

**STUDIES TO INVESTIGATE THE EFFECT OF PLANT GROWTH
REGULATORS, CARBON SOURCES AND MEDIA MODIFICATION ON *IN*
VITRO SEED GERMINATION AND CALLOGENESIS IN TOMATO**

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*to my mother Badriyah Hamad and father Muhammad Hamad Ameen, all my sisters,
brothers, and friends.*

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ABSTRACT

Tomato (*Lycopersicon esculentum* Mill.) is a major vegetable crop that has achieved tremendous popularity over the last century. However, its production faced many problems like diseases, therefore, establishing a good protocol to improve the quantity and the quality of the crops is a prerequisite nowadays. Moreover, tissue culture has provided rapid modes to develop desirable varieties of cultivated plant species. In this study, *in vitro* culture response was assessed in tomato (*Lycopersicon esculentum* Mill. c.v. King Stone) for *in vitro* germination and optimum callus induction. The highest germination frequency (84.44%) was obtained on MS media (maltose as carbon source). In addition, to optimize an efficient protocol for callus induction, MS medium were supplemented with different source of carbon (sucrose and maltose) and different concentration of 2,4-D (0, 0.25, 0.5, 0.75, 1) mg/l in combination with BAP 1.5 mg/l and GA₃ 0.5 mg/l. In cotyledon explants the highest response of callus induction frequency (81.25%) and callus induction rate (3.25) were in MS media, which was supplemented with sucrose as carbon source and plant growth regulators 2,4-D 0 mg/l, BAP 1.5 mg/l and GA₃ 0.5 mg/l (T1). while the maximum callus induction frequency (100%), callus induction rate (4.54) and explant productivity (17.32%) were obtained from hypocotyl explants which cultured on MS media in T1. In conclusion, maximum *in vitro* seed germination was observed on MS medium supplemented with maltose. While sucrose shows a significant role in callus induction. Callus induction was observed in both hypocotyl and cotyledon explants. Hypocotyls showed to be better explants for callogenesis. Maximum callogenesis was noted on MS medium supplemented with 2,4-D (0 mg/l), BAP (1.5 mg/l), GA₃ (0.5 mg/l).

ABSTRAK

Lycopersicon esculentum Mill. atau dikenali sebagai tomato merupakan hasil tanaman dan sayuran yang paling meluas akhir ini. Oleh kerana tomato penting dalam ekonomi, penanamannya semakin meningkat setiap hari. Namun begitu, penghasilannya menghadapi masalah seperti serangan penyakit. Oleh itu, teknik yang bersesuaian perlu diwujudkan untuk meningkatkan kualiti dan kuantiti tanaman. Tambahan pula, tisu kultur telah menyediakan cara yang pantas bagi menghasilkan variasi tanaman yang diinginkan. Dalam kajian ini, tindakbalas *in vitro* untuk percambahan dan penghasilan kalus secara optimum ke atas tomato tomato (*Lycopersicon esculentum* Mill. c. v. King Stone) telah dikaji. Percambahan tertinggi (84.44%) telah diperolehi di atas media MS (maltosa sebagai sumber karbon). Untuk mengoptimumkan penghasilan kalus, MS media telah ditambah dengan sumber karbon yang berbeza (sukrosa dan maltosa) dan hormon 2,4-D dengan kepekatan yang berbeza (0, 0.25, 0.5, 0.75, dan 1 mg/l) dengan kombinasi 1.5 mg/l BAP dan 0.5 mg/l GA₃. Frekuensi kalus yang tertinggi (81.25%) dan kadar penghasilan kalus (3.25) untuk eksplan kotiledon telah diperolehi atas media MS yang ditambah dengan sukrosa sebagai sumber karbon dan hormon 2,4-D (0 mg/l), BAP (1.5 mg/l) and GA₃ (0.5 mg/l) (T1), manakala frekuensi penghasilan kalus yang maksimum (100%), kadar penghasilan kalus (4.54) dan penghasilan eksplan (17.32%) diperolehi daripada eksplan hipokotil yang dikultur atas media MS T1. Kesimpulannya, hasil pemerhatian menunjukkan percambahan biji benih secara *in vitro* yang maksimum adalah di dalam media MS dengan penambahan maltosa, manakala sukrosa berperanan penting dalam penghasilan kalus. Penghasilan kalus dapat diperhatikan pada eksplan hipokotil dan kotiledon. Hipokotil didapati eksplan yang lebih berpotensi untuk penghasilan kalus. Penghasilan kalus yang paling maksimum dicatat pada media MS dengan penambahan 2,4-D (0 mg/l), BAP (15 mg/l), GA₃ (0.5 mg/l).

