

IN-VITRO PROPAGATION OF TOMATOES UNDER ASEPTIC CONDITION

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Abstract

*In this study the major focus was to regenerate and produce multiples of Tomato (*solanum lypersicon*), with other desirable traits; in the absence of seed. The apical Meristem of tomato seedling was used as ex-plants for in vitro callogenesis. The ex-plants were washed with distilled water and sterilized by treating with 70% ethanol for 2 minutes and 20% of sodium hypochlorite for 20 minutes, ex-plants were thereafter washed 3 times with distilled water and then sterilized ex-plants were placed on MS medium modified with 3.0, 3.5, 4.0, 4.5, 5.0 mg/L 2, 4-Dichlorophenoxy acetic acid (2, 4-D) and incubated in the dark for callus induction and regeneration. The effect of different concentration of 2, 4-D on the callus induction from apical Meristem of tomatoes was evaluated under in vitro condition, 3.0,3.5,4.0,4.5,5.0mg/L 2, 4-D and were tested. The best optimum number of callus induction was MS medium supplemented with 4.5mg/L 2,4-D with an average percentage callus induction of (95.8%) and degree of callus growth (XXX) followed by MS medium supplemented with 4.0mg/L 2,4-D with an average percentage callus induction of (70.8%) and degree of callus growth (XX). The least was MS medium supplemented with 3.5mg/L 2, 4-D with an average percentage of callus induction of (56.8%) and degree of callus growth (XX). From the best optimum number of callus induction on MS medium supplemented with 4.5mg/L 2,4-D, the percentage of callus formation in each bottle was sub-cultured on fresh MS medium having concentrations of Zeatin (1mg/L) and 2,4-D (1mg/L) for regeneration. They showed average percentages of regeneration of 62.2% . Negative factors were eliminated.*

Keywords: Tomatoes, ex-plants, in-vitro callogenesis, 2,4 Dichlorophenoxy acetic acid, MS medium, regeneration, callus induction, Meristem.

Introduction

Tomato is one of the important Solanaceae crop grown throughout the world (Rick, 1990). It is recognized as a highly valuable and nutritious food. Nowadays tomato is one of the major vegetable or fruit throughout the world. It is grown in tropical, sub-tropical and temperate areas. Several in vitro investigations have been conducted on tomato based on its relationship with tobacco, and on account of its consequently expected good work ability (Koblitz, 1992). Tomato is a favorable food crop for in vitro studies due to its low chromosomal number i.e. $2n=2X=24$ and due to comprehensive knowledge of tomato genetics (Chaudhry et al, 2001).

Productivity of tomato has been low due to many biotic and abiotic stresses. There are several common diseases of tomato crops Viz., bacterial wilt caused by *pseudomonas solanacearum* and bacterial scab, which is caused by *Xantomonan compestris*. Fungal disease, have resulted in decreased trend of its yield and powdery mildew caused by *Leveillula taurica*. Other main diseases are early blight, leaf spot, leaf mould and wilts etc. More over changes in insect's biotype and disease resistance are becoming a continuity threat to increased production. Tomato is an important crop species and is being used as a model plant in molecular studies (Ruf, S.M. et al 2001).

Tomato propagation deals with the multiplication and production of plants using propagules representing a specific genotype. Propagule is any tomato part used to produce a new tomato or a population of tomatoes. Specific propagules include seeds, cuttings, layers, buds, scion, and explants. To propagate, it is important to control the selection of the seeds parents or the vegetative source of propagules (Rick, 1998).

Regeneration ability of a number of tomatoes have been tested for their ability to produce callus and shoot induction in earlier studies (Casta et-al, 2000, Venkatachalam et-al, 2000). Plant regeneration in tomato is via shoot organogenesis from callus (leaf or cotyledon explants) or direct by inflorescence or another culture

under the influence of different phytohormones, but the condition of in vitro plant regeneration is still largely on empirical process and it is difficult to achieve for the same genotypes within a species (Compoton and Veilleux, 1991). The degree of response of explants have been by Durzan (1984) in the leaves, cotyledons, hypocotyls, where as Plastira and Perdikaris (1997) had reported in the order of hypocotyls, cotyledon and leaves; (Sani, 2007) had reported in the Apical and leaves. Tissue culture studies, with cultivars of tomato involving different combination of growth regulator have been reported (Dagmara et-al, 2005).

