



Standardization of Genetic transformation technique in Dolichos bean (*Lablab purpureus* (L.) sweet) for pod borer resistance .

Presented by

**Dr. S. V. Sawardekar,
Professor and In-charge
Plant Biotechnology Centre,
College of Agriculture, Dapoli**

**Dr. Balasaheb Sawant Konkan Krishi Vidyapeeth,
Dapoli (MAHARASHTRA) INDIA**



- **Problems associated with pulses**
- **Abiotic stresses – Drought , Temperature, Salinity**
- **Biotic stresses- Fusarium wilt , Ascochyta blight**

Gram pod borer

- **Objective:**

To study the standardization technique for genetic transformation of Dolichos bean (Konkan Bhushan).

- **Genotype- Konkan Bhushan**
- **Gene Construct:**

The *Cry I AaBbCc* gene construct produced from NRCPB, New Delhi.

Methodology



MT1-Explants were injected by *A. tumifaciens* under aseptic condition and co cultivated for 48 hrs.

MT2 - Explants injured mildly and dipped in *Agrobacterium* for 20 minutes under aseptic conditions and co cultivated for 48 hrs.

MT3 - Explants dipped for 30 minutes and co cultivated for 3 days.

MT4 - Explants Pre cultured in the medium supplemented with 0.5 mg/L TDZ for 2 days, bacterial suspension culture was injected and co cultivated for 48 hrs on same media.

Method	No. of Explants co cultivated	No. of plants regenerated	Survival (%)
MT ₁	550	147	26.72
MT ₂	600	181	30.18
MT ₃	600	18	3.00
MT₄	1250	434	34.72



Results

Table 1: Elimination of *A. tumefaciens* by different level of cefotaxime

Sr. No.	Cefotaxime conc. (mg/L)	No. of seeds inocul.	<i>Agrobacterium</i> reappearance	
			Reappe arance	Per cent reappearan ce
1.	0	50	50	100.00
2.	200	50	41	82.00
3.	400	50	35	70.00
4.	600	50	20	40.00
5.	800	50	10	20.00
6.	1000	50	0	0

Kanamycin Sensitivity Test

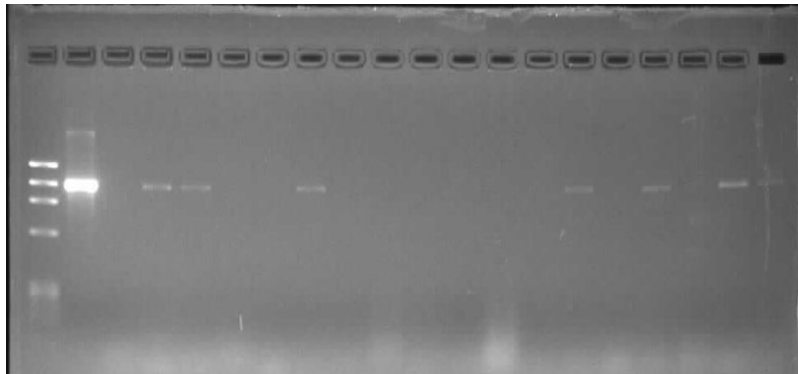


Concentration of kanamycin (mg/L)	Leaf blade
MS+0	++
MS+50	++
MS+100	++
MS+200	++
MS+400	++
MS+600	+
MS+800	+
MS+1000	0
MS+1200	0

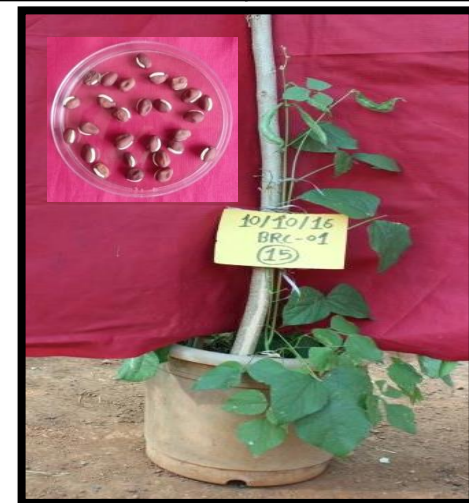
Frequency of putative transgenic



Method	No. of plants established	No. of Kanamycin resistant plants	PCR positive plants	Transformation frequency(%)
MT ₁	147	10 (6.80%)	0	0.00
MT ₂	181	12 (6.62%)	0	0.00
MT ₃	18	0 (0%)	0	0.00
MT ₄	434	30(16.12%)	20	1.6



PCR assay of +Ve plants



PCR Positive Plant



Future line of work

- Progeny analysis
- Incidence of secondary pests
- Movement of pollen grains
- Studies on biosafety issues

Thank you...