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LIST OF ABBREVIATIONS/ SYMBOLS

<i>et al.</i> ,	and friends
MS	Murashige & Skoog (1962)
2, 4-D	2,4- Dichlorophenoxyacetic acid
BAP	6- benzylaminopurine
GA ₃	gibberellin
PGR	plant growth regulator
e.g	example
c.v	cultivar
EtOH	ethanol
NaOH	sodium hydroxide
HCl	hydrochloric acid
L	liter
%	percentage
UV	ultraviolet
g	gram
g/l	gram per liter
min	minutes
v/v	volume per volume
°C	degree Celsius

Var	variety
ml	milliliter
mg	milligram
<i>sp.</i>	species
mg/l	milligram per liter
w/v	weight per volume
μm	micrometer
h	hour
No	number
T	treatment

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CHAPTER 1

INTRODUCTION

1.1 Background of study

Tomato is considered as a member of the family *Solanaceae*. The botanical name of tomato is *Lycopersicon esculentum* Mill. It is a diploid plant with $2n = 24$ chromosomes. Naturally, the tomato is a perennial plant, but it is cultivate annually because of having a great economical and commercial advantages. Moreover, tomato is considered as the 2nd greatest significant vegetable crops in the world after potato (Mohamed *et al.*, 2010).

Over the last century, tomato (*Lycopersicon esculentum* Mill.) as an important vegetable crop has attained a tremendous popularity. It can be grown in most places all over the world, like growing in the field, greenhouses and net houses. The tomato crop is grown and used for both fresh market and processing and it is an adaptable crop. Over the past 25 years, the demand on tomato recorded highly data in both producing and consuming and it has grown quite rapidly. Nowadays, tomato occupied an area about 3.9 million hectares all over the world for growing, and annually about 108 million metric tonnes of tomato will be produce for both fresh market and processing (Bhatia *et al.*, 2004).

Tomato has valuable vitamins for instance vitamin A and C and also it contains fibers, and is known as free in having cholesterol. Generally, the average size of tomato equal to (148g) boasts only 35 calories. Approximately 20–50 mg of lycopene/100g of fruit weight can be found in tomato. Lycopene is a member of the family of pigments, which called as carotenoids. This family have the ability to form colors in fruits and vegetables, naturally. Lycopene is the best powerful antioxidant in the carotenoid family and it prevents humans from free radicals that degrade many parts of the body, lycopene is also known to protect human from cancer. At present, tomatoes are utilized at a higher rate in the developed countries than in the developing countries and hence it may be referred to as a luxury crop (Bhatia *et al.*, 2004).

Tissue culture is a significant tool of biotechnology, which can be used to increase the productivity of crop through quick obtainability of superior planting stock. Plant tissue culture has developed greatly from the beginning in the 1930s, when the scientists used this technique to grow cells in culture. Presently, it is used for numerous different purposes like callus induction, anther culture, protoplast culture and somatic embryogenesis (Evans, 1989).

In vitro regeneration of cultivated tomato has been a topic of research because of the commercial worth of the crop and its flexibility for further improvement via genetic manipulation. Consequently, many studies on plant regeneration from a varied range of tissues and organs of wild and cultivated tomato germplasm have been conducted (Evans, 1989). Moreover, tomato breeding programs can extremely benefit of biotechnological tools, such as gene transfer technology, which accepts the introduction of foreign genes into a germplasm, without modifying the genetic background of elite varieties. However, a breeding program associated to biotechnological tools depends upon the development of an efficient *in vitro* plant regeneration system (Abu-El-Heba *et al.*, 2008).

