

## Supplementary Material

### Functional validation of the *Pi54* gene by knocking down its expression in a blast-resistant rice line using RNA interference and its effects on other traits

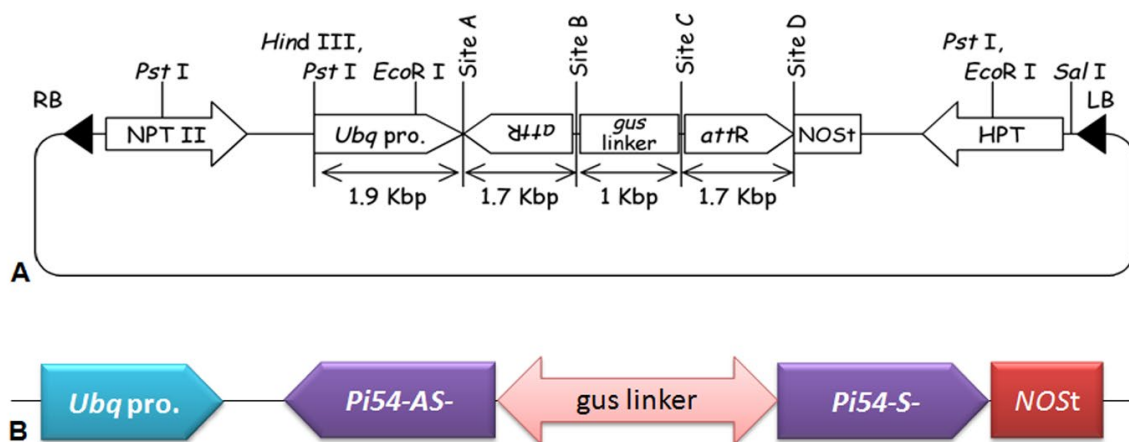
Kirti Arora<sup>A</sup>, Amit Kumar Rai<sup>A</sup>, Basavantraya N. Devanna<sup>A,B</sup>, Banita Kumari<sup>A</sup> and Tilak Raj Sharma<sup>A,C,D</sup>

<sup>A</sup>Indian Council of Agricultural Research (ICAR) National Research Centre on Plant Biotechnology, New Delhi-110012, India.

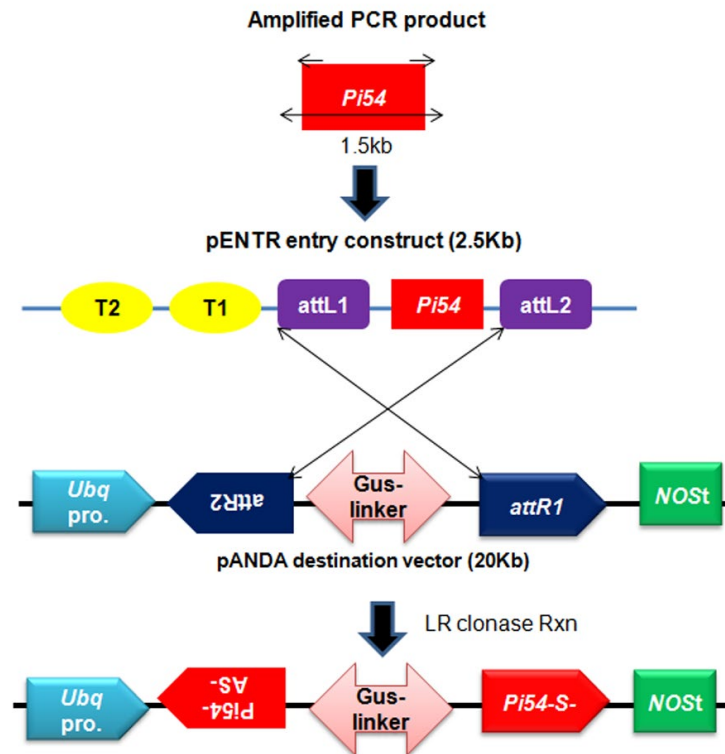
<sup>B</sup>ICAR National Rice Research Institute, Cuttack-753006, Odisha, India.

