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Original Research Article

Genetic and Molecular Analysis of Rice Recombinant Inbred Lines Under Saline Alkali Condition

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A B S T R A C T

Recombinant inbred lines developed involving parents CSR11and MI48 were evaluated in randomized complete block design with three replication in two sets one under salinity stress and other under control condition. The objectives for this study were (i) to work out the variability in RILs for various agro-morphological traits, (ii) to analyze the heritability and genetic advance, (iii) to find out the common Saltol QTLS in selected RILS. Observation were recorded on days to 50% flowering, plant height, panicles bearing tillers per plant, number of spikelets per panicle, number of grains per panicle, spikelet fertility (%), test weight (%) (g), sodium potassium ratio, biological yield (g), grain yield (g) and harvest index (%).The data obtained were analyzed for analysis of variance, mean performance of genotypes, coefficient of variability, heritability in broad sense and genetic advance in percent of mean. Experimental results revealed that analysis of variance for the design of experiment indicated highly significant differences among treatments for all the characters under both conditions. In general PCV was higher than GCV for all the characters. High heritability coupled with high genetic advance were recorded for yield and yield contributing traits under salinity alkali condition. The marker RM 123 and 140 exhibited polymorphism and validated its association with salinity QTLs.

Keywords

QTLs, *Saltol*, Heritibility, Correlation coefficient, Salt tolerance, Path coefficient, Genetic advance

Introduction

Rice (*Oryza sativa* L.) is the principal source of food for more than a third of the world's population and is one of the most widely grown crops in coastal areas inundated with sea water during high tidal period, although it is usually considered moderately susceptible to salinity.

80% of our country people depends fully (or) partially on rice as their main cereals food and stable diet. Nearly 6.73 million hectare soil in India a salt affected and categorized into 2-broad group: Alkali soils and Saline soils. The research to overcome salt related problems is based on either of the two approaches:change the growing

environment (make it normal) suitable for the normal growth of plants; or select the crop and/or change genetic architecture of the plant so that it could be grown in problem soil. The first approach involves major engineering and soil amelioration process which need lot of resources are often out of the reach of small and marginal farmers. The second approach i.e. breeding crop varieties with in-built salt tolerance is realized as the most promising, less resource consuming economical and socially acceptable approach. So the ability of the plant to tolerate the salt stress up to an extent is of paramount importance to manage the resources optimally and this is the reason to develop the tailored crops with higher salt tolerance suited to salt stress environments. Rice is found to be sensitive to soil salinity with salinity threshold level of 3.0dS m^{-1} and 12% reduction in grain yield per degree increase in electrical conductivity (EC) beyond this threshold, however it is moderately tolerant to sodicity (ESP 20–40%).

Soil salinity is serious constraint to increased production, in rice growing countries of the world (Gregorio, 1997). Molecular markers assisted selection (MAS) is man efficient and cost effective than the expensive and time consuming conventional screening under saline field condition. IRRI scientist has tagged *Saltol* gene on chromosome first of rice with the help of molecular markers (Gregorio, 1997).

Marker linked to *Saltol* gene now can be used effectively for screening of rice germplasm and breeding materials having the *Saltol* gene. DNA based molecular markers have been used extensively to assess the genetic diversity in most crop species. Due to high efficiency reproducibility, easy to use, co-dominance nature and high degree of polymorphism, microsatellites markers (or) simple sequence repeats (SSRs) are widely used as molecular markers for finger printing germplasm to assess genetic diversity, pedigree analysis, evolutionary studies and genome mapping.

Materials and Methods

Method for Sowing Seedling

The seeds of rice varieties were sown in nursery bed. After 25 days single seedling per hill were transplanted with 20 cm row to row and 15 cm plant to plant spacing in randomized complete block (RBD) design with three replications under salinity stress condition. The crop was maintained properly at 120: 60: 60 kg/ha NPK level. The experiment was initially grown under irrigated conditions.

Experimental Material

The material for this study consisted of 23 RILs developed by crossing of CSR-27 × MI-48 produced from the Department of Genetics and plant breeding, N.D.U.A. & T. Kumarganj, Faizabad.

Observation Recorded

Observation were recorded on randomly selected five plant from each entry line in each replication at maturity. These plants were harvested and threshed separately.

The data were recorded on following characters: days to 50% flowering, Plant height Panicle bearing tillers per plants, Spikelet per plant, Grains per panicle, Spikelet fertility (%),Test weight (g), Biological yield (g), Harvest index (%),Grain yield per plant(g), Na^+/K^+/ratio in both condition but Na^+/K^+/ratio not occur in irrigated/controlled condition.

