

**PLANTLET REGENERATION FROM MATURE ZYGOTIC EMBRYO CULTURE OF PIGEON PEA (*CAJANUS CAJAN* L. MILL SP) USING THIDIAZURON,**

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**ABSTRACT**

We report the plantlet regeneration of ICPL 87119 (Asha) Pigeon pea varieties from mature zygotic embryos cultured *in vitro*. Mature zygotic embryos were cultured on Murashige and Skoog's medium augmented with different concentrations of TDZ (1.0 – 6.0 mg/L) Mature Zygotic embryos were germinated and more number of multiple shoot buds are induced directly at 4.0 mg/L followed by 3.0 mg/L and 2.0mg/L TDZ. These multiple shoots were further sub-cultured on the same fresh medium containing 1.0 mg/L TDZ for further elongation. The micro-shoots were transferred onto rooting medium containing MS+0.5-3.0 mg/L IBA and 0.5–3.0 mg/L IAA alone. The micro-shoots were rooted (100%) on MS-medium fortified with 3.0 mg/L IBA. The regenerated plantlets were hardened in Plant Growth Chamber under controlled conditions (humidity 80%; temp.25<sup>0</sup>C). After 3 weeks, plants were transferred to field. The highest percentage of plantlets (95%) was survived under field conditions and they were found to be morphologically identical to the parental plants. Thus *in vitro* mature zygotic embryo culture plays a role in the production of plantlets that can be transferred to natural field.

## KEY WORDS

Mature zygotic embryos, Thiadizuron (TDZ), multiple shoots, *In Vitro* rooting

## INTRODUCTION

Pigeon pea (*Cajanus cajan* L. Mill sp) is one of the most popular legume grains in the world, especially in the Indian subcontinent. Due to its multiple uses, pigeon pea is widely used in intercropping systems in semi-arid regions. It provides the main source of protein for many of the poorest populations and plays an important role in reducing malnutrition to millions of people around the world. Pulses can furnish an eminent source of dietary protein constituents for human consumption as a big benefit in a balanced energy and protein diet for those who live in developing countries, especially when intake from animal or fish sources is limited or insufficient. It has multiple uses mainly under the newly reclaimed sandy soil, rather than the old cultivated soils [1]. Attempts to obtain stress-resistant genotypes of pigeon pea species by conventional breeding methods have not been successful because of limited genetic variation and sexual incompatibility with wild relatives [2]. *In vitro* plant regeneration in pigeon pea has been reported from cotyledons, leaf discs and stems through callus induction [3, 4 and 5]. Similarly, direct organogenesis from different explants circumventing the need for callusing has also been reported in pigeon pea by different workers [6, 7, 8, and 9]. Further somatic embryogenesis has also been reported in Pigeon pea [10].

The present study describes a reproducible protocol for plant regeneration from mature zygotic embryo explants in important cultivar cv ICPL 87119 (Asha) genotypes of pigeon pea.

## MATERIAL AND METHOD

Seeds of Pigeon pea (*Cajanus cajan* (L.) cv ICPL 87119 (Asha) were obtained from the ICRISAT Patancheru Hyderabad. Seeds were washed thoroughly under running tap water for 10-15 minutes and surface sterilized with 0.05% of Tween20 (Himedia, RM) for 2-3 minutes followed by rinsing in double distilled sterile water for 5-10 minutes. Later these seeds were sterilized by dipping in 0.1% fresh aqueous mercuric chloride ( $\text{HgCl}_2$ ) for 2 minutes and washed thoroughly with double distilled sterilized water for 5-10 rinses. The mature zygotic embryos were excised with intensive care from mature seeds with sterile forceps and scalpel under aseptic conditions.

### *Culture media and culture conditions*

The basal medium used throughout the experiments consisted of MS [11] inorganic salts,  $100\mu\text{Ml}^{-1}$  myo-inositol,  $0.4\mu\text{Ml}^{-1}$  Thiamine-HCl, 2%(w/v) of sucrose (w/v) (Himedia, Mumbai, India) and 0.8%(w/v) of agar (Himedia, Mumbai, India). The pH of the medium was adjusted to 5.8 either with 0.1 N HCl or 0.1 NaOH before autoclaving at  $121^\circ\text{C}$  for 15min under 15psi. The growth regulators were added to the MS sterilized medium in laminar-air-flow hood. Sterilized mature zygotic embryos were inoculated on MS medium and also MS medium supplemented with different concentrations of TDZ (1.0 mg/L - 6.0mg/L). All these cultures were incubated at  $25\pm 1^\circ\text{C}$  under white-cool fluorescent light ( $30-40\mu\text{mol m}^{-2}\text{s}^{-1}$ ) for 16/8 h photoperiod.

