform used for extraction followed by iso propanol precipitation.

The quality and quantity of DNA was estimated using UV eight channel spectrophotometer and DNA samples were diluted to concentration of 10ng/il. PCR reaction mixture contains 1 µl of 10X buffer with $MgCl_2$, 0.5 µl of dNTPs (2.5 mM), 1 µl (5 µ molar) each of forward and reverse primers, 1 µl *Taq* DNA polymerase 0.5 U/micro litre, 3 µl of template DNA (10 ng/ µl) and 2.5 µl of sterilized distilled water constitutes 10ul per reaction. Amplification of SSR was performed by using eppendorf thermo cycler. PCR amplification was carried out with initial denaturation at 94°C for 5 min followed by 35 cycles of denaturing at 94°C for 0.5 min, annealing temperature adjusted with marker at 55° C to 58°C for 0.5 min, extension at 72°C for 1.0 min and ending up with 7 min at 72°C for the final extension. The PCR products were resolved with ethidium bromide (10mg/ml) at 100 volts for 2 hrs in 1X TBE buffer. A 100 bp ladder (Banglore Genei) was used for appropriate sizing of the products. The gel image was captured under UV light using Ingenius gel doc system.

**Marker data analysis**

Amplified PCR products were scored sequentially from the largest to the smallest size band based on their position relative to the ladder. Each band that was amplified using each SSR primer was scored as 1 or 0 depending on its presence or absence.

The genetic associations among 7 rice varieties were evaluated using Jaccard's similarity co-efficient and clustered with unweighted pair-group method of arithmetical averages UPGMA analysis (NTSYS2.0). Polymorphic Information Content (PIC) values, genetic diversity were estimated using Power marker 3.25 package .

## RESULTS AND DISCUSSION

**Physiological Characterization**

Field screening of seven rice varieties for submergence tolerance at 15 days after transplanting for 10 days revealed that swarna sub recorded submergence tolerance score as 5, three varieties (MTU 7029, MTU 1075, MTU 1064) scored as 7 and three varieties (MTU 1010, MTU 1061, BPT 3291) as 9. None of the varieties showed seedling stage tolerance for submergence at 14 days after sowing for 14 days except swarna sub indicating scope for introgression of sub 1A gene into rest of genotypes under the study to enhance submergence tolerance at seedling stage.

Minimal shoot elongation under submergence is one of the important criteria to assess energy saving mechanism under submergence and this trait was observed as 9.2 cm (MTU 1064) followed by 12cm (swarna sub) indicating that these genotypes have fast recovery ability after de-submergence. Expression of alleles other than sub 1 A might be responsible for field level submergence in case of MTU 1064. Toojinda *et al*. (2003) reported that relative shoot elongation below 100 indicates use of minimal carbohydrates for survival under submergence. In our study, varieties MTU 1064 followed by MTU 1061 and MTU 1010 expressed relative shoot elongation less than 100 showing ability of energy saving mechanism (Table 1).

**Molecular Characterization**

Out of 218 amplified SSR markers, 86 markers were polymorphic among seven genotypes studied. These markers produced alleles ranging from 2 to 3 with an average of 1.96. These alleles were used to analyze the variation among the genotypes. A high degree of polymorphism was

Table 1. Physiological characterization of rice varieties for submergence tolerance.

| S.No | Genotype | Cross combination | Plant survival % at seedling stage | Submergence tolerance (0-9 score) | Total shoot elongation (cm) | Relative Shoot elongation |
|---|---|---|---|---|---|---|
| 1 | MTU 1010 (Cottondora sannalu) | Krishnaveni/IR64 | 0 | 9 | 36.3 | 72 |
| 2 | MTU 1075(Pushyami) | MTU 2716/MTU 1010 | 0 | 7 | 24.3 | 106 |
| 3 | MTU 1061(Indra) | PLA 1100/ MTU 1010 | 0 | 9 | 19.7 | 72 |
| 4 | MTU 1064(Amara) | PLA 1100/MTU 1010 | 0 | 7 | 9.3 | 57 |
| 5 | MTU 7029(Swarna) | Vasista / Mahsuri | 0 | 7 | 19.0 | 129 |
| 6 | Swarna sub | MTU 7029/ IR 49830 | 37.5 | 5 | 12.0 | 112 |
| 7 | BP 3291(Sonamahsuri) | Sona/ Mahsuri | 0 | 9 | 24.7 | 100 |

Fig 1: Dendogram showing genetic diversity in seven rice varieties at molecular level