Isolation of Genomic DNA from Leaves

Total genomic DNA from all rice varieties were isolated by CTAB method. CTAB was used to precipitate the nucleic acid at low salt concentration and low temperature. Firstly to collect the 50-100 mg fresh leaf of rice were taken and ground in liquid nitrogen with the help of pestle and mortar. Powdered leaves were taken in centrifuge tube and 4 mlof CTAB buffer was added. Then centrifuge tubes were heated in water bath at 65^0 C for 1 hour. After that centrifuge tube were cooled at room temperature and 3 ml of chloroform : isoamyl alcohol(24 : 1) is added. Mixed properly by inverting centrifuge tubes 20-25 times. Solution in centrifuge tubes were centrifuged at 6,000 rpm for 15 minutes at room temperature. Supernatant was transferred in fresh eppendrof tubes. In supernatant, half volume of 5 M NaCl and equal volume of iso-propanol were added and stored at 4^0C for overnight. Then, eppendrof tubes were centrifuged at 10,000 rpm for 20 minutes. Supernatant was discarded and pellet was washed with 70% ethanol. Pellet was re-suspended in 50 µl of TE buffer and stored at 4^0C.For purification of the genomic DNA firstly added RNAse was added to the DNA sample @ 5 µg/ µl and incubated at 37^0C for 1 hr. After 1 hr. 300 µl, 100% ethanol and 5 µl 3 M sodium acetate was added and kept in -70^0c for 30 minutes. Spined at 1000 rpm for 10 minutes. Supernatant was discarded and 100 µl 70% cold ethanol was added. Again spined at 1000 rpm for 10 minutes. Supernatant was discarded and pellet was dried for 30 minutes and dissolved in 45 µl TE buffer.

Agarose gel was casted in 0.8% 1X TAE (Tri Acetate EDTA) buffer containing ethidium bromide (2µl) and loaded 10 µl of DNA sample mixed with 5 µl loading dye. Gel was run at constant voltage (50 V for three hours). Gel was then visualized on U.V. using gel documentation system.

Dilution and Quantification of DNA

60 µl of autoclaved water was taken and 5 µl genomic DNA was added to make 50 to 100 ng per µl in each sample, based on their quantification volume. Genetic analysis of DNA sample took 1ml TE buffer in a cuvette and calibrated the spectrophotometer at 260 nm as well as 280 nm wave length. Added 5µl of DNA mixed properly and recorded the optical density (O.D) at both 260 and 280 nm and estimated the DNA concentration employing using the formula. For preparation of PCR reaction mixture (20.0µl) required DNA template (2.0 µl), 10 xTaq Buffer (2µl), dNTP mix (2 mM) (2 µl), $MgCl_2$(25 mM)(3 µl), Primer (F) (1 µl), Primer (R)(1 µl), Taq polymerase (5 u/ µl)(0.25 µl) and DDH_2O (8.75).19 µl of the mixture was added to 1 µl of dilute DNA and was used for amplification process.

The Cycling Parameters for SSR Marker

The initial incubation for 5 min. at 94°C, followed by 30 cycles, each consisting of denaturation step of 1 min. at 94°C, followed by an annealing step of 1 min. at 55°C and an extension of 2 min. at 72°C. The amplification reaction was concluded by a final extension at 72°C for 10 min. and then the temperature was decreased to 4°C until the reaction mixture was removed for PCR analysis. The amplified products were resolved on the 3% agarose gel and analyzed in the presence of ethidium bromide.

Agrose Gel Electrophoresisof the Amplified Products

In SSR 2.0% agarose gel was caste in 1 X TAE buffer and loaded 10 µl of amplified product mixed with 3 µl of loading dye.Gel

was run at constant voltage (40 V for three hour). Stained the gel with ethidium bromide solution (0.5 µl/ml) for 10 minute, washed with distilled water and visualized under UV light.Gel was then visualized on gel documentation system and photographed. After gel picture scoring was done for generation of genotype data. The marker which did not segregate for their alleles was recorded as monomorphic and the marker which segregate for their alleles was recorded as polymorphic.

Results and Discussion

Mean Performance of Genotypes

The mean performances of twenty five genotypes for eleven characters under salt and ten characters under control conditions are given in Table 1a and 1b. The highest grain yield was obtained in RILs- 18 and RIL-8. Among the high yielding genotypes five most promising genotypes in order of merit were RILs-18, RILs-8, RILs-17, RILs -23 and RILs -2 under salt conditions. Under control condition the highest grain yield was obtained in genotype 9.Five most promising genotypes in order of merit were RILs -9, RILs-5, RILs-1, RILs-4, and RILs-6. This indicate that the genotype performing better in non-stress condition not necessary perform better in stress condition. Therefore breeding varieties for different ecosystem recommended. RIL-17, RIL-18, RIL-19 and RIL-25 also exhibited good performance for biological yield, harvest index and grains per panicle. RILs-2 exhibited good performance for grains per panicle, plant height and spikelets per panicle under salt condition. RILs-25 exhibited above average mean performance for approximately all character under salt condition. RILs-23 had above average mean performance for grain yield, days to 50% flowering and panicle bearing tiller per plant under salt condition.

Beside, these selected genotypes few more genotypes which have desirable characters above CSR-27 were RILs-18, RILs-1, RILs-2 and RILs-6. These genotypes identified on the basis of desirable mean performance may be mentioned as elite lines for their probable genetic worth to be incorporate in hybridization programmes or cultivated in the salt and control condition after release.

Heritability in Broad Sense And Genetic Advance In Per Cent Of Mean

Estimates of broad sense heritability (h^2b) and genetic advance in per cent of mean for eleven characters under salt condition and ten characters under control condition in rice genotypes are depicted in Table 2 a and 2 b, respectively.Heritability estimates, which provides the assessment of transmissible genetic variability to total variability, happens to be most important basic factor that determine the genetic improvement or response to selection. However, the degree of improvement attained through selection is not only dependent on heritability but also on the amount of genetic variation present in the breeding material and extent of selection pressure applied by the breeder. The parameter, genetic advance in per cent of mean (GA) is a more reliable index for understanding the effectiveness of selection in improving the traits because its estimate is derived by involvement of heritability, phenotypic standard deviation and intensity of selection. Thus, heritability and genetic advance in per cent of mean, in combination provide clear picture regarding the effectiveness of selection for improving the plant characters.

