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# Effect of different plant hormones on *in vitro* plant regeneration via somatic embryogenesis in *Cajanus cajan* L. millsp.

**Manisha Sharma**
St Wilfred's P.G. College, Mansarovar, Jaipur, Rajasthan, India

**Abstract**
In the present study, regeneration of Pigeon pea (*Cajanus cajan* L. Millsp), belongs to family Fabaceae was achieved via somatic embryogenesis. Mature cotyledons were cultured on MS, $B_5$ and modified MS media ($MSB_5$). The media were supplemented with different concentrations of various auxins, *viz*, IAA/IBA/NAA/2, 4-D (1.0 to 6.0 mg/L) and cytokinins, *viz*, BAP/kn (1.0 to 6.0 mg/L) alone as well as in various combinations. Besides auxins and cytokinins, adenine sulphate was also incorporated into the media at various levels (50 to 150 mg/L). Embryogenic callus was induced on modified MS media augmented with 2, 4-D (3.0 mg/l) BAP (5.0 mg/l) and adenine sulphate (100 mg/l). At this particular combination, nodular embryogenic callus was obtained within 2-3 weeks. Further proliferation and maturation of these somatic embryos was achieved on $MSB_5$ medium containing reduced concentrations of hormones, i.e. 2, 4-D (1.5 mg/l), BAP (2.5 mg/l) and adenine sulphate (50 mg/l). In about 20-30 days, the somatic embryos depicted typical stages of embryo development. Germinating embryos showed well developed shoot, but meager root initiation. Therefore, these were further transferred on ½ MS media devoid of phytoharmones, containing activated charcoal (5%). After 10-15 days, rooting was achieved. *In vitro* regenerated shoots (5-6 cm long) with well-developed roots were transferred to pots containing vermiculite and soil (1:3) and maintained at high humidity.

**Keywords:** pigeon pea, somatic embryogenesis, modified ms media etc

## Introduction
Development of plants via tissue culture methods are based on hypothesis of totipotency. It is believed that each cell of plants has ability to differentiate, proliferate, and regenerate to produce a perfect plant (Feher A, 2019; Thorpe TA, 2007; Sugimoto L *et al.*, 2011) [4, 28, 27]. Generally, plants show regenerative capacity due to presence of high level of developmental plasticity in cells (Pulianmackal AJ *et al*., 2014; Ikeuchi M *et al.*, 2016) [16, 10]. Regeneration of plants in *in vitro* conditions can be achieved either by organogenesis or somatic embryogenesis. In organogenesis, new organ or whole plant is formed at the place of wound while in somatic embryogenesis, whole plant is produced from a single cell via formation of somatic embryo (Jain SM and Gupta PK, 2018) [12].
Somatic embryogenesis has become very useful method for development of important economic crops at large scale (Nic-Can GI *et al*., 2015; Horstman A *et al.*, 2017) [15, 9]. During development of embryo, various factors like media components, phytohormones, type of explant, light *etc.* have significant effects. Somatic embryogenesis can be achieved by using different kinds of explants such as leaf, trichome, haploid cells, stomatal cells, root, cotyledons *etc.* (Wang YH. and Bhalla PL, 2004; Chung HH *et al*., 2007; Iantcheva A *et al.*, 2005; Kim TD *et al.*, 2007; Soriano M and Li H, 2007) [30].
Grain legumes are the most important source of plant proteins and energy, and are cultivated throughout the world. But, there has been no significant increase in the production of pulses due to their low static yields and susceptibility to various fungal, viral and bacterial diseases. *Cajanus cajan* L. Millsp (Pigeon pea), belongs to family Fabaceae, is useful grain legume crop which is good source of dietary protein. Conventional methods of breeding have not been successful due to limited genetic variations, and sexual incompatibility with wild relatives. However, the use of biotechnological techniques over conventional methods of plant propagation and improvement show promising results. (Nic-Can GI *et al.*, 2015; Horstman A *et al*., 2017) [15, 9].
In the present investigation, effects of various phytohormones on plant regeneration via somatic embryogenesis have been studied using cotyledon segment of mature seeds as explants of *Cajanus cajan* L. Millsp.

