Effect of growth hormones on different gamma doses for direct shoot tip regeneration in wild Pea

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Abstract
The use of *in vitro* techniques such as anther culture, shoot organogenesis somatic embryo genesis and protoplast limitation in the application of mutation techniques in both seed and vegetatively propagated crops. *In vitro* culture in combination with induced mutation can speed up breeding programme, from the generation of variability through selection, to multiplication of the desired genotype. The expression of induced mutation in the pure homozygote obtained through microspore, anther or ovary culture can enhanced the rapid recovery of the desired traits.

Keyword: Growth hormones, gamma dose, Shoot inductions.

1. Introduction

Mutation techniques in combination with *in vitro* culture have become important tool in upgrading locally adapted cultivar. Mutation techniques also in combination with tissues culture and molecular method provide a powerful technology to improve clonally propagated plants such as banana, apple, pineapple, date palm, potato, sweet cassulla carnation chrysanthum, rose and tulips. Nearly all of these plants can be regenerated and multiplied *in vitro* allowing the production of large population, which would not have buds apical meristems regenerative callus culture, anther and microspores and somatic embryo provides miniaturized version of tree and seed in the Petri dish instead of the field.

Biotechnological and nuclear techniques have proved merit for the induction of genetic variability and mutant selection. Gamma irradiation of *in vitro* shoot tip culture can induced change in one or few characteristics of the treated explants without alternant their unique cultivars traits. Irradiation of cell cultures followed by regeneration through somatic embryogenesis open grate possibilities in relation to a high multiplication rate [1]. The importance of biotechnological approach such as the advantage of developing various tissues culture techniques has been incorporated in mutation breeding programme. Most of the mutants are now being evaluated for officially released preparation such as those in some ornamental crops such as banana and ginger.

Various aspects of *in vitro* combined with mutagenesis were studied for development species. Effect of gamma radiation on development yield and quality of micro tubers *in vitro* in *Solanum tuberosum* (L) [2].

An efficient and direct shoot bud differentiation and multiple shoot induction from seedling explants of pea have been achieved. The frequency of shoot bud regeneration was influenced by the type of explants of pea has been achieved .The frequency of shoot bud regeneration was influenced by the type of explants genotype and concentration of Cytokinine. Explants viz. epitocotyle hypocotyls, leaf cotyledon and cotyledon nodal segments from 7- day –old seedling were cultured on M.S medium augmented with different concentration of BAP /Kinetin. Among the various concentrations tested 2.0 mg/BAP or Kinetic was found to be the best for minimum shoot bud differential on percentage as well as the number of shoot per explants showing differentiation of shoot bud was higher on M.S media supplemented with BAP compared to Kinetin. Elongation of multiple shoot was obtained on M.S medium fortified with BAP in combination with NAA and GA3. The optimal BAP concentration for shoot elongation was 1.0 mg/ NAA increased the number of multiple shoot s as well as shoot elongation. Addition of GA3 along with BAP and NAA combination dramatically enhanced both multiple shoot proliferation and shoot elongation in all the explants. The elongated shoot was successfully rooted on M.S medium containing different auxins. Among them IBA at 0.2 mg/L induced maximum frequency of rooting followed by NAA and IAA. Regenerated plants were successfully established in soil whereas 90-95% of them developed into morphological normal and fertile plants. This method can thus be advantageously applied in the production of transgenic pigeon pea [3]. The possibility of producing ergonomically useful somaclonal via organogenesis and somatic embryogenesis from callus culture of pea *Pisum sativum* (L) [4]. Seedling was studied under the defined nutritional hormonal and environmental condition multiple shoot regeneration obtained from immature zygotic embryo (Using 2-4-D) or from shoot apices (Using picloram) of aseptically germinated seedling.

