THE EFFECTS OF MEDIUM MODIFICATION ON IN VITRO SEED GERMINATION AND CALLUS INDUCTION IN TOMATO

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This dissertation is dedicated to my family

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ABSTRACT

Tomato is one of the most important vegetable and it is part of human daily food. High world population growth rate demands to increase the tomato productivity. Tomato is chosen among the researchers due to its high anti-oxidant and mineral properties. In the present study, *in vitro* response of tomato c.v. *Rio Grande* was investigated. It was found that when sucrose was used as the source of carbon, the highest germination frequency of 85% was achieved in modified N_6 whereas the highest germination rate in modified $2N_6$ medium was 15.6. In medium supplemented with 1.5 mg/l GA_3, 1.5 mg/l BAP and 0.25 mg/l, 0.5 mg/l and 0.75 mg/l of 2, 4-D, the highest callus induction frequency of 39% was observed. In the same time, the highest induction rate of 1.3 was observed in medium supplemented with 1.5 mg/l GA_3, 1.5 mg/l BAP and 0.25 and 0.5 mg/l 2, 4-D. Analysis of variance showed significant effects of medium concentration on *in vitro* seed germination and plant growth regulators on callus induction in different tomato explants. Cotyledons showed better response compared to hypocotyls. These results suggested that sucrose would be a better carbon source either for *in vitro* germination or callogenesis and cotyledon would be better explants for tissue culture studies.

ABSTRAK

Tomato merupakan salah satu sayur-sayuran dan merupakan antara sayuran utama dalam pemakanan seharian manusia. Kadar pertumbuhan penduduk dunia yang tinggi menuntut untuk meningkatkan produktiviti tomato. Ciri-ciri tomato yang mempunyai komponen anti oksida dan kandungan mineral yang tinggi menyebabkan ia menjadi pilihan bagi pengkaji-pengkaji. Kajian ini mengkaji kesan pengubahsuaian media kultur, pengawalan tumbesaran tumbuhan dan penggunaan sumber karbon ke atas percambahan benih dan induksi kalus secara in vitro. Sukrosa telah digunakan sebagai sumber karbon dalam kajian ini. Dalam media N_6 frekuensi percambahan tertinggi ialah 85% manakala kadar percambahan bagi media terubahsuai $2N_6$ ialah 15.98. Dalam medium yang dibekalkan dengan 1.5 mg/l GA_3, 1.5 mg/l BAP dan 0.25 mg/l, 0.5 mg/l dan 0.75 mg/l 2, 4-D, frekuensi induksi kalus tertinggi ialah 39%. Padamasa yang sama, kadar percambahan tertinggi adalah sebanyak 1.3. telah didapati dalam medium yang dibekalkan dangan 1.5 mg/l GA_3, 1.5 mg/l BAP, dan 0.25mg/l dan 0.5mg/l 2, 4-D. Analisis varian menunjukkan kesan ketara kepekatan medium dalam percambahan benih *in vitro* dengan pengawalan tumbesaran tumbuhan dalan induski kalus dalam eksplan tomato yang berbeza. Kotiledon didapati lebih responsif daripada hipokotil. Kajian membuktikan bahawa sukrosa merupakan sumber karbon yang lebih baik sama ada untuk percambahan in vitro atau kalus genesis dan kotiledon adalah explan yang lebih baik untuk kajian kultur tisu.

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LIST OF ABBREVIATIONS / SYMBOLS

N6	-	Chu (1975)
MS	-	Murashige & Skoog (1962)
%	-	percentage
W/V	-	Weight per volume
V/V	-	Volume per volume
g	-	Gram
g/l	-	Gram per liter
°C	-	Degree celcius
et al.,	-	and friends
UV	-	Ultra violet
Var	-	Variety
cv.	-	Cultivar
mg/l	-	Mg per liter
NaOH	-	Sodium hydroxide
HCl	-	Hydrochloric acid
BAP	-	6-Benzylaminopurine
2, 4-D	-	2, 4-Dichlorophenoxyacetic acid
GA_3	-	Gibberellic acid
IAA	-	Indole-3-acetic acid

