UNIVERSITI PUTRA MALAYSIA

IMPACT OF SALINITY ON SEED GERMINATION AND ANTIOXIDATIVE PROFILE OF TOMATO LEAVES (Solanum lycopersicum L. cv.MT1)

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By

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IMPACT OF SALINITY ON SEED GERMINATION AND ANTIOXIDATIVE PROFILE OF TOMATO LEAVES (*Solanum lycopersicum* L. cv.MT1*)*

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Chairman : Rosimah binti Nulit, PhD
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Salinity is a serious problem in many parts of the world that limits the productivity of agricultural crops. Even though, it is one of the major abiotic stresses that affect almost every aspect of physiology and biochemistry of plants but at certain amount could be beneficial to plant. Many studies had been done to determine the effects of salt stress towards the morphology, physiology, biochemistry and productivity of tomato plant but study on germination and antioxidant activity on tomato plants is still limited. This work aimed to study the effects of NaCl on the germination, histological of seedling leaves, and antioxidant profiling of *Solanum lycopersicum* cv. MT1. A 20 surface sterilized of tomato MT1 seeds were germinated in sterilized petri dishes and treated with 6 ml of 1, 3, 5, 10, 15, 30 mM NaCl separately and deionized water used as a control to study the effects of NaCl on tomato seed germination. Plates were sealed with parafilm to prevent evaporation followed by placed in Random Block Design in the growth room at 25˚C and 16/8 h (light / dark) for 10 days. Seeds were considered germinated when radicle had extended at least 2 mm. The water uptake, germination percentage (% GP), seed vigor, salt tolerance, length of hypocotyl and radical, and biomass of seedlings were measured. Results found that the germination of MT1 seed adversely delayed and decreased as NaCl concentration increased. The water uptake, germination percentage, seed vigor and salt tolerance decreased when the concentration of NaCl increased except 3 mM NaCl showed the highest germination percentage (87.5%) and vigor (11.4). The water uptake, seed vigor and salt tolerance decreased when as the increased of NaCl. The response of hypocotyls and radical under NaCl treatment are different which is not significantly (ANOVA, $P<0.05$) affected the length of hypocotyl but significantly reduced the radical length (ANOVA, $P<0.05$) except in 3 mM NaCl. NaCl increase the seedling length up to 3 mM (13 cm). Increased NaCl concentration not significantly (ANOVA, $P<0.05$) reduced the shoot dry weight (ANOVA, $P<0.05$), on other hand, the root dry weight reduce significantly (ANOVA, $P<0.05$) as NaCl concentration higher. At day 10, seedling leaves were collected from control, 10 and 30 mM NaCl and fixed for histological studies. Histological of seedlings leaves highly affected by NaCl concentration. The entire structural of histological seedling leaves highly disrupted as NaCl concentration increased as can be compared between 10 mM and 30 mM NaCl. The structure of epidermis cells on both epidermal layers totally changed. Moreover, the arrangement of mesophylls tissues was also in disorder. A major reduction in veins tissue dimensions were observed and this lead on its ability to conduct water and nutrients into seedlings system. To study the effects of NaCl to the antioxidant profiling of tomato plant. Four different concentration of NaCl treatment which is 5, 10, 15 and 30 mM and deionized water as a control was used. 21 days old seedlings were planted in the pots containing 1 kg of mix soil (3 top soil: 1 sand). Each treatment has 6 replicates. The seedlings were watered with 100 ml of NaCl and alternate with 100 ml ultra-pure water every 3 days for six weeks. After 6 six weeks, total chlorophyll, total phenolic, flavonoid and proline and anti-oxidative activity were measured. Results showed that NaCl increase total chlorophyll, total phenolic, flavonoid and proline and anti-oxidative activity. Total chlorophyll found higher in 15 and 30 Mm NaCl which is 41.7 mg and 39.6 mg/0.1 g compared with control plant only 24.1 mg/0.1 g. Results showed an increasing of total phenolic, flavonoid, and proline content with increasing of NaCl. The highest amount of phenolic and flavonoid achieved at 15 mM NaCl higher than the control plant and other treatment. Results also found that total proline increase as NaCl increased. 15 and 30 mM NaCl show higher total proline content which is 0.041 and 0.044 mg/0.1g compared with control (0.016 mg/0.1g) and other NaCl treatment. The maximum of DPPH and reducing power activity were observed in the 5 mM NaCl while antioxidant reduced as NaCl increase up to 30 mM NaCl. As conclusion, raising the concentration of NaCl had adversely affected on the germination and early growth of seedlings (except 3 mM NaCl). The histological of MT1 seedlings leaves highly affected by NaCl. Treatment tomato plants with moderate concentration of NaCl able to increase the anti-oxidative properties and activity of tomato plant.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk Ijazah Master Sains

