MOLECULAR MARKER BASED IDENTIFICATION OF RESTORERS AND MAINTAINERS IN RICE

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- **Identification of restorers and maintainers from genetically diverse materials is a prerequisite for hybrid rice development.**
- **Generally restorers and maintainers are identified by evaluation of test cross hybrids for pollen and spikelet fertility. Phenotypic evaluations is a cumbersome, labour intensive and time consuming method.**
- **It was discovered that fertility restoration is controlled by two independent dominant nuclear genes with one stronger in action than the other (Young and Virmani 1984).**

- **Use of micro satellite markers tightly linked to fertility restorer genes (*Rf*) will help in rapid identification and utilization of parental lines and thereby increasing the breeding efficiency.**

- **A.K.Singh et al,(2005) identified a microsatellite marker,RM6100, linked with *Rf*4 gene in restorer lines at a distance of 6-7cM on chromosome 10.**

- **In our study a set of 165 breeding lines from IRRI were evaluated for presence of fertility restorer gene *Rf4* using an SSR marker RM 6100.**

- **Standard restorers and maintainers were used as checks. The lines were classified in to restorers and maintainers based on unique banding pattern in comparison with the checks.**

IRRI BREEDING MATERIALS USED IN THE STUDY

S.NO	Type of material	Number of lines
1	Aerobic lines	44
2	Lowland Irrigated	70
3	Salinity	13
4	Green Super Rice	13
5	New Plant Type-II	14
6	INGER entries	11
7	Total	165

METHODOLOGY

GENOMIC DNA ISOLATION AND PCR

- Total genomic DNA was isolated from fresh young leaves of individual plants using Cetyl-trimethyl-ammonium bromide(CTAB) method described by MURRAY and THOMPSON (1980), with some modifications.
- Polymerase chain reaction (PCR) was performed in 15ul reaction volumes containing 25 to 30ng of template DNA, 1x PCR reaction buffer(Banglore genei) 0.5 Units Taq polymerase, 200uM deoxyribonucleotides (dNTPS), and 5 Pico moles of each primer.

PCR PROGRAMME

The PCR conditions were an initial denaturation at 95°C for 5 min, followed by 30 cycles of denaturation (94°C for 15 Seconds), primer annealing (55°C for 30 Seconds) and extension (72°C for 45 Seconds), and a 6-min final extension at 72°C.

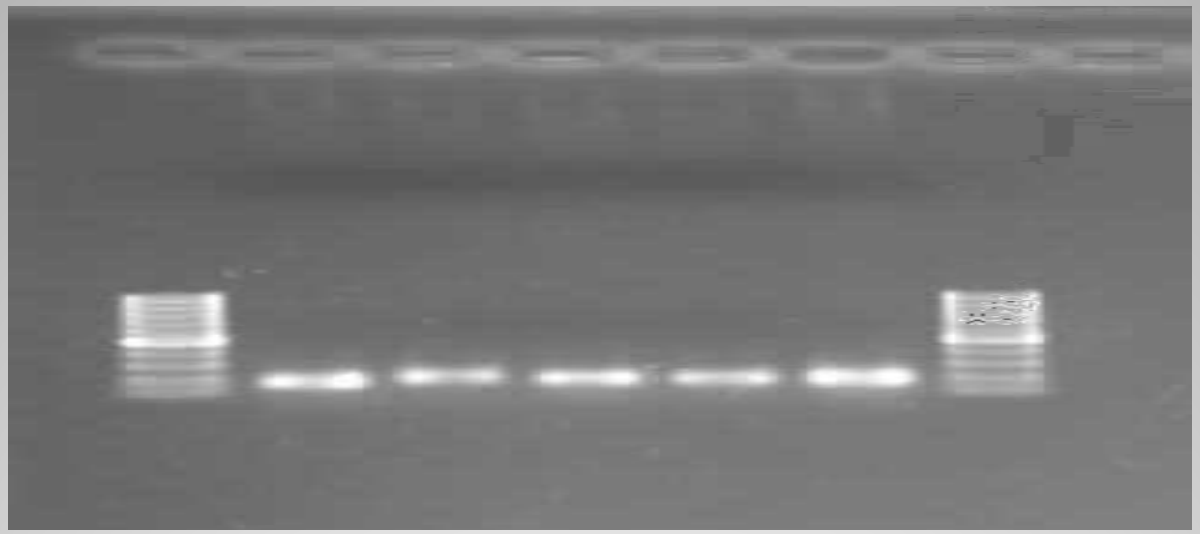
PRIMER SEQUENCE

RM6100

Forward: TCCTCTACCAGTACCGCACC

Reverse: GCTGGATCACAGATCATTGC

FIG: PCR AMPLIFICATION CHECK IN 1 % AGAROSE GEL



- **The PCR products were then resolved in 6% (w/v) Denaturing polyacrylamide gels. The gels were stained with Silver nitrate, documentation was carried out and the plates were scored.**

