

## Assessment polymorphism between parental lines Swarna and IC309814 using molecular markers to genotype the derived mapping population

### Abstract

Detection of molecular markers with parental polymorphism is a pre-requisite for QTL mapping and further identification of genomic regions. PCR based InDel markers tend to have a greater degree to show polymorphism among the parental genotypes for any specific cross. Polymorphic studies were carried out between the two parents Swarna (cultivated rice) and *Oryza rufipogon* IC309814 (Wild rice) using 192 randomly selected InDel markers and 40 gene specific markers for Iron and Zinc covering the entire 12 chromosomes. Results revealed that InDel markers were polymorphic between both the parents and highest percentage of polymorphism observed on chromosome 10 (75%) followed by chromosome 5 (60%) and chromosome 9 (55.6%) and lowest polymorphism percentage was shown in chromosome 3 (8.3%) with an overall 22.4 % polymorphism percentage among all the markers used in this study. Detected polymorphic (SSR) markers were for genotyping the mapping population derived from the cross between Swarna cultivar and *Oryza rufipogon* to map Quantitative Trait Loci (QTLs) associated with yield and grain quality traits.

**Keywords:** Swarna, Wild Rice, Parental Polymorphism, InDels and Biofortification

### Introduction:

Rice, botanically called as *Oryza sativa* L. and belongs to poaceace family, it serves as a primary food source as well as staple cereal crop for population globally. The crop is extensively cultivated in the Asian countries having major share in terms of production with 90% of the world's rice is cultivated from this part of the globe (Fukagawa *et al.*, 2019). Some wild relatives of rice found to have higher grain Fe and Zn concentrations compared with the cultivated rice germplasm (Garcia- Oliveira *et al.*, 2018). The AA-genome, wild progenitor of cultivated rice *O. rufipogon* is a rich source of natural allelic variation for several agronomic, grain quality and grain micronutrient trait. Wild accessions *O. nivara* and *O. rufipogon* have high concentrations of grain iron and zinc (Anuradha *et al.*, 2012a)

Genetic markers have helped in plant breeding methodologies by enabling precise tracking of trait inheritance and providing robust tools for genetic diversity

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assessment(Chakravarthi *et al.*,2006). These molecular markers are environment-independent in nature and helps to understand genetic similarities and characterization. By facilitating comprehensive genetic similarity analyses, these markers play a critical role in elucidating genetic relationships within and across species, ultimately enhancing the efficiency and precision of crop improvement strategies(Duzyaman, 2005). When such markers are very tightly linked to genes of interest, they can be used for the indirect selection of desirable alleles, which is known as marker-assisted selection (Yuet *et al.*, 2005).

Parental polymorphism is a prerequisite for implementing marker-assisted selection (MAS) and marker-assisted backcross breeding (MABB) methodologies. Molecular genetic analyses are essential to establish allelic diversity and detect polymorphic loci that facilitate introgression of quantitative trait loci (QTLs) from donor germplasm into recipient genetic backgrounds. Without demonstrable polymorphism across target genomic regions, the identification and subsequent selection of progenies harboring specific traits become fundamentally unachievable(Yerva *et al.*,2018). The genetic variation assessment provides the foundational framework for successful breeding strategies, ensuring the potential for trait introgression and genetic improvement in crop improvement programs(Graham *et al.*, 1999).

Polymorphism levels depends on the genetic diversity of genotypes and primer specificity during DNA sequence recognition. PCR and electrophoresis techniques reveal genetic variations by comparing DNA banding patterns across different samples. Monomorphic markers exhibit identical banding patterns, while polymorphic markers demonstrate distinct genetic variations between DNA samples. These molecular techniques enable precise genetic diversity assessment and characterization of genetic relationships among different genotypes. These markers are pivotal for linkage map construction, gene mapping, and marker-assisted selection (MAS)(Edwards and Batley, 2010; Gonzaga *et al.*, 2015).

In marker-assisted breeding, InDel markers offer advantages over SSRs and SNPs. These markers generate fewer shadow bands due to their conservative polymorphic regions, enabling more precise genotype characterization. The enhanced reliability and specificity of InDel markers make them a valuable tool for genetic mapping and breeding strategies (Mo *et al.*, 2024). QTL analysis allows detection of marker-trait associations, revealing genetic control of complex traits. By utilizing markers linked to QTLs, rice breeders can implement precise selection strategies, positively selecting beneficial elements and negatively selecting potentially

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undesirable components (Rathod *et al.*, 2021). QTL mapping of grain quality traits in rice provides a strategic forward genetic approach for marker-assisted selection and gene discovery. This method enables precise dissection of complex genetic regions, facilitating targeted breeding strategies (Swamy *et al.*, 2018).

