Transformation of castor with the developed gene cassettes for imparting grey mould tolerance

Castor transformation was attempted with triple as well as double gene constructs using meristem based transformation methods.. However, no putative transformed shoots could be recovered after third cycle of selection though about 18000 meristem explants were used for transformation. As an alternative, *in planta* experiments were carried out using different gene constructs.In planta transformation was carried out using 300 seedlings each with the double-gene (ERF1+BIK1) and triple gene (ERF1 + BIK1 + EBP1) cassettes, ERF1 single gene cassette as well as a concoction of ERF1 and *gus* gene cassettes. T1 progeny plants obtained from these were analysed for the presence and expression of the gene cassettes. Modified in planta transformation method as suggested by Dr. Rohini Sreevathsa, NRCPB, IARI, New Delhi was adopted with the cultivar 48-1 (Jwala) using different gene constructs including reporter gene (*gus*) construct. Totally about 625 T0 plants (as indicated in the table below) were established in the transgenic green house and the T1 seeds from each of these plants were harvested for further analysis.

Summary of the in planta transformation carried out

Constructs used	**No of seedlings co-cultivated**	**T_0 plants established**
GUS	100	50
ERF1 + GUS **	600	200
AtEBP1	400	150
BIK1	300	125
*AtEBP1 + BIK1***	300	100

**: Used as a concoction for co-transformation

In subsequent experiments, in planta transformation of castor (cultivar 48-1) with different constructs (as detailed in table below) was further carried out. About 470 T_0 plants were established in the transgenic green house and the T_1 seeds from each of these plants was harvested and subjected to analysis by growing the plants in transgenic greenhouse.

Summary of the in planta transformation carried out

Constructs used	**No of seedlings co-cultivated**	**T_0 plants established**
Multigene Construct (*AtEBP1- BIK1- ERF1*)	300	120
pCAMBIA 2301 –*ERF1*	250	100
AtEBP1	300	100
BIK1	250	100
AtEBP1 + BIK1(concoction)	200	50

Analysis of T_1 progeny (obtained through in planta transformation) castor plants :

Initially 15000 T_1 seeds from 300 To Castor in planta transformed plants with different constructs [*ERF1* + *GUS* (200), *GUS* (40), *AtEBP1*(60)] were raised in green house . Characterization of 1500 pooled samples of 10 plants each were done through PCR with gene specific primers. Only one T_1 plant showed presence of *ERF1* gene but the introduced gene cassette was not complete. Thus the frequency of transformation was negligible.

In order to increase the efficiency of identifying positive plants, Hygromycin selection was used to screen the T1 plants initially and only the seedlings that survived selection were transferred to soil and subjected to PCR analysis. About 10000 T_1 seedlings (3day old) from 200 T_0 Castor *in planta* transformed plants with different constructs were subjected to antibiotic screening (Hygromycin- 40 mg/lt). 600 plants that survived selection were subjected to PCR analysis with gene specific primers. Only one of the plants showed presence of *GUS* gene. Thus the frequency of transformation was found to be extremely low with *in planta* method of transformation.

T1 seeds from 500 T0 plants with different gene constructs from the second batch of in planta transformed were subjected to hygromycin selection. 22500 T_1 seeds from 300 T_0 plants (with different constructs) yielded 1000 plants that survived antibiotic selection. Out of these, 33 plants were PCR positive for *hptII* gene and of these, 30 plants were positive for EBP1 gene and two plants were positive for all two genes, *EBP1 and BIK1*. These putative plants will be further studied for the stability of the transgene(s) as well as for their expression and the imparted disease tolerance.