ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

In vitro propagation from nodal and internodal Explants of *Lathyrus sativus L*.

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ABSTRACT

In vitro propagation of *Lathyrus sativus* L., *Lathyrus sativus* L. commonly known as grass pea belongs to Fabaceae family. Here in the present study we developed an efficient micropropagation protocol from nodal and internodal explants of *Lathyrus sativus*. *In vitro* propagation is an alternative method of propagation of the threatened and endangered plant which can aid its conservation. The nodal and internodal explants were cultured on MS medium containing different concentration and combinations of growth regulators like 6-benzylaminopurine (BAP) and 3-indoleacetic acid (IAA). Multiple shoot buds were regenerated successfully from the nodal explants which were efficiently rooted on half strength MS medium supplemented with Indole-3-butyric acid (IBA). The regenerated plantlets were successfully transferred to the glasshouse, acclimatized and transferred to the field.

Keywords: *Lathyrus sativus* L., Grass pea, *in vitro* propagation, nodal and internode explants. MS Medium.

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ISSN: 0975-8585

INTRODUCTION

Grass pea *Lathyrus sativus* L. is well known for its recalcitrance to *in vitro* approaches [1]. It is an annual cool-season legume crop grown for food and feed all over the world [2, 3]. The grass pea of the Fabaceae is a grain legume with lysine-rich protein. The legume is cultivated in many parts of India and several other developing countries including Bangladesh, Nepal and Ethiopia. Although its cultivation requires the least crop management, the full potential of this crop has not been utilized because of the presence of the neurotoxic amino acid b-N-oxalyl-L-a,b-diaminopropionic acid which causes neurolathyrism in human beings on prolonged consumption. Conventional breeding practices and other agronomic approaches explored to date have not been successful in substantially reducing the toxin.

It can thrive well in adverse climatic conditions (drought, salinity and water-logging) and has substantially high protein content (25-30%) than other food legumes. The largest collections of grass pea genetic resources are in India followed by Syria and France. *In vitro* selection of low neurotoxin plants and gene transfer may offer genetic rectification in grass pea and make it suitable for consumption. An efficient *in vitro* culture system is a pre-requisite for success in isolation of somaclonal variants, or somatic mutants and also genetic transformation. In this context, somatic embryogenesis is gaining importance as the regenerates are derived from single somatic cells. But, legumes in general, are recalcitrant to regeneration *in vitro* [4, 5], reported that Organogenesis and somatic embryogenesis are under separate genetic control and therefore, genotypes of the same crop species are most likely to respond differentially with regard to morphogenetic potential even in an optimum regeneration medium [6], studied inheritance of the ability to form somatic embryos using a diallel cross among six different pure lines of pea (*Pisum sativum*). About 80% of the observed genotypic variation was due to additive effects. Analysis of the distribution of F3 family means from crosses between divergent lines indicated presence of a few major genes to control somatic embryogenesis in pea. Effect of genotype or genotype x medium interaction on callus induction and regeneration potential has been well documented in number of crop plants including grass pea [7, 8, 9 and 10]. Moreover successful callus induction does not necessarily correlate with induction of somatic embryogenesis and regeneration ability of a genotype.

In vitro shoot bud differentiation or plant regeneration via callus derived from shoot tips[11], axillary buds free of apical dominance[7], stem [12], leaf and root explants[13, 14] in *L. sativus* have been reported as well as direct shoot regeneration from epicotyl explants and seed cultures [15, 16]. However the efficiency of whole plant regeneration was low or not clearly stated in most of the above studies. Nevertheless they indicated that grass pea is amenable to in vitro shoot regeneration and that the regeneration-competence trait is present in a variety of explants.

Although, *in vitro* clonal propagation of grass pea has been reported using a variety of explants in various growth media [17, 18, 19, 20 and 21]; there is limited information available on optimization of sucrose concentrations, which is commonly used as carbon source in plant tissue culture media. It is well known that exogenous sucrose affects photosynthesis and biomass formation positively [22, 23]. Moreover, sucrose are also reported to have an osmotic role and act as source of energy to induce root and shoot regeneration in tissue cultured plants [24, 25, 26], by increased nutrient function [27, 28, 29, 30]. Previous studies further testify that sucrose also influence shoot regeneration and rooting during acclimatization. Rooting and root quality vary depending on genotype and sucrose levels [31]. The present study was carried out to standardize efficient protocols for micropropagation Medak genotype of *Lathyrus sativus* via direct somatic embryogenesis of leaf and internode explants.

MATERIALS AND METHODS

Material

Seeds of the *Lathyrus sativus* (L). were collected from wild plants located at Lingampally village, Pulkal Mandal, Medak district, Telangana, India. In addition to their use in *in vitro* studies, the seeds were also planted in the Botanical Garden of Department of Botany, Osmania University, Hyderabad and a plantation was raised in the form of a green hedge. *Lathyrus sativus* is a slow growing, perennial climber of tropical and subtropical regions leaves and stem spiral opposite petiolate leaves, entire, smooth shiny, varying in shape and

size according to their age. Flowers blue colored and small size. Further, a good protocol for micropropagation was developed to aid in its multiplication and conservation.