<sup>C</sup>National Agri-Food Biotechnology Institute, Mohali-140306, Punjab, India.

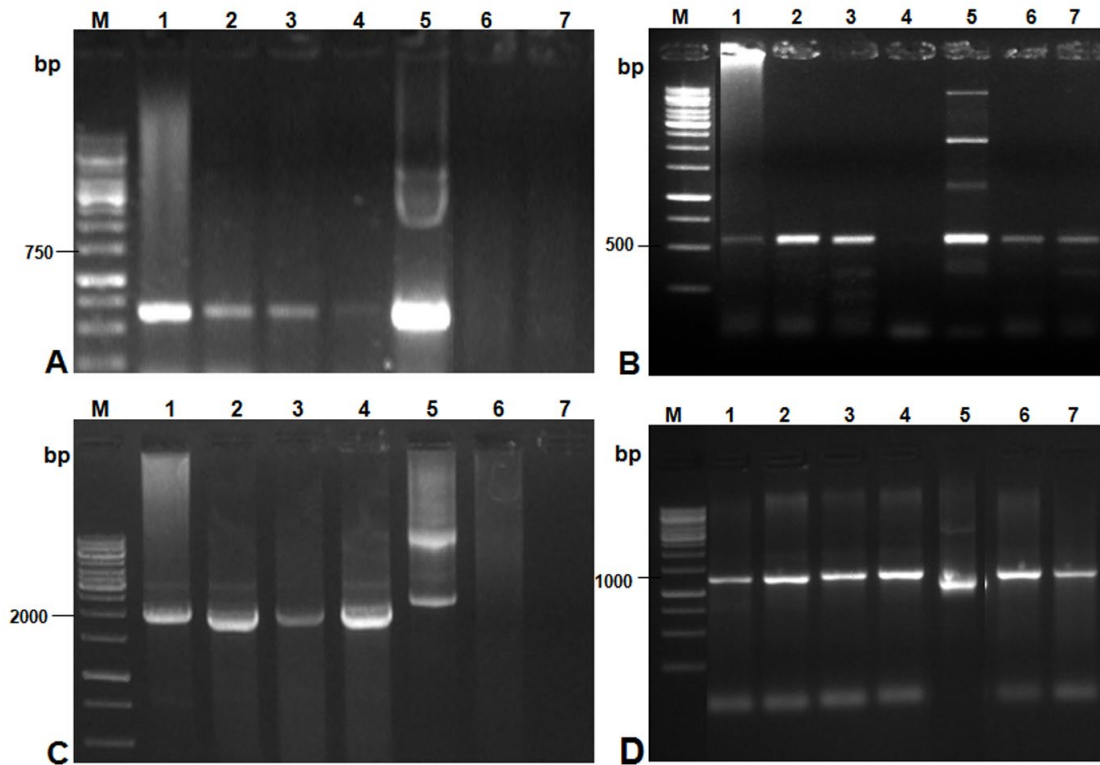
<sup>D</sup>Corresponding author. Email: trsharma@nabi.res.in