Table.1a Mean performance of different rice genotypes under salt condition

Character	Days to 50% Flowering	Plant Height (cm)	Paicle Bearing Tillers/ Plant	Spkilets/ Panicle	Grains/ Panicllle	Spikelet Fertility (%)	Test Weight (g)	Biological Yield (g)	Na+/K+ / ratio	harvest Index (%)	Grain Yield (g)
Line – 1	84.0000	72.1667	9.6333	94.0000	83.9667	89.2967	20.3667	45.9667	0.1063	21.4133	9.8333
Line – 2	85.6667	70.5000	9.0333	95.4000	84.6667	88.7367	19.3900	43.7333	0.1813	25.6267	11.2000
Line – 3	86.3333	65.8333	8.6000	95.2333	85.6333	89.9367	20.7100	32.0667	0.2667	29.4400	9.4333
Line – 4	84.0000	67.5000	8.5667	84.0333	76.6333	91.1900	19.8633	42.0000	0.1660	25.3500	10.6333
Line - 5	85.6667	67.6667	8.0333	86.8000	78.2000	90.1000	20.6033	41.9667	0.1720	25.8567	10.8333
Line - 6	89.0000	61.9667	10.1667	90.6667	81.9333	90.3667	20.7933	43.6000	0.0920	25.5000	11.1000
Line - 7	87.6667	59.3000	7.7667	91.7000	82.3667	89.7967	20.9233	37.0333	0.1203	26.8467	9.9333
Line - 8	85.6667	60.6000	9.8333	84.7667	76.3667	90.0300	22.0767	42.9667	0.2103	26.8533	11.5333
Line - 9	87.0000	65.5667	8.4667	82.3000	71.5333	86.8833	21.3233	38.3000	0.1170	25.4300	9.7333
Line - 10	89.6667	59.5667	8.9333	87.7667	78.0333	88.9033	19.9333	43.8000	0.3920	23.1633	10.1333
Line - 11	88.3333	64.6667	9.8333	86.0667	74.9000	87.0233	19.8400	43.7333	0.1670	22.8900	10.0000
Line - 12	88.0000	69.3667	8.2667	86.5000	75.9000	87.7100	20.9833	47.1000	0.1800	22.4900	10.6000
Line - 13	90.0000	62.3667	6.9667	89.4000	77.7333	86.9533	20.6333	40.3000	0.1323	23.7367	9.5667
Line - 14	89.0000	64.2667	10.8667	91.5333	79.9000	87.3000	21.5667	38.8333	0.0810	26.8000	10.4000
Line - 15	86.0000	63.1667	8.4000	91.3333	80.9000	88.5567	20.6967	40.2667	0.1680	23.8400	9.6000
Line - 16	82.0000	65.4000	10.5000	91.6333	81.5333	88.9633	20.3100	43.7333	1.1400	23.3700	10.2000
Line - 17	90.0000	64.7000	10.5333	86.1000	75.8000	88.0000	20.7333	45.5333	0.1870	25.1767	11.4667
Line - 18	88.0000	67.1333	11.7000	85.3667	74.4000	87.1033	20.5733	49.0667	0.2037	24.0433	11.8000
Line - 19	86.0000	65.8000	10.8000	87.1333	75.7333	86.9067	20.5367	48.8667	0.1270	22.7867	11.1333
Line - 20	85.3333	70.1667	10.9000	94.0333	84.7667	90.0933	21.0967	41.9667	0.1700	23.5633	9.8667
Line - 21	88.3333	60.4667	9.5000	86.4333	76.0333	87.9667	20.7767	42.2000	0.2157	25.1333	10.6000
Line - 22	88.3333	58.2333	10.9333	95.6333	85.3667	89.2300	20.5967	45.4333	0.2100	23.0367	10.4667
Line - 23	88.3333	61.0667	10.4667	91.8667	81.1333	88.3367	21.1467	43.7000	0.1437	25.8033	11.2667
Line - 24	88.0000	59.9667	11.2333	88.8000	80.1000	89.3833	20.7000	42.1000	0.3940	25.1767	10.6000
Line - 25	89.0000	62.4667	12.1333	94.7000	84.2667	88.9400	20.1300	38.7333	0.1743	28.6000	11.0667
Mean	**87.1733**	**64.3960**	**9.6827**	**89.5680**	**79.5120**	**88.7083**	**20.6521**	**42.5200**	**0.2207**	**24.8771**	**10.5200**
C.V.	1.6440	1.5556	8.1296	4.8318	6.3814	1.5456	2.5729	6.0496	1.1754	3.5078	5.2185
C.D. 5%	2.3528	1.6445	1.2923	7.1047	8.3299	2.2508	0.8723	4.2229	0.0043	1.4326	0.9013
C.D. 1%	3.1386	2.1938	1.7239	9.4777	11.1121	3.0026	1.1637	5.6333	0.0057	1.9111	1.2023
Range Lowest	82.0000	58.2333	6.9667	82.3000	71.5333	86.8833	19.3900	32.0667	0.0810	21.4133	9.4333
Range Highest	90.0000	72.1667	12.1333	95.6333	85.6333	91.1900	22.0767	49.0667	1.1400	29.4400	11.8000

