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# *IN VITRO* SHOOT REGENERATION FROM COTYLEDON OF REDGRAM (*Cajanus cajan* L. Var. LRG-41)

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## INTRODUCTION

Pigeonpea (*Cajanus cajan* L. Millsp) is one of the most popular legume grains in the world, especially in the Indian subcontinent. Pigeonpea plays an important role in food security, balanced diet and alleviation of poverty because of its diverse usages as food, fodder and fuelwood. Several biotic (Fusarium wilt, sterility mosaic and pod borer insects) and abiotic (drought, salinity and water logging) stresses, are serious challenges for sustainable pigeonpea production to meet the demands of the resource poor people of several semi-arid tropic regions of India and other countries. Keeping in view of the importance of the crop, it is imperative to have an efficient regeneration and transformation system in order to introduce novel traits in pigeonpea. In this report, we present an efficient protocol for rapid *in vitro* plant regeneration from cotyledon of redgram.

## MATERIAL AND METHODS

Seeds of LRG-41 were Surface sterilized with 0.1% $HgCl_2$ and soaked in sterile water for overnight. The imbibed seeds were decoated and two cotyledons were carefully separated. Excised cotyledon was transferred to Murashige and Skoog (MS) medium supplemented at different α-Napthalene acetic acid (NAA) 0 to 2mg/l and 6-Benzylaminopurine (BAP) 0 to 2mg/l concentrations. They were subsequently transferred into regeneration media containing BAP and NAA maintained in light for 20 days. Observations on reproducing explants and number of shoots were recorded 20 days after transferring to regeneration media. Elongated shoots were rooted on MS medium with different Indole-3-butyricacid (IBA) 0 to 2mg/l concentrations. After getting maximum root length the plantlets were transferred to soilrite for hardening.

## RESULTS

MS medium supplemented with various concentrations of BAP, which is most widely used for various legumes in combination with NAA. Among the various concentrations tested, 2.0mg/l BAP and 0.1 mg/l NAA were found to be the best for maximum shoot bud differentiation. Percentage, as well as the number of shoots per explant showing differentiation of shoot buds was higher on MS media supplemented with BAP and optimal BAP concentration for shoot regeneration was 2mg/l. Elongation of multiple shoots was obtained in MS medium with the concentration 0.4 mg/l gibberillic acid ($GA_3$). Regenerated green healthy shoots were seperated and transferred to rooting medium. Though different concentrations of NAA and IBA were supplemented to MS medium, IBA at 1.0 mg/l induced maximum frequency of rooting followed by NAA. Regenerated plants were successfully established in soil where 90 to 95% of them have been developed into morphologically normal and fertile plants.

## CONCLUSION

The present work proved that efficient regeneration could be done with cotyledon as explant in redgram variety LRG-41. This kind of regeneration system from cotyledonary explants would be useful for development of transgenics of redgram through agrobacterium mediated or particle bombardment transformation.

**Effect of concentration of MS medium on *in vitro* shoot regeneration of redgram**

| Hormone Conc (mg/l) | | | No of explants kept for Regeneration | No of explants Responding | Shoot buds per explant |
|---|---|---|---|---|---|
| BAP ( mg/l) | NAA ( mg/l) | $GA_3$ (mg/l) | | | |
| 0.0 | 0.0 | | | NR | - |
| 0.1 | 0.0 | | | NR | - |
| 0.5 | 0.0 | 0.4 | 7 | NR | - |
| 1.0 | 0.0 | | | 1 | - |
| 2.0 | 0.0 | | | 2 | 2 |
| 0.0 | 0.1 | | | NR | - |
| 0.1 | 0.1 | | | NR | - |
| 0.5 | 0.1 | 0.4 | 7 | NR | |
| 1.0 | 0.1 | | | 2 | 4 |
| **2.0** | **0.1** | | | **7** | **15** |
| 0.0 | 0.5 | | | NR | - |
| 0.1 | 0.5 | | | NR | - |
| 0.5 | 0.5 | 0.4 | 7 | NR | - |
| 1.0 | 0.5 | | | 2 | 5 |
| 2.0 | 0.5 | | | 3 | 5 |
| 0.0 | 1.0 | | | NR | - |
| 0.1 | 1.0 | | | NR | - |
| 0.5 | 1.0 | 0.4 | 7 | NR | - |
| 1.0 | 1.0 | | | 2 | 3 |
| 2.0 | 1.0 | | | 4 | 6 |
| 0.0 | 2.0 | | | NR | - |
| 0.1 | 2.0 | | | NR | - |
| 0.5 | 2.0 | 0.4 | 7 | NR | - |
| 1.0 | 2.0 | | | 3 | 2 |
| 2.0 | 2.0 | | | 3 | 4 |

NR: Not Responding

Explant with dome like structure after three weeks of culture

Multiple shoots developed from cotyledonary segment

Isolated elongated shoot cultured for rooting

## ACKNOWLEDGEMENTS

The authors are thankful to Acharya N.G. Ranga Agriculture University for providing research facilities at Institute of Frontier Technology and financial support for fullfilment of master degree.

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