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Identification of Salt Tolerance Genotypes Using SSR Markers in Rice.

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ABSTRACT

The genotypes that were salinity tolerant in Rice were indicated in a Rice population. The salinity markers RM 219, RM 234, RM 315 and RM 263 in Chromosome 1 were selected for 26 local landraces of Rice. The selection of the 26 local Landraces (LR) of Rice samples were done. Followed by the process of extraction of the DNA from the rice samples and the samples were run on SSR markers. Gel documentation of the samples was done to get a band. Later, through cluster analysis a dendro gram was constructed and cluster analysis of the rice genotypes based on SSR data the genotypes were divided into 2 categories which indicated that the 2 genotypes LR 25 and LR 5 are highly diverse and can be utilized for breeding for salinity.
Keywords: Salanity markers, SSR markers, Dendrogram. Rice population, Landraces

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INTRODUCTION

Rice is one of the widely consumed foods in the world [1]. It is grown in the tropical areas as an annual plant [2]. Abiotic stresses like salinity, drought and high-salt are the most common problems being suffered in plant agriculture [3]. Salinity can affect crop production which can lead to reduced productivity and restrict plant growth. Among all the cereal grains rice is the most condign crops for saline soils. In spite of the improved rice varieties and new production techniques, it is strongly affected by salinity [4-5]. Attempts are made to develop salt- tolerant genotypes in Rice in different varieties [6-7]. This is done by using DNA based molecular markers to evaluate the genetic diversity in most of the crop species. The gene that is saline is linked to the DNA of the particular Rice variety. To evaluate the Genetic diversity, Gene Mapping, evolutionary studies the SSR markers (Simple Sequence Repeats) are widely used to find out the degree of polymorphism, reproducibility and co-dominance [8-9]. The SSR Rice markers were basically used to find out the polymorphism between and within Rice population [10-12].

The main objective of the present study was to (i) Identify the genes/ genomic regrow associated with salinity tolerance in Rice, (ii) Genotypes that are tolerant to salt in seedling stage.

MATERIALS AND METHODS

Extraction of DNA by CTab method:

Young and healthy levels of 5g from 25 days old plants were collected and the leaves were cut into pieces and homogenized completely with liquid nitrogen with mortar and pestle. The Leaf powder that was obtained was transferred to 50ml falcon tube containing 6ml ice cold extraction buffer. The samples were incubated for 30 mins at 65°C after the addition of 1ml of 10% SDS and during incubation and they were shaken at regular intervals. After incubation, 3ml of ice cold 5/3 potassium acetate was added and samples were vigorously rotated and incubated on ice for 30 mins. After that lead to the centrifugation of the samples for 10 mins at 4000rpm at 4°C.

The supernatant was filtered through Mira cloth into another centrifuge tube. To that 6ml of chilled ethanol was added and then incubated at RT for 10 mins to precipitate the DNA. Then again it was centrifuged for 10 mins at 4000rpm. The supernatant was discarded and the DNA pellet was dried. The dried DNA pellet was dissolved in 200-400µl TE buffer depending on the size of the DNA pellet and transferred into the 2ml Eppendorf tube and incubated for 20mins at 37°C after adding RNAse and mixing. To that 400µl of CTAB buffer was added and the tubes were incubated for 15mins at 65°C (the tubes shaken periodically). To that Dried DNA pellet 800µl of chloroform/ isoamyl alcohol (24:1) was added and the tubes were incubated in minifuge for 10mins at 4°C. The supernatant was transferred to fresh eppendorf tube, add equal volumes of chilled absolute ethanol (96%) and incubate mixture at RT for 15mins for DNA precipitate.

The tubes were centrifuged for 10mins in a minifuge and the supernatant was discarded. The DNA pellet was washed with 70% ethanol and then the ethanol was removed

and the pellet was dried. Finally, the DNA pellet was dissolved in 200µl of TE buffer and stored at -20°C until use. Here 26 local land races of Rice were used named as LR 1, LR 2…….LR 26 and 4 SSR Rice primers RM 219, RM 234, RM 263 and RM 315.

PCR Reaction

To perform the PCR reaction a reaction mixture was prepared. The reaction mixture contained 1µl of DNA, 0.8µl of primer both forward and reverse each, 1µl of Taq buffer, 1µl of DNTP's, 0.5µl of Taq Polymerase and 4.9µl of water. After 4 hrs of PCR reaction 2µl of dye was added and it was subjected to Gel electrophoresis.

Gel Electrophoresis

10.5 GM of agar was added to 300ml (6ml TAE + 294ml of sterile water) of water in a beaker. It was liquefied at 3°C in oven. To that 1 drop of ethidium bromide was added. The gel was added to the plate and was made to solidify for 30mins. After solidification of the gel the tapes were removed and the gel along with the plate was placed in the buffer tank. It is a semisolid gel called a slab gel with depressions at the top which is called as wells. Into that well 12µl of the sample was loaded. After loading the samples an electric field was supplied at 100 volts. After 1 and a half hour the gel was visualized under UV rays under Trans-illumination i.e. BIO-RAD.

