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# SOMATICEMBRYOGENESIS AND PLANTLET REGENARATION FROM LEAF EXPLANTS OF *CAPSICUM ANNUUM* USING THIDIAZURON

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**ABSTRACT**

Somatic embryogenesis and plantlet regeneration from the leaf explant of red pepper (*Capsicum annuum*.L) cv.Pusajwala a commercial important plant in reported. Embryogenic callus was induced from leaf explants on Murashige and Skoog's medium fortified with 0.5-5.0 mg/L 2.4.D+0.5mg/L TDZ. High frequency of somatic embryo formation was found at 3.0mg/l 2.4-D+0.5mg/LTDZ in leaf explants, respectively. Secondary somatic embryogenesis was also observed in primary somatic embryos were sub cultured on the same somatic embryo induction medium, well developed cotyledonary stage embryos were germinated on MS medium supplemented with 0.5mg/L Indole-3-acitic acid (IAA) +1.0-6.0mg/L TDZ. Maximum percentage75% of somatic embryos germinated and plantlet formation was found at 0.5mg/L IAA +3.0mg/L TDZ, the post transplantation survival rate of plants was 65-80% Plants and flowers formed were morphologically similar to mother plants. The present protocol can be used for genetic transformation experiments in *Capsicum annuum*. cv. Pusa jwala

**Key words:** *Capsicum annuum* cv Pusajwala, Somatic embryos and Regeneration.

## INTRODUCTION

Red pepper (*Capsicum* spp.) is an economically important vegetable and spice crop. Sixty percent of this crop is produced in Asia, and India is the leading producer in area and production (Berke and Shieh 2000). Concerted breeding efforts over the past three decades in India have resulted in the development of high yielding varieties and hybrids suited to diverse agro-climatic situations. However, vulnerability of the improved genotypes to a multitude of pathogens restricted their potential yields. Globally, the most important aspect of pepper breeding is to incorporate resistance to viral, fungal and bacterial diseases and nematode infestations, while retaining high yielding capacity (Galmarini 1997). Alternative approaches like alien gene transfer and biotechnological innovations are envisaged for genetic upgrading of *Capsicum* (Kim *et al.* 1997 Mihalka *et al.* 2003). An efficient and reproducible plant regeneration protocol is the first step in utilizing the power and potential of this new technology.

Plant regeneration via organogenesis and somatic embryogenesis has been reported from diverse explants of *Capsicum* spp. (Fari and Andrasfalvy 1994 Christopher and Rajam 1996; Steinitz *et al.* 1999, Ochoa-Alejo and Ramirez-Malagon 2001). Most transformation experiments have utilized the direct shoot organogenesis procedure to raise transformed plantlets (Steinitz *et al.* 1999, Pena and Dabauza, 2003). Despite these attempts, occurrence of leafy shoots (Liu *et al.* 1990), lack of root pole and a low regeneration frequency of transformants (Steinitz *et al.* 1999, Ochoa- Alejo and Ramirez-Malagon 2001) does not permit its practical use in breeding programmes. Meristematic explants may be ideal for use in transformation experiments because meristematic cells and tissues are already programmed for shoot development (Sairam *et al.* 2003, Weber *et al.* 2003). Few procedures for plant regeneration from shoot meristem explants of pepper using cytokinin like, thidiazuron (TDZ: N-phenyl-N-thiadiazol-1,2,3– 5,ylurea), BA and Kn either alone or in combination with various auxins have been reported (Fari and Csillery, 1983; Phillips and Hubstenberger 1985; Agrawal *et al.* 1988; Sun and Wang 1990; Fortunato and Tudisco 1991; Madhuri and Rajam 1993; Ebida and Hu 1993; Christopher and Rajam 1994; Daubaza and Pena 2001). However, the rate of multiplication is very low. In the present communication, we report reproducible method for *in vitro* plant regeneration via Somatic embryogenesis in *Capsicum annuum*, cv Pusajwala from Leaf explants using various cytokinins especially thidiazuron (TDZ).

## MATERIALS AND METHODS

### PLANT MATERIAL

Seeds of *Capsicum annuum* L. cv. Pusa jwala obtained from Regional Agricultural Research Station, Lam, Guntur, A.P. Seeds were soaked in sterile distilled water for 24 hrs. Later, these were cleaned with 5% teepol followed by through washing with running tap water 3-4 times. Now, the seeds were surface sterilized with 0.1%

$HgCl_2$ for 3-4 minutes followed by rinsing with sterile distilled water for 3 to 4 times and were germinated aseptically on MS (Murashige and Skoog 1962) basal medium.

