RESEARCH ARTICLE

In vitro direct multiple shoots regeneration through mature seeds of Pigeon pea (*Cajanus cajan*)

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ABSTRACT

Our research work has been mainly focused on development of regeneration protocol for *In Vitro* direct multiple shoots regeneration in pigeon pea (*Cajanus Cajan)* by using explants as a pre soaked mature seeds of *Cajuns cajan* (Cv. Maruti). This protocol includes washing of overnight pre soaked mature seeds of pigeon pea with laboline and rinsed with tap water and then agitated with bavistin fungicide for 5-6 min. After that surface sterilization of explants by 0.1% of Hgcl2 followed by 70% ethanol. Surface sterilized explants were inoculated on MS media supplemented with different hormonal concentrations of BAP (3mg/l, 5mg/l, 7mg/l, 8mg/l) in combination with NAA (0.5mg/l) and IAA (1.5mg/l). The media MS+BAP (7mg/l) gives best results of multiple shoots induction with standard deviation of (3.1 ± 1.3901) after 25 days of inoculation and (3.2 ± 1.4349) after 50 days of inoculation. We got maximum 7-8 no of multiple shoots on this standardized media by comparing with the control media (MS+ without hormone).We standardize this media i.e. MS +BAP (7mg/l)by comparing with the another two, first is MS+BAP (7mg/l) +IAA (1.5mg/l) which gives standard deviation of (2 ± 0.8968) after 50 days of inoculation and second is MS+BAP (7mg/l) +NAA (0.5mg/l) which gives standard deviation of (1.8 ± 0.8071) after 50 days of inoculation. We also overcome the problem of phenolic compounds secretions by replacing the solidifying agent in our media i.e. agar by clarigel (2.5%). This research paper deals with the progress made on direct multiple shoot induction from mature seeds of pigeon pea, which helps in fulfillment of all the future research threats.

Key words- *Cajanus Cajan,* Multiple shoots regeneration, MS Media, BAP, *In Vitro* culture.

INTRODUCTION

Pigeon pea belongs to the family fabaceae which is an important high-protein grain legume of the semi-arid tropics and caters to the protein requirement of a population in Indian sub-continent. The planting of pigeon pea also replenish soil nutrient and controls soil erosion (ICRISAT

1998). Due to its high nutritive value and special protein content, it can be utilized to combat malnutrition in children. But there is a low per capita consumption of 40g as against the requirement of 80 to 100g due to the shortage in production. The main constraints leading to low yield in pigeon pea include factors such as lack of tolerance to pests, diseases and stress, heavy dependence on rainfall (Lal and Chandra, 1987). Improvement of pigeon pea cultivars possessing resistance to pests and diseases, tolerance to abiotic stresses and low allergic proteins in seeds is therefore desirable for improvement in yield of pigeon pea. The best option currently available for control of insect and pest are through use of chemical insecticides that are expensive and not affordable for most farmers in India. At this stage, development of biotechnological approaches like cell and tissue culture techniques in pigeon pea would be of help to overcome some of these constraints by allowing wide hybridization for inducing genetic variability for yield as well as resistance to biotic and abiotic stresses. Due to high demand in market pigeon pea becomes commercial target for micropropogation and tissue culture, and it can be used for large scale production of pigeon pea. The technique of tissue culture provides reliable system for the rapid multiplication of genetically uniform disease free plant.

MATERIALS AND METHODS

Plant material and explants preparation-

The required experimental plant material of pigeon pea (matured seeds of Cv. Maruti) was taken from the plants available at farm of Shri. Shivaji College of Agril Biotechnology Amravati. These mature seeds (Healthy & uniform) were used as explants. These seeds were thoroughly washed under tap water and soaked for overnight. Then these soaked seeds of pigeon pea washed properly with laboline followed by mixing with bavistin solution (fungicide) to avoid future fungal contamination, for 5-6 min and wash properly with distill water. After that firstly these explants treated with 0.1% mercuric chloride for 4-5 min and followed by two washings of distill water. Secondly it's treated with 70% alcohol for 2 min followed by 2-3 washings of distill water. Then the explants were inoculated on MS media supplemented with different hormonal concentration i.e. BAP (3mg/l, 5mg/l, 7mg/l, and 8mg/l) without any other hormonal combination, then BAP (3mg/l, 5mg/l, 7mg/l, and 8mg/l) in combination with IAA (1.5mg/l) and NAA (0.5mg/l) and then placed under standard growth conditions i.e. 16 hours light and 8 hours dark photoperiod at 25±2ºc temperature.

