*Int.J.Curr.Microbiol.App.Sci* (2018) Special Issue-7: 388-391

*International Journal of Current Microbiology and Applied Sciences*
*ISSN: 2319-7692* **Special Issue-7 pp. 388-391**
Journal homepage: http://www.ijcmas.com

**Original Research Article**

# *In vitro* Seed Germination and Regeneration Potential of *Capsicum annum* L. (Faizabadi Kala) for the Production of Disease Free Chilli Plant

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**A B S T R A C T**

Faizabadi Kala belongs to the member of *Solanaceae* is local land cultivar native to UP East region and has been marketed to West Bengal, Bihar region. It is a very pungent chilli, fruit is large in size and black in color. It takes around six to eight month in fields which effects the farmer for the cultivation of other crops in there favorable season. As this variety of chilli is also highly susceptible to fungal and viral pathogens. *In vitro* plant regeneration is essential for the rapid multiplication of disease-free planting materials and is an imperative for the application of biotechnology tools to plant breeding and genetic improvement. The present study demonstrates *In vitro* regeneration of *C. annuum* callus derived from a leaf, cotyledon and shoot tip of chilli as explants for regeneration on MS medium supplemented with different concentration and combinations of auxin and cytokinine. Best results were shown by shoot regeneration from shoot tip explants. The highest callus was induced from Shoot tip in a combination of BAP (2.0 mg/$l^{-1}$) with IAA (2.2 mg/$l^{-1}$). The absence of light was favorable for callus formation. The callus induction, as well as shoot initiation, was higher in shoot tip than cotyledon for plant regeneration in Capsicum. It reduces the multiplication time and potentially offers an efficient system for regenerating and propagating plants with relatively high genetic uniformity. *In vitro* regeneration consequently will promote the application of plant tissue culture technology in the areas of genetic transformation.

**Keywords**

*In vitro*, *capsicum annum*, chilli plant, *solanaceae*

## Introduction

Faizabadi Kala is a chilli belongs to the member of *Solanaceae* is local land cultivar native to UP East region and has been marketed to West Bengal, Bihar region. It is a very pungent chilli; fruit is large in size and black in color. It takes around six to eight month in fields which effects the farmer for the cultivation of other crops in there favorable season. As this variety of chilli yield is very high but it is also highly susceptible to fungal and viral disease mainly leaf curl virus disease. As *in vitro* plant regeneration is essential for the rapid multiplication of disease-free planting materials and is an imperative for the application of biotechnology tools to plant breeding and genetic improvement.

## Materials and Methods

The present study on regeneration potential of local cultivar *Capsicum annum* Faizabadi kala cultivar (local land cultivar native to UP East region) was conducted in the

Department of Plant Molecular Biology & Genetic Engineering Narendra Deva University Agricultural Technology, Faizabad, and Uttar Pradesh during 2016-2017.

## Establishment of Axenic seedlings aseptically

Seeds of Fresh and healthy ripe fruits were obtained from NDUAT Kumarganj area Faizabad, India. Mature seeds were washed with tap water and 3 times with distilled water. Surface Sterilization with 70% ethanol for 1 min followed by 3 immediate washes with sterile distilled water.

After, they were treated with Tween -20 (0.1%) for 4-5 minutes with continuous shaking and then rinsed again with sterile distilled water. Surface sterilization is done by $HgCl_2$ for 5 minutes. The surface-sterilized seeds were inoculated in Petri plates containing sterile filter paper for blotted dry and sown in 250ml flasks containing 50 ml of MS basal medium 3% (m/v) sucrose and 0.6% (m/v) agar (Murashige and Skoog 1962). pH of the medium was adjusted to 5.8 with 1N KOH incubated in the culture room for at 25±2 °C after 4-5 week old *in vitro* germinated seedlings.