NAA	-	Naphthalene acetic acid
min	-	Minute
h	-	Hour
PGR	-	Plant growth regulator

CHAPTER 1

INTRODUCTION

1.1 Back ground of study

Tomato belongs to the family Solanaceae by its nature of a perennial plant but is commercially cultivated as an annual crop. Tomato (*Lycopersicon esculentum* Mill.) is the second most popular vegetable crop next to potato in the world (Bhatia *et al*., 2005). Tomato is planted in almost 4 million hectar worldwide. Lycopene is part of the pigments known as carotenoids which are natural substances that generate colors of fresh fruits and vegetables. Lycopene defends people from free radicals that degrade many parts of the body it is the most powerful anti-oxidant in the carotenoid family and it; moreover, lycopene is also known as an anti cancer (Rao and Agarwal, 2000). At present, tomato vegetables are used at a higher rate in developed countries compared to developing countries, thus, it may be referred as a luxury crop. It is grown in tropical, sub-tropical and temperate areas. It is one of the most important protective

foods as it possesses significant quantities of minerals and vitamins and sometime termed as poor person's orange (Devi *et al*., 2008).

Plant tissue culture techniques are identified as valuable instrument in crop improvement. In tomato, *in vitro* culture is used in tomato in several biotechnological applications such as development of virus free plants (Moghaib *et al.,* 1999) and genetic transformation (Park *et al.,* 2003). Moreover, Plant tissue culture method is used for highest callus induction and improved plantlet regeneration (Amini and Ehsan pour, 2006). Plant regeneration is a key facilitator component in genetic transformations, using *Agrobacterium tumefaciens*, electroporation and particle bombardment (Amini and Ehsan pour, 2006).

In vitro regeneration of cultivated tomato has been a topic of research because of the commercial value of tomato plants and its potential for additional improvement via genetic manipulation (Evans, 1989). Other quality components e.g. color, acidity and flavors can be enhanced by introgression of genes (Chaudary *et al.,* 2001).

The successful application of plant tissue culture presupposes the establishment of an efficient culture system, consisting of a competent genotype, explant source as well as optimal culture conditions (Chaudary *et al.,* 2001). Different explant sources can be used for callogenesis and regeneration. Studies about the effect of variety and plant growth regulators on callus proliferation and regeneration of three tomato cultivars has been reported (Chaudhry *et al.,* 2007). Shoot apex, nodal segments and root segments were successfully used for callus induction and regeneration (Jatoi *et al*., 2001). Various hormonal combinations are

used to induce callus and regeneration like BAP and IAA, IAA and Kin (Chen *et al.,* 1999).

1.2. Problem statement

Tomato (*Lycopersicon esculentum* Mill.) is a major vegetable crop that has achieved tremendous popularity over the last century and it is grown in almost every country of the world (Abu-El-Heba *et al.,* 2008). However, Tomato production is adversely affected by wide ranges of biotic and abiotic stresses (Osman *et al.,* 2010) such as disease, high temperature, draught, salinity and its vulnerability to frequent insect and pest attacks. Diseases infestations are notorious factors that reduce crop yields and inflate production costs (Ishag *et al*, 2009). These diseases include viral, bacterial and fungal (Chaudhry *et al.*, 2010).

To attain sustainable tomato productions, such above mentioned constraints have been addressed by conventional breeding and enhanced management but it has resulted in limited commercial success (Osman *et al.*, 2010). Plus, the improvement of plant through conventional breeding methods is slow, time-consuming and labor-intensive (Moghaieb *et al.*, 1999). The integration of modern biotechnology like tissue culture into breeding programs may provide powerful tools to overcome these limitations.

1.3. Objectives of study

The objectives of this study are:

1. To investigate the effects of different strength of modified N_6 medium namely 1/2 N_6, N_6 and $2N_6$ on *in vitro* seed germination.
2. To assess the effects of carbon source and plant growth regulators on callus induction from cotyledon in tomato.
3. To study the effects of plant growth regulator and carbon source on callus induction from hypocotyl in tomato.

1.4. Aim of study

The aim of this work was to produce information regarding the effects of different strength of modified N_6 medium on *in vitro* seed germination in tomato. Moreover, the present study was conducted to explore the callogenic potential of hypocotyls and cotyledon segments so as to establish a reproducible protocol for callus induction from tomato (*Lycopersicon esculentum* Mill.) cultivar *Rio Grande* by using different sources of carbon and different combination and concentration of plant growth regulators.

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