Culture Medium and Conditions-

Sterilized glassware was used for media preparation. The MS media was prepared with 3% (w/v) sucrose supplemented with different hormonal concentration i.e. Firstly only BAP (3mg/l, 5mg/l, 7mg/l, and 8mg/l) without any hormonal combination, secondly BAP (3mg/l, 5mg/l, 7mg/l, and 8mg/l) in combination with IAA (1.5mg/l) and BAP (3mg/l, 5mg/l, 7mg/l, and 8mg/l) in combination with NAA (0.5mg/l). All media were adjusted to pH of 5.8 prior to the addition of clarigel (2.5%) and autoclaved at 121ºc temperature, 15 lbs pressure for 20 minutes. After preparation of growth media the surface sterile explants were inoculated on it under aseptic conditions. Inoculated bottles were placed in growth room (culture room) under standard growth conditions i.e., 25±2ºc temperature, 16 hours light and 8 hours dark photoperiod, 2000-3000 Lux light intensity provided by cool white fluorescent light.

We get direct multiple shoots from explants of *Cajanus cajan* (mature seeds of Cv. Maruti) after 15 days of inoculation. These multiple shoots with explant were sub cultured on new fresh but same multiple shoot regenerating media after every 15 days for its proper maintenance by future experimental point of view.

RESULTS AND DISCUSSION

Surface sterilized seeds culture directly on MS media showed 75-80% seed germination and development of single shoot, and then explants with single shoot sub cultured on fresh multiple shoot regeneration medium. And the multiple shoot regeneration was observed directly from the mature seeds of *Cajanus cajan* after 2-3 weeks in the regeneration medium (MS) of various concentrations of growth hormones. Successful regeneration of legume species has been greatly aided by species- specific determination of critical parameters, such as explants source, genotype and media concentrations (Geetha et al., 1998). We obtained healthy multiple shoots of *Cajanus cajan* (Cv. Maruti) along with greenish color.

Table 1: Effect of different concentration of BAP on multiple shoot generation in *Cajanus cajan:*

Sr. No.	Concentration of hormone (mg/l)	Multiple shoot induction observed after 25 days.	Multiple shoot induction observed after 50 days.
Control	MS + without growth regulator	No Response	No Response
1.	MS + BAP (3mg/l)	1.4 ± 0.6278	1.5 ± 0.6726
2.	MS + BAP (5mg/l)	1.6 ± 0.7174	2.0 ± 0.8968
3.	MS + BAP (7mg/l)	3.1 ± 1.3901	3.2 ± 1.4349
4.	MS + BAP (8mg/l)	Swelling of seeds	Swelling of seeds

Table 2: Effect of different concentration of BAP in combination with IAA on multiple shoots generation in *Cajanus cajan*:

Sr. No.	Concentration of hormone (mg/l)	Multiple shoot induction observed after 25 days.	Multiple shoot induction observed after 50 days.
Control	MS + without growth hormone.	No Response	No Response
1.	MS + BAP(3mg/l)+IAA(1.5mg/l)	0.8 ± 0.3587	1.0 ± 0.4484
2.	MS + BAP(5mg/l)+IAA(1.5mg/l)	1.6 ± 0.7174	1.8 ± 0.8071
3.	MS + BAP(7mg/l)+IAA(1.5mg/l)	1.8 ± 0.8071	2.0 ± 0.8968
4.	MS + BAP(8mg/l)+IAA(1.5mg/l)	0.6 ± 0.2690	0.8 ± 0.3587

