

## Whole Genome Analysis of Mahsuri Rice and Its Blast-Resistant Mutant for Understanding Resistance Mechanisms

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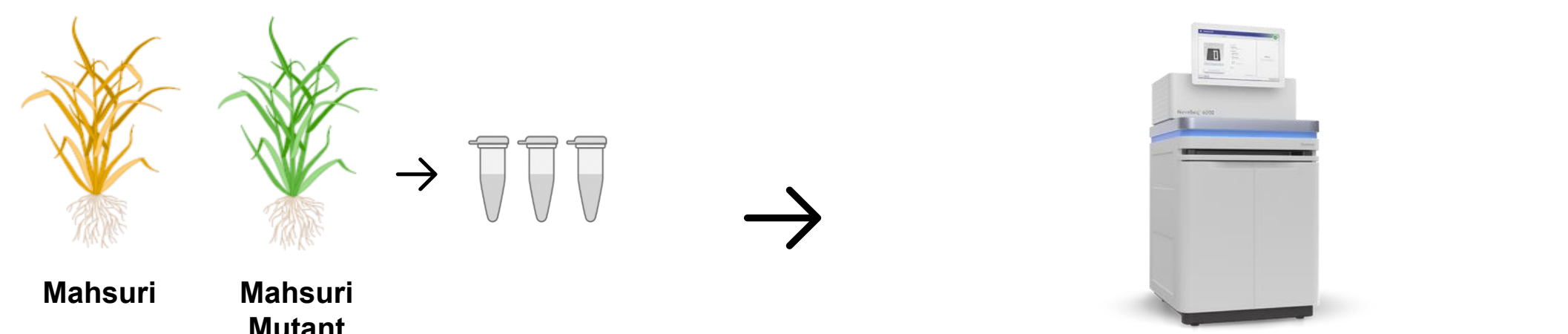
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### INTRODUCTION & AIM

Rice (*Oryza sativa* L.) is a staple food for over 40% of the global population, especially in Asia. The traditional high-yielding cultivar 'Mahsuri', developed by the Food and Agriculture Organization (FAO) through japonica-indica hybridization, is susceptible to blast disease. Blast disease can affects all above ground parts of the rice plants, with initial symptoms white to grey-green lesions with brown borders. To address this, the 'Mahsuri Mutant' was created via mutational breeding to enhance blast resistance while retaining desirable traits. However, the specific molecular mechanisms and genetic mutations driving this resistance remain unexplored as the lack of molecular markers found that associated with specific SNPs in mutant line. Therefore, this study aims to conduct a genomic profiling of the susceptible 'Mahsuri' and its blast-resistance mutant line against a high-quality reference genome to identify genetic variants underlying blast resistance. By developing these genomic tools, these research aims to accelerate the development of improved rice cultivars which directly contributing to achieving several United Nations Sustainable Development Goals (SDGs) by 2030.

### METHODOLOGY



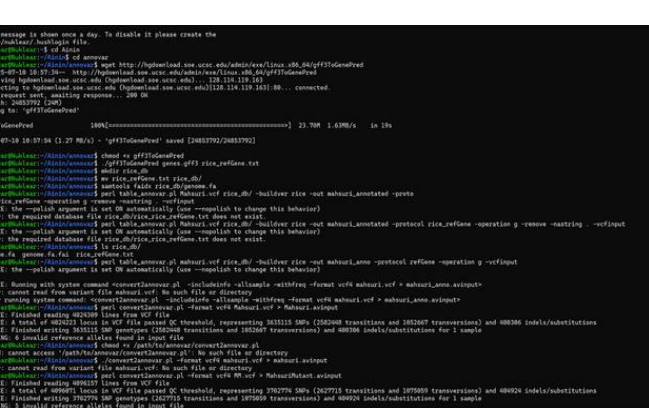
#### Sample Preparation and DNA Extraction

- Mahsuri seeds from Malaysian Agricultural Research and Development Institute (MARDI).
- Mahsuri Mutant seeds from Malaysian Nuclear Agency.
- Extract two weeks old of young leaves using QIAGEN DNAeasy Plant Extraction Kit.