Table.1b Mean performance of different rice genotypes under control condition

Character	Days to 50% Flowering	Plant Height (cm.)	Panicle Bearing Tillers/ Plant	Spikelets/ Plant	Grains/ Panicle	Spikelet Fertility (%)	Test Weight (g)	Biological Yield/ Plant(g)	Harvest Index (%)	Grain Yield (g)
Line – 1	95.6667	97.1333	17.2667	122.7333	113.3667	92.3533	24.1333	72.0000	21.6700	15.6000
Line – 2	97.6667	95.4000	14.1333	117.5667	107.9333	91.7900	24.1100	60.0000	22.0000	13.2000
Line – 3	94.3333	87.7700	13.8900	125.3333	115.9333	92.4933	23.3667	64.6667	20.7367	13.3333
Line – 4	93.6667	91.3667	17.4667	118.4667	112.7333	95.1433	24.0700	63.3333	22.0567	13.7333
Line – 5	90.0000	92.2033	17.7000	114.0667	104.7667	91.8567	23.9500	61.6667	25.3800	15.2000
Line – 6	98.3333	89.3633	15.7333	121.9667	114.3667	93.7733	23.8033	56.3333	24.6600	13.7033
Line – 7	97.3333	91.5833	20.2333	135.9000	126.9333	93.3967	22.8567	63.0000	20.8700	13.0433
Line – 8	91.3333	92.6933	19.4000	111.1000	103.7333	93.3800	22.3567	46.6667	28.7567	13.2900
Line – 9	92.0000	95.6967	21.2000	107.2667	99.1333	92.3700	23.2933	65.0000	24.3067	15.5333
Line – 10	98.0000	93.0333	16.2000	111.1000	103.0667	92.7367	23.7800	57.3333	22.3267	12.7333
Line – 11	89.6667	93.3667	16.5667	113.6333	106.2667	93.4533	23.7000	53.3333	22.5567	11.9667
Line – 12	92.0000	98.2000	16.2000	97.6467	88.5133	90.6500	22.9967	67.0000	20.6400	13.7000
Line – 13	92.6667	96.6467	19.4667	121.7000	112.3000	92.2500	22.5467	57.3333	23.2067	13.2667
Line – 14	93.0000	96.4500	19.8667	97.1133	88.4467	91.0833	24.6933	52.0000	23.7100	12.3333
Line – 15	90.0000	96.5867	16.0667	97.8000	89.1000	91.0933	23.5433	60.0000	22.6867	13.6000
Line – 16	90.6667	94.7933	16.2667	118.7000	110.6333	93.2200	23.3467	57.6667	21.5367	12.2667
Line – 17	90.3333	89.0600	15.4000	105.2667	98.3000	93.3900	24.0667	51.6667	24.1200	12.4667
Line – 18	94.0000	88.2233	16.4000	105.7667	95.6000	90.3767	24.2800	49.3333	22.2667	10.8000
Line – 19	92.6667	93.2867	15.8000	129.1333	118.8667	92.0600	23.9700	53.3333	21.4433	11.4333
Line – 20	93.0000	91.4200	18.2667	126.2000	116.7333	92.5033	23.9733	55.0000	22.4233	12.3000
Line – 21	90.3333	90.3033	20.0333	117.2333	108.4333	92.4667	23.6767	53.3333	23.7333	12.6667
Line – 22	91.3333	86.3933	18.1333	128.2667	119.0000	92.7233	22.8133	60.6667	19.2900	11.7000
Line – 23	91.6667	85.7200	14.1000	117.1000	106.3333	90.8100	23.6300	46.3333	24.7433	11.3667
Line – 24	98.3333	91.0667	18.4000	121.5000	113.0667	93.0233	23.5033	56.6667	22.5800	12.8000
Line – 25	98.6667	86.0433	15.5000	128.4667	118.6667	92.3600	22.6700	56.6667	23.0533	13.0000
Mean	**93.4667**	**92.1521**	**17.1876**	**116.4411**	**107.6891**	**92.4303**	**23.5652**	**57.6133**	**22.8301**	**13.0015**
C.V.	1.8169	1.9920	15.3695	4.5555	5.6372	1.0257	2.7783	13.8190	9.2999	9.5515
C.D. 5%	2.7879	3.0135	4.3367	8.7083	9.9661	1.5564	1.0748	13.0704	3.4856	2.0387
C.D. 1%	3.7191	4.0200	5.7852	11.6168	13.2947	2.0763	1.4338	17.4359	4.6498	2.7196
Range Lowest	89.6667	85.7200	13.8900	97.1133	88.4467	90.3767	22.3567	46.3333	19.2900	10.8000
Range Highest	98.6667	98.2000	21.2000	135.9000	126.9333	95.1433	24.6933	72.0000	28.7567	15.6000