Table 3: Effect of different concentration of BAP and NAA on multiple shoot generation in *Cajanus cajan*

Sr. No.	Concentration of hormones (mg/l)	Multiple shoot induction observed after 25 days.	Multiple shoot induction observed after 50 days.
Control	MS + without Growth Regulator	No Response	No Response
1.	MS + BAP(3mg/l)+NAA(0.5mg/l)	0.6 ± 0.2690	0.8 ± 0.3587
2.	MS + BAP(5mg/l)+NAA(0.5mg/l)	1.2 ± 0.5351	No Response
3.	MS + BAP(7mg/l)+NAA(0.5mg/l)	1.6 ± 0.7174	1.8 ± 0.8071
4.	MS + BAP(8mg/l)+NAA(0.5mg/l)	Swelling of seed	Swelling of seed

Fig. 1. Inoculation of explants (mature seed) on the MS media under aseptic condition

Fig. 2. The best result of multiple shoots obtained at the media concentration of (7mg/l BAP) from the treatments given in table 1

Fig. 3. The best result of multiple shoots obtained at media concentration of (7mg/l BAP ± 1.5mg/l IAA) from the treatments given in table 2.

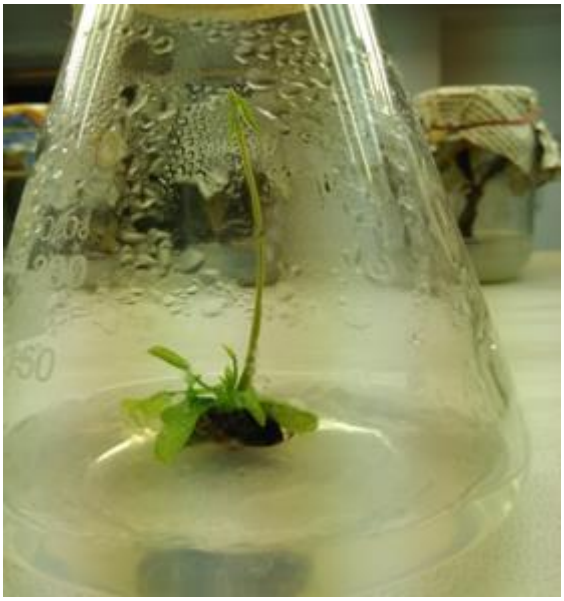

Fig. 4. The best result of multiple shoots obtained at the media concentration of (7mg/l BAP + 0.5mg/l NAA) from the treatments given in table 3.

As given in table 1 mature seeds of pigeon pea were inoculated on MS media supplemented with 4 different concentrations of BAP (3mg/l, 5mg/l, 7mg/l and 8mg/l) from which the best results of multiple shoot (7-8) regeneration were obtained on media supplemented with 7mg/l BAP which comes out as the best standardized media for multiple shoot regeneration.

We obtained our standardized media (7mg/l BAP) by inoculating our explants on three different media with three different hormonal combinations. First is only BAP without any other hormonal combination (as given in table 1), second is BAP + IAA (1.5mg/l) (as given in table 2) and lastly BAP + NAA (0.5mg/l) (as given in table 3). All the results obtained in above three tables were compared with the explants which inoculated on control media (MS + without any hormonal combination). From these all three different hormonal combinations we tried in our experiment the best results of multiple shoots were obtained on the MS media which is supplemented with only BAP.

CONCLUSION

From our present study, the combination of MS + BAP (7mg/l) showed higher multiple shoots formation (S.D. 3.2 ± 1.4349), observed after 50 days. We try our level best to form the plant regeneration protocol for *Cajanus cajan* which act as a pre-requisite for the exploitation of various biotechnological techniques. A remarkable progress can be made in *Cajanus cajan* improvement through combination of conventional and biotechnological approaches. This protocol can be of help in aspect of other biotechnological experiments.

Conflicts of interest: The authors stated that no conflicts of interest.

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