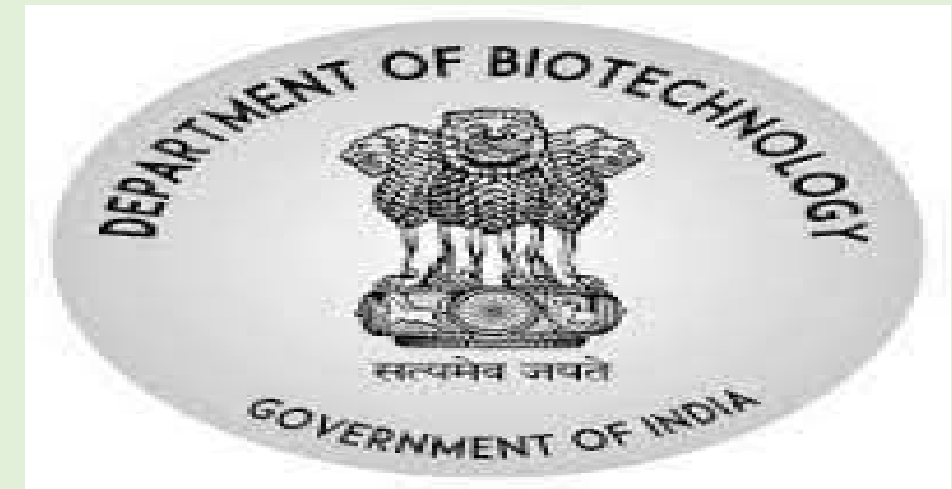


Elucidating the mechanism controlling the plant architecture through CRISPR/Cas9 based editing of *Ideal Plant Architecture 1 (IPA1)* in hexaploid wheat



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INTRODUCTION

- ❖ Ideal Plant Architecture 1 (IPA1) gene, reported as a negative regulator of tiller number in rice.
- ❖ The sequence of IPA1 candidate genes were highly conserved across the genomes of 15 wheat cultivars available on the Ensembl Plants database.
- ❖ Rice IPA1 orthologues in wheat can act as strong candidates for genes regulating tillering in wheat.

