Research Article
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In-vitro organogenesis in tomato (*Solanum Lycopersicum*) using kinetin

Abstract

This research was undertaken for optimizing simple and reproducible protocol on indirect regeneration in tomato using kinetin. The effect of kinetin for efficient callus production was studied and its results were interpreted. Hypocotyls and cotyledons were used as explants and effect on callus derived regeneration was greatly influenced by addition of kinetin in media. The individually positioned explants on MS media supplemented with two cytokinins BAP (2.0mg/L) and kinetin (1.75mg/L) proved to produce good embryogenic calli and developed into whole plantlets. Lower and higher kinetin concentrations produce lesser calli and results derived using mean values in this experiment suggest closely equal concentrations of kinetin to other cytokinin can be a potential calli inducer in tomato regeneration. Multiple shoot induction data was recorded and its mean values interpreted. Shoots transferred in shooting elongation media MS+ IAA (0.1mg/L) + Zeatin (2.0mg/L) gave better shooting response (89.4%) and after two weeks these elongated plantlets were subjected to rooting. Therefore kinetin with combination of BAP majorly influences in-vitro organogenesis in tomato plant regeneration.

Keywords: cotyledon, hypocotyls, BAP, kinetin

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Introduction

Tomato (*Solanum Lycopersicon*) being a perennial crop clutches its second position in consumption and is well appropriated in Solanaceae family for remaining prevalent due to its higher nutritive values.[1] Cultivation status of tomato includes tropical and subtropical areas across the world and annual production in India reaching 18227000 metric tonnes in year 2012-13.[2] In daily diet, tomato consumption remains as a major source due to its beneficial constituents like β- carotene, lycopene and other essential vitamins. It has been substantiated for its anticipatory nature in various types of cancer[3] and cardiovascular diseases.[4] In India, Arka vikas tomato variety is majorly preferred for cultivation due to its special adaptation characteristics and good production yields. Some aspects of this genotype like light and preconditioning effects has been previously studied[5] and consequences on direct regeneration using growth regulators has been readily investigated[6] of need to be studied in detail. Obtaining plantlets using callus induction from plant tissue culture techniques has motivated many researchers to select tomato as model species for advancement of several genetic characters. Vital factors like plant genotype and media composition is most subjective during plant regeneration through organogenesisin study of tomato cultivars and this factor is considered for experimenting in arka vikas cultivar. Considering the accessible evidences till date on morphogenesis, tomato tissue culture still need better refinement for *in-vitro* mass propagation among various commercially available cultivars.

Cytokinins alone or in combination with other auxins has improved callus induction in many tomato cultivars[7] and its necessity in media has been already proved in many Indian cultivars like Arka Ahuti, Punjab upma[8] and Pusa ruby.[9] Among cytokines, Benzyl amino purine, Zeatin and Thidiazuron along with Indole acetic acid, Naphthalene acetic acid are frequently used in tomato regeneration. The combination of two cytokinins BAP with TDZ (thidiazuron) was studied earlier and till today these two cytokinins are studied extensively used in tomato plant tissue culture for callus induction and regeneration, whereas studies using kinetin have been slightly seldom. Kinetin (N-2-furanylmethyl-1H-purine-6-amine), an adenine type cytokinin which is equally potent phytohormone to zeatin also plays a major role for callus induction in tomato. Among cytokinins, zeatin is highly preferred for tomato organogenesis due its good shoot forming capacity[10] and hence activity of kinetin needs to be addressed in tomato organogenesis. The presence of cytokinin and its role in shoot multiplication is well studied[11] whereas the callus induction using kinetin has not been studied thoroughly in local tomato cultivars for efficient regeneration protocols. The influence of kinetin on callus induction and regeneration in local cultivar Arka vikas was evaluated in this experiment and results obtained will be extremely helpful in study of major genetic transformation experiments using this cultivar. Establishment of efficient *in-vitro* regeneration protocol for tomato through organogenesis is the fundamental step in any genetic transformations[12] and this experiment was conducted for genetic transformation of tomato using Bt-cry2A gene.