Micropropagation studies

Lathyrus sativus plants were subjected to *in vitro* propagation and a good protocol for micropropagation was developed to aid in its multiplication and conservation. The micropropagation studies comprised the culture of nodal and internodal explants on different culture media under standard growth conditions. The nodal and intermodal explants were collected from mature and healthy field grown plants. They were washed under running tap water for 15 min followed by soaking in 0.1 % (v/v) liquid detergent Tween-20 for 5 min and then subsequently washed with tap water. The explants were then soaked in 70% ethanol (C_2H_5OH) for 5 min followed by washing with water. Finally the explants were surface sterilized with 0.1% solution of mercuric chloride ($HgCL_2$) for 3 to 5 min followed by thorough rinsing in sterile distilled water (DH_2O). A total of thirty explants were inoculated in culture tubes containing MS medium (Murashige and Skoog medium) augmented with 2 % sucrose and 0.8 % agar and different combinations and concentrations of various plant growth regulators. The experiment was carried out in triplicates. Prior to that, the pH of the medium was adjusted to 5.8, autoclaved at 121^{o}C for 15 lbs / cm^{2} for 15 min and allowed to cool before inoculation. The culture media comprised of the following: MS + BAP (0.5, 1.0 and 1.5 mg/l) and MS + BAP (0.5, 1.0 and 1.5 mg/l) + IAA (0.5 mg/l). All the inoculated cultures were incubated in sterile growth room under controlled conditions of 22 ± 1^{o} C temperature, 75 % humidity and 2000 lux illumination of 16 hr / 8 hr L/D cycle. The 2 cm long regenerated shoots were transferred to root inducing media comprising half MS medium supplemented with IBA (0.5, 1.0 and 1.5 mg/l). The regenerated plantlets were later transplanted to pots containing a mixture of soil and vermicompost in the ratio of 2:1. The plantlets were gradually acclimatized on the laboratory bench by covering with a plastic bag with holes (to maintain high humidity), which were opened up gradually over a period of one week. The plants in the pots were moved to the glasshouse to a shaded area and gradually acclimatized.

RESULTS AND DISCUSSION

The present study contributes valuable information of an efficient micropropagation protocol was developed with a high percentage of shoot regeneration and multiple shoots (fig.1-B to C). The highest response of production of multiple shoots was recorded with MS + BAP (1.5 mg/l) followed by in MS + BAP (1.0 mg/l) (Table-1). The explants proliferated by 5-8 days and shoot regeneration was observed by 10-15 days (fig.1-C). Shoots of about 2 cm with 2-3 nodes were produced by 20 days. These were cultured on root induction media containing different concentrations of IBA (0.5, 1.0 mg/l) to induce roots. The higher concentration of IBA (1.0 mg/l) produced better rooting efficiency of (Fig.1-D; Table-2). The regenerated plants were transferred to the glasshouse for acclimatization (fig.1-E, F). Out of a total of 625 explants (pooled from triplicates) inoculated, 350 explants could regenerate shoots and 160 shoots were inoculated on rooting media for root induction out of which 130 shoots could develop roots to enable 110 plants to be transplanted out of which 94 plants survived in pots. In the present study, different concentrations of BAP and BAP with IAA were used to induce regeneration [18, 19].

However reported the regeneration of *Lathyrus sativus* through the use of BAP and KN and TDZ individually and combined with NAA. The present results agree well with the above report with supplementation of BAP individually or in combination but a higher frequency of regeneration was obtained with BAP (1.5 mg/l) presently [18, 19] reported the plant regeneration of *Lathyrus sativus* from cotyledons and nodal explants on MS medium supplemented with BAP (2.0 mg/l), KN(3.0 mg/l) and TDZ (0.1 mg/l) whereas, in our present report use of BAP individually produced the highest shoot regeneration frequency without any additional supplementation of IAA and IPA. In the present study it was observed that MS + IBA combination produced efficient rooting compared to other reports where they achieved rooting on MS medium supplemented with IAA. This efficient high frequency plant regeneration protocol developed presently can be taken up for large scale micropropagation for its multiplication and conservation.

ISSN: 0975-8585

Table 1:- Efficiency of shoot regeneration and production of multiple shoots from nodal explants of *Lathyrus sativus* (L). on different culture media

Culture medium	No. of explants with shoot induction
MS + BAP (0.5 mg/l)	56
MS + BAP (1.0 mg/l)	60
MS + BAP (1.5 mg/l)	68
MS + BAP (0.5 mg/l) + IAA (0.5 mg/l)	51
MS + BAP (1.0 mg/l) + IAA (0.5 mg/l)	52
MS + BAP (1.5 mg/l) + IAA (0.5 mg/l)	54

The value was calculated as the percentage of nodal explants that have produced shoots out of a total number of inoculated explants (90).

Table 2:- Percentage of root induction from multiple shoots regenerated from nodal explants of *Lathyrus sativus* (L).

Culture medium	No. of shoots with root induction
MS + IBA (0.5 mg/l)	50
MS + IBA (1.0 mg/l)	60

The value was calculated as the percentage of shoots with root induction out of a total number of inoculated shoots.

Figure 1: A-F: A. *Lathyrus sativus* plant, B. Shoot regeneration from nodal explants, 12 days after inoculation, C. Multiple shoots, 25 days after inoculation, D. Rooting from regenerated shoot, 15 days after inoculation of shoot, E. Acclimatization of regenerated plantlet, F. Regenerated plant transferred to the field.

ISSN: 0975-8585

CONCLUSION

In conclusion the protocol reported *in vitro* propagation of *Lathyrus sativus* (L)., *Lathyrus sativus* is very efficient for the production of a high frequency of adventitious shoot regeneration across a wide range of *Lathyrus sativus* genotype where the site of shoot differentiation is predictable and occurs in a short spon of 2 weeks. The present protocol describes on *in vitro* propagation from Nodal and internodal explants of *Lathyrus sativus.* High frequency regeneration of multiple adventitious shoots in over 90%of nodal and intermodal explants that are devoid of preexisting meristems. Such a generally applicable to biotechnological improvement of *Lathyrus sativus*, an important legume crop.

ACKNOWLEDGEMENTS

The financial support of UGC-NON–NET Fellowship. We express deep thanks to Department of Botany, Osmania University, Hyderabad.

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