2. Material and Methods

2.1 Plant material and explants preparations

The Set of ten seeds from wild Pea, control and each gamma irradiated doses i.e 5Kr, 10Kr, 15Kr, 20Kr, 25Kr and 30Kr were washed with sterile distilled water. Seed was then surface sterilized with 0.1% (w/v) aqueous mercuric Chloride solution for 2min which was followed by rinses of sterile distilled water. They were allowed to germinate aseptically on a sterile Petri plates on moist filter paper for 2 to 4 days. Thus 4 day old embryo, leaf bit and seed from each set along with control were used as explants.

The optimization of growth medium i.e. *in vitro* regeneration and multiple shoot formation by the concentration of auxin and cytokines were done to propagate the wild Pissum variety. The explants i.e. embryo, leaf bit and seeds were used for study on *in vitro* shoot and root regeneration on MS medium with specific hormones combination in twenty sets.

2.2 Chemicals

The chemical used were macronutrients, macronutrients glucoses, Vitamins amino acids and hormones used for composition of Murashige and Skoogs medium. The chemical used for sterilization was 0.1-1%, $HgCl_2$ 70% alcohol absolute and double glass distilled water.

2.3 Culture medium and condition

The culture medium was that of M.S with 3% (W/v) sucrose. This medium was augmented with 1mg/l, 2mg/l, 3 mg/l 4mg/lit and 5 mg/lit concentration of 6 benzylaminopurine (BAP) along with 0.1 mg/lit, 0.5mg/lit, 1.0mg/lit and 1.5 mg/lit of naphtalane acetic acid (NAA) in 20 combination for shoot induction. The Ph of the medium was adjusted to 5.8 prior to adding 8 g/lit phytagel. The medium was autoclaved at 121c under 15/bs pressure for 15 min. The cultures were maintained 27c +_2c under 16 hrs day photoperiod.

2.4 Method of media preparation and sterilization

In the present study Murashige and skoogs (MS) medium through the experiment the composition of M.S medium is given in table 11. The media was prepared using different volumes of different stock solution for the preparation of M.S media the 50 ml of stock I was used whereas 5ml of stock II, stock III, IV each was added followed by addition of sucrose ie 3% as a carbon source.

Different hormonal combination treatment were used for the induction of multiple shoots using ie embryo, leaf bit and seeds as explants for standardization on M.S medium cytokines as BAP (1,2,3,4 and 5 mg/lit) was individually for induction of multiple shoot and auxin. NAA (0.1, 0.5, 1.0, and 1.5 mg/Lit) was taken as supplement along with BAP in each multiple combination.

The media was prepared in double distilled water. The PH of the media was adjusted to 5.8 and finally phytagel (0.25 l) was added as gelling agents. The media was then boiled to melt the phytagel. The medium was taken poured in tissues culture test tube were plugged with non-absorbent cotton plug wrapped with muslin cloth. The tubes were sterilized by autoclaving at 121 for 15 psi pressure. The prepared was checked for contamination for at least 3 days. If the contamination appears tubes were discarded for decent. The checked tubes were further used for inoculation purpose.

2.5 Standardization for surface sterilization of explants

Before inoculation of explants on nutrition media the surface sterilization with HgCl2 was optimized. Different concentrations of HgCl2 solution are used for different time period to standardize the surface sterilization system. The Concentration of HgCl2 was ranging from 0.1 to 1% and time for incubation of explants was studied from 0.5 min to 5min duration.

The explants immersed in 0.1% Mercuric Chloride (HgCl2) for 0.5 min for shoot tip as well as embryo and 1.0% for 1 min for seed as explants shows good result i.e no browing and contamination, thus the Same concentration has concentration of HgCl2 and different time duration shows either contamination or browning or damage to the tissues. The different disinfection used for Sterilization of plant material, but the choice of disinfectant is depend upon the water type, microbial load and type of explants used [5].