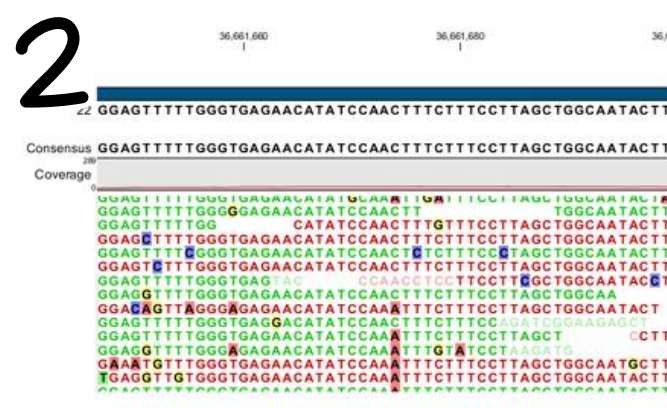
#### Whole-Genome Resequencing

- Illumina Novaseq 6000 with a depth 30x coverage, 150 bp paired end.
- Data NCBI: SRR24388814 (Mahsuri) and SRR22952097 (Mahsuri Mutant).

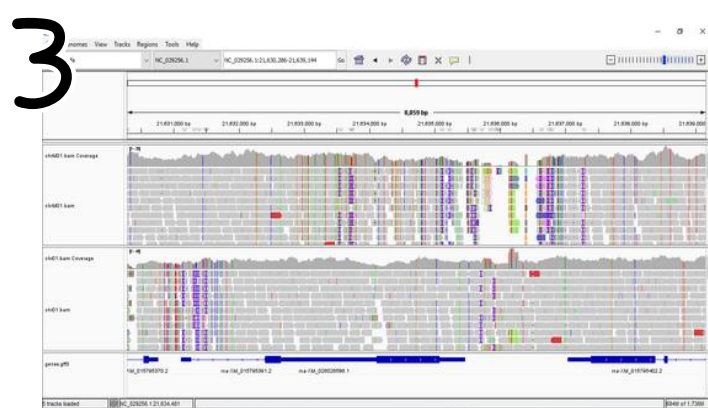
#### Bioinformatics Analysis



**Pre-processed Short Reads Mapping**  
(BWA v0.7.12 - *Oryza sativa* IRGSP-1.0)



**Variant Calling and Variant Effects of SNP and InDels**  
(SAMtools to BAM, BCFtools SNPs and InDels, ANNOVAR)



**Resistance (R) Gene Annotation and Visualization**  
(IGV, Rap-DB validation of resistance genes associated to unique SNPs)

### CONCLUSION

The whole-genome sequencing of 'Mahsuri' and its blast-resistant mutant, were successfully sequenced to the *Oryza sativa* Japonica reference genome. By identifying genome-wide Single Nucleotide Polymorphisms (SNPs) and Insertions/Deletions (InDels), this study highlight the key genetic variants potentially linked to important agronomic traits, including disease resistance. These findings have resulted in findings the specific molecular markers to Mahsuri and Mahsuri Mutant, and will provide valuable insights for Malaysian rice varieties. In the future, functional validation will be emphasized to validate the particular functions of discovered blast resistance (R) genes using targeted gene expression analysis and transgenic research, providing a foundation for developing climate-resilient rice cultivars that support global food security goals.