Aseptically generated seedlings were used as explants. A leaf, cotyledon, and axillary shoot tip were obtained from the seedlings apices (0.5 - 1 cm length) trimmed were cultured on MS medium supplemented with combinations of growth regulators with BAP, KIN, Zea or TDZ with three concentrations (0, 1.0, 2.0 mg/l) combined with IBA or NAA. Three concentrations (0, 0.2, 0.5 mg/l) of IBA or NAA were applied to enhance shoot formation. Sterilization was performed by autoclaving at 121 ºC for 20 min. pH was adjusted at 5.8 before adding 0.6% (w/v) agar and 0.4% (w/v) activated charcoal. Explants were aseptically cultured and placed horizontally in each vial containing 50 ml of the induction medium and gently pressed into the surface of culture medium. The flasks were closed with autoclaved cotton plugs and sealed with household plastic foil for a period of 4-5 weeks and then transferred to the same conditions for the direct regeneration of shoots.

## Parameters studied

### Callus induction (%)

Number of explants formed callus recorded and the percentage of callus induction was calculated as:

$$\text{Callus induction (\%)} = \frac{\text{Number of explants showing callus}}{\text{Number of explants inoculated}} \times 100$$

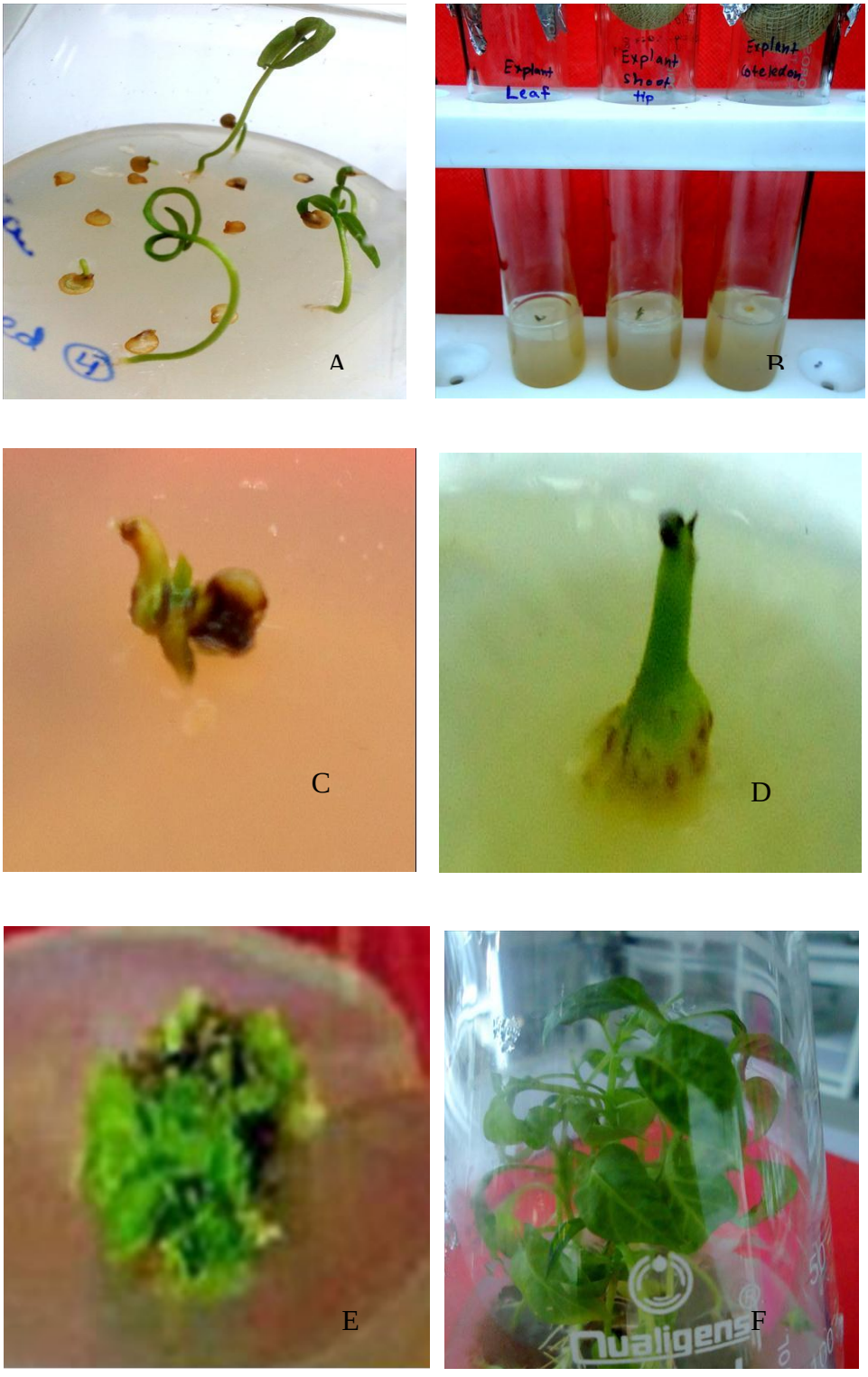
### Days to callus initiation

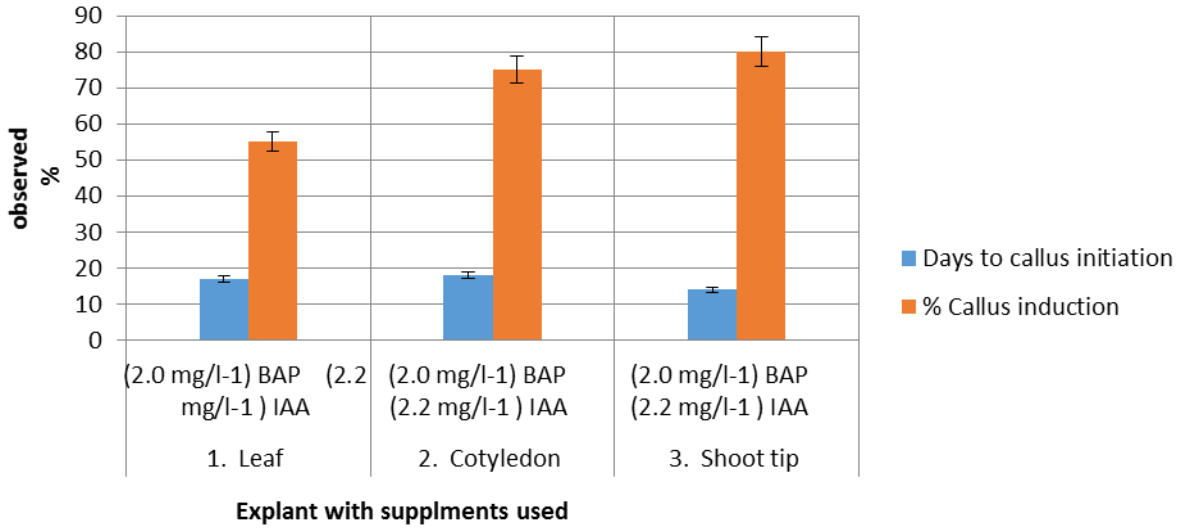
Number of days required for initiation of callus from the day of inoculation was recorded. The number of callus initiated over a number of days recorded. The mean value of data provided the days required for callus initiation.

