Induction of embryogenic callus and plantlet regeneration in Indian cassava cultivars

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INTRODUCTION

- Induction of somatic embryos in cassava (Stamp and Henshaw (1987a)
- Development of transgenics 1990's necessitated development of efficient tissue culture based regeneration systems
- 11 cassava varieties released from CTCRI
- Several traits like, modification of starch quality, enhancement of protein content, CMD resistance needs the application of biotechnological interventions for genetic improvement

- Regeneration of transgenics either through regeneration from FEC or cotyledons of somatic embryos
- Majority of the transgenics developed are from a highly responsive clone TMS 60444
- Varying *in vitro* morphogenic response between even closely related cultivars reported in cassava
- Development of regeneration system in Indian cassava varieties essential for the development of transgenics

OBJECTIVE

- To identify popular Indian cassava varieties with high embryogenic response
- To develop reproducible regeneration protocol that can be used in future transgenic programmes in India.

MATERIALS & METHODS

GNOTYPES : 21

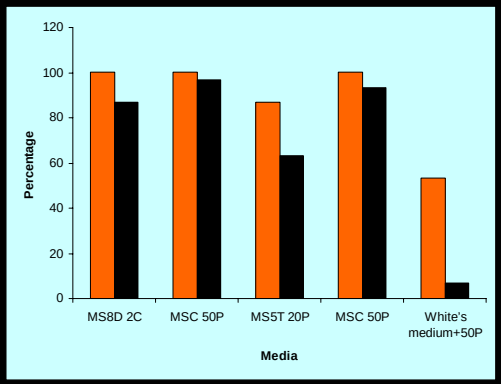
H 97, H 165, H 226, Sree Sahya Sree Visakham, Sree Prakash, Sree Harsha, Sree Jaya, Sree Vijaya, Sree Rekha, Sree Prabha , MNga1, 9 elite landraces

EXPLANTS USED

Unopened leaf lobes, meristems, Petiole , Axillary meristem , Internode

MEDIA : Murashige & Skoog + sucrose (20 g l-1)+ Agar (8 g l-1)+ hormones(2,4D, Picloram,TDZ) GY medium

RESULTS



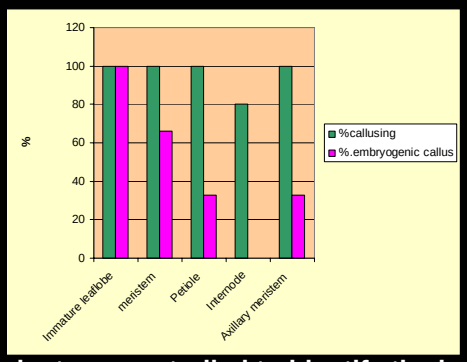
- Induction of embryogenic callus was significantly higher in MS medium with picloram (50µM) than with 2,4D (8mg l^{-1})

RESULTS

- Lowering of Picloram level to 20μM reduced the percentage of embryogenic induction to 63.3%.
- Addition of casein hydrolysate (500mg/l) favoured the induction of PEC.
- No significant effect with TDZ

RESULTS

EXPLANTS



- Five explants were studied to identify the best explant for induction of PEC
- Number of somatic embryos/explant higher in unopened leaf lobes
- Compact pale yellow embryogenic callus in all the eleven varieties within a period of 21 days of inoculation

DEVELOPMENT OF SOMATIC EMBRYOS

Unopened leaf lobes **Meristem**

Comparison of induction of embryogenic callus in different Indian cassava varieties

Variety	% of callusing	% of embryogenic callus	No. of somatic embryos/ explant
H-97	40	35	7
H-165	65	65	19.6
H-226	80	77.5	20.8
Sree Sahya	35	35	11.8
Sree Visakham	80	75	16.6
Sree Prakash	62.5	62.5	18.4
Sree Harsha	67.5	55	11
Sree Jaya	42.5	40	7.2
Sree Vijaya	62.5	60	13.4
Sree Rekha	65	57.5	19
Sree Prabha	75	75	20
CD	15.20	13.74	3.99

High embryogenic potential

Released varieties : H 226
H 165
Sree Prakash
Sree Visakham
Sree Prabha

Landraces : M4
Ambakkadan

- **Sree Harsha, the triploid variety has moderate response**
- **MNga1 –Poor response**

REGENERATION

Media Used

MS+Sucrose (20g/l)+hormones

NAA, BAP, TDZ

- **Use of NAA for maturation did not have any significant effect**
- **Use of BAP and TDZ favoured regeneration**
- **The effect of TDZ/BAP varied with genotypes**

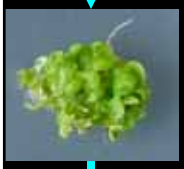
PROTOCOL FOR REGENERATION IN CASSAVA THROUGH SOMATIC EMBRYOGENESIS

Induction of PEC

Unopened leaf lobes/meristems

21 days

MS+sucrose (20g/l)+Agar 8g/l)+Picloram (50µM)



Maturation & shoot development

21 days

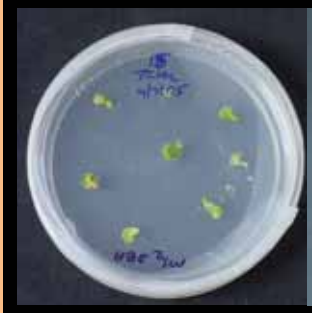
MS+sucrose (20g/l)+Agar 8g/l) +BA (2µM)+NAA(1µM)

Rooting of plantlets

MS+sucrose (20g/l)+ Agar 8g/l)

MATURATION AND REGENERATION OF SOMATIC EMBRYOS

Sree Prabha



MATURATION AND REGENERATION OF SOMATIC EMBRYOS

Sree Vijaya

MATURATION AND REGENERATION OF SOMATIC EMBRYOS

Sree Prakash

REGENERATION OF SREE HARSHA

MS2 + BA(2µM)

REGENERATION OF H-165

In vitro plants of H 226 regenerated from somatic embryos

ROOTING : MS without hormones

FRIABLE EMBRYOGENIC CALLUS(FEC)

Gresshoff and Doy medium with 20 % sucrose and picloram @50µM)

- ❖FEC response : H 226 and Sree Prakash
- ❖The FEC of Sree Prakash had better regeneration efficiency as compared to H 226.

To Summarise….

- The present study could identify H 226 and Sree Prakash as the ideal genotypes for the development of transgenic cassava in India
  H-226 is the most popular released variety in India. Sree Prakash has high leaf retention

- **Protocol for Indian genotypes**
  Induction and multiplication of embryogenic callus in MS+50 µM Picloram follwed by maturation of somatic embryos in MS+BA(2 µM) and normal plant development in MS without hormones