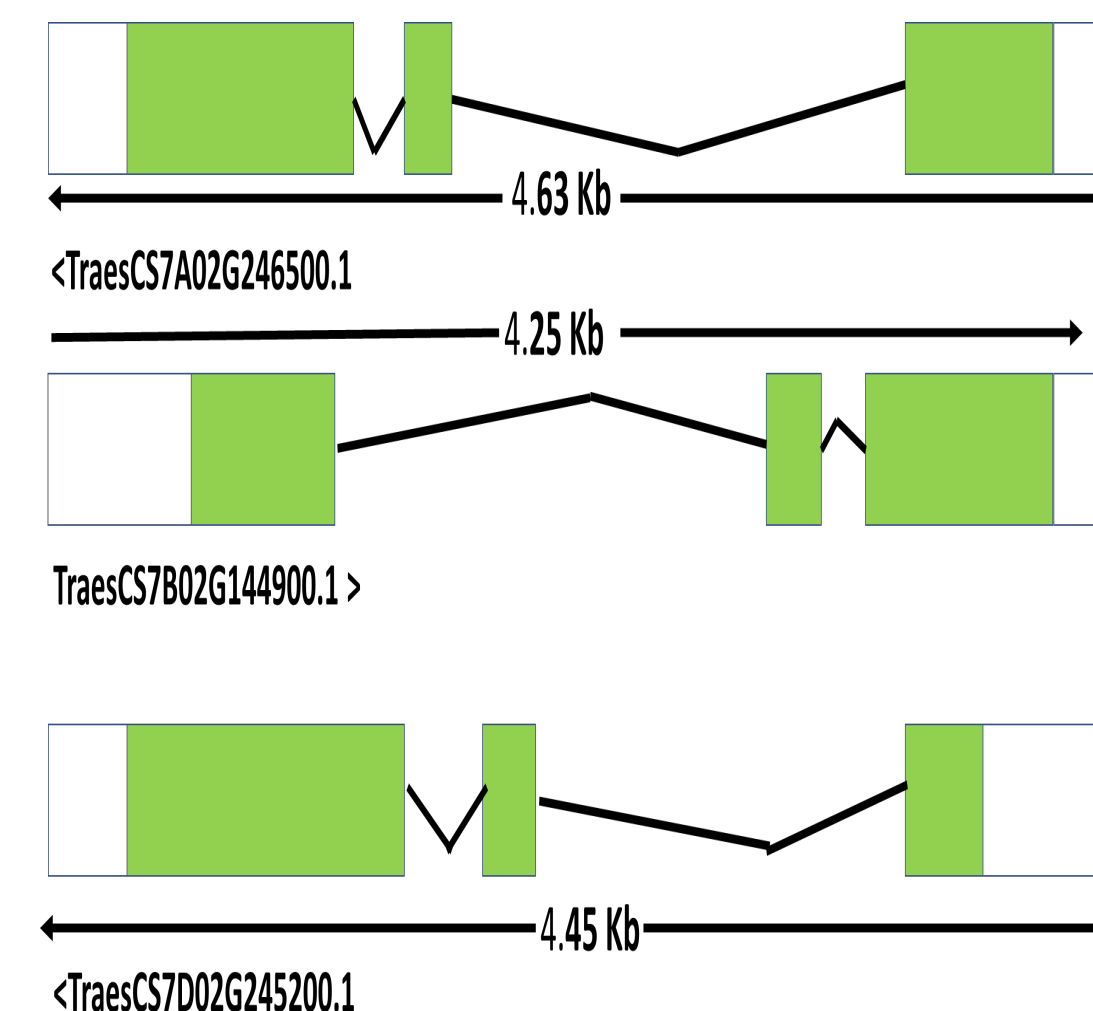
HYPOTHESIS

- ❖ To elucidate the regulatory network of *TaIPA1* for better understanding of molecular mechanisms controlling the wheat plant architecture.
- ❖ *TaIPA1* may provide a powerful way to manipulate wheat architecture for yield increases, and provide new markers and targets for molecular wheat-breeding programs.

OBSERVATIONS

EXPERIMENT 1

- ❖ In silico identification of *IPA1* orthologs in hexaploid wheat



- ❖ Potential orthologues for *IPA1* in wheat:
 - TraesCS5A02G265900
 - TraesCS5B02G265600
 - TraesCS5D02G273900
 - TraesCS7A02G246500
 - TraesCS7A02G260500
 - TraesCS7B02G144900
 - TraesCS7D02G245100
 - TraesCS7D02G245200

Figure 1. Conservation pattern of *IPA1* gene among the subsets of wheat genome on chromosome 7