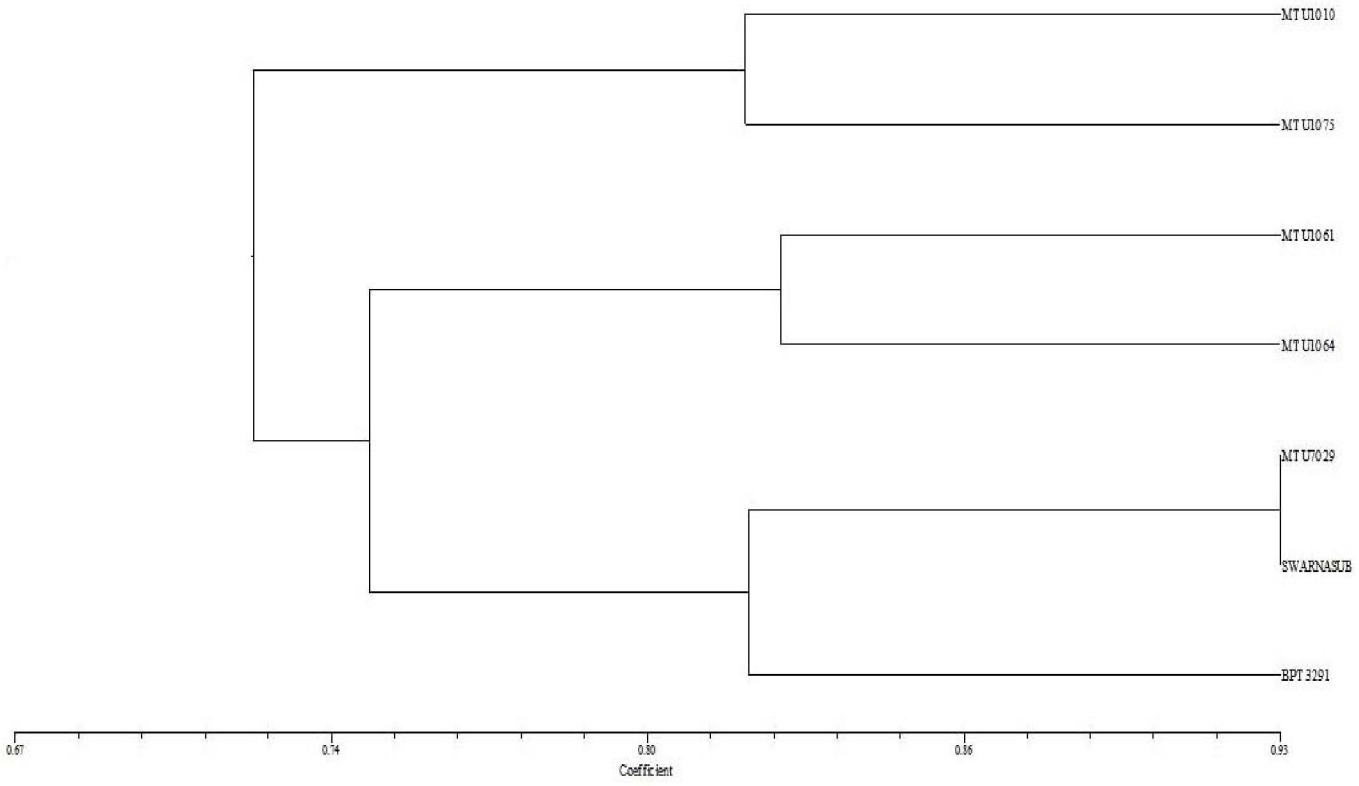

Table 2. Details of polymorphic SSR markers.