**CULURE MEDIA AND CULTURE CONDITIONS**

Leaf (6 week old) explants from axenic seedlings were placed on MS medium supplemented with (3.0 gm/L) sucrose along with different combinations of 2,4-D (0.5-5.0 mg/L) + (0.5 mg/L) TDZ for maturation in the second culture phase, these somatic embryos developed from leaf explants were transferred to MS medium fortified with (0.5 - 5.0 mg/L) 2,4-D + (0.5 mg/L) TDZ respectively. The pH of the medium was adjusted to 5.8 prior to autoclaving at 121s°C for 15-20 min. All the cultures were incubated under 16/8hr light/dark photoperiod at 25 ± 2°C. A light intensity of 40 mol $m^{-2}s^{-1}$ was provided by cool white fluorescent tubes.

The cultures were transferred to fresh medium after an interval of 4 weeks. For germination and plantlet formation somatic embryos were transferred to MS medium supplemented with (0.5mg/L) IAA + (1.0-5.0mg/L) TDZ and incubated under the same culture conditions. (Table-2).

**RESULTS AND DISCUSSION**

Results on somatic embryogenesis in *Capsicum annuum* L. cv. Pusa jwala are presented in (Table 1).Tender leaves cultured on various concentrations of 2,4-D (0.5-5.0 mg/L) in combination with (0.5mg/L) TDZ become Swollen and generally dedifferentiated and developed friable callus after 8-10 days of culture. Maximum number of somatic embryos / explant and higher percentage of response for somatic embryos formation have been found at 3.0 mg/L 2, 4-D + 0.5 mg/L TDZ in leaf explants of *Capsicum annuum* L. cv. Pusa jwala (Plate I fig a) with the increase of 2, 4-D concentration up to 5.0 mg/L with (0.5 mg/L) TDZ,with in 25-30 days of culture, globular, cotyledonary, and heart shaped embryos have formed directly on the surface of callus (Plate I fig b). But when the concentration of 2,4-D was increased above (3.0 mg/L) percentage of response and somatic embryo induction were decreased it was found that at higher concentration of 2,4-D (5.0 mg/L) in combination with (0.5 mg/L) TDZ. When the explants of primary somatic embryos were cut in to fragments and cultured on the same induction medium secondary somatic embryos were induced with in two weeks.

Thus proliferation of somatic embryos occurred in two ways:

1. Multiplications of somatic embryos from the explants through primary. Somatic embryogenesis and
2. Proliferation of secondary somatic embryos from already formed

**GERMINATION OF SOMATIC EMBRYOS**

For germination, globular, heart and torpedo shaped embryos (a mixture) were transferred to MS medium supplemented with different concentration of auxin such as IAA (0.5mg/L) in combination with TDZ (0.5-5.0mg/L).Highest (75%) frequency of embryo germination was noticed on a medium containing 0.5mg/L IAA in combination with 3.0mg/L.While TDZ and IAA was better for germination of somatic embryos in the cultivar Pusa jwala of *Capsicum annuum*. When the concentration of TDZ was increased above (3.0 mg/L) percentage of response and somatic embryo germination

**Table -1 Effect of various concentrations of 2,4-D and 0.5 mg/L TDZ on Somatic embryo genesis in Leaf explant culture of *Capsicum annuum* cv. Pusa jwala cultured on MS medium after 8 weeks of cultures.**

| Growth regulators (mg/L) | % of cultures responding | % of response for somatic embryo formation | Average number of somatic embryos / explant (S.E)* |
|---|---|---|---|
| **2,4-D + TDZ** | | | |
| 0.5 + 0.5 | 70 | 50 | 5.3 ± 0.35 |
| 1.0 + 0.5 | 75 | 52 | 6.8 ± 0.36 |
| 1.5 + 0.5 | 85 | 66 | 8.0 ± 0.25 |
| 2.0 + 0.5 | 86 | 65 | 12.0 ± 0.25 |
| 2.5 + 0.5 | 89 | 70 | 15.0 ± 0.35 |
| 3.0 + 0.5 | 92 | 86 | 27.0 ± 0.36 |
| 3.5 + 0.5 | 68 | 80 | 20.0 ± 0.45 |
| 4.0 + 0.5 | 65 | 65 | 18.0 ± 0.32 |
| 4.5 + 0.5 | 60 | 62 | 16.0 ± 0.42 |
| 5.0 + 0.5 | 55 | 60 | 12.0 ± 0.43 |

*** Mean ± Standard Error.**

frequencies were decreased it was found that at higher concentration of TDZ (5.0 mg/L) in combination with (0.5 mg/L) IAA. Maximum number of somatic embryos germination and higher percentage of response for somatic embryos germination have been found at 3.0 mg/L TDZ + 0.5 mg/L IAA in leaf explants derived Somatic embryogenesis of *Capsicum annuum* L. cv. Pusa jwala (Table 2) (Plate I fig c and d).

