Application of *S5* functional marker system in inter-subspecific hybridization for improving level of heterosis

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Introduction

Heterosis

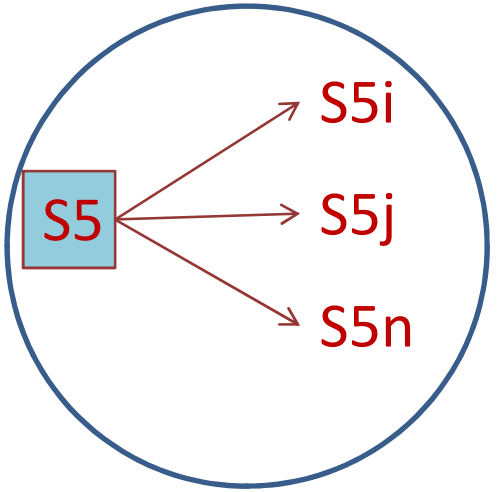
Inter-subspecific hybridization

Hybrid sterility

(Kato *et al, 1928)*

Major break through

- Discovery of Wide Compatible Varieties (WCVs), Ikehashi & Araki (1984)
- WC genes -1986
- Genetic model- Wide compatibility
- Single locus allelic interaction model
- S5i/S5n , S5j/S5n – Fertile
- S5i/S5 j - Semi sterile



WCVs -Wide compatible varieties

India	China	Japan	Philippines
N22	02428	CPSLO 17	Moroberekan
Dular	Varylava	Calotoc	BPI 76
Jaldidhan	Lemont	KetaNangka	Fossa HV
Gharbharan	Pei-ai 64	Norin PL-9	Palawan
	LunHui422	Padi Bujag	Lambayeque1
		Pendak	

Identification of WCVs

- Conventionally- Test crossing
- Evaluating spikelet fertility of F_1
- Tedious, Time consuming, often inconclusive
- Use of molecular markers can overcome these limitations

Sterility mechanisms- (*I* x *J* hybrids)

1. Female gamete abortion (Embryo sac abortion)
2. Pollen sterility
3. Reduced affinity between the uniting male & female gametes

S5n allele - overcomes embryo sac sterility of I x J crosses

With the availability of MM, saturated linkage maps, and genome sequence in rice and considering the importance of HS &WC