The objective of this study is to identify polymorphic InDel markers showing genetic variation between the cultivated rice variety (Swarna) and the wild rice accession (*Oryza rufipogon* IC309814). These polymorphic markers would be crucial for mapping quantitative trait loci (QTLs) in the advanced backcross population derived and further to identify the genomic regions associated with specific traits.

### Materials and methods:

The experimental material constitutes of two rice genotypes i.e., most popular mega cultivar Swarna and accession IC 390814 of wild species *Oryza rufipogon* available at Indian Institute of Rice Research (IIRR). From both the genotypes genomic DNA was isolated using CTAB method (Doyle and Doyle, 1987). In the procedure, 0.1g of young and tender leaves were used for grinding using mortar and pestle (Maxprep) with CTAB buffer (4% CTAB, 100 mM Tris HCl, 20 mM EDTA, 1.4 M NaCl). The DNA concentration is quantified at OD values of 260 and 280 nm using nanodrop to check the purity and quantity of genomic DNA.

In all, 192 randomly selected InDel markers covering all the 12 chromosome and 40 gene specific markers (Table no.) linked to Iron and Zinc concentration were used in the polymorphic studies between Parent 1 (Swarna) and Parent 2 (*O. rufipogon* IC 309814). The primer sequence and chromosomal location information were retrieved from Hechanova *et al.*, 2022.

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**Table 1. Characteristics of Parental lines used in this study**

Feature	Swarna (Cultivated)	<i>Oryza rufipogon</i> IC309814 (Wild)
Origin	India	Native to Southeast Asia

<b>Genetic</b>	Cultivated species	Wild species
<b>Background</b>	( <i>Oryza sativa</i> )	( <i>Oryza rufipogon</i> )
<b>Morphology</b>	Semi-dwarf, robust plants	Typically shorter to tall, variable in size
<b>Plant Height</b>	Typically 90-120 cm	Generally shorter, around 60-100 cm
<b>Leaf Color</b>	Dark Green, broader leaves	Narrower, often darker green
<b>Growth Habit</b>	Semi-dwarf, erect	More variable, can be erect or prostrate
<b>Grain Color</b>	White to pale yellow	Dark brown to reddish-brown
<b>Grain Shape</b>	Long and slender	Shorter, often irregular
<b>Grain Length</b>	6.5-7.5 mm	5-6 mm
<b>Grain Breadth</b>	2.0-2.5 mm	1.5-2.0 mm
<b>Kernel Length</b>	6.0-7.0 mm	5.0-6.0 mm
<b>Kernel Breadth</b>	2.0-2.4 mm	1.4-1.8 mm
<b>L/B Ratio</b>	2.8-3.0	3.0-4.0
<b>Disease Resistance</b>	Moderate	High, evolved resistance to major biotic stresses
<b>Yield Potential</b>	High, suited for cultivation	Low, variable in wild habitats
<b>Adaptation</b>	Prefers flooded conditions	Adapted to diverse environments
<b>Genetic Diversity</b>	Pure line developed through selective breeding	High, significant genetic variability
<b>Conservation</b>	Widely cultivated, not endangered	Often threatened due to habitat loss

**Source:** Swarna (Haug *et al.*, 2004, IRRI 2017), *Oryza rufipogon* (Wang *et al.*, 2008, Vaughan *et al.*, 1997)

**Parent 1** Swarna

**Parent 2** *Oryza rufipogon* IC 390814



**Fig 1: Difference in the plant and grain phenotype of Parent1 (Swarna) and Parent 2 (*Oryza rufipogon* IC 390814)**

PCR reaction was carried out in thermal cycler (Biorad Thermal cycler) with a final reaction volume of 10 $\mu$ l containing 30ng of genomic DNA, Master mix, 10 pmol of forward and reverse primers. The programme followed for PCR products as: initial denaturation at 94°C for 7 min followed by 35 cycles of 94°C for 30 s, 55°C (58°C for gene-specific markers) for 30 s, 72°C for 1 min, and a final extension of 10 min at 72°C. Amplified products were resolved in 2.5 % agarose gel prepared in 1X TAE buffer and electrophoresed at 150 V for 1 hour. Gels were stained with ethidium bromide and documented using a gel documentation system (Vilber- BIO-PRINT cx4 Edge- Fixed pad Container, France).