### REFERENCES

- [1] Abdelhameed, A. A., Ali, M., Darwish, D. B. E., AlShaqhaa, M. A., Selim, D. A.-F. H., Nagah, A., & Zayed, M. (2024). Induced genetic diversity through mutagenesis in wheat gene pool and significant use of SCoT markers to underpin key agronomic traits. *BMC Plant Biology*, 24, 673. <https://doi.org/10.1186/s12870-024-05345-5>
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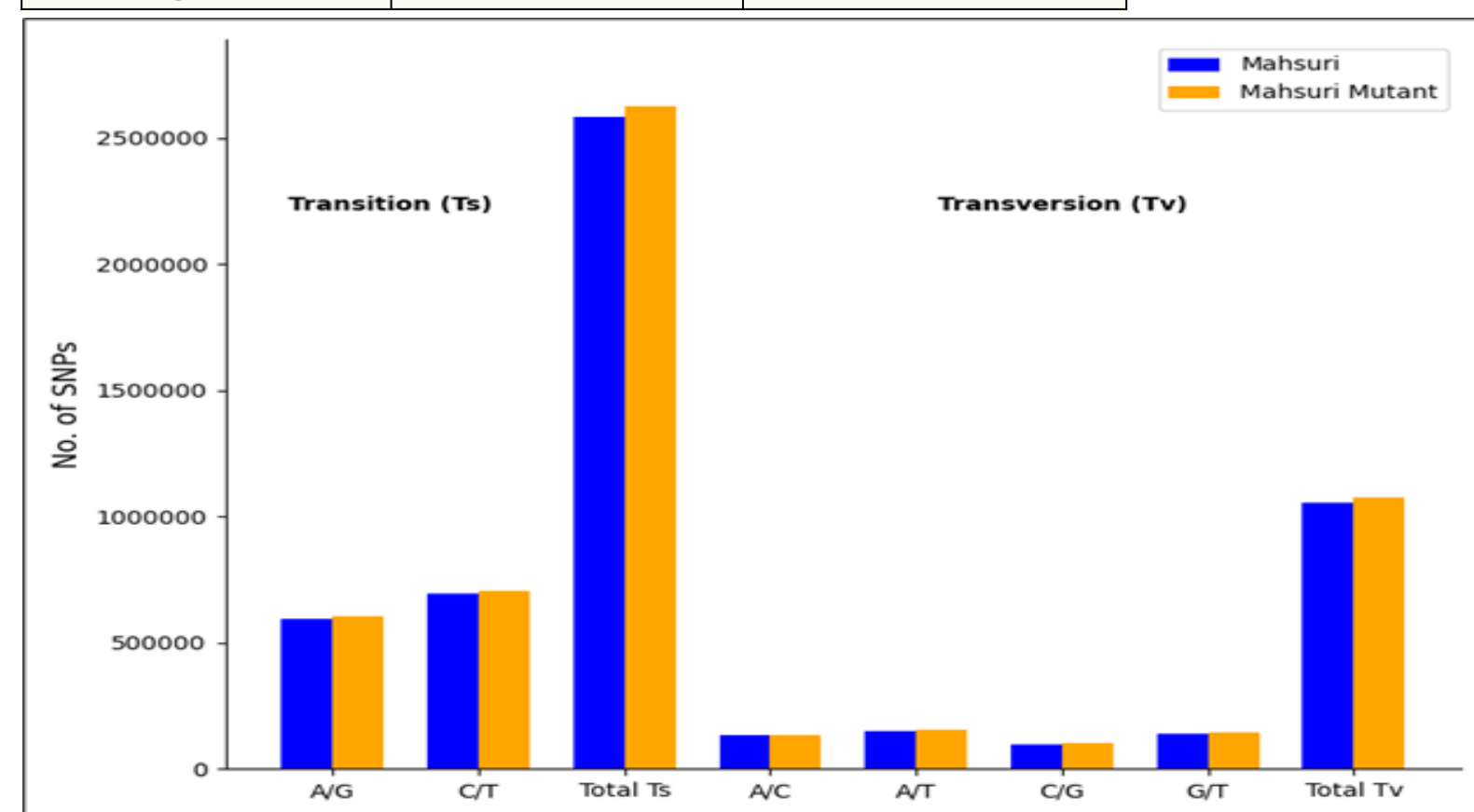
### RESULTS & DISCUSSION

**Table 1** Mapping statistics data of short reads from Mahsuri and Mahsuri Mutant rice variety.

| Variant             | Mahsuri             | Mahsuri Mutant      |
|---------------------|---------------------|---------------------|
| Total reads         | 55,510,645          | 60,907,677          |
| Mapped reads        | 54,976,618 (99.04%) | 60,365,342 (99.11%) |
| Properly paired     | 51,784,952          | 57,173,975          |
| Singleton           | 534,027 (0.96%)     | 542,335 (0.89%)     |
| Sequencing coverage | 21.76               | 23.91               |

