Indian Journal of Fundamental and Applied Life Sciences ISSN: 2231-6345 (Online)
An Online International Journal Available at http://www.cibtech.org/jls.htm
2011 Vol. 1 (4) October-December, pp.203-208 /Supe and Roymon.
.***Research Article***

MICROPROPAGATION OF RADIATED SEEDS OF *PISUM SATIVUM*

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ABSTRACT
An efficient protocol was developed for high frequency shoot regeneration from the radiated seed explants of *Pisum sativum*. *Pisum sativum* is the fourth important cultivated legumes next to soybean, groundnut, and beans in the world. It is a cheap source to meet the protein requirements of a large majority of the population. The use of radiations is an alternative for improvement of desired characters in agricultural important crops such as in *Pisum sativum*. Seeds of *Pisum sativum* were used as the source material for the establishment of mutagenic shoots and roots. Seeds are treated with different radiations at various ranges (0.6-2.0KR). Murashige and Skoog (MS) medium supplemented with BAP (3mg/l) in combination with Kn (1mg/l) or NAA (0.5mg/l) was found to be most effective in initiating multiple shoots. Both shoot and root developed in the same medium. Microshoots rooted best in *vitro*, in half strength MS medium supplemented with IAA (0.1mg/l). Regenerated plantlets were successfully established in soil with a survival rate of 95%. The main aim of doing this work was to study the effect of irradiations on the *in vitro* culture of *Pisum sativum*.

Key Words: *Pisum sativum,* Micropropagation, Radiation, Auxin, Cytokinin

Abbreviations: BAP-6-Benzylaminopurine, NAA-1-Napthalene acetic acid, Kn- Kinetin. IBA- Indole butric acid, IAA- Indole acetic acid, MS Medium- Murashige and Skoog medium

INTRODUCTION
Pea (Pisum sativum L.) is an important crop in Northern Europe. It is grown for its seeds, which are considered as a high quality and relatively cheap source of protein, used for cattle feed and in the human diet. Resistance to viruses, lowering of anti-nutritional factors, and improving protein composition and quality, are important goals in pea breeding. Improvement of these goals can probably only be accomplished with genetic modification, since peas' natural variation is limited (Christou, 1997).
During the last few years, rapid and large scale propagation of medicinal plant has been significantly increased using the micropropagation techniques (Patnaik *et al*., 1996). Micropropagation is an alternative to the conventional method of vegetative propagation with the objective of enhancing the rate of multiplication (Murashige, 1990; Bala *et al.*, 2010).
The availability of efficient seed germination system is crucial in achieving successful mutagenesis. The major advantage of inducing mutations under *in vitro* conditions is that many varieties can be exposed to mutagens for reliable screening in a relatively small space which can save time, money and space compared to growing thousands of plants in the greenhouse or field (Griga, 2000).
The advantage of use of radiations is to enhance variation, induced by tissue culture technique and to obtain agronomically desirable mutant crops. Appropriate dosages of radiation in *in vitro* cultured tissues of plants induce changes in one or a few characteristics of the treated explants without altering their unique traits (Otroshy *et al.*, 2011).
Since medicinal and pesticidal plants are now being overexploited from natural population and thereby leading to a depletion of plant resources (Gantait and Mandal 2010) therefore, it is important to develop a protocol for its multiplication through micropropagation of this highly valuable plant. The present study was aimed to develop a tissue culture method for regeneration of plants from seed segments of *Pisum-sativum*. Mutation breeding is also used as an alternative for improvement of desired characters in

agricultural crops. This is based on creation of variations, selection, evaluation and ultiplication of desired genotypes. The use of nuclear techniques directed for inducing mutations is one of the
most important ways to achieve the objective and their use has become an established technology for breeding of new varieties. Many crops with improved economic value have been obtained using induced mutation. Besides the economic benefits, some mutants also play an important role in the study of genetics and plant development. Although advantages are evident, there are surprisingly few reports describing induced mutations from seeds under in vitro conditions. Mutagens have been applied to suspension cultures, callus, and embryo cultures in many species including carrot, maize, rice, wheat, and tobacco.