***In vitro* rooting**

Healthy micro-shoots from mature zygotic embryo cultures were transferred onto MS medium fortified with different concentrations of IBA (1.0– 3.0mg/L) / IAA (1.0 – 3.0mg/L) + 4.0mg/LTDZ for healthy shoot elongation. For *in vitro* rooting MS medium was solidified with 0.8 % (w/v) agar agar.

***Acclimatization of the plantlets***

After *in vitro* rooting, the plantlets were taken out and washed with sterile distilled water. These plants were transferred to plastic pots (7cm x 8cm size) containing sterile vermiculite and kept in plant growth chamber 60-65% RH and 25-30°C for acclimatization. After 3 weeks of acclimatization, they were transferred to pits of size 50cm<sup>3</sup> and filled with garden soil and farmyard manure (1:1) in the open field. The percentage of survival was found to be 95% and the plants were morphologically identical to the parental plants.

**RESULTS**

The mature zygotic embryos of Pigeon pea (*Cajanus cajan* (L.) cv ICPL 87119 (Asha) were cultured on MS medium supplemented with various concentrations of TDZ (1.0, 2.0, 3.0, 4.0,5.0 and 6.0 mg/L) at (1.0 mg/L) TDZ 55% cultures responded with (2.0 ± 0.35 shoot buds/ explant) at 2.0 and 3.0 mg/L BAP

showed 62 and 70% cultures were responded with 3.8 ± 0.25, and 4.0 ± 0.35 shoot buds/ explant. The number of shoots was considerably increased also increasing the concentration of BAP. Whereas maximum number of shoot buds (4.3 ± 0.53 shoots/explant) (Fig I- a) were developed from the embryo culture on MS medium fortified with 4.0 mg/L TDZ. As the concentration of TDZ was increase from 4.0 to 5.0 and 6.0 mg/L the number of shoots were considerably reduced. At 5.0 and 6.0 mg/L showed 65 and 50% of culture responding and produced (3.0 ± 0.49 and 2.2 ± 0.47 shoot buds/ explant ) (Fig I- b) (Table -1). Whereas germinated seedlings were healthier at 2.0 – 5.0 mg/L and cotyledons were expanded well. But at high concentrations, the germination percentage was found to be decreased. After eight weeks complete plantlets are formed. (Fig I- c, d and e)

***In vitro* rooting**

The micro-shoots were cultured on MS medium containing 3% sucrose supplemented with different concentrations of IBA/IAA along with 4.mg/L TDZ. Profuse rooting was found on MS medium fortified with 2.0mg/L IBA and followed by 2.0mg/L IAA compare to more number of roots per shoot at 2.0mg/L IAA. While at high concentrations of IBA/IAA, roots formation

Table – 1

***Direct shoot bud proliferation from Mature Zygotic embryo explants of Pigeon pea (Cajanus cajan (L.) cv ICPL 87119 (Asha) cultured on MS medium supplemented with different concentrations of TDZ.***

Hormone conc. (mg/L) TDZ	% of cultures responding	Average number of shoots/ explants (S.E)*
1.0	55.0	2.0±0.35
2.0	62.0	3.8±0.25
3.0	70.0	4.0±0.35
4.0	73.0	4.3±0.53
5.0	65.0	3.0±0.49
6.0	50.0	2.2±0.47

\* Mean ± Standard error

Table – 2

In vitro rooting ability of Zygotic embryo regenerated shoots on MS media fortified with various concentration of IBA and IAA in Pigeon pea (*Cajanus cajan* (L.) cv ICPL 87119 (Asha).

Hormone conc. (mg/L)	% of shoots rooted	Average number of roots / shoots (SE)*
<b>IBA</b>		
0.5	64.0	4.0±0.2
1.0	89.0	6.0±0.4
2.0	72.0	6.8±0.5
3.0	60.0	3.0±0.4
<b>IAA</b>		
0.5	60.0	2.0±0.6
1.0	70.0	5.0±0.7
2.0	78.0	8.4±0.2
3.0	56.0	4.8±0.4

\* Mean ± Standard Error.