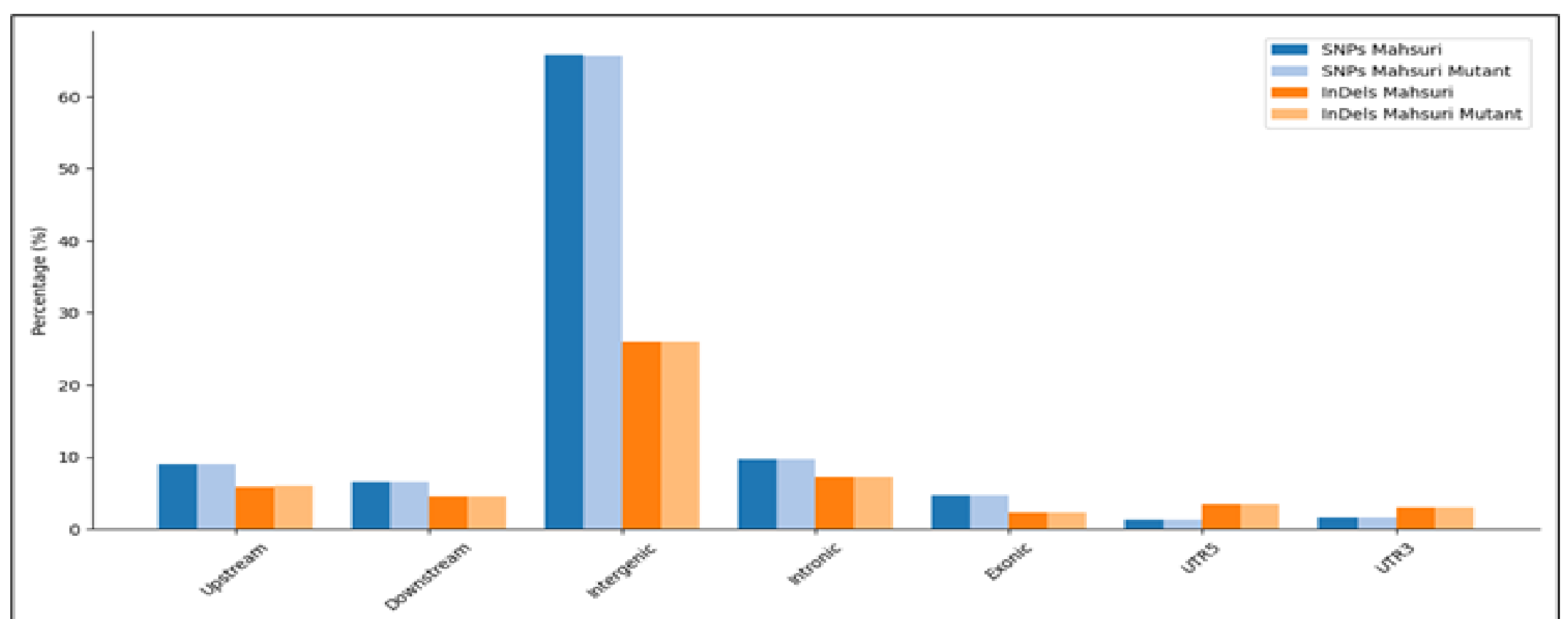
**Table 2** Number of SNPs and InDels identified in Mahsuri and Mahsuri Mutant rice varieties.

| Variant | Mahsuri   | Mahsuri Mutant |
|---------|-----------|----------------|
| SNPs    | 3,629,220 | 3,696,685      |
| InDels  | 395,003   | 399,386        |
| Total   | 4,024,223 | 4,096,071      |