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MATERIAL AND METHODS

Seeds of the *Pisum sativum* were used as explants. The seeds were collected from Indira Gandhi Agricultural University, Raipur and experiments were carried out in St. Thomas College, Bhiali in 2006. Explants were washed thoroughly under running tap water (10 minutes) followed by treatment with 1% teepol solution for 3 minutes.Then explants were washed under tap water to remove detergent and finally surface sterilized for two minutes with 0.1% mercuric chloride. Finally explants were washed three times with sterile distilled water to remove traces of mercuric chloride

The seeds were placed on MS medium (Murashige and Skoog's, 1962) containing 3% sucrose, 0.8% agarose supplemented with various concentrations of cytokinins namely BAP and Kn (1-3mg/l) were added individually or in combinations. The pH of the medium was adjusted to 5.7 before gelling with agar and autoclaved at 121^{o}c for 30 minutes under 1.06kgcm3 steam pressure. All the cultures were raised 25x150mm culture tubes plugged with Aluminium foil and were incubated in growth room at temperature of 24±2^{o} c and relative humidity 70±5%, a photoperiod from cool white florescent tubes giving 2000lux at culture level. The cultures were maintained by regular subculture at 6 week intervals on fresh medium with the same composition. Growth parameters were recorded at an interval of 10 days from the date of inoculation.

For root induction, excised shoots 2cm long with 4 or more leaves were transferred to half strength MS medium fortified with IBA, IAA and NAA (0.1-1mg/1) individually or in combinations. Plantlets with well-developed roots were removed from culture medium and gently washed with sterile double distilled water. For each treatment minimum 20 replicates were used and experiment was repeated thrice. The mean value (average) of shoots and roots produced per explant was calculated from the replicates of each treatment.

RESULTS AND DISCUSSION

Treated seeds were evaluated for lethality from different doses of irradiations. It was observed that seed germination was independent of dose of rays and was mainly affected by the germination capability of the genotypes. Radiations had an insignificant effect on germination of genotypes having germination frequency of 10% to 90% increase in germination was recorded at 180 Gy g irradiations compared to the control. In contrast, a decrease in germination was recorded from Bolero and Sprinter with all doses of irradiations compared to theThe radiated seeds germinated on MS medium after 4 days of inoculation. The higher multiple shoot initiation was found after 10-11 days of inoculation in 0.06 KR treated seeds. The seeds of higher range of radiation gave less number of multiple shoots when compared with increased observation days (Table 1).

The initiation of multiple shoots was observed within 6 days of culture in MS medium supplemented with 3mg/l of BAP. However, in MS medium supplemented with BAP 1mg/l the multiple shoots initiation was observed with maximum number of shoots (6.8).. This is confirmatory with earlier experiments reported in medicinal plant species including *Weddelia calendulacea* (Emmanuel *et al*., 2000). In *Aloe barbadensis* (Supe, 2007) maximum shoots were produced in medium supplemented with BAP (4 mg/l).

Within 2 weeks, an average number of shoots ranging from 1.5 to 6.8 were formed from each seeds of *Pisum sativum* when they were cultured on MS supplemented with 0.5-3mg/l of BAP (Fig.1A). The addition of Kn and NAA, at a lower concentration (0.5-1mg/l), into MS medium containing BAP, however, did not show significant influence on multiple shoots formation from the seeds. This observation suggested that the induction of multiple shoots formation of *Pisum sativum* depended only on the presence of BAP in the culture medium. The explant cultured on basal MS medium without any growth regulator produced only a single shoot. All the multiple shoots formed on MS medium supplemented with BAP 0.5-3mg/l and Kn (1mg/l) or NAA(0.5-1 mg/l)formed small clusters. Similar result was reported in *Spilanthes acmella* (Purabi and Kalita, 2005) but, Urbina *et al*., in 2008 observed that BA had no significant effect on shoot induction in Agave.

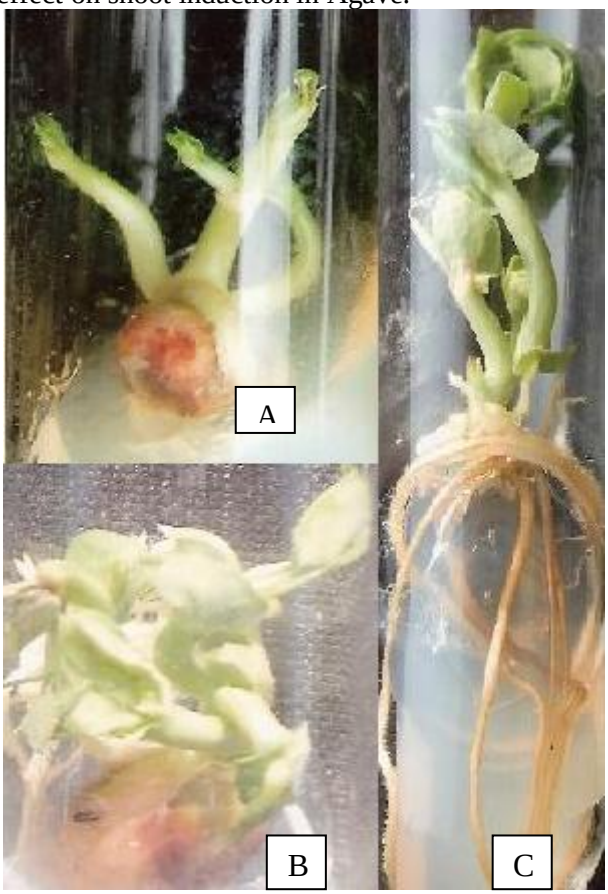

Fig.1. A. Induction of Multiple shoots.
B. Mass multiplication of shoots.
C. Root induction in shoots.