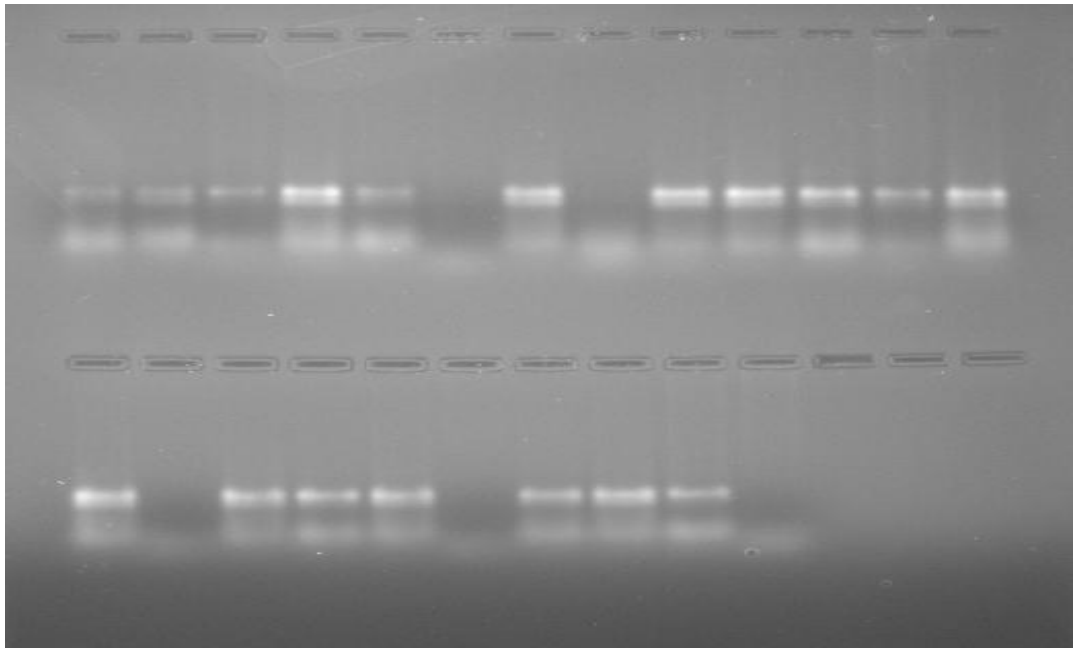
Table.2a Estimates of grand mean, C.D., C.V., range, phenotypic (PCV) and genotypic (GCV) coefficient of variation (%), heritability in broad sense (h^2b) and genetic advance in per cent of mean (GA%) for 11 characters in rice genotypes under salt condition

Characters	Grand mean	C.D. 5%	C.V.	Range		PCV (%)	GCV (%)	h^2	GA in % of mean
				Min.	*Max.*				
Days to 50% flowering	87.17	2.35	1.64	82.00	90.00	2.70	2.15	63	3.51
Plant height (cm)	64.40	1.64	1.55	58.23	72.16	6.09	5.89	98	11.73
Panicle bearing tillers per plant	9.68	1.29	8.12	6.96	12.13	15.31	12.97	72	22.63
Spikelet per panicle	89.57	7.10	4.83	82.30	95.63	5.92	3.43	33	4.09
Grains per panicle	79.51	8.32	6.38	71.53	85.63	7.23	3.40	22	3.29
Spikelet fertility(%)	88.71	2.25	1.54	86.88	91.19	1.89	1.10	33	1.30
Test weight(g)	20.65	0.87	2.57	19.39	22.07	3.48	2.34	45	3.25
Biological yield per plant (g)	42.52	4.22	6.04	32.06	49.06	10.12	8.11	64	13.40
Na^+/K^+/ratio	0.22	0.00	1.17	0.08	1.14	93.40	93.39	100	192.37
Harvest index (%)	24.88	1.43	3.50	21.41	29.44	8.26	7.48	82	13.94
Grain yield per plant (g)	10.52	0.90	5.21	9.43	11.80	7.73	5.71	54	8.67

Table.2b Estimates of grand mean, C.D., C.V., range, phenotypic (PCV) and genotypic (GCV) coefficient of variation (%), heritability in broad sense (h^2b) and genetic advance in percent of mean (GA%) for 10 characters in rice genotypes under control condition:

S. NO.	Character	Grand mean	C. D. 5%	C.V.	Range		PCV (%)	GCV (%)	h^2	GA in % Of mean
					Min.	Max.				
1	**Days to 50% flowering**	93.46	2.78	1.81	89.66	98.66	3.55	3.05	74	5.39
2	**Plant height (cm)**	92.15	3.01	1.99	85.72	98.20	4.30	3.81	79	6.97
3	**Panicle bearing tillers per plant**	17.18	4.33	15.36	13.89	21.20	17.26	7.85	21	7.35
4	**Spikelet per panicle**	116.44	8.70	4.55	97.11	135.90	9.68	8.55	78	15.53
5	**Spikelet per panicle**	107.68	9.96	5.63	88.44	126.93	10.53	8.89	71	15.47
6	**Spikelet fertility(%)**	92.43	1.55	sss1.02	90.37	95.14	1.45	1.02	50	1.49
7	**Test weight(g)**	23.56	1.07	2.77	22.35	24.69	3.38	1.93	32	2.26
8	**Biological yield per plant(g)**	57.61	13.07	13.81	46.33	72.00	15.69	7.42	22	7.24
9	**Harvest index (%)**	22.83	3.48	9.29	19.29	28.75	11.28	6.38	32	7.43
10	**Grain yield per plant (g)**	**13.00**	**2.03**	**9.55**	**10.80**	**15.60**	**12.12**	**7.46**	**38**	**9.46**

