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BIOTECHNOLOGY GUIDED PRODUCTION OF L-DOPA

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ABSTRACT

Transformed callus culture of *Mucuna italies* (Fabaceae) were obtained by infecting explants (epicotyls, hypocotyls and cotyledons) obtained from aseptically germinated seedlings and 4 weeks old callus of epicotyl on MS media supplemented with NAA (1.0 ppm) + BAP (1.0ppm) with *Agrobacterium tumefaciens* (MTCC-431, MTCC-2250 and MTCC-2251). The oncogenic strains had different level of virulence on different types of explants. The main difference was found in the nature of galls formed and in their subsequent morphological competence. The comparative callus induction efficiency of various strains was evaluated. Various transformed callus grew axenically in the absence of exogenous plant growth regulator in Murashige and Skoog's medium containing 3% w/v of sucrose. The best result was found with epicotyl explants with *A. tumefaciens* strain (MTCC-2250). The highest transfection efficiency was observed with MTCC 2250. MTCC 431 also showed satisfactory results, while MTCC 2251 failed to transfect any of explants. The suspension culture of transformed callus were initiated and maintained successfully. The gall and cell suspension obtained synthesizes various normal constituents of plant out of which, L-Dopa was isolated, identified and estimated. The transformed callus showed callus induction efficiency (85% epicotyl callus transfected with MTCC 2250), viability (84% callus and 76% cell suspension), growth index (4.92) and productivity of L-DOPA (4.37% by Cell DW in callus and 7.88% by Cell DW in cell suspension) and it was found higher than the intact *Mucuna pruriens* seeds (3.61% by Cell DW).

Keywords: L-DOPA, callus culture, *Agrobacterium tumefaciens* and *Mucuna* sps.

INTRODUCTION

Mucuna italies[1] is a reputed drug in various systems of medicine and considered as a rich source of L-DOPA, which is being used for the treatment of various ill conditions, especially Parkinsonism[2]. The chemical synthesis of L-DOPA is also carried out but it is costly and the product does not meet the quality parameters.

Inspite of vigorous experimental work, L-DOPA production remains a difficult task and search for suitable convenient protocol for the production of the same through tissue culture techniques.

The present work is undertaken with a view to achieve the objective of establishment of commercially viable method for the bioproduction of L-DOPA at cost effective level through plant tissue culture and genetic transformation.

EXPERIMENTAL

1. The germination of seeds started on third day and upto 80% germination was observed on 6th day.
2. The viability of seeds was checked by 1% aqueous solution of 2,3,5-triphenyl tetrazolium chloride, about 70% seeds (embryo) developed an intense red colour[3].
3. Viable seeds were aseptically germinated over surface of sterilized filter paper absorbent cotton wool padding, secondly on the MS medium solidified with 0.6% agar.
4. Seedlings from aseptically germinated seeds were used as explants for genetic transformation by *A. tumefaciens* (MTCC 431, MTCC 2250 and MTCC 2251)[4] on plain Murashige and Skoog's media[5].
5. Tumorous growth was seen after 5-7 days. The percentage transformation was higher

in epicotyl explants transformed with MTCC 2250.

6. Opines were detected in tumorous calli. This was a firm indication of transfer of plasmid DNA to plant genome.
7. The tumorous callus obtained by the transformation of epicotyl explants by *A. tumefaciens* strain MTCC 2250 was used for the establishment of the suspension culture[6].
8. Qualitative and quantitative estimation for L-DOPA in transformed cultures were done[7].

RESULTS

Following observations were concluded on the basis of various experimental work:

1. The percentage viability of the seeds by chemical method was found to be more than 75%.
2. The callus induction efficiency was best observed (96%) with MTCC 2250 strain/ epicotyl explants.
3. Viability of the transformed callus was found to be 84% and 89% in cell suspension culture by fluorescent microscopy.
4. Confirmation of transformation was done by opine assay.
5. Suspension culture of transformed callus was successfully established.
6. Growth index of transformed callus was found to be 3.12.
7. L-DOPA content in transformed culture was found to be 3.37% by cell CDW and 7.88% by cell CDW respectively in callus and suspension culture is higher than the intact seeds (3.61% by cell CDW) and in normal callus (2.62% by cell CDW).

Conclusion

The results obtained after the various genetic transformation studies were encouraging and showed the potential of genetic transformation as a tool for the bioproduction of secondary metabolites (L-DOPA). This is concluded that the genetic transformation of *Mucuna italies* using *Agrobacterium tumefaciens* can be manipulated for the large-scale production of L-DOPA and work should be done in this direction.

Future prospects

This was the first ever approach to develop transformed callus and suspension culture of transformed calli and the attempt was made in order to exploit the L-DOPA production.

This is the very first successful tissue culture approach on Indian variety of white seeds of *Mucuna italies* for the production of L-DOPA and the results obtained indicates the potential of these methodologies for large scale production of L-DOPA and may lead to development of commercially viable system for production of the drug.

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