Identification and Mapping

> 50 genes HS & WC

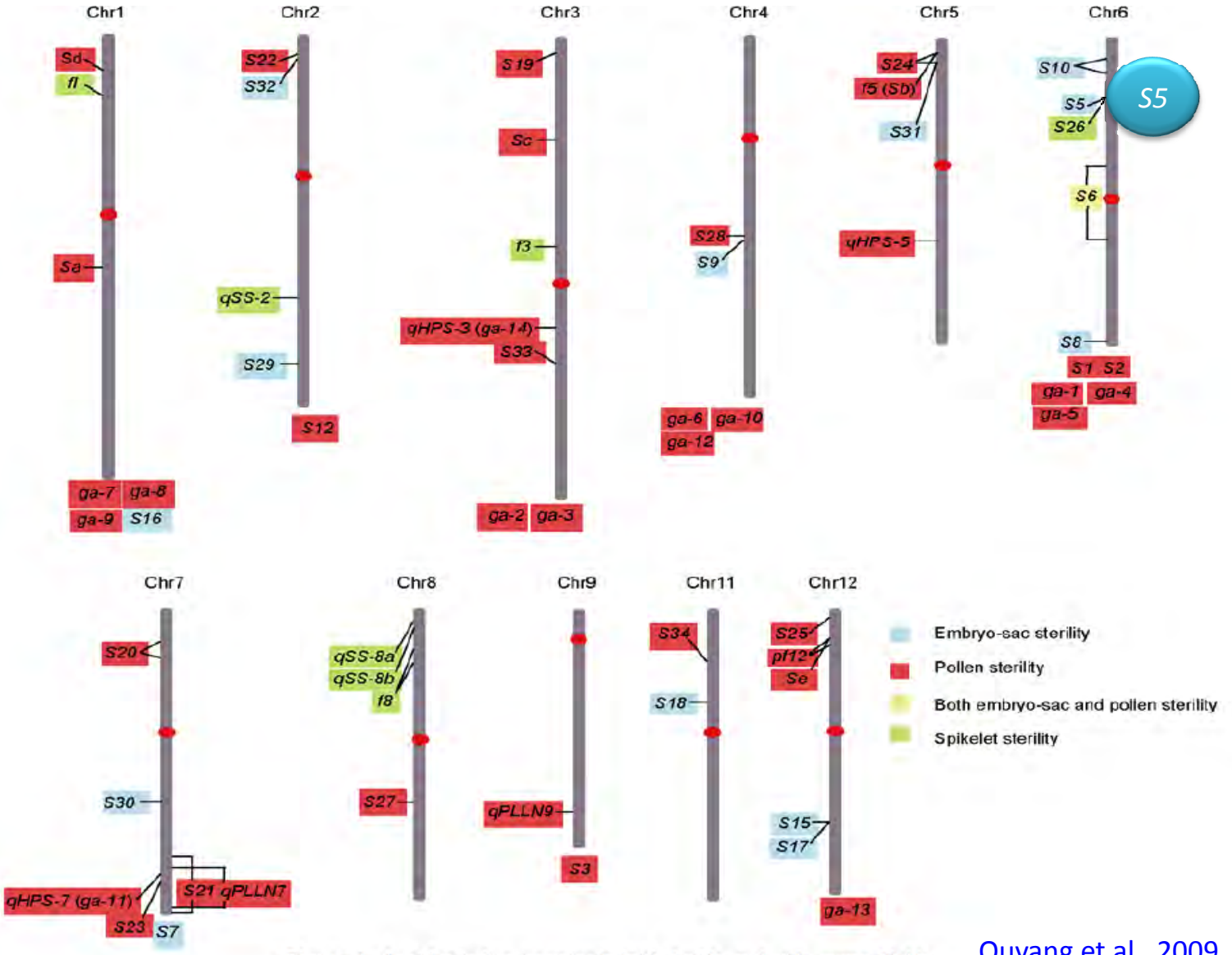


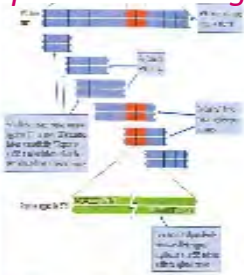
Figure 1 Loci for *indica-japonica* hybrid sterility and wide-compatibility.

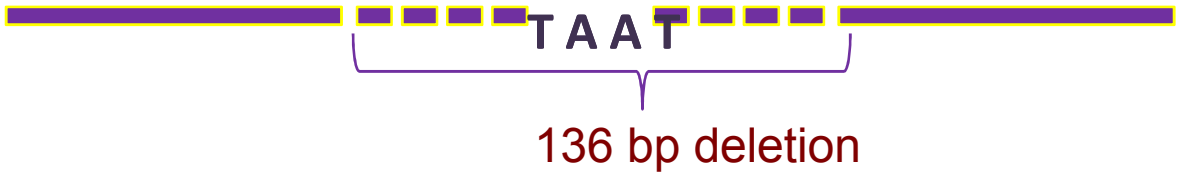
Ouyang et al., 2009

S5 major locus

- S5 cloned - Map based cloning (Chen et al.,2008)
- Encodes- Aspartic protease

Map based cloning



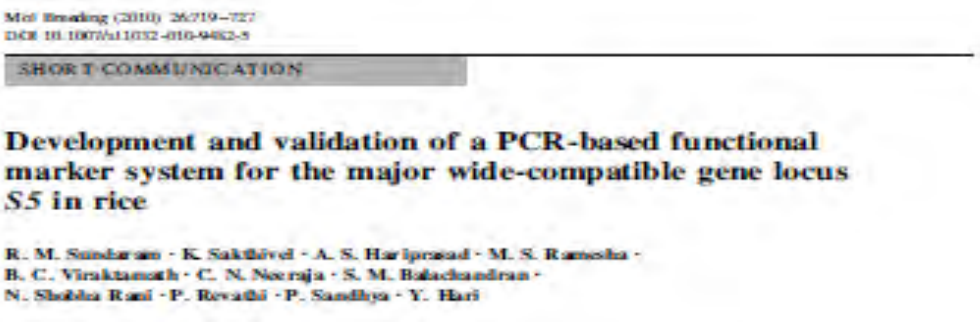


- $S5^n$- a discontinuous 136 bp deletion separated by TAAT motif in the 1st exon of gene coding for AP.
- S5i/j alleles differed by two nucleotides , aa substitution in the protein ,
- Large deletion - sub cellular mislocalization of the protein, non functionality of $S5^n$

Functional Marker- S5

- CAPS markers & SNPs
- Tedious,
- Costly
- High throughput SNP genotyping requirement