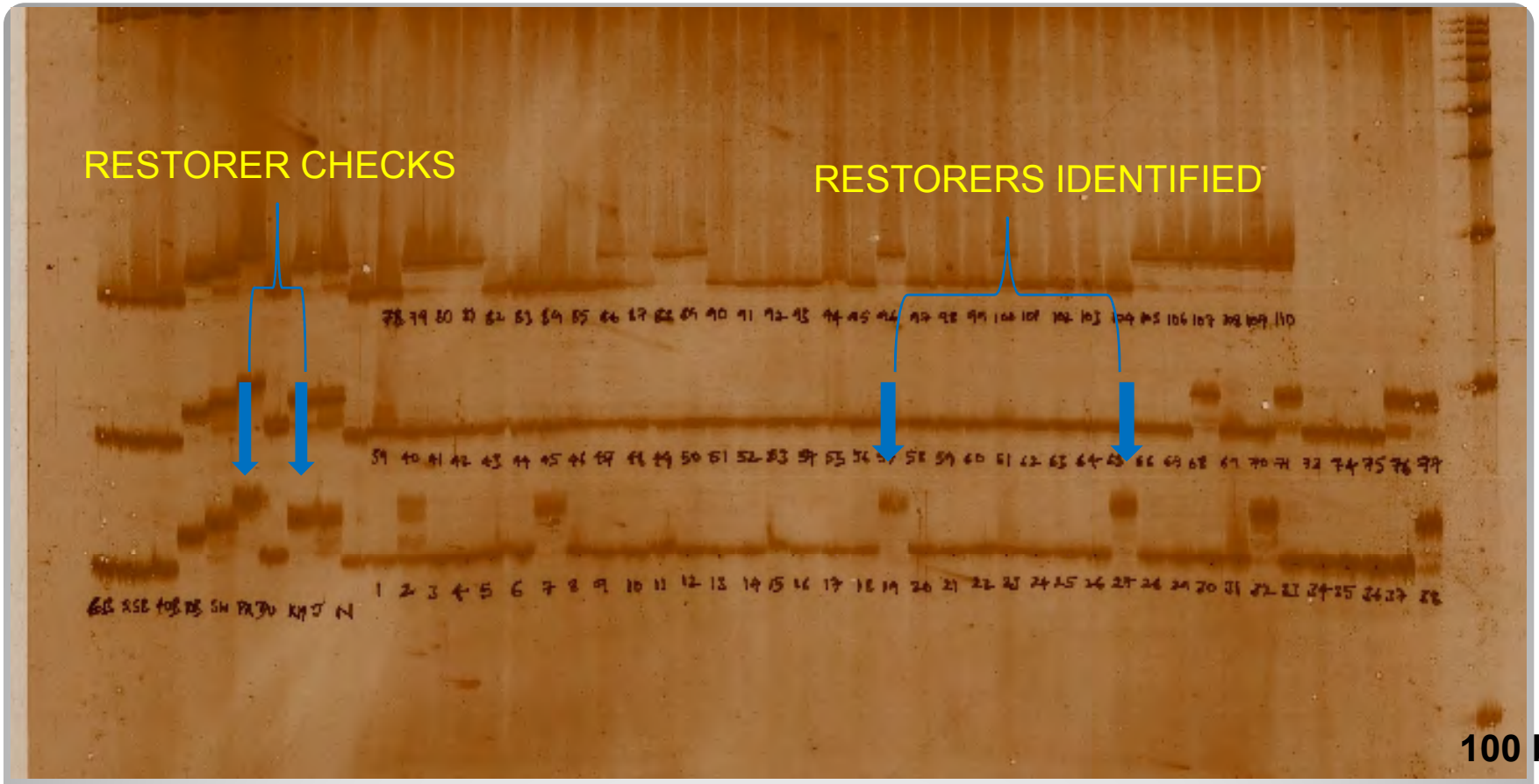


Fig: Evaluation of presence of fertility restorer gene *Rf4* *using SSR marker RM*6100 in the lines based on unique banding pattern in comparison with standard checks.

FIELD EVALUATION

- 165 test cross hybrids involving IR 58025A and IRRI breeding lines were produced.
- All Test cross hybrids (15-20 plants/cross) were evaluated in the field along with the parents
- Test cross progenies with incomplete panicle exertion were subjected to pollen study and entries with 98-100 % pollen sterility were classified as maintainers
- Test cross entries with complete panicle exertion and more than 80% spikelet fertility were classified as restorers

RESULTS

- The molecular data were compared with spikelet fertility data of test cross hybrids evaluated in the field.
- Out of 165 lines evaluated, 50 restorers and 72 maintainers showed one to one correspondence with molecular data.
- The primer RM6100 showed 94.28% selection accuracy for R.M.Sundaram (2009) et al in mapping population and 97% for singh et al, (2005)

FREQUENCY OF RESTORERS IDENTIFIED

MATERIAL	PERCENTAGE
NPT-II	71
GSR	69
LOW LAND	36
AEROBIC	7
SALINITY	15
INGER	18

FREQUENCY OF MAINTAINERS IDENTIFIED

MATERIAL	PERCENTAGE
INGER	73
SALINITY	62
AEROBIC	55
GSR	15

CONCLUSION

- As marker RM6100 is closely linked to the *Rf* locus and it could be useful in routine screening of germplasm to identify potential restorers.
- Prediction based on a single marker is 74 % accurate and use of one more marker linked to fertility restorer gene will be very useful for increasing the accuracy.
- Marker based identification of hybrid parental lines will be less time consuming and enhance the breeding efficiency and thereby accelerating the development of heterotic hybrids in the near future.

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THANK YOU