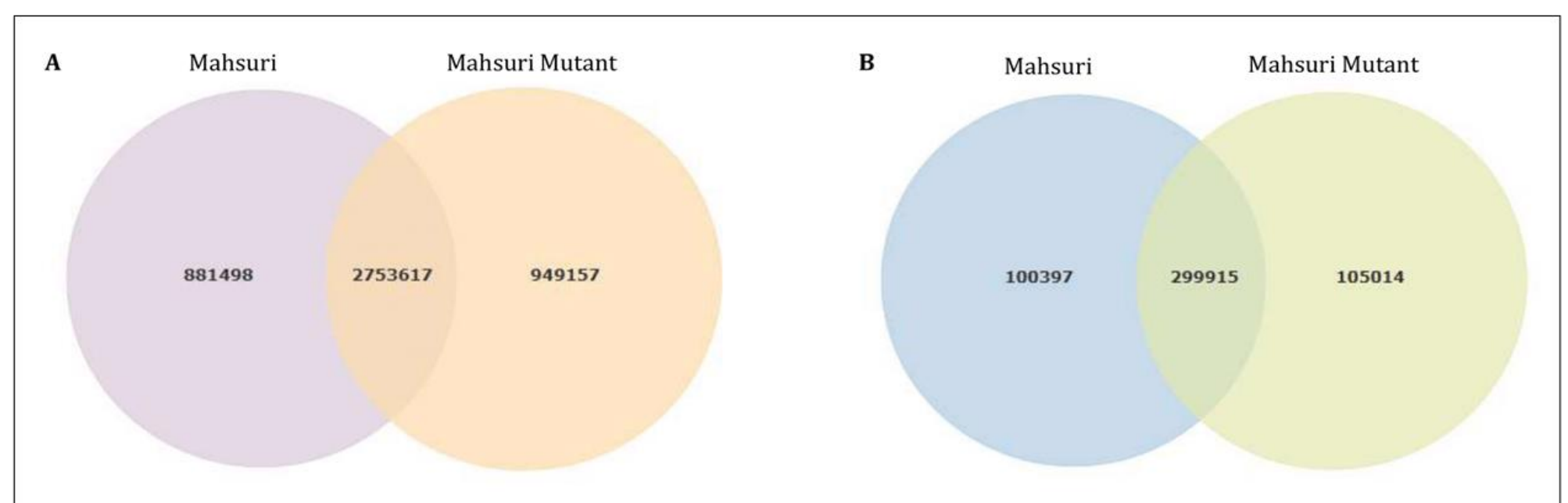


**Figure 1** Transition (Ts) and transversion (Tv) substitutions in SNPs in Mahsuri and Mahsuri Mutant rice varieties.

- Whole-genome resequencing generated high-quality data, with over 99% mapping efficiency to the *Oryza sativa* Japonica reference genome.
- The sequencing depth of 20-30x ensures reliable SNP and InDel discovery.



**Figure 2** Distribution of SNPs and InDels in different genomic regions for Mahsuri and Mahsuri Mutant rice variety.



**Figure 3** Number of shared and unique SNPs (A) and InDels (B) among Mahsuri and Mahsuri Mutant rice variety.

- The increased SNPs frequency suggested that 'Mahsuri Mutant' received new point mutations during mutagenesis, which may alter or disrupt the existing genes' functions (Abdelhameed et al., 2024).
- The 'Mahsuri Mutant' showed a higher count of unique SNPs (949,157) and InDels (105,014), are strong candidates for the genetic basis of improved traits (Bohry et al., 2021).

**Table 3** Summary of the identified blast resistance gene and its location in Mahsuri Mutant.

| Gene ID      | Gene Name                               | Chromosome | Gene Length | Location Number         |
|--------------|---|------------|-------------|-------------------------|
| Os01g0149500 | <i>Pyricularia oryzae resistance T</i>  | 1          | 4,937       | 2,681,220 – 2,686,364   |
| Os01g0781200 | <i>Pyricularia oryzae resistance 64</i> | 1          | 4,411       | 33,098,082 – 33,103,904 |
| Os01g0782100 | <i>Pyricularia oryzae resistance SH</i> | 1          | 4,636       | 33,136,846 – 33,136,846 |
| Os04g0401000 | <i>Pyricularia oryzae resistance 21</i> | 4          | 1,183       | 19,835,206 – 19,836,892 |
| Os06g0286700 | <i>Pyricularia oryzae resistance Z</i>  | 6          | 2,673       | 10,387,793 – 10,390,465 |
| Os11g0689100 | <i>Pyricularia oryzae resistance KM</i> | 11         | 3,777       | 27,984,697 – 27,989,128 |
| Os12g0281300 | <i>Pyricularia oryzae resistance TA</i> | 12         | 4,094       | 10,606,359 – 10,611,917 |

Unique SNPs within these loci can be used as molecular markers for Marker-Assisted Selection (MAS).