1 2 3 4 5 6 7 8 9 10 11 CSR 27 MI -48



14 15 16 17 1 8 19 20 21 22 23 24 25

Fig.1 Agarose gel sowing PCR amplification products using salt specific SSR primer RM 140

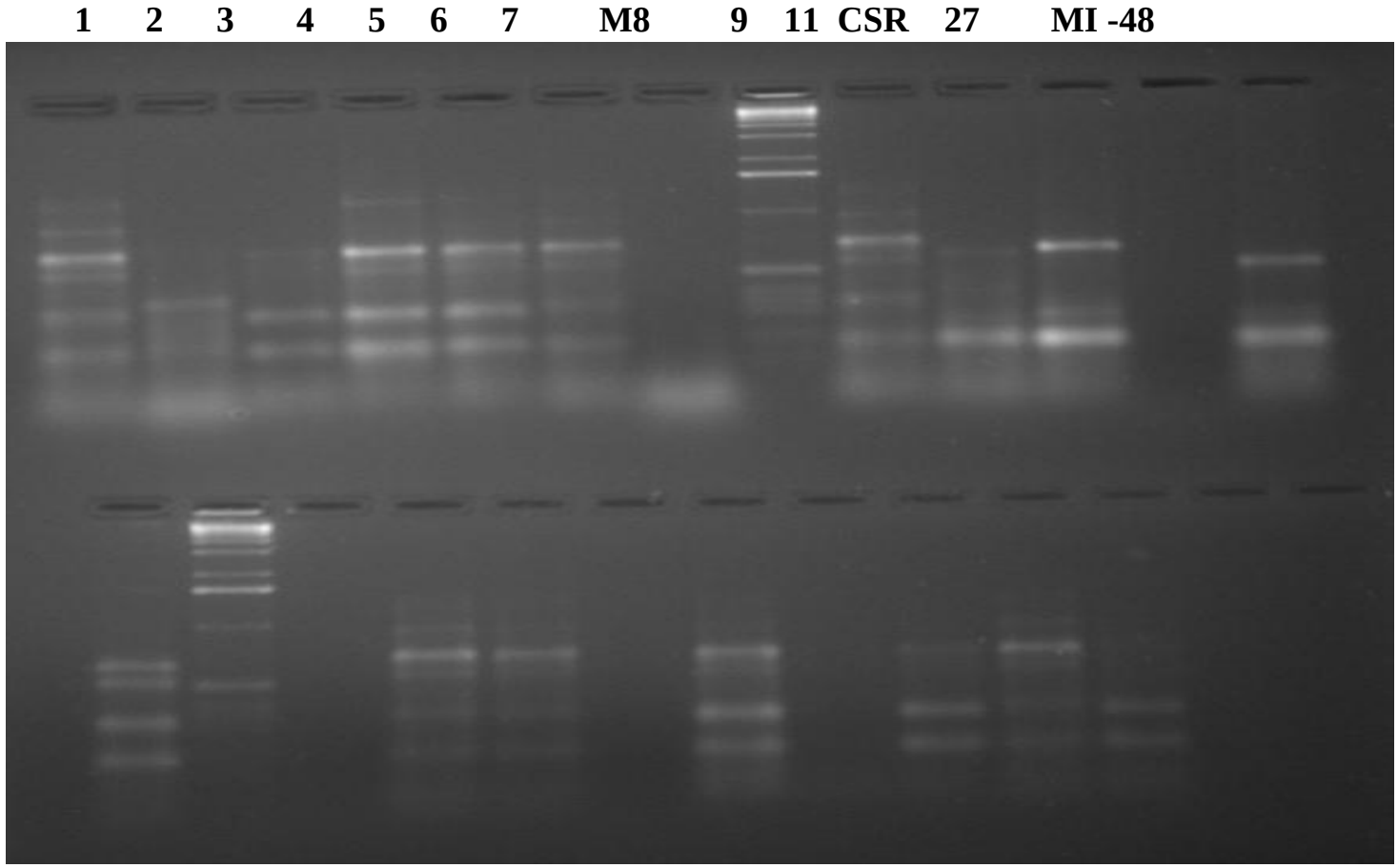


Fig.2 Agarose gel sowing PCR amplification products using salt specific SSR primer RM 493