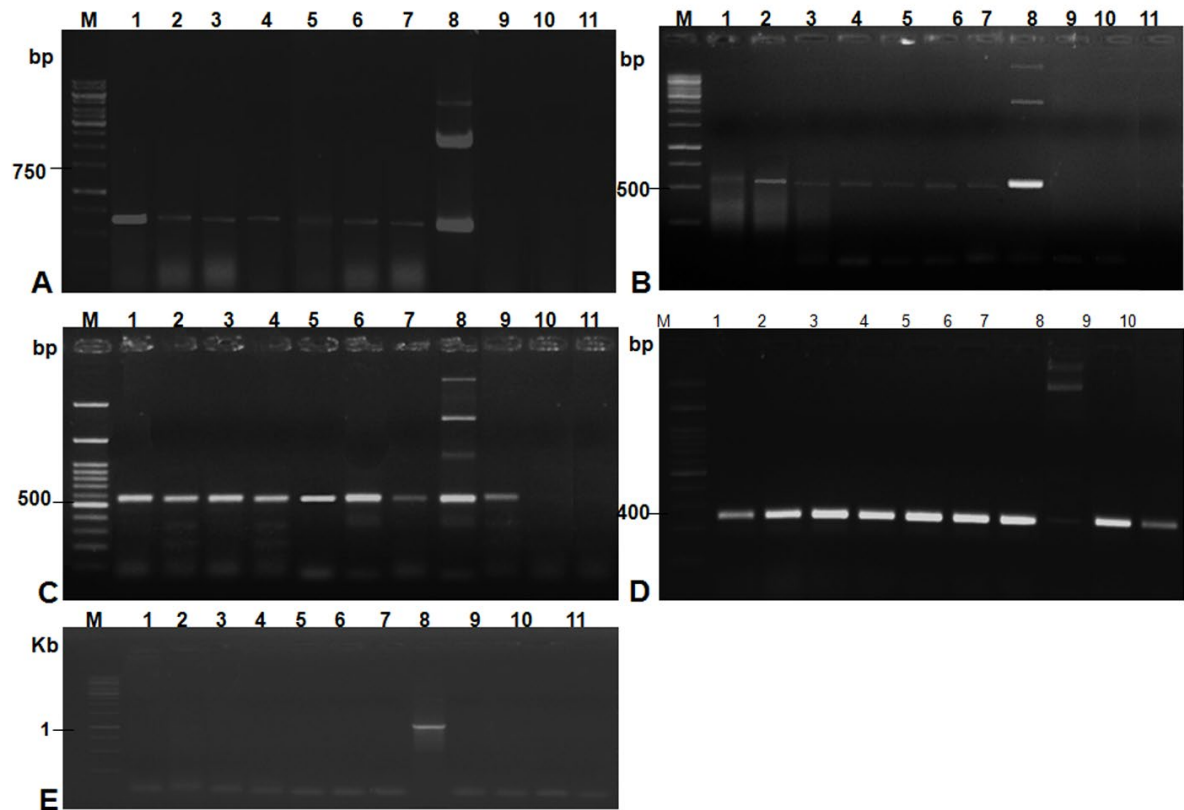
**Supplementary Figure 1. Schematic diagram of pANANDA vector based *Pi54* siRNA antisense gene cassette. A: pANANDA vector; B: *Pi54* antisense gene cassette.**



**Supplementary Figure 2.** Flowchart depicting development of RNAi construct using gateway technology.



**Supplementary Figure 3.** PCR screening of molecular transformants with different sets of primers (A) PCR amplification with gus linker primers with desired size of 636 bp; (B) PCR amplification with kanamycin specific primers with desired size of 550 bp; (C) PCR amplification with Ubiquitin specific primers with desired size of 1.9Kb; (D) PCR amplification with *Pi54* specific primers with desired size of 1086 bp. Lane M- 1kb DNA ladder, lane 1 to lane 4- molecular transformants screened to be positive, lane 5- RNAi construct, lane 6- Empty vector, lane 7- TP-Pi54-2.



**Supplementary Figure 4.** RT-PCR of molecular transformants with different sets of primers (A) PCR amplification with gus linker primers with desired size of 636 bp; (B) PCR amplification with bar gene specific primers with desired size of 550 bp; (C) PCR amplification with kanamycin specific primers with desired size of 550 bp; (D) PCR amplification with Ubiquitin specific primers with desired size of 300bp; (E) PCR amplification with *Pi54* specific primers with desired size of 1086 bp. Lane M- 1kb DNA ladder, lane 1 to lane 7- molecular transformants screened to be positive, lane 8- RNAi construct, lane 9- Empty vector, lane 10- TP-Pi54-2, lane 11- no template control