3. Results

3.1 Effect of BAP and NAA for induction of multiple shoots

This experiment was designed to compare two cytokines BAP and NAA, with respect to their effect on multiple shoot induction and callus formation. The three types of explants viz. shoot tip, embryo and seeds were used. From control and mutants were cultured on MS medium supplemented with different concentration of BAP and NAA (1, 2, 3, 4 and 5mg/l and 0.1, 0.5, 1.0, 1.5 mg/l) respectively. After 3weeks of culture, the number of explants forming shoots bud and callus induction was counted. Each experiment was replicated thrice.

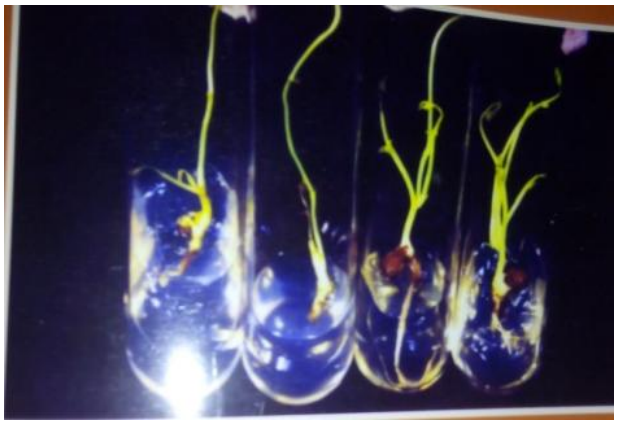
Table: Effect of BAP and NAA for induction of shoots and Callus by explants

Sr No	MS+growth hormone (mg/lit)	MS+growth hormone (mg/lit)	Regeneration of explants Shoot tip	Embryo	Seed
1	BAP(0)	NAA(0)	-	-	-
2	BAP(1)	NAA(1.5)	-	-	-
3	BAP(2)	NAA(1)	-	-	-
4	BAP(2)	NAA(1.5)	-	-	-
5	BAP(3)	NAA(0.5)	-	+	+
6	BAP(3)	NAA(0.1)	++	+	+
7	BAP(4)	NAA(0.5)	++	++	++
8	BAP(4)	NAA(1.5)	++	++	+++
9	BAP(4)	NAA(0.5)	+++	+++	++
10	BAP(4)	NAA(1.0)	++	++	++
11	BAP(4)	NAA(1.5)	++	++	++
12	BAP(5)	NAA(0.1)	+++	++	++
13	BAP(5)	NAA(0.5)	++	++	++

-No induction, ++ moderate induction, +++ highest induction

All the 3 types of explants from control and sets of seeds were screened for the various combinations of BAP and NAA for induction of shoots and callus, shown in Table.

Shoo tip (a): (BAP 5mg/lit+0.1 NAA mg/lit)



Embryo (b): (BAP4mg/lit+0.1 NAA mg/lit) Seed

Seed (c): (BAP4mg/lit+0.1 NAA mg/lit)

4. Discussion

An induced mutation technique is a valuable tool not yet fully exploited. Tissues culture makes it more efficient by allowing the handling of large population and by increasing mutation induction efficiency, possibility of mutant recovery and speediness of cloning selected and variants. Same vegetative propagated species are recalcitrant to plant regeneration which can be limit for application of gene transfer biotechnology, but not for mutation breeding.

Mutagenesis offer the possibility of altering only one or a few character of an already fast rate cultivar, while preserving the overall characteristics [6]. The combination of *in vitro* culture and mutagenesis is relatively in expensive, simple and efficient [7]. The availability of suitable selection method could improve its effectiveness and potential application [8].

5. Conclusion

Thus the present study involves optimization of medium for *in vitro* propagation of wild *Pisum* and its mutants induced by variable doses of gamma. The three explants were used in study viz. Shoot tip, embryo and seeds. The induction of multiple shoot, callus and height of explants were taken into account. The study confined to search out, the typical rate of propagation, variability in phenotypic characters and efficient mutant explants which inheritance the mutant traits.

6. References

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