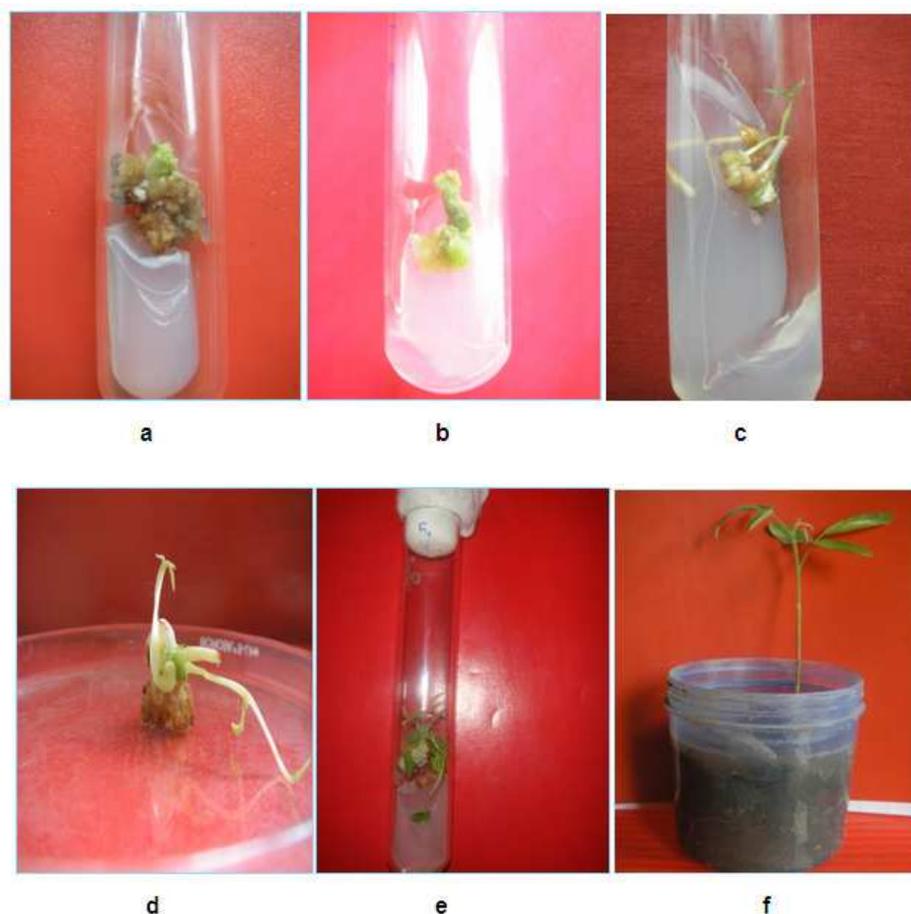


Figure 1

Plantlet regeneration from mature zygotic embryo explants culture of Pigeon pea: a) multiple shoot bud induction on MS medium supplemented with 4.0 mg/L TDZ b) shoot bud induction on MS medium supplemented with 5.0 mg/L TDZ c) Germination of multiple shoot buds on MS medium supplemented with 4.0 mg/L TDZ d) Separations of Germinated multiple shoot buds on MS medium supplemented with 4.0 mg/L TDZ e) Germination of multiple shoot buds on MS medium supplemented with 4.0 mg/L TDZ after 8 weeks of culture f) Regenerated plant derived from mature zygotic embryo explants culture established in a pot.

was decreased. At lower concentrations of the IBA and IAA low frequency number of roots was induced.(Table-2).

### **Acclimatization of plantlets:**

The regenerated plantlets were acclimatized in plant growth chamber. After 3 weeks, these plants were transferred to pits of size 50cm<sup>3</sup> and filled with garden soil and farmyard manure (1:1). Later these were transferred to open field. Most of the plantlets (95%) were survived under field conditions and were found to be morphologically identical to the parental plants. (Fig I-f. )

## **DISCUSSION**

The success of tissue culture largely relies on the selection of a suitable explant for use as the starting material for the experiment. Recently, there has been an increased interest in using mature zygotic embryos as an experimental plant material to reinforce the technique of micropropagation in a number of plant species that are difficult to regenerate including pigeon pea [12] Hungarian vetch [13], *Vigna unguiculata* [14] and lesser burnet [15].

Similarly it was also reported in *Prunus armeniaca* L.cv Hacıhaliloglu (Apricot) where an average of 2-4 shoots was produced from the initial seedlings after 14 days of culture and *in vitro* developed plantlets were successfully acclimatized and transferred to soil.

The present investigation, *in vitro* regeneration protocol the direct regeneration

and the rapid neoformations (about 10 days culture) were beneficial for the ability of *in vitro* plants. This success of *in vitro* regeneration makes the use of appropriate genetic transformation program of *Cajanus cajan* (L.) cv ICPL 87119 (Asha) possible, particularly for var-Dh transformation, in order to develop a new resistant variety, especially to virus.

## **CONCLUSIONS**

The importance of Pigeon pea (*Cajanus cajan* (L.) cv ICPL 87119 (Asha) as a commercial plant is growing up substantially with increasing and stronger reports in support of its multiple uses. The present study on TDZ-based multiple shoots induction and plantlet regeneration from mature zygotic embryos system is the first report on large-scale multiplication in a short period of time for the mass propagation and conservation of an economic important species Pigeon pea (*Cajanus cajan* (L.) cv ICPL 87119 (Asha)

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