The explant cultured on MS medium supplemented with various concentrations of BAP and Kn or NAA in which BAP 3.0mg/l and Kn 1.0mg/l combination induced maximum number of multiple shoots (Fig.1B). Both shoot and root development was noticed in the same medium. combination of auxin and cytokinin was advantageous for the production of multiple shoots in Rice (Rashis *et al*., 2001`), *Spilanthes maurtiania* (*Bais et al*., 2002 *Spilanthes acmella* (Saritha *et al*., 2002) and *Spilanthes maurtiania* (Ang *et al*., 2003).

Table 1: Germination percentage of irradiated seeds in MS medium without growth regulators after 10 days of inoculation

S. No.	Days of observation	Treatment of x rays(KR)				
		control	0.06	1.00	1.25	2.00
1	0-5	10%	50%	45%	40%	47%
2	5-10	12%	60%	58%	50%	55%
3	10-15	50%	95%	82%	70%	90%
4	15-20	40%	80%	72%	60%	70%
5	20-25	45%	90%	80%	65%	82%
6	25-30	15%	70%	62%	51%	67%

Table 2: Effect of various growth regulators on multiple shoots induction from the radiated seeds of the *Pisum sativum.*

Growth regulators(mg/l)			Average no. of shoots per explant(S.D.)
BAP	KIN	NAA	
0			0.0
0.5	-	-	2.0 ±1.2
1	-	-	3.5 ± 0.1
2.0	-	-	1.5 ± 0.3
3.0	-	-	6.8 ±1.2
-	1	-	3.5 ± 0.7
1	1	-	2.4 ± 0 .4
2	1	-	3.0 ± 0.2
3	1	-	6.7 ± 0.4
3	-	0.5	4.5 ±1.1
3	-	1	1.6 ±0.9

In most of the members of *Asteracea*, *in vitro* microshoots were successfully rooted either in the medium containing an auxin or a combination of auxin and cytokinin (Roy and Kabir, 2007). In *Pisum sativum* multiple shoots were successfully rooted on half strength MS medium containing either IBA (1.0mg/l) or IAA (1.0mg/l) or NAA (1.0mg/l). However higher percentage of rooting was obtained in the medium containing a combination of IBA (0.5mg/l), NAA (1.0mg/l), and IAA (0.1mg/l) (Fig. 1C) The well-

developed plants were transferred to plastic pots containing sterilized vermiculite and sand in the ratio 3:1 and sprayed with liquid MS medium every day for 2 weeks. Plantlets were maintained in glass house set at 25^{o}C. After 4 weeks these plantlets were transferred to garden soil and kept under shade. About, 95% of the hardened plantlets were survived and showed normal growth without any morphological variations These were enabled to induce mutation in *Pisum sativum* through *in vitro* mutagenesis by treating the explants with four doses of gamma radiation.

CONCLUSION

Pea production is being seriously threatened by several biotic/abiotic causes and resistant/tolerant cultivars are urgently required. In view of the difficulties in conventional breeding, mutagenesis *in vitro* can offer as a feasible tool to achieve these goals. Evaluation of the effects of the mutagen (gamma-rays in present investigations) on seed is imperative and essential. The investigations indicated that increasing the dose of gamma-rays resulted in a corresponding decrease in the growth of the explants. It is thus possible to obtain live plant materials even at high doses thereby increasing the probability of higher frequency of mutations. Lower doses of gamma-irradiation had an enhancing effect on the *in vitro* multiple shoot cultures. It can be speculated that the observed aberrations in plant morphology were due to the irradiation induced disturbances in the normal physiological functioning.

Nevertheless, the use of *in vitro* regeneration techniques for mutation induction in Pea is advantageous over the *in vivo* system because it offers (i) a high propagation rate facilitating proper chimera separation, (ii) generation of large plant populations for screening, (iii) the possibility of exposing the *in vitro* cultures to higher irradiation doses

and thereby expecting a high frequency of mutations, (iv) reduction in time and space requirement, (v) the optional facility of *in vitro* selection and (vi) better chances of selecting dominant or desired mutations. The results of the present studies will serve as useful hints for designing *in vitro* mutagenesis

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