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# *In Vitro* Propagation of Yellow Color *Capsicum annuum*

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## Abstract
A simple and efficient protocol was developed for in vitro propagation of yellow color *capsicum annuum*. Seeds of *capsicum annuum* were decontaminated and placed in MS medium and kept it for germination. Leaf explants excised from one-month old aseptic seedlings and were cultured on MS medium containing different hormone with different concentrations and combinations of auxins and cytokines. The highest callus was obtained from leaf explants with NAA(2.0mg/L) and with kinetin(5.0mg/L). Calli derived from leaf explants were transferred to the shooting media. The media containing(2.5.mg/L) had the best shoot regeneration. Regenerated plants with six to eight leaves were potted protected by gaze and kept about one week in the culture room. Then transferred to the green house and grown to maturation.

**Keywords**
*capsicum annuum*, leaf explant, plant growth regulator, callus induction.

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**INTRODUCTION:**
*Capsicum annuum* is a genus of the family Solanaceae. *Capsicum annuum* consist of approximately 20-27 species. The fruits of capsicum have a variety of names like chill pepper, yellow pepper, sweet pepper, bell pepper, miniature paprika. The various colors in Capsicum are due to mixture of esters capsorubin, zeaxanthin, crytoxanthine, capsinthin and other carotenoids. (Sherya Swamy et al., 2014). Capsicum annuum has been a part of human diet because they contain numerous biochemical such as volatile oil, fatty oils, capsaicinoids, caratinoids, proteins, vitamins, fibre and mineral element *Capsicum annuum* is an excellent source of vitamin A, B, C and E (Daood et al.,2006). Beta carotenoids and vitamin A and C are powerful antioxidants that destroy free radicles (Simonne et al., 1997) (Marine et al., 2004). This plant is well known for its pungent character which is contributed by naturally occurring lipophilic chemical capsaicin (Howardet al., 2000). The susceptibility of *capsicum annuum* as sweet pepper to many pathogens including viruses, fungi, bacteria and nematodes constitute the main problem for the cultivation of this species. The most several incidence and economic impact are due to viral diseases which may destroy entire harvest. The propagation through seeds is further restricted by

short span of viability, low germination rates of seeds, high risk of infections by various disease, low productivity (Sanatombai and sharma, 2006) In addition some of the limiting factors for the production of *capsicum annuum* such as extreme climatic conditions particularly temperature (Agrawal et. al., 1988).

In accordance to facilate and improve the propagation of the commercial cultivars of the species and to meet the increasing demand of crop and overcome the plant's lack of natural vegetative production these tissue culture methods provide a better way for the asexual multiplication of *capsicum annuum* plants. Methods for improvements of resistance to diseases that are unsatisfactorily controlled by chemicals. This selection is important for economic progress and will suit human needs. In our study the main purpose was to elaborate an adapted protocol of in vitro plant regeneration of *capsicum annuum.*

**MATERIALS AND METHODS:**

**Collection of seeds:**

Seeds of *Capsicum annuum* were obtained from Syngenta seeds India Limited (Pune).

**Media preparation for seed germination:**

Murashige and skoog (MS) media was used for seed germination. Sucrose was used as a carbon source 3% (W/V), 1.8% 9W/V) agar as a gelling agent and media was adjusted to $p^H$5.8. It is sterilized by autoclaving at 121◦ C for 10 min at 15 psi pressure.

**Preparation of Explants:**

Seeds were thoroughly washed in running tap water and subsequently were surface sterilized with a 70% ethanol solution for 60 sec. Seeds were rinsed with sterile distilled water 2-3 times to remove excess amounts of ethanol and then dipped in 1% $Hgcl_2$ for one minute then seeds were washed with sterile distilled water 2-4 times as excess amount of $Hgcl_2$ is harmful or toxic for the growth of plants. Then seeds were inoculated in bumper test tubes containing 25 ml MS medium $p^H$ was adjusted to 5.8. Seeds were maintained for 10 to 15 days for germination at 23-+°c and 16hr. Photo period.

**Media preparation for callus induction:**

MS. Medium containing plant growth regulators such as NAA, IAA, BAP, Kinetin at different concentration were used. Sucrose was used as a carbon source 3% (W/V), 1.8% 9W/V) agar as a gelling agent and media was adjusted to $p^H$5.8. It is sterilized by autoclaving at 121◦ C for 15 min at 15 psi pressure.