The percentage of polymorphism calculated using the formula:

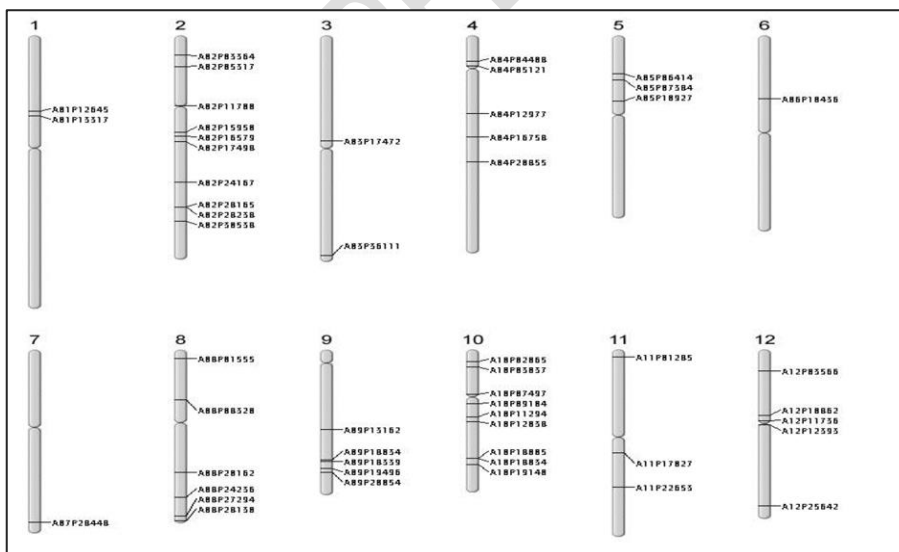
$$\text{Polymorphism \%} = \frac{\text{RM Primers with Polymorphism}}{\text{Total number of RM primers used}} \times 100$$

### Results and Discussion

The quantity of isolated DNA samples checked using Nanodrop from both the parents its concentration ranged between 2108 to 2765 ng/ $\mu$ l, were it is further diluted in a ratio of 1:20 for PCR reaction. Ratio of UV absorbance of DNA at OD 260/280 ranges from 1.80 to 1.97 and hence the DNA samples are standard and good. Using the diluted genomic DNA of the both parents, we have screened 192 InDel markers and 40 gene specific markers for Fe and Zn concentration across all the chromosomes, 52 InDel markers were polymorphic between Swarna and *O rufipogon* IC 309814 with 22.4 % of polymorphism percentage. None of the gene specific

markers are polymorphic among the parents. The polymorphic markers along with their chromosomes depicted in **Table 1 and Fig 2**. Similar type of studies were done previously by Shankar *et al.*, (2023) and identified 85 polymorphic markers between parents Jyoti and Chuvannamaran. In another study focused on iron and zinc content, Shivani *et al.*, (2020) reported 52 polymorphic SSRs among the 171 markers used for iron and zinc content between the Swarna and type 3, Bimponget *et al.*, 2011 reported 40.5 percent of polymorphism among the *O. sativa* cv. IR64 and *O. glaberrima* parents using 464 markers. Above findings reveals that the varying degrees of genetic diversity that can be detected between different rice varieties using SSR markers, with polymorphism rates ranging from approximately 30-41% across these different parental combinations.

Swamy *et al.*, 2018 have reported 100 polymorphic markers between Swarna /*O. nivara* (IRGC81832), Hu et al 2014 also reported 108 polymorphic markers between Xieqingzao B (*O. sativa*) / *O. rufipogon*. 101 polymorphic markers were identified by Anuradha *et al.*, 2012b between Madhukar/ Swarna. Ilango and Sarla, (2010) have studied parental polymorphism between 5 parental lines Swarna, BPT5204, Madhukar, Jalmagna, and IR64 using 112 RM markers out of which 33 polymorphic markers were reported.