Scoring

Scoring aims at finding out the banding pattern with binary variables. Only clear fragments were recorded. The genotypes were classified according to the amplification done. The genotype having amplification was marked at 1, 2, 3 and 4 and no amplification was marked as 0. According to the scoring pattern the validation was done to see how many genotypes are resistant to salinity.

RESULTS

According to the dendogram as shown in figure 9, among the 26 local Landraces of Rice, LR 26 and LR 5 were highly diverse genotype and which can be utilized for breeding for salinity. There are 3 major clusters and under the first cluster there are 2 genotypes, the second cluster consists of 3 genotypes and the third cluster consist of 9 linkages. The linkage distance between the first clusters is 1, the second cluster 1.4 and the third cluster is 1.65. The 2 genotypes that are LR 25 and LR 5 can be chosen for breeding. The results which were visualized and the bands that are obtained are shown in the following figures for the 4 primers used i.e. RM 219, RM 234, RM 263 and RM 315.

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Primers	RM 234	RM 219	RM 315	RM 263
LR 1	1	1	1	1
LR 2	2	1	1	1
LR 3	1	2	1	1
LR 4	1	3	1	1
LR 5	1	1	1	1
LR 6	2	1	1	1
LR 7	2	1	1	1
LR 8	2	1	1	1
LR 9	1	1	1	1
LR 10	2	3	1	1
LR 11	2	1	1	1
LR 12	2	1	1	1
LR 13	2	3	1	1
LR 14	2	3	1	1
LR 15	2	3	2	1
LR 16	2	3	2	1
LR 17	2	4	2	1
LR 18	2	1	3	1
LR 19	2	2	3	1
LR 20	1	3	2	1
LR 21	2	3	2	1
LR 22	2	2	1	1
LR 23	2	0	2	2
LR 24	2	4	2	1
LR 25	0	2	3	1
LR 26	2	2	4	1