Materials and methods

Seed material and sterilization

This experiment was performed in Division of Biotechnology, Indian Institute of Horticultural Research (IIHR), Hessaraghatta, Bengaluru, India. The genuine breeder seeds of tomato cv. Arka vikas were obtained from Seed Division, Indian Institute of Horticultural Research (IIHR), Hessaraghatta, Bengaluru, India. Initially seeds were treated with 0.1% CAP-50 (a commercially available fungicide) before surface sterilization. After fungicide treatment the seeds were washed under running tap water and were air dried in room temperature. During surface sterilization, the seeds are rinsed with 70% ethanol for 30-45seconds. This was followed by washing twice with autoclaved sterile distilled water. Later the seeds were rinsed with 4% NaoCl (Sodium hypochlorite) for 6-8 minutes and were washed

thrice with autoclaved sterile distilled water. The seeds were blot dried on autoclaved sterile blotting paper and placed on half-strength MS[13] media and were allowed for germination.

Callus induction from hypocotyl and cotyledons

Cotyledons and Hypocotyl explants of ten to twelve old day seedlings were selected for excision and were placed on callus induction medium. The composition of callus induction medium include full strength MS with 2.0mg/L benzyl amino purine and 1.5mg/L to 2.25mg/L Kinetin. Intense observations among percentage callus induction, percentage shoot initiation and shoot regeneration till rooting were recorded and calculated systematically. Using petridishes the explants were incubated under dark conditions at 24±2°C temperatures. Six replications with each petridish containing 28 sections of hypocotyls and cotyledons were used for evaluating best media composition for callus induction.

Callus induced regeneration

After fifteen days, good embryogenic calli is derived from the explants at both ends of cotyledon and hypocotyls (Figure A) (Figure B). This callus was excised and was individually placed in a fresh petridish containing same medium (Media used for obtaining calli) for multiple shoot induction and its proliferation. These individually separated cultures were incubated under dark (Figure C) conditions at 24±2°C temperatures. After ten days of incubation the petridishes with multiple shoots were obtained (Figure D) and were transferred to light conditions with same temperature conditions (Figure E). The multiple shoots obtained were transferred to shoot elongation medium full strength MS+IAA (0.1mg/L)+Zeatin (2.0mg/L) and were allowed for elongation (Figure F).

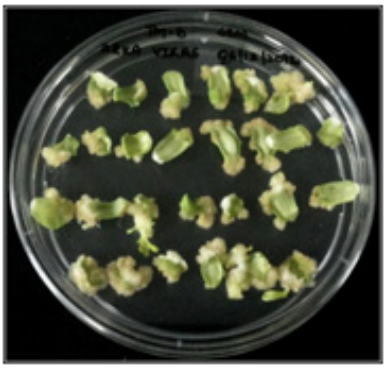

Figure A Callus induction in cotyledons.

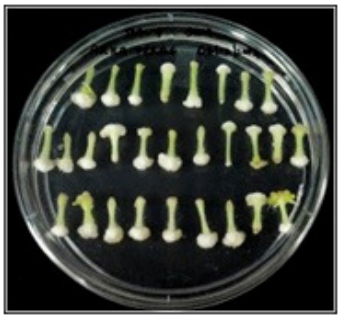

Figure B Callus induction in hypocotyls.

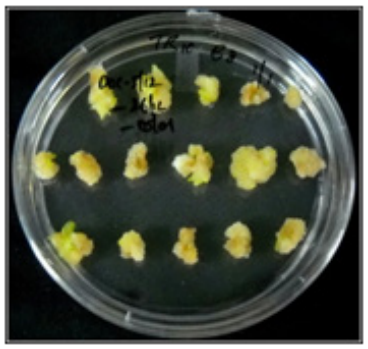

Figure C Separately placed embryonic callus.

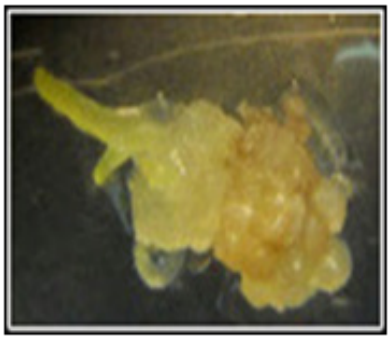

Figure D Microscopic observation of shoot formation from embryogenic calli.