## Materials and methods
### 1. Explant sources
For the present study, mature cotyledons excised from soaked seeds of *Cajanus cajan* L. Millsp to use as the explant. To obtain the explant, seeds were surface sterilized with mercuric chloride ($HgCl_2$) for 2-3 minutes. Thereafter, seeds were soaked in liquid MS media containing BAP (2.0 mg/L) for 48 hrs and cotyledons were excised after removing the seed coat.

### 2. Culture media
Mature cotyledons were cultured on MS, $B_5$ and modified MS media ($MSB_5$). The media were supplemented with different concentrations of various auxins, *viz*, IAA/IBA/NAA/2, 4-D (1.0 to 6.0 mg/L) and cytokinins, *viz*, BAP/kn (1.0 to 6.0 mg/L) alone as well as in various combinations. Besides auxins and cytokinins, adenine sulphate was also incorporated into the media at various levels (50 to 150 mg/L).

**3. Culture conditions**

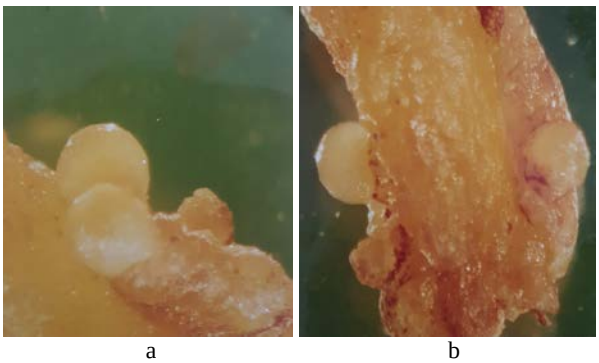
All the cultures were incubated at 26±2°C temperature and 50-60% relative humidity under 16-hrs photoperiod using cool white fluorescent light (3000-4000 lux).

**Results & Discussion**

During the present study, embryogenic callus was induced on modified MS media i.e. $MSB_5$ augmented with 2, 4-D (3.0 mg/l) BAP (5.0 mg/l) and adenine sulphate (100 mg/l). At this particular combination, nodular embryogenic callus was obtained within 2-3 weeks. These nodules denoted the embryonic initials which was developed into conspicuous globular embryos (Figure 1a-1b). Further proliferation and maturation of these somatic embryos was achieved when this embryogenic clump was sub-cultured on $MSB_5$ medium containing reduced concentrations of hormones, i.e. 2, 4-D (1.5 mg/l), BAP (2.5 mg/l) and adenine sulphate (50 mg/l). In about 20-30 days, the somatic embryos depicted typical stages of embryo development (Figure 2a-2c). Complete plantlet via somatic embryo germination was also formed (Figure 3) in the study. Germinating embryos showed well developed shoot, but meager root initiation. Therefore, these were further transferred on ½ MS media devoid of phytoharmones, containing activated charcoal (5%). After 10-15 days, rooting was achieved (Figure 4). *In vitro* regenerated shoots (5-6 cm long) with well-developed roots were transferred to pots containing vermiculite and soil (1:3) and maintained at high humidity (Figure 5a-5b). However transplantation rate was low (20-25%).