Table 1: Scoring by banding pattern with binary variables

Figure 1: RM 219

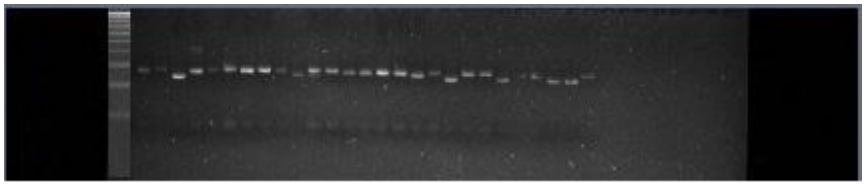

Figure 2: RM 219 INVERT

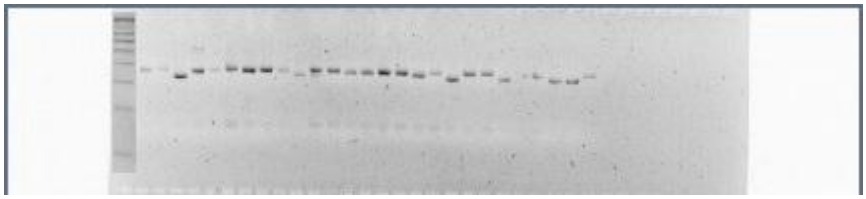

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Figure 3: RM 234

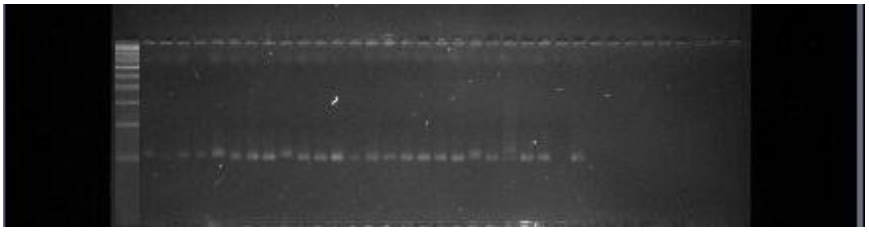

Figure 4: RM 234 INVERT

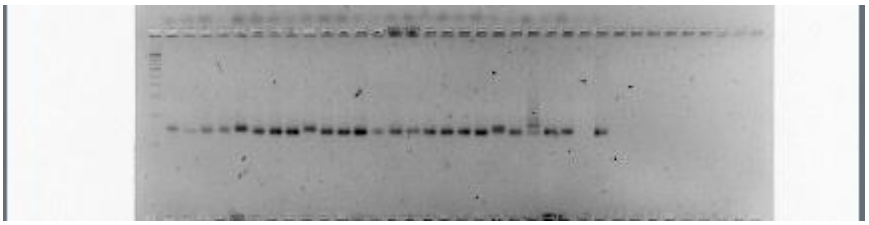

Figure 5: RM 263

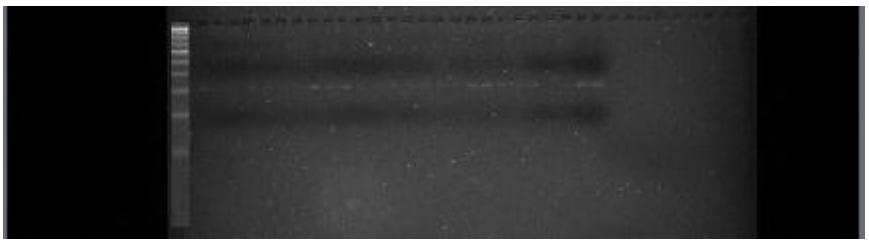

Figure 6: RM 263 INVERT

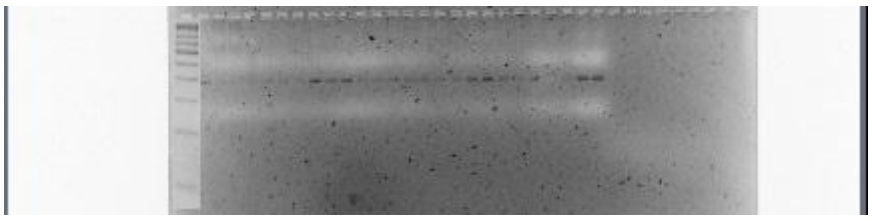

Figure 7: RM 315

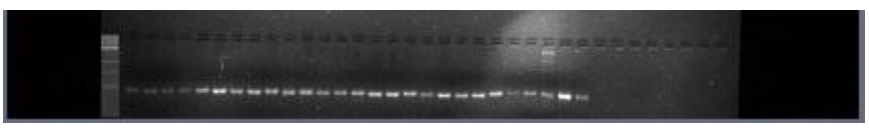

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Figure 8: RM 315 INVERT

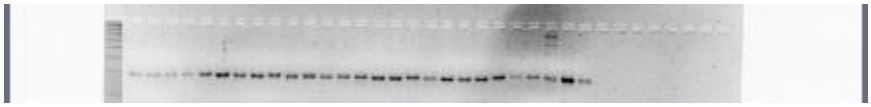
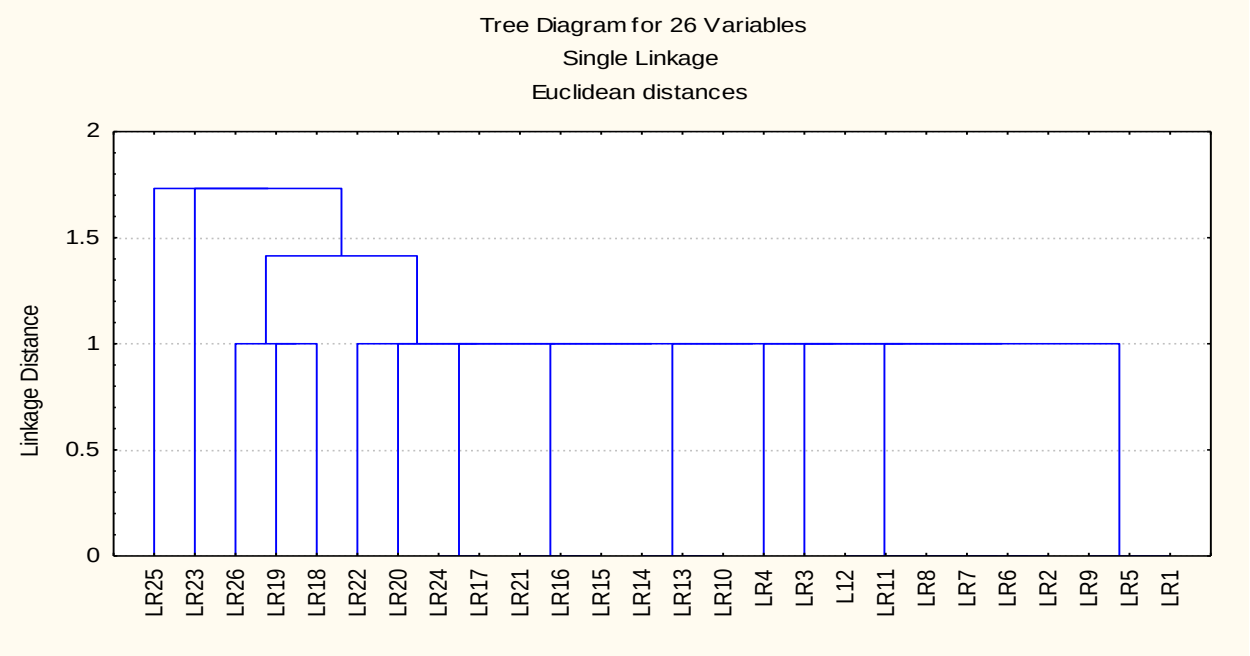


Figure 9: Dendogram: Cluster Analysis



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