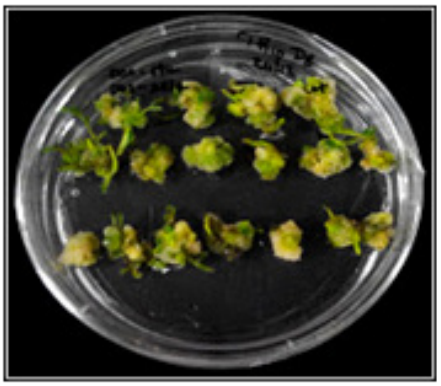

Figure E multiple shoot formation from calli.

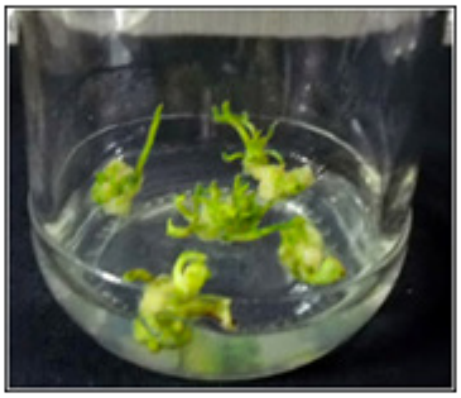

Figure F developing shoots in elongation media.

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Rooting and acclimatization

The appropriately elongated shoots (4-5cm long) were then transferred to rooting medium for root induction and its development. The composition of root induction medium include half strength MS with 0.5mg/L indole-3-butyric acid (IBA) and well rooted plants were acclimatized using soilrite mixture and were then transferred to green house.

Results and discussion

In this study, it was clearly proved that use of kinetin with the combination of BAP produced good embryogenic calli from tomato explants. Among the four media combinations tested, the media combination two was found efficient callus production and multiple shoot initiation. The performance by these two explants selected for callus production was altered with different media concentrations. The media combination with MS+BAP (2.0mg/L)+Kinetin(1.75mg/L) was found ideal and superior results were obtained from cotyledons in comparison with hypocotyls in all four combinations with cotyledons producing greater mean callus percentage than hypocotyls. The data documented after 15days period on callus propagation indorses respectable increase in embryogenic calli production with cotyledons in comparison with hypocotyls. Investigations using the statistical resources showed a significant difference at α=5% between explant callus production means differ with several media combinations. The average mean values for all media combinations used were deliberated and media combination with BAP (2.0mg/L)+Kinetin(1.75mg/L) proved well for both explants in cotyledons having (22.83) whereas (18.67) in hypocotyls (Table 1). A one□way ANOVA between subjects proved a significant difference among the four conditions in test scores with F values (3,15)=3.74, $p < .05$ (3.59) for cotyledons and, F values (3,15)=14.97, $p<.05$ (4.16) for hypocotyls. The results obtained by performing one way ANOVA reveals major substantial differences between the treatments ($p<0.05$) and has been mentioned in Table 2. Since the p-value is 0.000141, which has much lesser value than the significance level of 0.05, the null hypothesis can be strongly rejected and can be concluded that all media combinations has different means. The multiple shoots obtained from embryogenic calli were transferred in shoot elongation media MS+ IAA (0.1mg/L)+Zeatin (2.0mg/L) and overall growth response found to be 89.4%. Among the results obtained, the Table 3 graphically represents the mean values of media-2 proved good for callus induction out of four different media combinations attempted and is highly effective for indirect regeneration in this tomato cultivar.