KESAN KEMASINAN KE ATAS PERCAMBAHAN BIJI BENIH DAN PROFIL ANTIOKSIDAN DAUN TOMATO (*Solanum lycopersicum* L. cv. MT1)

Oleh

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Kemasinan merupakan masalah serius di kebanyakan tempat di dunia dimana menghadkan produktiviti tanaman. Walaupun, kemasinan salah satu faktor abiotik yang memberi kesan kepada hampir setiap aspek fisiologi dan biokimia tumbuhan tetapi pada amaun tertentu bermenfaat kepada tumbuhan. Kesan tegasan kemasinan terhadap morfologi, fisiologi, biokimia dan produktiviti ke atas tomato telah banyak tetapi kajian ke atas percambahan dan aktiviti antioksidan pada tanaman tomato masih terhad. Kajian ini bertujuan untuk mengkaji kesan NaCl pada percambahan, histologi anatomi, dan profil antioksidan tomato (*Solanum lycopersicum* cv. MT1). Satu siri kepekatan NaCl iaitu 1, 3, 5, 10, 15, 30 mM dan air ternyahion sebagai kawalan telah digunakan untuk mengkaji kesan NaCl ke atas percambahan benih tomato. Sejumlah 20 biji benih tomato MT1 dimana permukaannya disteril terlebih dahulu disemai di dalam piring petri dan diberi rawatan dengan 6 ml larutan NaCl pada kepekatan 1, 3, 5, 10, 15, 30 mM NaCl dan air ternyahion digunakan sebagai kawalan. Semua piring petri dimeterai dengan parafilm untuk mengelakkan penyejatan dan ditempatkan secara blok rawak dalam bilik kultur pada suhu 25°C dan 16/8 h (terang/gelap) selama 10 hari. Biji benih dianggap bercambah apabila radikel telah tumbuh sekurang-kurangnya 2 mm. Peratusan percambahan (% GP), vigor bijibenih, toleransi terhadap kemasinan, panjang hipokotil dan radikel serta biomas anak benih diukur. Kajian mendapati, percambahan dan vigor biji benih MT1 tomato adalah lambat dan menurun dengan meningkatnya kepekatan NaCl kecuali pada rawatan 3 mM NaCl dimana peratus percambahan (87.5%) and vigor adalah yang tertinggi (11.4). Kajian juga mendapati gerakbalas hipokotil dan radikel terhadap NaCl berbeza dimana NaCl tidak memberikan kesan yang signifikan (ANOVA, $P<0.05$) ke atas kepanjangan hipokotil dan biomas pucuk tetapi mengurangkan pemanjangan radikel dan biomas akar (ANOVA, $P<0.05$) kecuali pada rawatan 3 mM NaCl. Pada hari ke sepuluh, daun anak-anak benih daripada kawalan, 10 mM dan 30 mM NaCl telah di awet untuk kajian histologi. Hasil kajian ini juga mendapati histologi daun anak benih sangat dipengaruhi oleh kepekatan NaCl. Keseluruhan struktur histologi daun anak benih tomato berubah apabila kepekatan NaCl meningkat seperti yang ditunjukkan pada kepekatan 10 mM dan 30 mM NaCl. Struktur dan bentuk sel-sel epidermis pada kedua-dua lapisan epidermis juga berubah. Kajian juga mendapati susunan kedua-dua jenis tisu mesofil tidak teratur. Di samping itu, pengurangan saiz tisu veina sangat ketara pada rawatan 10 mM dan 30 mM NaCl dan ini mengurangkan keupayaan pengangkutan air dan nutrient ke dalam anak benih. Untuk mengkaji kesan NaCl ke atas profil antioksidan pokok tomato, empat kepekatan NaCl iaitu 5, 10, 15 dan 30 mM dan air ternyahion sebagai kawalan digunakan. Anak benih yang berumur 21 hari ditanam di dalam pot yang mengandungi 1 kg tanah campuran (3 tanah atas:1 pasir). Setiap rawatan mengandungi 6 replikasi. Anak-anak benih disiram dengan 100 NaCl dan diselangi dengan 100 ml air ternyahion setiap 3 hari selama 6 minggu. Jumlah klorofil, jumlah fenolik, flavonoid, prolina dan aktiviti antioksidan diukur. Hasil kajian mendapati rawatan NaCl meningkatkan kandungan klorofil, fenolik, flavonoid dan prolina. Kandungan klorofil didapati tinggi pada rawatan 15 dan 30 mM NaCl iaitu 41.7 mg/0.1g dan 39.6 mg/0.1g berbanding tumbuhan kawalan hanya 24.1 mg/0.1g dan lain-lain rawatan. Jumlah tertinggi fenolik dan flavonoid ditunjukkan pada 15 mM NaCl. Hasil kajian mendapati kandungan prolina meningkat dengan peningkatan NaCl (ANOVA, $p<0.05$) dimana 15 dan 30 mM NaCl menunjukkan kandungan prolina 0.041 mg/0.1g dan 0.044 mg/0.1g berbanding kawalan hanya 0.016 mg/0.1g. Hasil kajian menunjukkan bahawa aktiviti DPPH dan kuasa penurunan adalah tertinggi dalam 5 mM NaCl dan semakin menurun pada kepekatan NaCl yang tinggi. Kesimpulannya, peningkatan kepekatan NaCl menyebabkan percambahan dan pertumbuhan awal anak benih lambat dan menurun (kecuali pada 3 mM NaCl). NaCl menjejaskan histologi daun anak benih tomato MT1. Rawatan garam dapat meningkatkan nilai antioksidan dan juga aktiviti pokok tomato MT1.