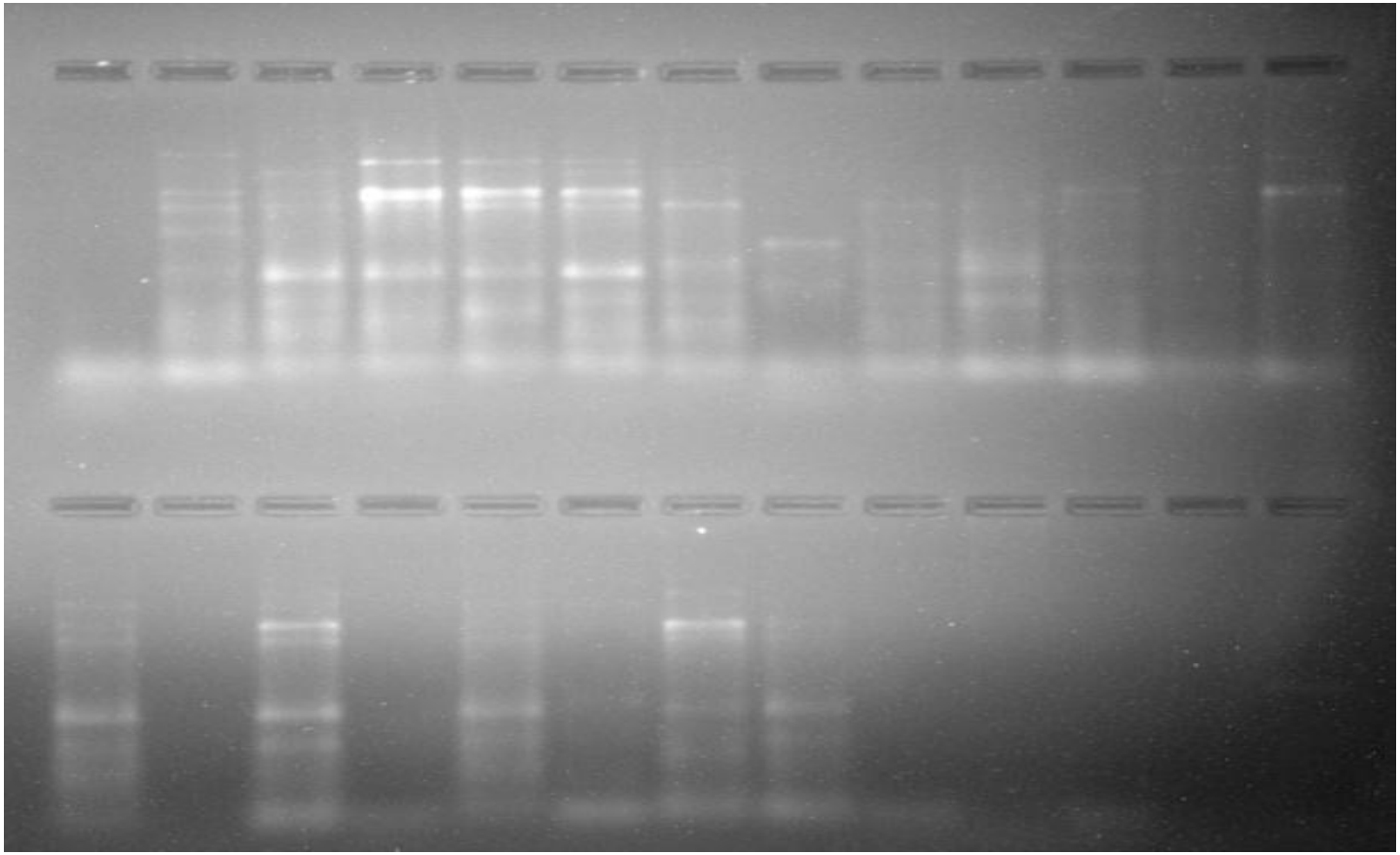


Fig.3 Agarose gel sowing PCR amplification products using salt specific SSR primer RM 123

Under salt condition high heritability coupled with high genetic advance were estimated for Na^+/K^+/plant, plant height, biological yield, harvest index, panicle bearing tillers per plant, and panicles per plant. Under control conditions high estimates of heritability coupled with high genetic advance were estimated for plant height, spikelet per panicle, biological yield, number of grains per panicle, days to 50% flowering, panicles bearing tiller per plant and grain yield per plant, which represents ideal situation for obtaining very high response through selection. Similar findings were also reported by Chand*et al.,* (2004) and Suman *et.al.* (2005), Tarannum and Dwivedi (2006) evaluated 46 rice genotypes and observed high heritability and high genetic advance as per cent of mean for total number of tillers per plant, seedling vigor, number of filled grains per panicle, biological yield, harvest index and number of spikelets per panicle.

Molecular Analysis for Validation Salt QTLs

The DNA amplification was done with RM 113, RM 140, RM 3412 and RM-493. Out of four primers RM 140 show monomorphic (Figure 1) and RM 113 and RM 493 exhibited polymorphism (Figure 2 and 3). RM 3412 failed to amplify DNA. All RILs exhibited similar band to CSR-27 (salt tolerance) and having different alleles to MI-48 (salt susceptible).The RILs having similar band to CSR-27 are identified as salt tolerant genotype.

In general, marker detecting greater number of alleles per locus detected more number of unique alleles in accordance with the earlier reports (Dhar *et al.,* 2012; Lodha*et al.,* 2011; Titov *et al.,* 2009; Kanawapee *et al.,* 2011). The presence of unique alleles indicated that the materials used in this studyare useful as a rich source of genetic diversity for their purposeful and effective utilization in rice breeding.