| Marker | Ch.No | Allel No | GD | PIC | Marker | Ch.No | Allele No | GD | PIC |
|---|---|---|---|---|---|---|---|---|---|
| RM6464 | 1 | 2 | 0.24 | 0.2149 | RM5473 | 4 | 2 | 0.5 | 0.375 |
| RM3530 | 1 | 3 | 0.54 | 0.4598 | RM470 | 4 | 2 | 0.24 | 0.2149 |
| RM6334 | 1 | 2 | 0.49 | 0.3698 | RM335 | 4 | 2 | 0.34 | 0.28 |
| RM1297 | 1 | 2 | 0.41 | 0.3249 | RM307 | 4 | 2 | 0.49 | 0.368 |
| RM9 | 1 | 2 | 0.24 | 0.2149 | RM252 | 4 | 2 | 0.5 | 0.375 |
| RM3340 | 2 | 2 | 0.28 | 0.2392 | RM3524 | 4 | 2 | 0.46 | 0.3538 |
| RM1211 | 2 | 2 | 0.46 | 0.3538 | RM2636 | 4 | 2 | 0.49 | 0.3698 |
| RM106 | 2 | 2 | 0.44 | 0.3457 | RM3476 | 5 | 2 | 0.5 | 0.375 |
| RM5460 | 2 | 2 | 0.49 | 0.3698 | RM163 | 5 | 2 | 0.46 | 0.3538 |
| RM263 | 2 | 2 | 0.5 | 0.375 | RM334 | 5 | 2 | 0.41 | 0.3249 |
| RM240 | 2 | 2 | 0.34 | 0.28 | RM169 | 5 | 2 | 0.22 | 0.1948 |
| RM6911 | 2 | 2 | 0.49 | 0.3698 | RM2998 | 5 | 2 | 0.46 | 0.3538 |
| RM138 | 2 | 2 | 0.41 | 0.3249 | RM8107 | 6 | 2 | 0.34 | 0.28 |
| RM3865 | 2 | 2 | 0.34 | 0.28 | RM5957 | 6 | 2 | 0.49 | 0.3698 |
| RM3501 | 2 | 2 | 0.46 | 0.3538 | RM340 | 6 | 2 | 0.15 | 0.1411 |
| RM5578 | 2 | 3 | 0.57 | 0.5015 | RM225 | 6 | 2 | 0.46 | 0.3538 |
| RM4108 | 3 | 2 | 0.28 | 0.2392 | RM549 | 6 | 2 | 0.46 | 0.3538 |
| RM6931 | 3 | 2 | 0.49 | 0.3698 | RM400 | 6 | 2 | 0.5 | 0.375 |
| RM1350 | 3 | 2 | 0.41 | 0.3249 | RM510 | 6 | 2 | 0.49 | 0.3698 |
| RM168 | 3 | 2 | 0.24 | 0.2149 | RM253 | 6 | 2 | 0.32 | 0.2688 |
| RM231 | 3 | 2 | 0.24 | 0.2149 | RM2353 | 6 | 2 | 0.13 | 0.1239 |
| RM241 | 4 | 2 | 0.49 | 0.3698 | RM589 | 6 | 2 | 0.49 | 0.3698 |
| RM8101 | 6 | 2 | 0.41 | 0.3249 | RM566 | 9 | 2 | 0.44 | 0.3457 |
| RM5711 | 7 | 2 | 0.46 | 0.3538 | RM3700 | 9 | 3 | 0.49 | 0.4235 |
| RM182 | 7 | 2 | 0.5 | 0.375 | RM6100 | 10 | 2 | 0.41 | 0.3249 |
| RM8006 | 7 | 2 | 0.5 | 0.375 | RM216 | 10 | 2 | 0.34 | 0.28 |
| RM5211 | 7 | 2 | 0.5 | 0.375 | RM5348 | 10 | 2 | 0.49 | 0.3698 |
| RM125 | 7 | 2 | 0.46 | 0.3538 | RM1812 | 11 | 2 | 0.41 | 0.3249 |
| RM295 | 7 | 2 | 0.46 | 0.3538 | RM3701 | 11 | 2 | 0.5 | 0.375 |
| RM427 | 7 | 2 | 0.49 | 0.3698 | RM206 | 11 | 3 | 0.58 | 0.5174 |
| RM455 | 7 | 2 | 0.49 | 0.3698 | RM524 | 9 | 2 | 0.24 | 0.2149 |
| RM8015 | 7 | 2 | 0.24 | 0.2149 | RM3909 | 9 | 2 | 0.49 | 0.3698 |
| RM22250 | 8 | 2 | 0.34 | 0.28 | RM219 | 9 | 2 | 0.46 | 0.3538 |
| RM1309 | 8 | 2 | 0.24 | 0.2149 | RM 464 | 9 | 2 | 0.46 | 0.3538 |
| RM281 | 8 | 2 | 0.34 | 0.28 | RM286 | 11 | 2 | 0.13 | 0.1239 |
| RM8019 | 8 | 2 | 0.13 | 0.1239 | RM202 | 11 | 2 | 0.24 | 0.2149 |
| RM8266 | 8 | 2 | 0.28 | 0.2392 | RM209 | 11 | 2 | 0.46 | 0.3538 |
| RM404 | 8 | 2 | 0.41 | 0.3249 | RM2136 | 11 | 2 | 0.46 | 0.3538 |
| RM72 | 8 | 2 | 0.13 | 0.1239 | RM1880 | 12 | 2 | 0.49 | 0.3698 |
| RM5122 | 9 | 2 | 0.46 | 0.3538 | RM247 | 12 | 2 | 0.48 | 0.3648 |
| RM5899 | 9 | 2 | 0.34 | 0.28 | RM1246 | 12 | 2 | 0.28 | 0.2392 |
| RM23865 | 9 | 2 | 0.44 | 0.3457 | RM5939 | 12 | 2 | 0.47 | 0.3589 |
| RM23869 | 9 | 2 | 0.46 | 0.3538 | RM1227 | 12 | 2 | 0.46 | 0.3538 |
| | | | | | Mean | | 1.96 | 0.37 | 0.2918 |