**Fig 1: Regeneration via somatic embryogenesis in leaf explant culture of *Capsicum annuum* cv Pusajwala.**
a) Formation of somatic embryos on MS+3.0mg/L 2.4D+0.5mg/LTDZ
b) Formation of Globular, Cotyledonary and Heart shaped embryos on MS+3.0mg/L 2.4D+0.5mg/LTDZ observed on Stereo microscope
C) Conversion of somatic embryos in to plant lets on MS+0.5mg/L IAA+3.0mg/L TDZ
d) Conversion of somatic embryos in to plant lets on MS+0.5mg/L IAA+3.0mg/L TDZ after 8 weeks

**DISCUSSION:**

In the present investigation, the results on somatic embryogenesis have shown that auxin such as 2,4-D along with cytokinin TDZ are essential for inducing the somatic embryogenesis from leaf explants of *Capsicum annuum*.L. A major factor for somatic embryogenesis is the nature of growth regulators used in the induction medium. The type of auxin or auxin in combination with cytokinin used in the medium can

greatly influence somatic embryo frequency. The requirement of cytokinin in addition to auxin was observed to *Sapindus trifoliatus* (Desai *et al.* 1986), *Terminelia arjuna* (Kumari *et al.* 1998) and *Psoralea corylifolia* (Sahrawat *et al.* 2001) as it was observed in the present studies. Somatic embryo genesis was induced on medium containing NAA alone in *Solanum melongena* (Matsuoka and Hinata 1979; Gladdie *et al.* 1983 Sharma and Rajam, 1995).

Somatic embryogenesis is also preferred because it allows production of plant without somaclonal variation and in efficient cloning and genetic transformation (Sharp *et al.* 1980). Synthetic seeds can also be developed by encapsulating somatic embryos in sodium alginate complexed with calcium chloride as it was developed in *Solanum melongena* (Lakshmana Rao and Singh 1991). Whereas Binzel *et al.* (1996) reported that

**Table 2: Effect of 0.5 mg/L IAA in combination with various concentration of TDZ on the conversion of Somatic embryoids into plantlets in *Capsicum annuum* cv. Pusa jwala cultured on MS medium after 8 weeks of cultures.**

| Growth regulators (mg/L) | % of cultures responding | Germination of frequency (S.E)* |
|---|---|---|
| IAA + TDZ | | |
| 0.5 + 0.5 | 60 | 10.0 ± 0.32 |
| 0.5 + 1.0 | 62 | 16.0 ± 0.46 |
| 0.5 + 1.5 | 64 | 18.0 ± 0.37 |
| 0.5 + 2.0 | 68 | 20.0 ± 0.43 |
| 0.5 + 2.5 | 70 | 22.0 ± 0.32 |
| 0.5 + 3.0 | 75 | 30.0 ± 0.32 |
| 0.5 + 3.5 | 68 | 26.0 ± 0.37 |
| 0.5 + 4.0 | 66 | 22.0 ± 0.36 |
| 0.5 + 4.5 | 55 | 18.0 ± 0.27 |
| 0.5 + 5.0 | 50 | 10.0 ± 0.37 |

***Mean ± Standard Error.**

The entire process of induction and maturation of the embryos was completed on the same MS medium containing auxins and cytokinins (2,4-D + TDZ) in *Capsicum annuum* L. cv. Pusa jwala as it was observed the requirement of both the hormones in the present investigations. Similarly somatic embryos maturation on MS medium containing the combination of auxins (NAA) and cytokinins (BAP) was observed in *Cajanus cajan* (Mallikarjuna *et al.* 1996), *Prunus axivum* (Garin *et al.* 1997) and *Hardie wickiabinate* (Chand and Singh 2001).

In conclusion, for induction of *in vitro* somatic embryo genesis the type of primary explant, choice of genotypes and hormonal concentration plays on important role (Patel *et al.* 1994).

During the present investigations, it was found that the high concentration of auxin in combination with less concentration if cytokinin induced the somatic embryogenesis and maturation of somatic embryos in *Capsicum annuum* L. cv. Pusa jwala

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