**Fig 2:** Map image showing physical location and distribution of 52 polymorphic markers

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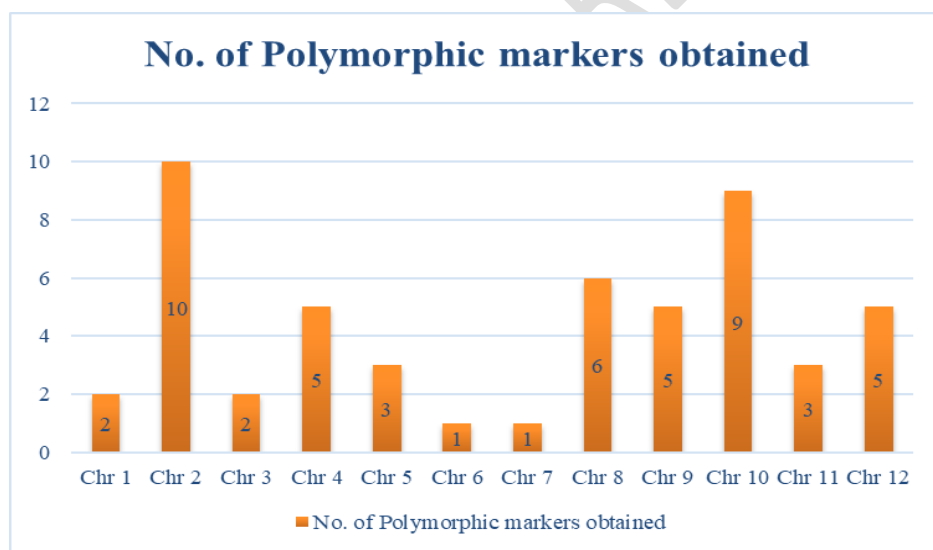
Chromosome wise polymorphism percentage of InDelmarkers is showed in the **Table 2**. Among the 192 markers Chromosome 10 had the highest polymorphism percentage i.e., 75% (9 markers- A10P02065, A10P03037, A10P07497, A10P09104, A10P1129, A10P12038, A10P18005, A10P18034, A10P1910) followed by chromosome 4 with 60% (5 markers- A04P04408, A04P05121, A04P12977, A04P16758, A04P20855), Chromosome 9 with 55.6% (5 markers- A09P1312, A09P1803, A09P18339, A09P19496, A09P20054) and lowest percentage of polymorphism was observed in chromosome 3 with 8.3 % (2 markers- A03P17472, A03P36111). Frequency distribution of polymorphic markers are shown in **Fig no. 3**. Polymorphic studies conducted by Yervaet *et al.*, 2018., Shankaret *et al.*, 2020, and Singh *et al.*, 2022 in rice consistently demonstrated that molecular markers exhibit varying frequencies across different rice chromosomes which are reported in their studies.

Table 2 List of Polymorphic markers between the Swarna and *O. rufipogon* IC 309814

S.No.	Marker	Chr No.	Amplicon Size	S.No.	Marker	Chr No.	Amplicon Size
1	A01P12645	1	173	27	A08P20162	8	213
2	A01P13317	1	138	28	A08P24236	8	177
3	A02P03364	2	204	29	A08P27294	8	376
4	A02P05317	2	164	30	A08P28130	8	118
5	A02P11700	2	132	31	A09P13162	9	134
6	A02P15950	2	133	32	A09P18034	9	219
7	A02P16579	2	276	33	A09P18339	9	309
8	A02P17498	2	288	34	A09P19496	9	224
9	A02P24167	2	279	35	A09P20054	9	146
10	A02P28165	2	140	36	A10P02065	10	189
11	A02P28238	2	261	37	A10P03037	10	163
12	A02P30538	2	170	38	A10P07497	10	164
13	A03P17472	3	406	39	A10P09104	10	147
14	A03P36111	3	187	40	A10P11294	10	161
15	A04P04408	4	366	41	A10P12038	10	255
16	A04P05121	4	256	42	A10P18005	10	173
17	A04P12977	4	274	43	A10P18034	10	180
18	A04P16758	4	656	44	A10P19140	10	179
19	A04P20855	4	220	45	A11P01285	11	213
20	A05P06414	5	276	46	A11P17027	11	237
21	A05P07384	5	352	47	A11P22653	11	291
22	A05P10927	5	208	48	A12P03566	12	230
23	A06P10436	6	281	49	A12P10862	12	212
24	A07P28448	7	127	50	A12P11736	12	222
25	A08P01555	8	160	51	A12P12393	12	193
26	A08P08320	8	131	52	A12P25642	12	334