At Directorate of Rice Research



Mol Breeding (2010) 26:719–727
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SHORT COMMUNICATION

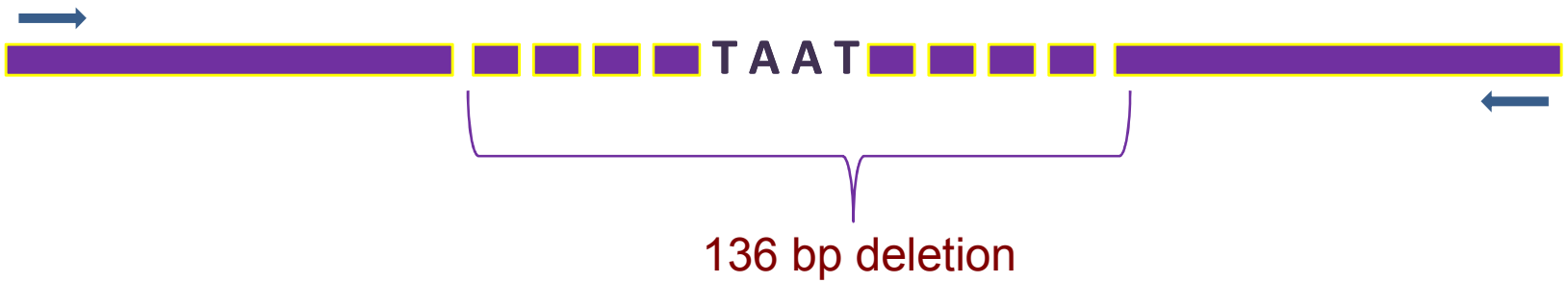
Development and validation of a PCR-based functional marker system for the major wide-compatible gene locus *S5* in rice

R. M. Sundaram · K. Sakthivel · A. S. Hariprasad · M. S. Ramesha · B. C. Viraktamath · C. N. Neeraja · S. M. Balachandran · N. Shobha Rani · P. Revathi · P. Sandhya · Y. Hari

Development of PCR based functional marker-S5 MMS & its applications

S5 Multiple Marker system-which clearly distinguish *indica, japonica* and *S5* neutral allele of S5 locus.

S5-InDel primer - S5 Neutral allele



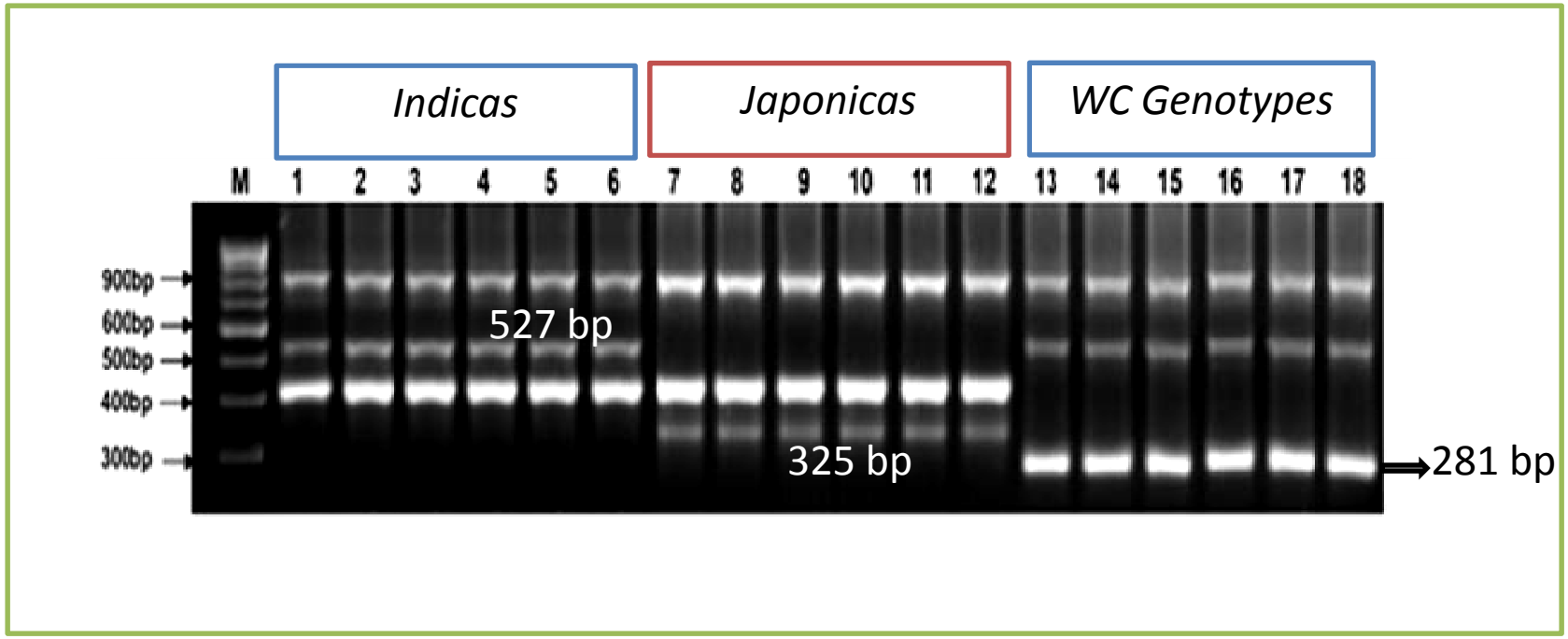
By targeting SNP between I /J, C ⟶ A primers for indica & japonica alleles