Ch No. Chromosome number, GD: Genetic diversity, PIC: Polymorphic Information Content.

obtained with markers RM 5578, RM 206 and RM 3530 with 3 alleles and most of the markers with two alleles. All these markers showed an average polymorphic information content (PIC) value of 0.2918 with a range of 0.1239 – 0.5174 and genetic diversity ranged from 0.15 – 0.54 with a mean of 0.37 (Table 2).

Jaccard's similarity coefficients among 7 varieties were calculated to establish genetic relationships. The similarity index values varied from 0.000 to 1.000 indicating the presence of wide range of genetic variability at molecular level among the 7 genotypes. The dendrogram (fig.1) generated using pooled data divided the genotypes into two clusters at about 67% similarity level.

Varieties MTU 1075 and MTU 1010 were grouped into cluster I, MTU 1061, MTU 1064, MTU 7029, Swarna sub and BPT 3291 into cluster II. Grouping of MTU 1075 and MTU 1010 in cluster I shows more genotypic contribution of parent MTU 1010 in development of MTU 1075. Clustering of varieties MTU 1061 and MTU 1064 into sub group of cluster II is appropriate as they were derived from the same parentage. Genotypic contribution of mahsuri as parent in development of varieties MTU 7029, Swarnasub, BPT 3291 might be reason for sub grouping in cluster II. Swarna sub, a submergence tolerant variety derived from MTU 7029 through marker assisted selection showed 95% similarity level. Prasad *et al.* (2011) reported genetic diversity and molecular characterization of genotypes for submergence tolerance.

Dendogram branching pattern of seven genotypes correlates involvement of parentage Mahsuri/Vijaya in development of PLA 1100, MTU 2716. The same parentage was involved in development of sowbhagya, one of the parents of Krishnaveni used to develop MTU1010. Inheritance of adaptable characters from parents transmitted to widely cultivated varieties in the process of pedigree method of breeding might be the reason for the observed clustering pattern of seven varieties. Correlation of pedigree and marker data in characterization of these seven rice varieties paved a way to use marker assisted selection in further improvement for specific traits like submergence tolerance.

Physiological and molecular characteri zation of seven rice varieties revealed that there is an ample scope of introgression of sub 1 A from Swarna sub into widely cultivated rice varieties through marker assisted selection for enhancing productivity under adverse climatic conditions.

## LITERATURE CITED

**IRRI (International Rice Research Institute) 2002** Standard Evaluation System for Rice. http://www.knowledgebank.irri.org/ses/SES.htm.

**Neeraja C N, Maghirang R, Pampolana A M, Heuer S, Collard C Y, Septingish E M, Vergara G, Sanchez D, Ismail A M and Mackill D J 2007** A marker assisted back cross approach for developing submergence tolerant rice cultivars. Theoretical Applied Genetics 115:767-776.

**Prasad B D, Thapa G, Baishya S and Sahni S 2011** Biochemical and molecular characterization of submergence tolerance in rice for crop improvement. Journal of Plant Breeding and Crop Science 3(10) : 240-250.

**Septinguish E M, Pampalona A M, Sanchez D L, Neeraja C N, Vergara G V, Heuer S, Ismail A M and Mackill D J 2009** Development of submergence tolerant cultivars: the Sub 1 locus and beyond. Annals of Botany 103(2):151-160.

**Toojinda T, Siangliw M, Tragoonrung S and Vanavichit A 2003** Molecular Genetics of submergence tolerance in rice: QTL Analysis of key traits. Annals of Botany 91:243-253.

**Xu K, Xu X, Fukao T, Canlas P, Maghirang Rodriguez R, Heur S, Ismail A M, Bailey-Serres J, Ronald PC and Mackill D J 2006.** Sub 1 A an ethylene responsive factor like gene that confers submergence tolerance to rice. Nature 442(7103):705-708.

**Zheng K, Subudhi P K. Domingo J, Magantay G and Huang N 1995** Rapid DNA isolation for marker assisted selection in rice breeding. Rice Genet News Letter 12:255-258.

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