**Table3** Chromosome wise polymorphism percentage of RM markers

Chromosome no.	Total No. of SSR marker used	No. of Polymorphic markers obtained	Polymorphism (%)
Chromosome 1	14	2	14.3
Chromosome 2	25	10	40.0
<b>Chromosome 3</b>	<b>24</b>	<b>2</b>	<b>8.3</b>
Chromosome 4	20	5	25.0
Chromosome 5	5	3	60.0
Chromosome 6	8	1	12.5
Chromosome 7	7	1	14.3
Chromosome 8	23	6	26.1
Chromosome 9	9	5	55.6
<b>Chromosome 10</b>	<b>12</b>	<b>9</b>	<b>75.0</b>
Chromosome 11	19	3	15.8
Chromosome 12	26	5	19.2
<b>Total</b>	<b>192</b>	<b>52</b>	<b>22.4</b>



**Fig 3.** Frequency distribution of polymorphic markers across the chromosomes

Amplicon size of markers ranged from 117 bp to 656 bp, and polymorphic markers were 52 out of 232 and the remaining weremonomorphic as showed in **Fig 4**. Similar type of banding

pattern were reported by Chandu *et al.*, 2020, Rathiet *et al.*, 2021, Majhi *et al.*, 2022 and Shankar *et al.*, 2023 for the markers used in this study.

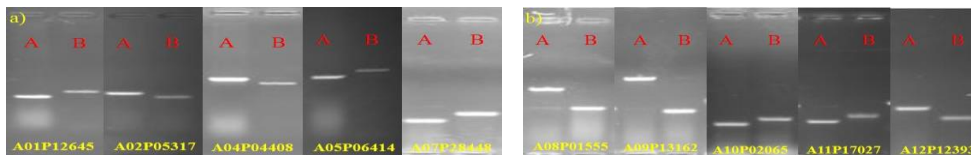


Fig 4 :Polymorphic markers between the parents

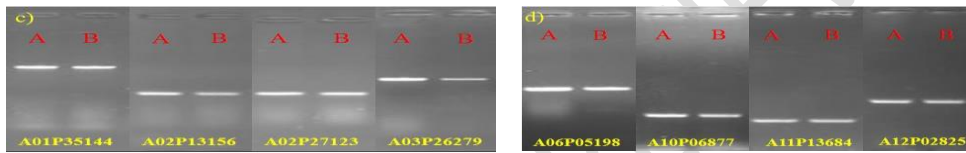


Fig 5 :Monomorphic markers between the parents

Genotypic data using polymorphic markers are useful for linkage studies and diversity analysis for a number of linked characteristics in rice using the detected polymorphic molecular markers. Polygenic traits, which were previously difficult to analyze, using traditional breeding methods, would now be easily tagged using molecular markers. The screening of markers for parental polymorphism among the rice cultivars forms the basis for tagging of the desired gene, fine mapping of the gene in the rice chromosome and in the subsequent Marker Assisted Selection (MAS) programmes. The characterization and physical mapping of polymorphic InDel markers represents a significant advancement in molecular breeding, by establishing a well-defined set of these markers, which serve as genotyping of mapping populations to the identification and analysis of quantitative trait loci. Additionally, their application in marker-assisted selection significantly fasten the breeding process, allowing for more precise and efficient selection of desired traits.

It ultimately contributing to the acceleration of rice variety development and crop improvement programs. The polymorphic rice markers can be further used in fine mapping for iron and zinc rich micronutrient genes in the mapping population obtained from these parents (Welch and Graham, 2004). The present work gives the preliminary understanding the genetic

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polymorphism exist in the population for biofortification of micronutrient such as iron and zinc in cereals like rice.

### Conclusion

This study detected 52 polymorphic markers out of 192 InDel and 40 gene specific markers for iron and zinc were found with a polymorphic percentage of 27.1%, these polymorphic markers can be used in QTL mapping for yield and grain quality traits. Additional polymorphic studies and detection of genome-wide polymorphic markers uniformly covering all chromosomes are required to identify precise detection of effective QTLs for target traits.

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