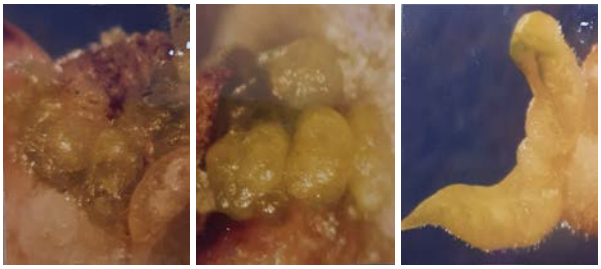
For successful induction of somatic embryogenesis several factors have been found to be responsible, *viz*., explants and its physiological status, culture medium, growth hormones, sucrose concentration, complex and sequential treatment and environmental conditions (Wang YH and Bhalla PL, 2004; Martin *et al.*, 2001) [30, 13]. Somatic embryogenesis has been induced from different types of explants like excised embryos, and hypocotyl (Soriano M and Li H, 2013; Steward *et al.*, 1984) [23, 25]. Similarly other reports are also available on the induction of embryos from excised embryos & cotyledon (Xiao JN *et al*., 2004) [32]. However, during the present study pre conditioned mature cotyledons proved to be the most desired explants for raising embryogenesis. Somatic embryos have been grown on a wide range of media from relatively dilute media like white's medium, (White, 1963) [31] to the most concentrated media formulation like that of MS media (Murashige and Skoog 1962) [14]; $B_5$ media (Gamborg *et al*., 1968) [6] and SH media (Schenk and Hildebrandt., 1972) [21]. However, during the present study culture of mature cotyledons on $MSB_5$ media give optimal embryogenic response. Similarly, several reports are available on successful induction of somatic embryogenesis a wide range of plant species using MS media as such or with slight modifications (Stephan and Jayabalan., 2001, Sarasan *et al*., 2002; Xiao JN *et al*., 2004) [24, 32]. The role of hormones in the induction of somatic embryos is of great interest since it interacts directly with a number of factors. However in case of predetermined embryogenic cells somatic embryos have been obtained even in the absence of exogenous plant growth regulators (Vardi *et al*., 1975; Rose RJ, 2019) [29, 19]. Whereas, in case of cultures derived from undifferentiated tissues, growth regulators in the medium appear to be essential for growth and induction of somatic embryos. Auxins have proved to be the most essential constituent for successful somatic embryo induction in most of the plant species (Rao and Lakshmi-Sita, 1996; George and Eapen, 1994; Geovanny I *et al*., 2016) [17, 7, 8]. Moreover the superiority of 2,4-D amongst all the auxins has also been well established ( Eyer L *et al*., 2016). Nevertheless, cytokinins have also proved to be quite important for the induction of somatic embryos (Fujimura and Komamine, 1975; Budimir and Vajicic. 1992; Eyer L *et al*., 2016) [5, 3].

Whereas in other cases cytokinin is required together with auxin for the induction of embryogenesis (Jadhav and Hedge, 2001) [11]. Similarly during the present study (2,4-D) in combination with BAP was employed for the obtainment of somatic embryogenesis. Moreover, in some plant species, somatic embryos may be induced, proliferated and matured on the same culture medium (Su YH and Zhang XZ, 2014)[26]. However, in certain plant species change in medium at various stages of development has also been required (Geovanny I *et al*., 2016) [8]. Similarly, during the present study nodular embryogenic callus was obtained when mature cotyledons were cultured on $MSB_5$ media along with 2, 4-D (3.0 mg/l), BAP (5.0 mg/l) and adenine sulphate (100 mg/l). At this combination initial stages of embryo formation was observed, for further development of these embryos this embryogenic callus was sub-cultured on $MSB_5$ medium supplemented with a reduced level of hormones that is 2, 4-D (1.5 mg/l), BAP (2.5 mg/l) and adenine sulphate (50 mg/l). Development of mature somatic embryos into complete plantlets generally requires the absence of auxin from the culture medium (Razdan, 1993). Similarly it was recorded in present study that when embryo germination was tried on the same medium meager roots were developed. However, their transfer to hormone free ½ MS medium containing 0.5% activated charcoal led to complete plantlet formation with well -developed shoot and roots. Similar observation recorded by Reddy and Reddy (1993) [18]. During the present study embryogenic mass was not induced on medium without sucrose. The induction percentage increased with an increase in the sucrose concentration maximum being at 3.0%. However, further increase in the sucrose concentration showed a decline in embryogenic percentage. However, higher as well as lower concentration of sucrose concentration showed a decline in embryogenic percentage. However, higher as well as lower concentration of sucrose was found to be very effective (George and Eapen, 1994) [7]. Light is another important factor, which influences somatic embryogenesis. The best response was obtained at 16-hour photoperiod. This is in consonance with Singh *et al* (2021) [22]. In some other cases, complete darkness supported embryo maturation (Ammirato, 1974) [1]



a b

**Fig 1a-1b:** Globular embryo [$MSB_5$ + 2, 4-D (3.0 mg/L) + BAP (5.0 mg/L) + adenine sulphate (100 mg/L)].



a b c

**Fig 2a-2c:** Various stages of embryo development [$MSB_5$ + 2, 4-D (1.5 mg/L) + BAP (2.5 mg/L) + adenine sulphate (50 mg/L)].

**Fig 3:** Germinated Somatic embryo with shoot differentiation.

**Fig 4:** Complete regenerate plantlet via somatic embryo showing well developed roots on ½ MS + activated charcoal (0.5%).

**Fig 5a-5b:** Acclimatization and hardening of germinated plantlets.

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