Primers for S5 locus

Primer name	Target	Sequence (5'-3')	Melting temp (^{0}C)	Expected product size (bp)		
				Indica	*Japonica*	Neutral
S5-InDel F	Neutral allele-specific deletion	cctacgtttgactgcctgcctg	61.0	417	417	281
S5-InDel R		ctacacgcggcttcgggaaagc	63.4			
S5-ELSP F	Indica-specific SNP	gacagcagcatcaacgacttcc	59.1	527	No amp	527
S5-IASP 2		tcgtcagtgggcaagcagtagctg	63.3			
S5-JASP 1	Japonica-specific SNP	accctgatattctgagttacaaggcatta	57.4	No amp	325	No amp
S5-ELSP R		gctcttgatgtccggtgatacc	58.1			

InDel-insertion/deletion; ELSP-External Locus Specific Primer; IASP-Indica Allele Specific Primer; JASP-Japonica Allele Specific Primer.

S5 Multiplex Marker System

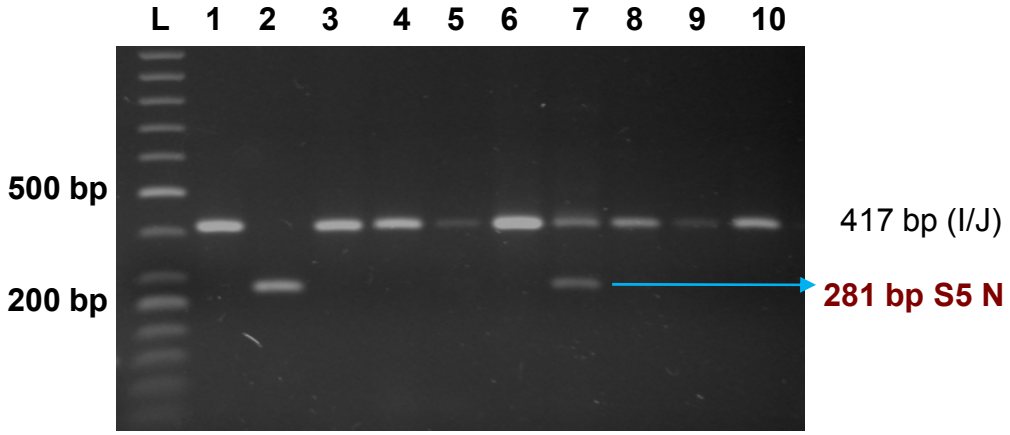


Mining rice germplasm for $S5^n$

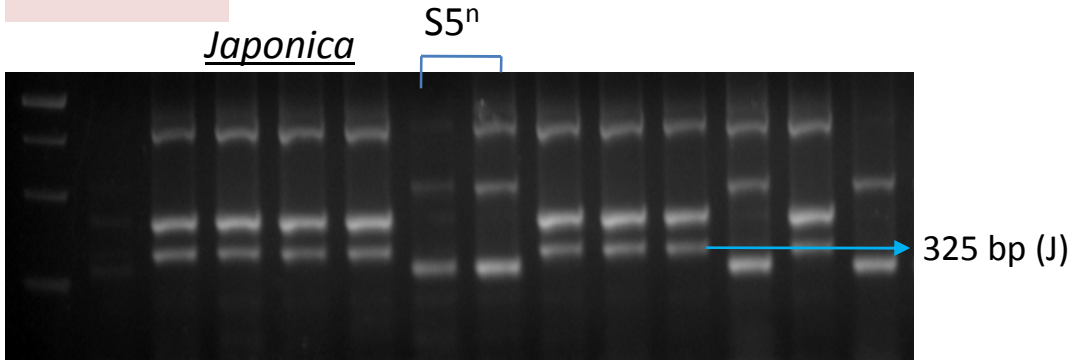
325 *japonica* lines(*T.japonica*)- 90 genotypes identified to carry $S5^n$