Many varieties of tomato are widely used and developed in an optimized culture condition for callus induction and multiple shoot induction. Tomatoes that have been modified using genetic engineering have been developed, and although none are commercially available at the moment they have been in the past. The first commercially available genetically modified food was a variety of tomato named the Flavr Savr, which was engineered to have longer shelf life. Scientist are continuing to develop tomatoes with new traits not found in natural crops, such as increased resistance to pests or environmental stresses. Other projects aim to enrich tomatoes with substances that may offer health benefits or provide better nutrient.

When small pieces of plants tissue are put in sterile culture on a solid medium, the cell proliferate and a callus is formed, and if some plant hormones are present in the correct amount, shoots will be formed. Shoots normally arise from small groups of rapidly dividing cell within the callus (Jablonski, and Skoog, 1994).

The project aims to develop efficient in-vitro callus induction and regeneration (propagation), using an organ, or any part of tomato plant (as explants)

Materials and Methods

The experiment was conducted at Jigawa Research Institute, under the Biotechnology unit (plant tissue culture), opposite the Police Station, Daura road, Kazaure Local Government. Kazaure is Located in the Northern part of Jigawa State about 87KM away from Kano City. Geographically located between latitudes 11.00° and 13.00° North and longitude 8.00° and 10.35° East and covers about 2.24100 hectares of land, which is (5KM). The area is covered with non-marshy soil that favors agricultural practice in the area (Ibe, 2003).

UC-82-B tomato seed which is commercially known were used in the study. Its hermetically sealed seed was treated with chemicals and freshly packed. It contained a REF: J033900JAJ with expiry date 12/2012. The seeds were planted in the soil (conventionally), after 5 days the seeds germinated. They were kept under proper watering (3 times/day), and after they spent about 4-5 weeks, they were physically identified as tomato plant. They were used as the source of explants.

The media used for this study was Murashiga and Skoog (MS) based medium supplemented with 3% sucrose and solidified with 8% agar.

❖ Composition of Murashiga and Skoog (MS)

A. Micro Salts.

KNO ₂	- 19.00g/L
NH ₄ NO ₃	- 16.5g/L
MgSO ₄ .7H ₂ O	- 3.74g/L
CaCl ₂	- 4.4g/L

B. Micro Salts

MnSO ₄ .4H ₂ O	-	223mg/L
ZnSO ₄ .H ₂ O	-	860mg/L
H ₃ BO ₄	-	620mg/L
NaMnO ₄ .2H ₂ O	-	25mg/L
CuSO ₄ .7H ₂ O	-	2.5mg/L
CoCl ₂	-	2.5mg/L

C. Vitamins

Nicotinic Acid	-	50mg/L
Thiamine Hcl	-	50mg/L
Pyridoxine	-	10mg/L
Glycine	-	200mg/L

D. Iron Solution

FeSO ₄ .7H ₂ O	=>	5.57g/L
NaEDTA	=>	7.45g/L

E. Carbon Source

Sucrose	=>	30g/L
Agar	=>	8g/L

Apical portion of the week old seedling were obtained and used as explants for in vitro callogenesis. The explants were washed with distilled water and sterilized by 20% commercial bleach (3.5 sodium hypochlorite) for 20 minute. The explants were there after sterilized 3 times with distilled water.

Sterilized explants were placed on MS medium (Murashiga and Skoog 1962) modified with 3.0, 3.5, 4.0, 4.5, 5.0 mg/l 2, 4 – Dichloro phenoxy acetic acid (2, 4-D and -Naphthalene acetic acid (NAA). Three culture bottles were used for each treatment and repeated three times. The experiment was laid in a completely randomized design (CRD). Callus formation was observed after 3 weeks of incubation in the dark.

Data collected were days to callus formation, number of responding explants and degree of callus formation, The data were expressed and the sample percentage were presented in tables.

After formation of callus, the optimum number of callus induction from the following MS medium modified with 3.0, 3.5, 4.0, 4.5, 5.0 mg/L 2, 4 –D (2, 4 – Dichloro phenoxy acetic acid) were sub-cultured on fresh MS medium having concentration of Zeatin (1mg/L) and 2, 4 – D (1mg/L), incubation at growth chamber for regeneration. The percentage of growth differentiation and shoot formation was observed after 2 weeks

Results

The results showing the optimum response of eight (8) tomato explants (apical Meristem). Eight (8) explants were placed in each culture bottle. Five concentrations (3.0, 3.5, 4.0, 4.5, 5.0 mg/L) of 2, 4 – D (Dichlorophenoxy acetic acid) was evaluated for callus induction.

