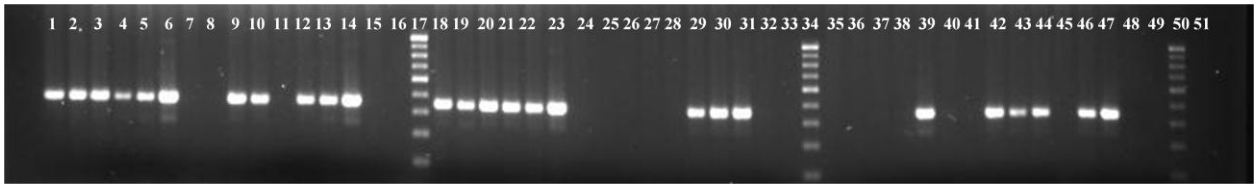
Tobacco transformation to validate PTGS constructs

Owing to the low levels of transformation frequencies achieved in castor, to test different gene constructs in castor by developing the transgenics with multiple gene constructs is a difficult task. Therefore, a model system was developed to test different post transcriptional gene silencing technologies so that the most efficient silencing construct could be identified which could later be deployed in castor.

Tobacco was transformed with full length ricin promoter-*gus*, Truncated ricin promoter-gus constructs. Analysis of the transgenic tobacco plants developed using these vectors, indicated the endosperm specific expression pattern of the isolated Ricin promoter.

Tobacco transgenic plants expressing ricin gene under 35S promoter were developed and confirmed at molecular level. Also, tobacco was transformed with Full length ricin Promoter (FP)-ricin and Truncated ricin Promoter (TP)-ricin constructs and the resultant plants were characterized at PCR level for the presence of the gene cassettes.

Analysis of T_1 and BC_1 tobacco plants carrying full length ricin gene under the 35S promoter indicated that the gene cassette was transmitted and expressed stably in the progeny plants. A representative gel picture of the PCR analysis with the T1 progenies of six T0 transgenic plants expressing full length ricin gene is shown below. Expression of the ricin gene in these plants was confirmed by the RT-PCR.



Confirmation of the presence of ricin gene cassette in the T_1 progeny plants of six T_0 tobacco transgenic plants expressing full length ricin gene

Lane1-8:AER3; Lane9-16:AER5; Lane 18-25:AER13; Lane 26-33:AER17; Lane 35-41:AER21; Lane 42-49:AER35 Lanes6,14,23,31,39,47:Positive control; Lanes 7,15,24,32,40,48: Negative control; Lanes8,16,25,33,41,49:blanks; Lanes17,34,50:100bp ladder

PCR Analysis of the T1 generation tobacco plants carrying the silencing vectors demonstrated stable Mendelian inheritance pattern for silencing vectors. RT-PCR showed that plants with *ihp* constructs did not show ricin transcript while plants carrying SHUTR constructs showed ricin specific transcripts.

Plants carrying single insert of the ricin transgene were identified based on the progeny analysis and were crossed with transgenic plants carrying the five silencing constructs independently. The

progeny plants would be analyzed for the segregation of the cassettes and its effect on the expression of ricin.