## Results and Discussion

The present study demonstrates *In vitro* plantlet regeneration of *C. annuum. C*allus derived from a leaf, cotyledon and shoot tip of chilli as explants for regeneration on MS medium supplemented with different concentration and combinations of auxin and cytokinine. After 2-3 weeks of culture on the shoot bud induction medium, about 2-4 buds multiple shoot buds developed from the shoot-tip explants derived from *in vitro* germinated seedlings.

**Fig.1** (A) In vitro Seedling preparation aseptically; (B) Seedlings apices (0.5 - 1 cm length) trimmed; (C), (D) Initiation of premature callus; (E) Proliferation of shoots; (F) Elongated disease free shoots






All cultures were maintained in a growth chamber at a temperature of 25±2 °C and 16-h photoperiod provided by white fluorescent tubes. However, Absence of light was also favorable for callus formation initially. Best results were shown by shoot regeneration from shoot tip explants. The highest callus was induced from Shoot tip in a combination of BAP (2.0 mg/$l^{-1}$) with IAA (2.2 mg/$l^{-1}$).

In conclusion, the present study significant it reduces the multiplication time and potentially offers an efficient system for regenerating from explants of *Capsicum annum* L. and propagating disease-free plants with relatively high genetic uniformity. This Protocol involved *in vitro* regeneration consequently will promote the application in the areas of genetic transformation and other transgenic experiments in this species.

## Acknowledgment

This study was funded by Department of PMB & GE. The Facilities provided by Tissue Culture Laboratory, NDUAT, Faizabad is acknowledged.

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