IRGC ID	Japonica genotypes
IRGC137	Sunbonnet
IRGC138	Bluebonnet
IRGC143	Rexoro
IRGC144	Texas patna
IRGC289	Azmil85
IRGC238	Azucena
IRGC1715	Rexoro
IRGC1797	Texas patna
IRGC1811	Bluebonnet-50
IRGC1943	Rexar rgue
IRGC1972	Rexark rogue
IRGC3255	Mojito colorado
IRGC3764	Asse-y-pung
IRGC3849	Kinastano
IRGC4020	Pilawan
IRGC4122	Iguape cateto

S5-InDel



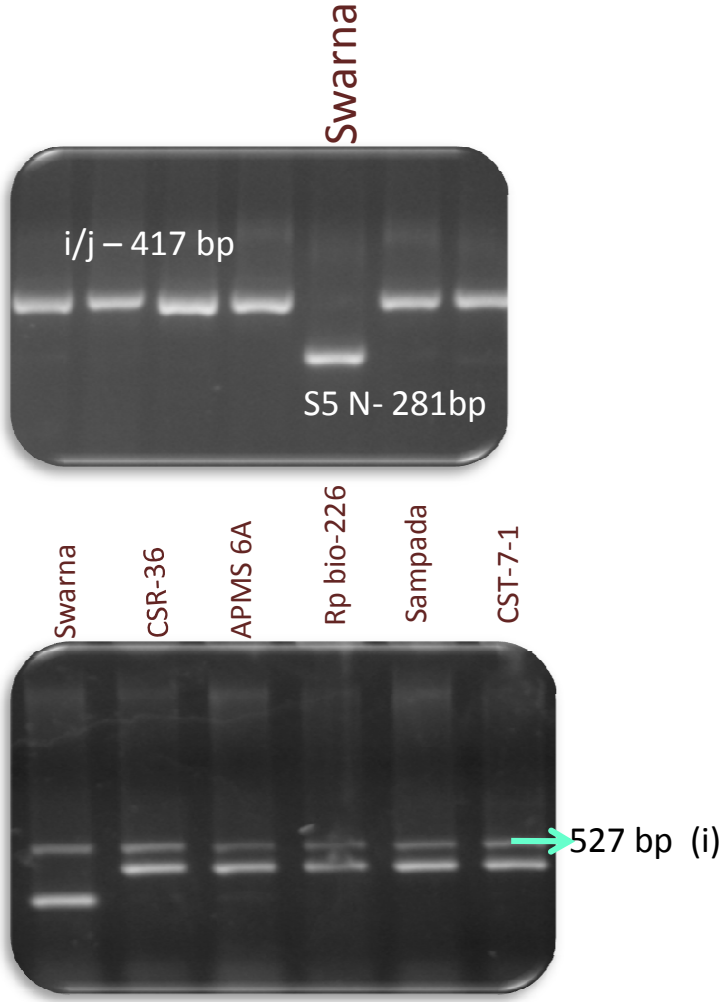
S5- MMS



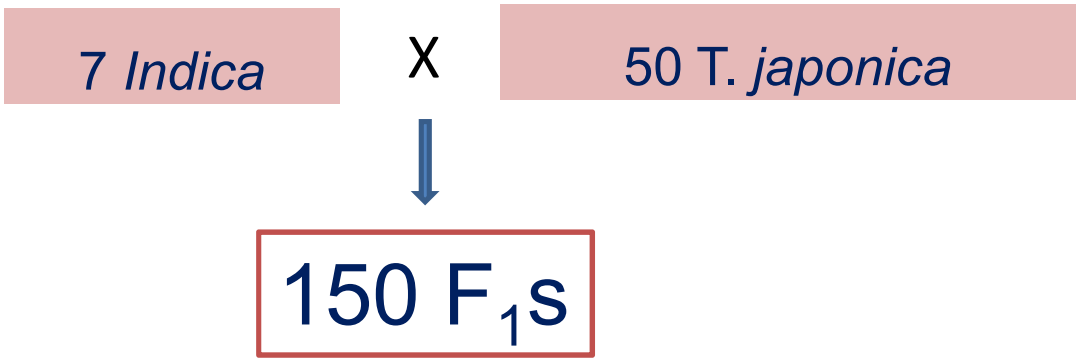
Mining rice germplasm for WC

Mining *indica* for $S5^n$ allele

Indica genotypes	S5 Neutral allele	I/J allele
Swarna	$S5^n$	I
APMS 6B	Absent	I
APMS 6A	Absent	I
Sampada	Absent	I
CST 7-1	Absent	I
Rp-Bio 226	Absent	I
CSR 36	Absent	I