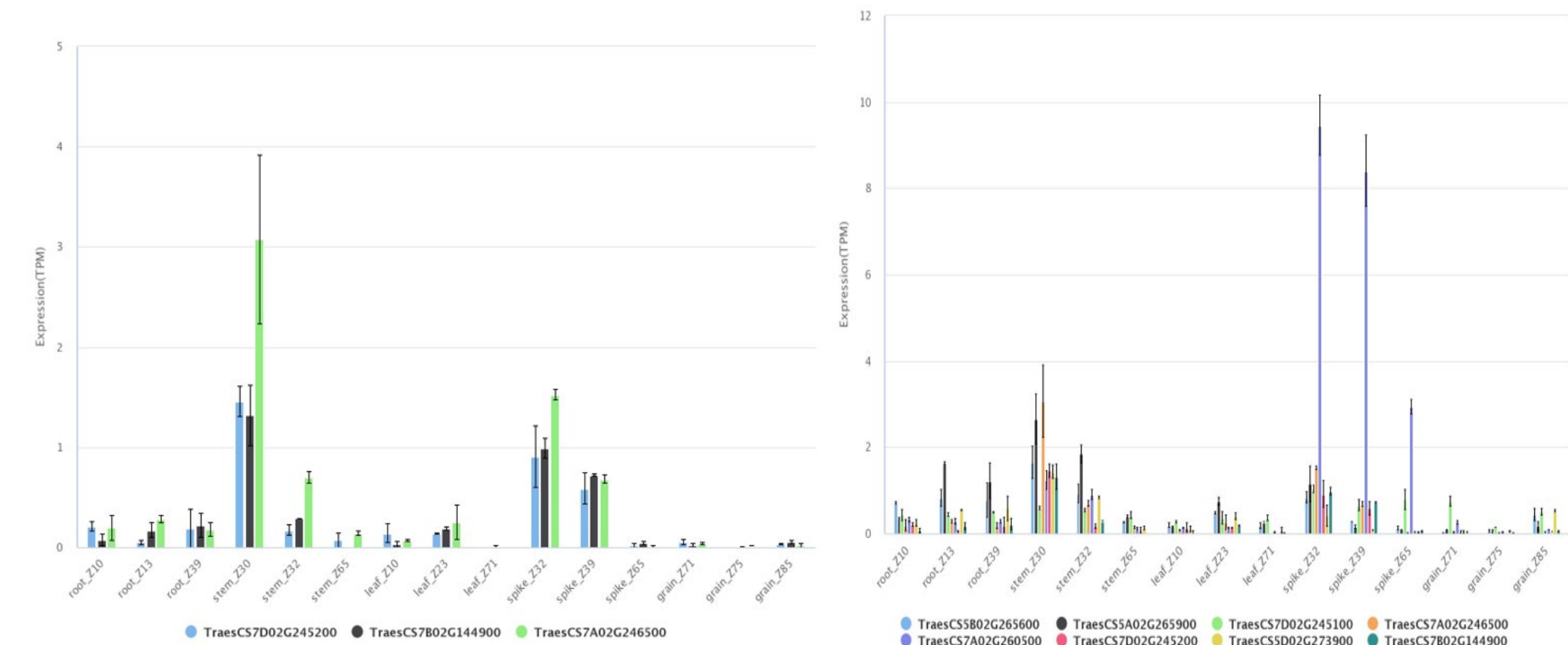


Figure 2. Transcriptome analysis of *IPA1* orthologs in wheat

CONCLUSION AND FUTURE PROSPECTS

- ❖ After transformation the genotypic and phenotypic analysis of mutants will be carried out for the presence of targeted mutation in *IPA1* orthologs in wheat.
- ❖ The selected mutants will be phenotypically characterized to access the effect of *IPA1* knockout in wheat and data on various agro-morphological traits will be recorded.

Reference Hayta S, Smedley MA, Demir S U, Blundell R, Hinchliffe A, Atkinson N and Harwood W A (2019) An efficient and reproducible *Agrobacterium*-mediated transformation method for hexaploid wheat (*Triticum aestivum* L.). *Plant Methods*, 15(1): 1-15.

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Figure 3. Homology based structure prediction could identify presence of SBT domain between 70-150aa