***In Vitro* Callus induction:**

Leaves without petioles were excised from one-month old seedlings and cut into 1-2 cm long segments and explants were inoculated immediately in order to prevent drying of cut edges of explants. The abaxial sides of explants were placed down to contact the regeneration medium. Leaf explants were cultured on MS medium containing different hormones with different concentrations and combinations of hormones like BAP, NAA, IAA and kinetin. Leaf explants were cultured on a MS medium supplemented with BAP (1.0, 2.0, 3.0, 4.0, 5.0 mg/L) and kinetin (3.0, 4.0, 5.0 mg/L) and leaf explants were also cultured on combination of kinetin and BAP each with a concentration of 2.5 mg/L also with a combination of IAA and BAP with concentration of (3.0, 3.0 mg/L) (4.0, 2.0 mg/L) and (2.0, 1.0 mg/L). All these inoculated explants supplemented with hormones were placed under controlled temperature 23 -+°c using 16hrs. photoperiod. Callus were maintained at 23°c and 16hr.photo period.

**RESULTS:**

**Callus induction:**

Leaf explants were cultured on MS medium supplemented with different hormones with different combinations and concentrations started swelling of plant tissues after one week i.e. callus initiation step is started. After 3 weeks explants were covered with greenish yellow callus and other with whitish yellow callus. The highest callus was obtained from leaf explants with NAA (2.0 mg/L). The other highest callus induction was observed with the growth regulator kinetin. (3.0 mg/L). The combination of BAP and kinetin (2.5+2.5 mg/L) also shows complete growth of callus. The growth of callus is also found in IAA+BAP (2.0+1.0 mg/L). The smallest callus formation was observed with growth regulator BAP 5.0 mg/L). See figure ,2.

**Shoot regeneration:**

Calli derived from leaf explants were transferred to the shooting media supplanted with the2.0mg/BAP in combination with NAA and IAA and also with BAP and kinetin (1.5+1.5 mg/L). The media containing BAP (2.0 mg/L) had the best shoot regeneration. Medium supplemented with BAP (.0 mg/L) and IAA (1.0 mg/L) had the poorest performance where the calli became rusty in color. The rusty coloration of the calli might be due to the inhibitory effect of IAA on shoot regeneration. Regenerated plants with six or eight leaves were potted protected by gaze and kept about one week in the culture room subsequently they were placed in a surrounding culture room atmosphere. Then transferred to the

green house and grown to maturation. See figure 1 and 2.

**Table 1. Effect of different hormone concentration on leaf explants for callus induction.**

| Sr.no. | Hormone | Concentration (mg/L) | Callus initiation | Complete growth Of callus | Callus Characteristics |
|---|---|---|---|---|---|
| 1. | NAA | 2.0 | One week | Three weeks | Green, compact embryogenic |
| 2. | Kinetin | 3.0 | Twelve days | Twenty-five days | Green, compact, embryogenic |
| 3. | BAP+ kinetin | 2.5+2.5 | One week | Three weeks | Whitish yellow compact embryogenic |
| 4. | IAA+ BAP 2.0+1.0 | 2.0+1.0 | One week | Twenty days | Whitish yellow compact embryogenic |
| 5. | BAP | 5.0 | Two weeks | Three weeks | Friable brownish green non-embryogenic |
| 6. | Kinetin | 3.0 | Three weeks | One month | Whitish yellow compact embryogenic |

**Table-2 Effect of hormone concentration on callus for shoot formation.**

| Sr.no. | Hormone | concentration(mg/L) | Callus derived shoots | Days required |
|---|---|---|---|---|
| 1. | NAA | 1.0 | 0 | - |
| 2. | Kinetin | 1.5 | 1 | 15 |
| 3. | BAP+ Kinetin | 1.5 | 2 | 18 |
| 4. | IAA+ BAP | 2.0 | 3 | 22 |
| 5. | BAP | 2.5 | 5 | 28 |
| 6. | Kinetin | 1.5 | 4 | 20 |

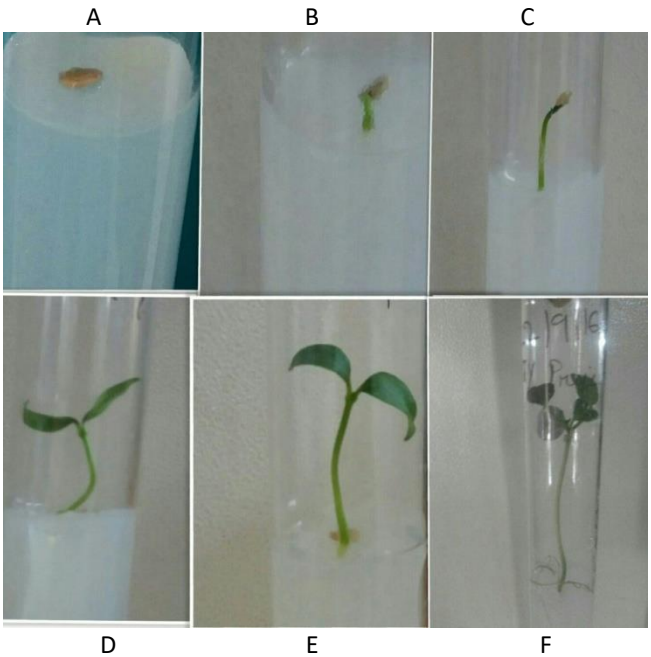


**Fig. 1(-A) Seed inoculation of *capsicum annuum*, (B) five days old shoot initiation (C) twelve days old shoot (D) Twenty days old seedling (E) twenty-five days old seedling (F) one-month old seedling.**