Improvement in plant regeneration protocol via organogenesis becomes first priority for a researcher to achieve optimistic outcomes during any genetic transformations, necessary studies in regeneration procedures and many other biotechnological aspects. The influence of genotype plays a significant role in callus production[14] and hence selection of cultivars should be given prime importance for establishing regeneration protocols via organogenesis. The consequences of organogenesis in tomato for both hormones with several combinations were studied in detail using local cultivar Arka Vikas. Cotyledons and hypocotyls of tomato can be used successfully to demonstrate the role of cytokinin and *in-vitro* organogenesis in tomato using kinetin. High concentration levels of cytokinins with lower levels of auxins have always improved callus induction[15] and hence response of tomato in absence of auxins supplemented with additional cytokinin was evaluated. Lower and higher kinetin concentrations produce lesser calli and results derived using mean values in this experiment suggest closely equal concentrations of kinetin to other cytokinin can be a potential calli inducer in tomato regeneration. Earlier studies using benzyl amino purine alone or with kinetin has substantiated direct regeneration in tomato[12] and using the same hormones, the effect on indirect regeneration studies with different combinations were performed. Studies in PKM-1 variety of tomato,[16] Arka meghali[12] proved the influence of kinetin on direct regeneration in tomato and indirect regeneration was examined and outcome of this research was elucidated. Virtuous callus incidence in tomato cultivar helps in obtaining good plantlet regeneration by simplifying the regeneration protocol in tomato. This study provides the base for *in-vitro* organogenesis and improving research in genetic transformation experiments using Arka Vikas tomato cultivar.

Table 1 The average mean values for all media combinations.

Hormones - BAP + Kinetin	No of Cotyledons	Mean calli Production	Percentage Callus Production	Average Shoot Induction per Calli
Media A-2.0mg/L+1.5mg/L	28	18	64.28	3.12
Media B- 2.0mg/L+1.75mg/L	28	22.83	81.54	5.23
Media C- 2.0mg/L+2.0mg/L	28	17.16	61.3	4.09
Media D- 2.0mg/L+2.25mg/L	28	15.16	54.1	3.41
Hormones -BAP + Kinetin	**No of Cotyledons**	**Mean calli production**	**Percentage callus production**	**Average shoot Induction per calli**
Media A-2.0mg/L+1.5mg/L	28	18	64.28	3.12
Media B- 2.0mg/L+1.75mg/L	28	22.83	81.54	5.23
Media C- 2.0mg/L+2.0mg/L	28	17.16	61.3	4.09
Media D- 2.0mg/L+2.25mg/L	28	15.16	54.1	3.41

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Table 2 ANOVA reveals major substantial differences between the treatments

ANOVA Details for Cotyledons				
Source	SS	df	MS	F
Treatment between groups	190.45	3	63.4861	13.74
Error	69.29	15	4.6194	
Ss/Bl	41.2	5		
ANOVA Details for Hypocotyls				
Source	SS	df	MS	F
Treatment between groups	279.79	3	93.2639	14.97
Error	93.45	15	6.2306	
Ss/Bl	17.35	5		

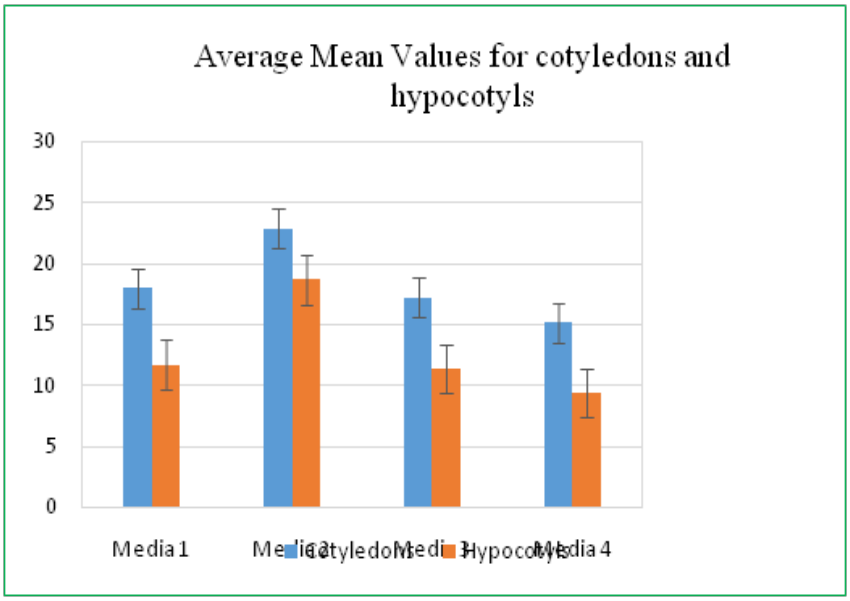

Table 3 Average mean values for cotyledons and hypocotyls.

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None.

Conflict of interest

The author declares no conflict of interest.

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