EXPERIMENT 2

- ❖ Sequencing *IPA1* orthologs in wheat cv. Unnat PBW550 and designing of gRNA

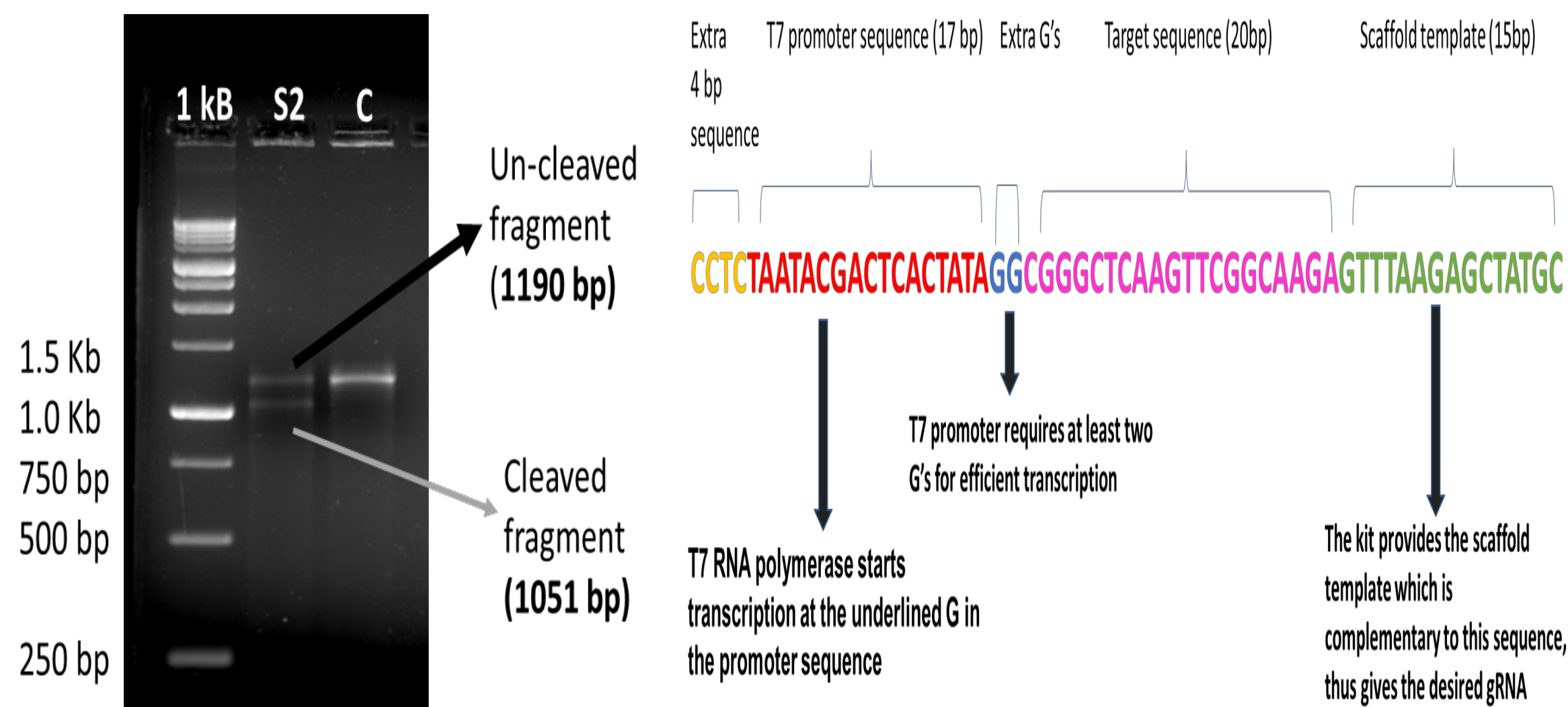


Figure 4. In-vitro cleavage of target by gRNA; S2 is Cas9 treated sample; C is the untreated sample

EXPERIMENT 3

- ❖ Generating the knockout mutants of *IPA1* gene orthologs in wheat
 - Cloning and assembling the gRNAs into pRGE31/ Cas 9 vector
 - Delivery of the plasmid into wheat immature embryos using *Agrobacterium* mediated transformation method

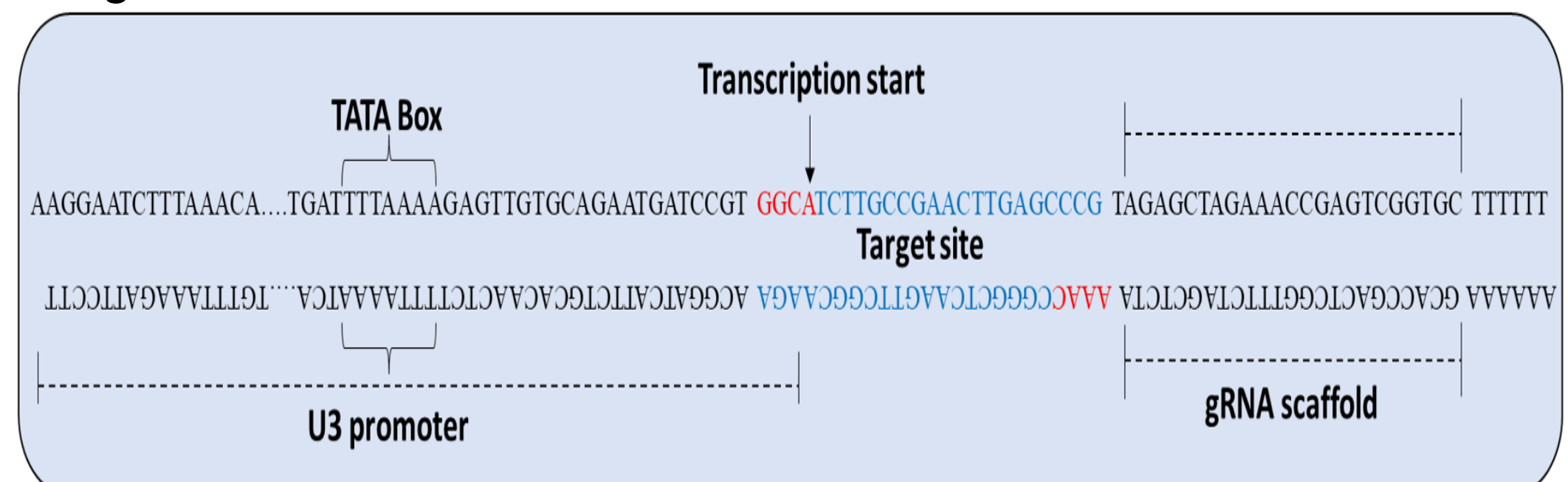


Figure 5. The single guide RNA construct with pRGE31 vector



Figure 6. Wheat immature embryo infected with cas9 target cassette transformed in AGL-1 strain of *Agrobacterium*