Inter subspecific hybridization

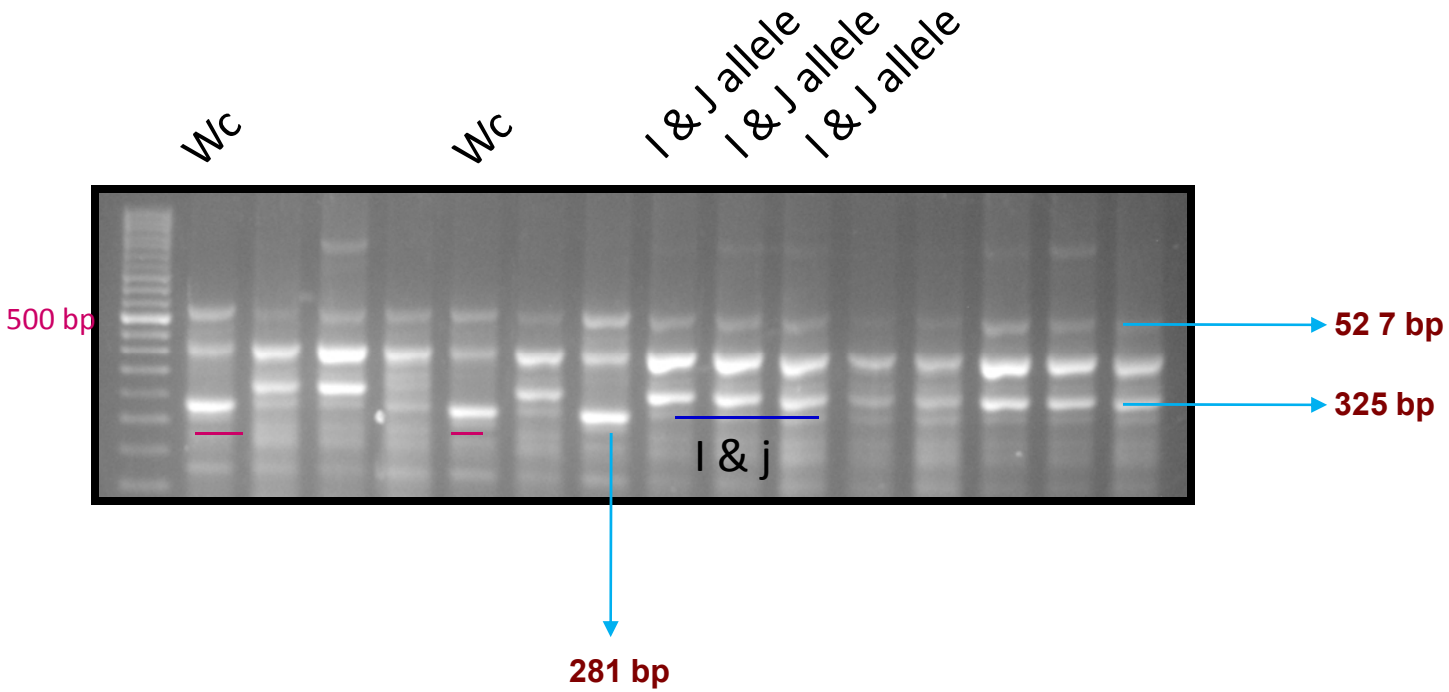


- Phenotyped - SPF %
  (4 to 97 %)

I X J Cross	Fertile	Sterile	SF%
1774	969	30	97
1902	34	877	4
1914	777	30	96
2113	937	557	63
2177	695	92	88

Genotyping of F_1s

S5 Multiplex marker system



I X J hybrids with $S5^n$

I X J hybrid	SPF %	Phenotypic evaluation	S5 Locus
SWARNA X TJP362	97	F	$S5^n$
SWARNA X TJP 278	92	F	$S5^n$
CSR-36 X TJP139	77	F	$S5^n$
SAMPADA X TJP173	87	F	$S5^n$
RP bio 226 X TJP 120	76	F	$S5^n$
Rp bio 226 X TJP 324	78	F	$S5^n$