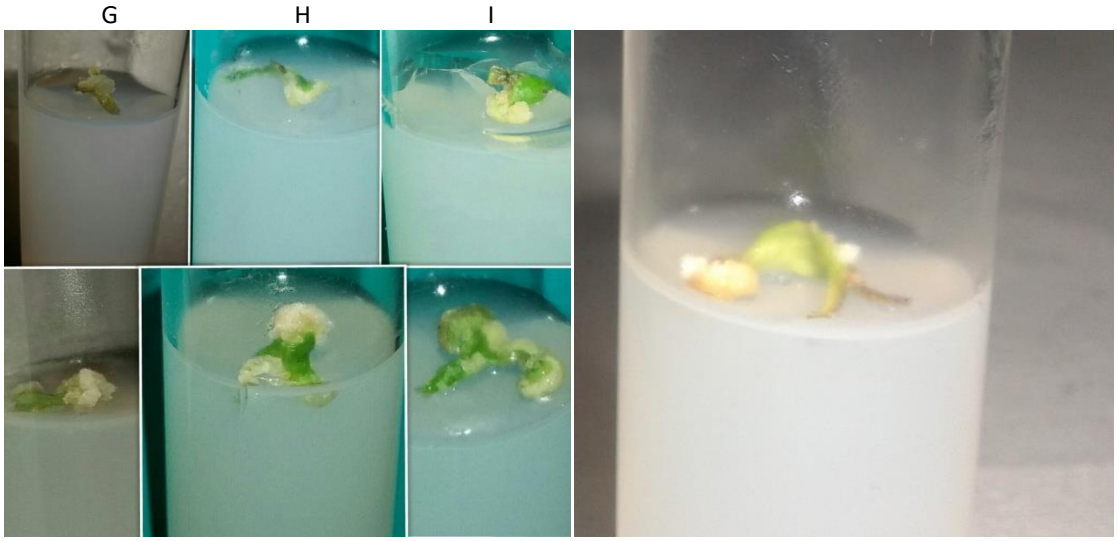


**Fig. 2 (G) Callus derived from leaf explants cultured on MS kinetin 3.0mg/L, (H) MS+BAP (5.0mg/L) (I)MS+IAA+BAP (2.0mg/L+1.0mg/L,) (J) MS Kinetin+BAP (2.5mg/L+2.5mg/L) (K) MS kinetin (5.0mg/L (L)), MS+NAA (2.0mg/L. (M) shoot derived from leaf explants callus on Ms+BAP (5.0mg/L).**

**DISCUSSION:**

In this paper we demonstrate regeneration of *capsicum annuum* (yellow color) plant from leaf explants. Highest callus formation was obtained at NAA(5.0mg/L) and the media containing BAP(2.5mg/L) had the best shoot regeneration. Leaf explants was more amenable to regeneration of adventitious shoots. Various explants obtained from one-month old plant seedlings such as leaf, petiole stem and root, were tested for the shoot induction on a MS medium containing different concentration of TDZ. Among the best, only the leaf explant showed shoot induction and other explants formed only callus (JUYeon song et., al). Similar observations were also made in capsicum species indicating that the leaf explants were more amenable to regeneration of adventitious shoots than other explants (Agrawal et,.al1989; Christopher and Rajam,1996; venkataiah and Subhash 2001). Three types of explants viz cotyledon and hypocotyls were used hypocotyls explants was the highest in 5.0 mg/L BAP+0.1 mg/L NAA (M. Ashra fuzzamav, et., al). Hypocotyls of mature zygotic embryos of the Tunisian pepper Varity are an efficient organ genic explants. However, germinated seedlings as explants failed in regeneration (Arous et, al.1998) Such young tissue seems to have the best response to in vitro regeneration and it was successfully used by many authors in peppers (Binzel et al.,1996, Harini and Lakshmi,1993) and other species.

**CONCLUSION:**

In the present study, one-month old seedlings were used for callus induction. The highest callus was obtained from leaf explants with NAA(2.0mg/L). Calli derived from leaf explants were transferred to the shooting media. The media containing BAP (2.0mg/L) had the best shoot regeneration. The present research was successful to obtain callus from variety of different hormonal concentrations and shoot elongation from callus.

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