In addition, the success in tomato regeneration response has been found to depend largely on explants, genotype and plant growth regulator used in culture medium. Plant growth regulators have effect on morphogenic of tomato cultures. Many kinds of plant growth regulators have been used with varying concentrations for tomato regeneration. Different types of cytokinin and auxin combinations could induce callus induction in tomato from different source of explants (Mohamed *et al.*, 2010). Furthermore, the hormones auxin and cytokinin have a multitude of complex interactions, which control plant development. The hormonal balance between auxins and cytokinins can regulate the formation of roots, shoots, and callus tissue *in vitro*. The way of interaction between auxins and cytokinins mostly depend upon the type of tissue and on the plant species in which the interaction occurs (Abu-El-Heba *et al.*, 2008). Another kind of PGRs is known as gibberellins, which are naturally occurring in plants, they have affect on cell enlargement and division which leads to internodes elongation in stems. They have a dwarf reversing response e.g., it allows certain dwarf cultivars to grow to normal height when treated with gibberellin. It has also a different influence on developmental processes, particularly those controlled by temperature and light for instance seed and plant dormancy, germination, seed stalk and fruit development are controlled by GA₃ (Afroz *et al.*, 2009).

1.2 Problem statement

Owing to high food value, the request and demand on tomatoes is rising day after day. However its production is affected by many types of stresses (biotic and abiotic stress) like diseases that caused by fungi, bacteria, viruses and nematodes. There are many environmental stresses, which influence negatively on the growth and production of crops (Afroz *et al.*, 2010). Moreover, there are various factors, which limited the production and growth of tomato such as high temperature, draught, salinity and its vulnerability to frequent insect and pest attacks. Diseases infestations are well known factors that decrease crop yields and expand production costs (Ishag *et al.*, 2009). The

mentioned diseases and factors (biotic and abiotic factors) also help to cause to the decline of hybrid seed recovery from the field. The development and improvement of stress tolerance of crops are main objectives for plant molecular and genetic breeding (Afroz *et al.*, 2010). Genetic engineering techniques can play an important role in the development of disease resistant cultivars. In addition, the use of disease resistant cultivars might be a successful method of controlling the above diseases (Bhatia *et al.*, 2004).

Diseases infestation is mostly controlled by using of chemicals, which occasionally reaches the level of toxicity. So, currently it is evident that improvement of this crop is a critical task to overcome the constraints of tomato production. Conventional breeding techniques were attempted to develop the properties of agronomic significance in tomato, but it was not so successful because of high degree of self-pollination and non-availability of suitable wild germplasm. Beside this, conventional breeding technique needs longer time, extending from seven to eight years, which include crossing and selection of desirable traits. (Amitav, 2011). A good *in vitro* plant regeneration system can help in further improvement of the commercially essential cultivars for disease resistance through genetic engineering (Bhatia *et al.*, 2004) and also through plant biotechnological techniques which have been effectively and successfully applied for developing abiotic (Wangxia *et al.* 2003) and biotic stress resistance through genetic transformation in different crops (Islam, 2006).

1.3 Objectives of study

The objectives of this study are:

- i. To study the effects of MS strength and carbon sources on *in vitro* seed germination of tomato.
- ii. To investigate the effects of plant growth regulators and explants on callus induction.
- iii. To investigate the effects of carbon sources on callus induction.

1.4 Significant of study

The purpose of this study was to establish a tissue culture protocol for callus induction in tomato (*Lycopersicon esculentum* Mill), also this study was to determine the effect of MS strength and carbon sources on *in vitro* seed germination. The other goal of this study was to analyse the effect of different plant growth regulators (different combination and concentration) and different carbon sources (sucrose and maltose) on callus induction in tomato by using MS media. Moreover, this protocol provided a basis for future studies on genetic improvement and could be applied to study large-scale multiplication systems. It has also benefit to establish an efficient tissue culture protocol to provide an important tool to be used in tomato breeding program.

1.5 Scope of study

Plant tissue culture is a major tool of biotechnology, it has a great role in improving productivity of crops through rapid availability of superior planting stock (Mohamed *et al.*, 2010). Plant biotechnology may also assist plant breeders by producing and manipulating genetic variability. The impact of plant biotechnology in plant breeding involves improving both crop quantity and quality. This improvement can be obtain by controlling infection which caused by pathogens and parasites and also increasing the plant tolerance against biotic and a biotic stress (Abdel-Rahim *et al.*, 2007).

This study includes the effect of carbon source (maltose and sucrose) and strength of MS media on *in vitro* seed germination of tomato plant. Consequently, cultured hypocotyls and cotyledons explants on MS media at different concentration and combination of PGRs such as 2,4-D (dichlorophenoxyacetic acid) (auxin), BAP (6-benzylaminopurine) (cytokinin) and GA₃ (gibberellin) under sterile conditions and also to evaluate the effect of carbon sources on callus induction in tomato (*Lycopersicon esculentum* Mill).

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