Table 1 -mg/L 2, 4 - D

S/N	No. of explants	No. of explants forming callus	% callus	Degree of callus growth
1	8	3	37.5	XX
2	8	4	50	XX
3	8	5	62.5	XXX
4	8	3	37.5	XX
5	8	2	25	X
6	8	5	50	XX

The maximum number of explants forming callus was five (5) from bottle number 3, and minimum number of explants was 2 from bottle number 5. Average number of explants forming callus was 3.5% explants when subjected to 3.0mg/L 2, 4 – D.

The maximum percentage of callus formation was 62.5% from bottle number 3 of number of explants forming callus and minimum is 25% from bottle number 5 of number of explants forming callus. Average percentage number of callus was 43.8%.

In degree of callus growth, maximum was XXX, and minimum was X. average growth was XX. Degree of callus growth is represented by X.

Table 2:3.3mg/L 2, 4 - D

S/N	No. of explants	No. of explants forming callus	% callus	Degree of callus growth
1	8	4	50	XX
2	8	5	62.5	XXX
3	8	6	75	XXX
4	8	5	62.5	XXX
5	8	3	37.5	X
6	8	4	50	XX

The maximum number of explants forming callus was six (6) from bottle number 3, and minimum number of explants was 3 from bottle number 5. Average number of explants forming callus was 4.5% explants when subjected to 3.5mg/L 2, 4 – D.

The maximum percentage of callus formation was 75% from bottle number 3 of number of explants forming callus and minimum is 37.5% from bottle number 5 of number of explants forming callus. Average percentage number of callus was 56.8%.

In degree of callus growth, maximum was XXX, and minimum was X. average growth was XX.

Table 3:4.0 mg/L 2, 4-D

S/N	No. of explants	No. of explants forming callus	% callus	Degree of callus growth
1	8	8	100	XXX
2	8	7	87.5	XXX

S/N	No. of explants	No. of explants forming callus	% callus	Degree of callus growth
3	8	3	37.5	XXX
4	8	7	87.5	X
5	8	4	50	XX
6	8	5	62.5	XXX

The maximum number of explants forming callus was eight (8) from bottle number 1, and minimum number of explants was 3 from bottle number 3. Average number of explants forming callus was 5.6% explants when subjected to 4.0mg/L 2, 4 – D.

The maximum percentage of callus formation was 100% from bottle number 1 of number of explants forming callus and minimum is 37.5% from bottle number 3 of number of explants forming callus. Average percentage number of callus was 70.4%.

In degree of callus growth, maximum was XXX, and minimum was X. average growth was XX.

Table 4: 4.5 mg/L 2, 4-D

S/N	No. of explants	No. of explants forming callus	% callus	Degree of callus growth
1	8	8	100	XXX
2	8	7	87.5	XXX
3	8	8	100	XXX
4	8	8	100	XXX
5	8	8	100	XXX
6	8	7	87.5	XXX

The maximum number of explants forming callus was eight (8) from bottle number 1, 3, 4, and 5 and minimum number of explants was 7 from 2 and 6 bottles. Average number of explants forming callus was 7.7% explants when subjected to 4.5mg/L 2, 4 – D.

The maximum percentage of callus formation was 100% from bottle number 1, 2, 3 and 5 of number of explants forming callus and minimum was 87.5% from bottle number 2 and 6 of number of explants forming callus. Average percentage number of callus was 95.8%.

In degree of callus growth, maximum was XXX, and minimum was not. Average growth was XXX.

Table 5:5.0 mg/L 2, 4-D

S/N	No. of explants	No. of explants forming callus	% callus	Degree of callus growth
1	8	7	87.5	XXX
2	8	6	75	XXX
3	8	7	87.5	XX
4	8	8	100	X

S/N	No. of explants	No. of explants forming callus	% callus	Degree of callus growth
5	8	5	62.5	XX
6	8	3	37.5	XX

The maximum number of explants forming callus was (8) from bottle number 4 and minimum number of explants was 3 from bottle number 6. Average number of explants forming callus was 6% explants when subjected to 5.0mg/L 2, 4 – D.

The maximum percentage of callus formation was 100% from bottle number 4 of number of explants forming callus and minimum was 37.5% from bottle number 6 of number of explants forming callus. Average percentage number of callus was 75. %.

In degree of callus growth, maximum was XXX and minimum was X. average growth was XX.X.