I x J Hybrids with high SPF%, without $S5^n$

I X J hybrid	SPF %	Phenotypic evaluation	S5 Locus
CSR-36 X TJP 190	77	F	-
RP BIO 226 X TJP287	76	F	-
SAMPADA X TJP 76	79	F	-
SAMPADA X TJP 290	83	F	-
CST-7-1 X TJP 235	93	F	-
APMS 6 B X TJP 287	91	F	-

Reason: some other neutral allele / few tropical *japonica* lines identified to carry *indica* allele instead of japonica allele by S5 MMS marker system

I X J hybrids with $S5^n$, high sterility

I X J hybrid	SPF %	Phenotypic evaluation	S5 Locus
RPBIO226 X TJP139	40	PF	S5
RPBIO226 X TJP144	22	PS	S5
SAMPADA X TJP275	37	PS	S5
SAMPADA X TJP 184	34	PS	S5

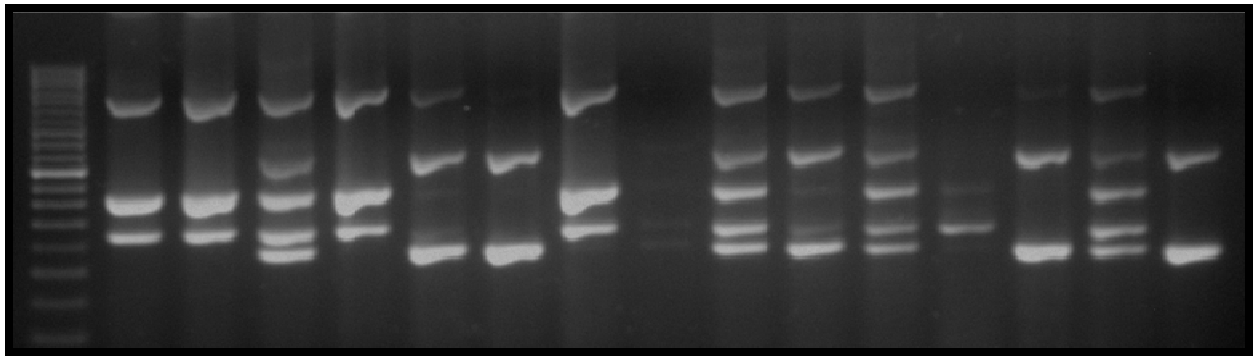
Indicating existence of other than embryo sac sterility mechanism of I X J crosses, since *S5n* overcomes only embryo sac hybrid sterility & it is not sufficient for producing I xJ hybrids with normal fertility

Genotyping – F_2s Swarna X TJP 190

S5^n

S5 MMS

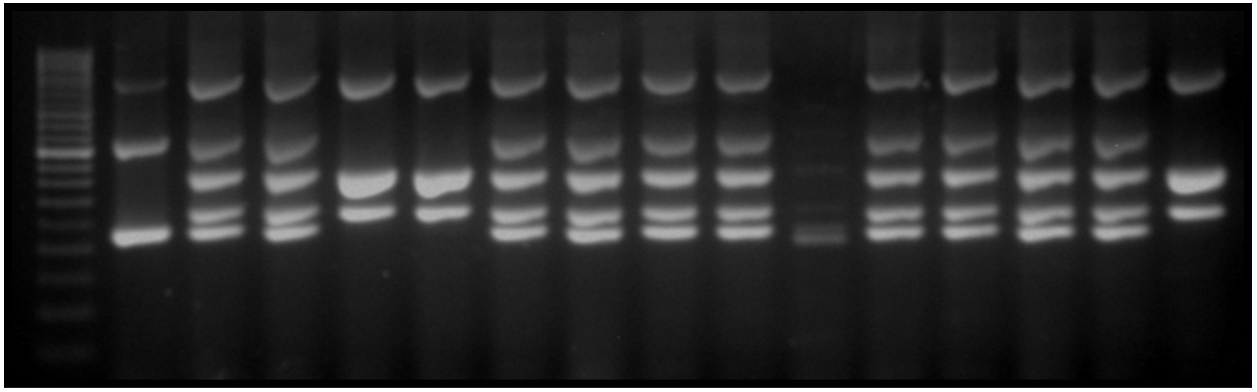
P1 P2 H



Genotypic ratio:1 : 2 : 1

Indica&S5n: hetrozygous: japonica

38: 76: 41 (155 plants)