The present investigation 23 RILs along with two checks CSR-27 and MI-48 were evaluated under salt and control conditions. For identification and validation of QTLs associated with salt, the introgression lines were scan with microsatellite markers.The markers RM 113 and 493 exhibited polymorphism and is related with salt tolerance. The data analysis for heritability in broad sense, genetic advance in per cent of mean, correlation coefficient, path analysis and genetic divergence. Experimental results revealed that analysis of variance for the design of experiment indicated highly significant differences among treatments for all the characters. In general PCV was higher than GCV for all the characters. High heritability coupled with high genetic advance were recorded for plant height, spikelets per panicle, biological yield, grains per panicle, spikelet fertility, panicle bearing tillers per plant, days of 50% flowering, harvest index and grain yield under control condition. Plant height, biological yield, spikelet fertility, days of 50% flowering, harvest index and grain yield under salt condition. This indicated that these characters are likely to provide good response to selection and correlation coefficient at phenotypic level showed biological yield was positively and highly significantly correlated with spikelet fertility and no.of grain per panicle under salt conditions while in control condition grain per panicle and grain yield per plant exhibited highly significant and positive correlation with biological yield and spikelet per panicle. The path coefficient analysis indicated that biological yield, test weight and harvest index had maximum direct effect on grain yield at phenotypic under salt condition. Biological yield and harvest index had maximum direct effect on grain yield at phenotypic level under control condition.

Biological yield exerted very high positive indirect effect on grain yield at phenotypic under control conditions *via* harvest index.

References

Agnihotri, R.K., Palni, L.M.S. and Pandey, D.K. (2006). Screening of landraces of rice under cultivation in Kumaun Himalaya for salinity stress during germination and early seedling growth.*Indian J. of Plant Physiol.*. 11(3):266-272.

Akbar, M., and Ponnamperuma, F.N. (1972). Saline soils of south and south east Asia as potential land. *IRRI Los Banos Laguna, Philippines.*

Anand, G., Amirthadevarathinam, A., and Rogbell, J.E. 1998. Variability and correlation in rice (*Oryza sativa* L) under cold condition. *CropRes.* (Hisar), 15 (1):104-107.

Anbanandan,V., Saravanan K., and Sabesan, T. (2009). Variability, heritability and genetic advance in rice (*Oryza sativa* L.). *International J. Plant Sci.,* (4)1 *: 61-63.*

Babar, M., Khan, A.A., Arif, A; Zafar, Y. and Arif, M. 2009. Path analysis of some leaf and panicle traits affecting grain yield in double haploid lines of rice (*Oryza sativa* L), *J. Agri. Res*., 45 (4): 245-252..

Babu, S., Anbumalarmathi, J., Yogameenakshi, P., Sheeba, A. and Rangaswamy, P. 2003. Genetic divergence studies in rice. *Crop research*., 25 (2): 280-286.

Bagali, P.G.; Hittalmani, S. and Shashidhar, H.E. (1999). Character association and path coefficient analysis in *indica* X *Japonica* doubled haploid population of rice. *Oryza*, 36 (10): 12.

Borbora, T.K., Hazarika, G.N., and Medhi, A.K. 2005. Correlation and path analysis for panicle characters in rice. *Crop Research*, 30 (2):215-220.

Burton, G.W. and De Vane, E.M. 1953. Estimating heritability in all foscue (*Festuca arundica*) for replicated clonal material. *Agron. J.*,45: 478-487.

Dhar P, Ashrafuzzaman M, Begum SN, Islam MM, Chowdhury MMH 2012. Identification of salt tolerant rice genotypes and their genetic diversity analysis using SSR markers. *Int. J. Biosci.*,2(9): 40-50.

Gregario, G.B. 1997. Tagging salinity tolerance genes in rice using amplified fragment length polymorphism (AFLP), *Ph.D. Thesis University Philippines Los Banos Laguna Philippines*.

Gregario, G.B., Senadhira, D., Mendoza, R.D., Manigbas, N.L., Roxas, J.P. and Guerta, C.Q. 2002. Progress in breeding for salinity tolerance and associated abiotic stress in rice. *Field Crop Res.* 76 (2/3): 91-101.

Jha, S.K. Awasthi, L.P. and Maurya, D.M. (1999). Genetic diversity in rice germplasm of eastran U.P. India *Oryza* 36 (2):157-158.

Johnson, H.W., Robinson, H.F. and Comstock, R.E. 1955. Estimates of phenotypic and genotypic correlation in soybean and their implication in selection. *Agronomic Journal*, 47 : 477-482.

Kanawapee N,Sanitchon J, Srihaban P and Theerakulpisut P2011.Genetic diversity analysis of rice cultivars differing in salinity tolerance based on RAPD and SSR markers.14(6): 1-15.

Kempthorne, O. and Curnow, R.N. 1961. The partial diallel cross. *Biometrics*, 17: 229-250.

Lodha T, Karmakar J, Roychoudhuri R and Dey N 2011. Assessment of genetic diversity of some commonly grown rice genotypes of South Bengal using microsatellite markers associated with the saltol QTL mapped on 1st Chromosome. J.plant Sci., 5(1):35-39.

Titov*et al.,* (2009). Phenotypic and genotypic screening of rice genotypes at seedling stage for salt tolerance. *Revista UDO Agrícola*, 9 (4): 770-775.

Yadav, S., Saini, N., Rana, P., Jain, S. And jain, R.K. 2009. Identification of SSR markers for salt tolerance in rice variety CSR 10 by selective genotyping. *J Plant Biochem. & Biotechnology* 18 (1): 87-91.