Table 6: Regeneration of Tomato Plant

The result showing the achieved regeneration of tomato from each percentage of callus formation on optimum number of callus induction from MS medium supplemented with 4.5mg /L 2, 4-D (tab. 4), after sub-cultured on MS medium supplemented with Zeatin (1mg/L) and 2, 4_D (1mg/L).

S/N	% callus	% Growth differentiation	% shoot formation
1	100	80	72
2	87.5	66	60
3	100	78	70
4	100	84	68
5	100	72	69
6	87.5	63	52

The average percentage of growth differentia from each percentage of callus formation was 73.8% and average shoot formation from each percentage of growth differentiation was 62.2%. In-culture negative factors were eliminated.

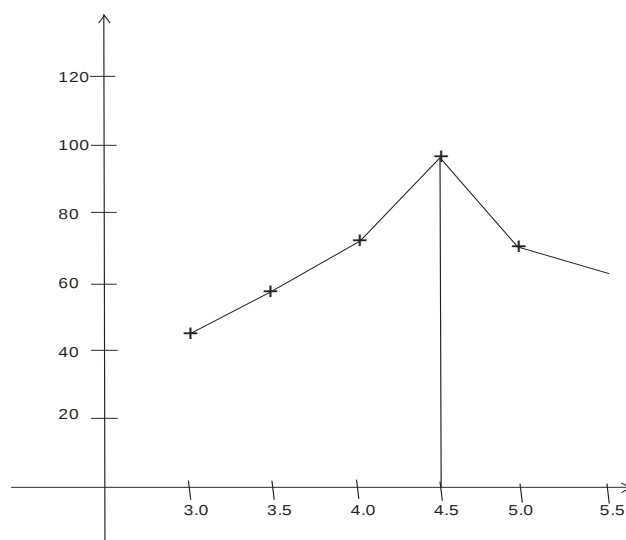


Chart showing the optimum percentage of callus induction for tomatoes regeneration.

Discussion

In previous reports, supplementing MS with 4.5 mg 2, 4-D was proved to be the optimum concentration for callus induction from apical Meristem of tomatoes (*Solanum lycopersicum* L.) and regeneration on MS medium supplementing with Zeatin (mg/L) (Sani, L.A 2007).

MS medium supplemented with 4.5 mg/L 2, 4-D showed the best optimum concentration for callus induction from the Apical Meristem of tomatoes (*Solanum lycopersicum* L.) with highest average percentage callus growth (XXX) (Table. 4) and then MS medium supplemented with 4.0 mg/L 2, 4-D with an average percentage callus induction of (70.8%) and degree of callus growth (XX) (Table. 3). The least was MS medium supplemented with 3.5 mg/L 2, 4-D, with an average percentage callus induction of (56.8%) and degree of callus growth (XX) (Table. 2). The other MS medium supplemented with 3.0, 5.0 mg/L 2, 4-D with less average percentage callus induction and degree of callus growth.

The average percentage of regeneration was achieved on MS medium supplemented with 4.5 mg/L 2, 4-D on fresh MS medium supplemented with Zeatin (1mg/L) and 2, 4-D (1mg/L) with an average percentage of shoot formation of (62.2%) (Tab.6). The implication of the practical, after sub-cultured of the callus formation on fresh MS medium, there must be the presence of number of Necrosis and powdery callus which will not regenerate (shoot).

The application of the practical is production of tomato in sterile container that allows them to be moved with greatly reduced chances of transmitting diseases, pest and pathogens.

Conclusion

Callus induction was observed in Apical meristem of tomatoes (*Solanum lycopersicum* L.) and was evaluated under in-vitro condition after which 3.0, 3.5, 4.0, 4.5, 5.0 mg/L 2,4-D was tested. The MS medium supplemented with 4.5 mg/L 2, 4-D showed to be the optimum and better explants for callogenesis and regeneration on MS medium supplemented with Zeatin (1mg/L) and 2, 4-D (1mg/L) was noted, of about (95.8%) and degree of callus growth (XXX). Average regeneration from the MS medium Zeatin (1mg/L) 2, 4-D. This study is a baseline to carry further research on this tomato variety for improvement by using gene transfer technology.

Recommendation

It is recommended that plant tissue culture being an important tool in Biotechnology and facilitator system for genetic transformation be adopted as it is essential to arrive at the common in-vitro system which can

suit most variety available in the country. More so as it is a modern technique that scientists are using to propagate plants under aseptic condition and/or using genetic engineering to propagate plant or alter the position of that particular plant.

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