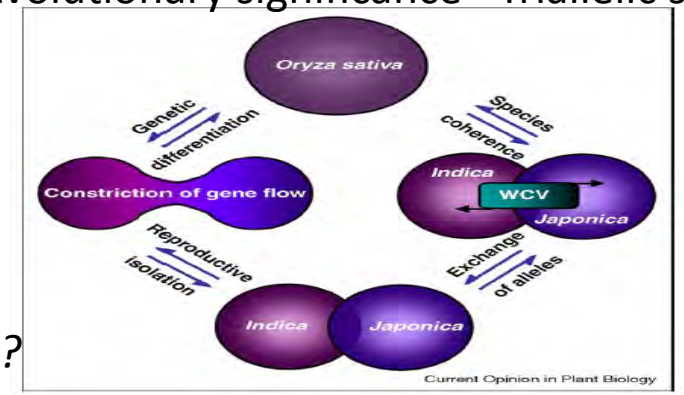
Search for T. *japonica restorers*

S.No:	Pedigree	SPF %	Phenotypic evaluation	PRESENCE OF S5
1	APMS 6A /TJP 189	32	PS	-
2	APMS6A /TJP 194	35	PS	-
3	APMS6A/TJP10	57	PF	S5n
4	APMS6A/TJP10	31	S	-
5	APMS6A/TJP101	12	S	-
6	APMS6A/TJP120	33	PS	S5 n
7	APMS6A/TJP143	34	PS	-
8	APMS6A/TJP143	21	PS	-
9	APMS6A/TJP144	12	S	S5n
10	APMS6A/TJP145	4	S	-
11	APMS6A/TJP151	23	S	-
12	APMS6A/TJP158	47	PS	-
13	APMS6A/TJP172	28	PS	-
14	APMS6A/TJP190	15	S	S5 n
15	APMS6A/TJP234	24	S	-
16	APMS6A/TJP235	17	PS	-
17	APMS6A/TJP289	35	PS	-
18	APMS6A/TJP323	31	PS	S5n
19	APMS6A/TJP38	31	PS	-
20	APMS6A/TJP76	9	S	-
21	APMS6A/TJP83	26	PS	S5n
22	APMS6A/TJP90	38	PS	-

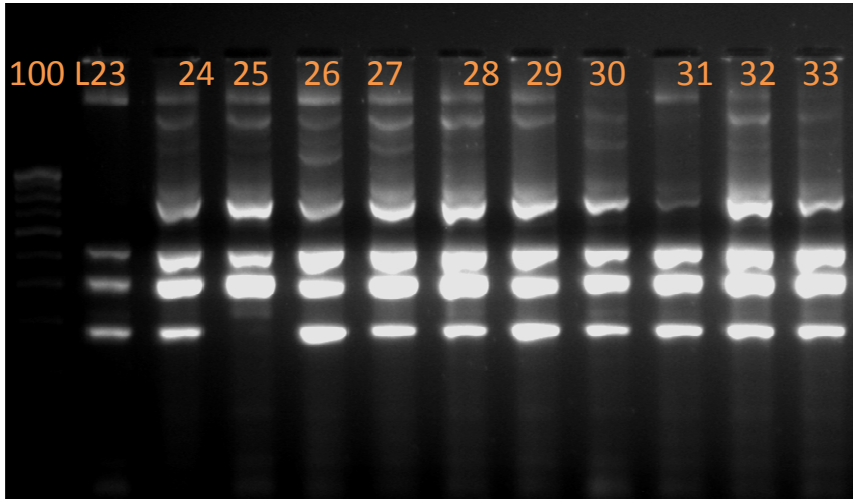
Screening O.*rufipogan* accession for S5^n

S5^n evolved from **O.*rufipogan*** wei et al.,2010

Evolutionary significance –Triallelic system



S5n allele exists in wild populations of O. rufipogon ???



- *Oryza sativa and O. nivara had the S5n gene in the* homozygous state (*S5nS5n),*
- *Whereas O. rufi pogon had it in the* heterozygous state (*S5nS5i or S5nS5j).*
- *O. sativa, O. rufi pogon, and O. nivara were closely related with each other. (Yang et al.2012)*

Conclusion

- S5 MMS is a powerful PCR based low cost ,highly efficient (100%) marker system for rapid & precise identification of germplasm containing S5n.

- Saves one year of breeder's valuable time in inter-subspecific hybridization.

Acknowledgement

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Thank you