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I certify that a Thesis Examination Committee has met on 28 January 2016 to conduct the final examination of Asma Abubaker A Shanab on her thesis entitled "Impact of Salinity on Seed Germination and Antioxidative Profile of Tomato Leaves (*Solanum lycopersicum* L. cv. MT1)" in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The Committee recommends that the student be awarded the Master of Science.

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This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Master of Science. The members of the Supervisory Committee were as follows:

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%	percentage
˚C	degree Celsius
µl	Microliter
$AlCl_3$	Aluminium chloride
ANOVA	Analysis of Variance
Ca^{2+}	Calcium ion
Cl^-	Chloride ion
Cm	Centimetre
CO_2	Carbon Dioxide
Cu	Copper
DPPH	2, 2- diphenyl-1-picrylhidrazy
DPX	Diputal petroleum xylene
DW	Dry weight
Fe	Iron
FW	Fresh Weight
G	Gram
Mg	Milligram
Mg^{2+}	magnesium ion
$MgCl_2$	Magnesium chloride
mg/µl	milligram per microliter
mg/l	milligram per liter
ml	Milliliter
mM	miliMolar
Na^+	Ammonia
Na_2co_3	Sodium carbonate
NaCl	Sodium Chloride
NADH	nicotinamide adenine dinucleotide reduced
Nm	Nanometre
PSI	Photosystem I
ROS	Reactive Oxygen Species
v/v	volume per volume
w/v	weight per volume

CHAPTER 1

INTRODUCTION

1.1 Background of study

Nowadays, the phenomenon of the salinity is one of the most serious problems facing soil and also effecting seed germination and plant growth. It is becoming one of the issues that hamper the economy in most countries of the world. Salinity is a common term used to describe the presence of elevated levels of different salt content such as sodium chloride, magnesium and calcium sulphates in soil and water. It has become a major problem in agriculture as high concentration of minerals in soil that causes salt stress to plant. Salinization of soil is one of the major factors limiting crop reproduction particularly in semi-arid and arid regions of the world (Ahmed, 2009). Salt stress can be defined as excess in ions especially in sodium ions (Na^+) and chloride ions (Cl^-). This salt stress reduces the ability of the plant to take up water which can reduce the growth rate as well as causes metabolic changes similar to water stress conditions (Munns, 2002).

Salinity is a common environmental challenge all around the world. Soil salinity significantly reduces the productivity of economical important plants. Salts in the soil water prevent the growth of plants because of two reasons. First, the presence of salt in the soil solution reduces the ability of the plant to absorb water, and consequently growth rate will be decreased. This is called the osmotic or water-deficit effect of salinity. Secondly, if excessive amounts of salt enter the plant in the transpiration stream, the cells can be damaged in the transpiring leaves and this may cause more reductions in growth. This is called the salt-specific or ion-excess effect of salinity (Yao & Fang, 2009).

In general, seed germination is considered as an important stage in plants growth cycle. In fact, it controls the beginning of seedling growth. Salinity factor's has a critical influence on the seed germination which is the most sensitive step in plant growth (Ahmad *et al*., 2009) and the seeds exposed to unfavorable conditions like salinity may have to compromise the seedlings development (Albuquerque & Carvalho, 2003).

Tomato (*Solanum lycopersicum*) belongs to the family *Solanaceae* that includes several additional economically important crops such as potato, pepper and eggplant. Tomato is native to Southern North America, Central and South and it is the most popular and second important frequently consumed vegetable crop grown throughout the world after potato (Dorais *et al*., 2008). It is recognized as a highly valuable and nutritious food. These days, tomato is one of the major vegetable throughout the world. It is grown both in the open and green house. Moreover, it is fruit contain a number of health-beneficial compounds, such as high potassium content, iron, vitamins A, B, C and lycopene (Preedy, 2008).

1.2 Problem Statements and Objectives of Study

The main aim of this work is to study the effects of salt stress on tomato plant. Many studies had been conducted regarding the effects of salt stress towards growth, physiology and productivity of tomato plant but study on the effect of salinity on seed germination and antioxidant activity of tomato plant in response to salt stress is still limited.

Thus, this present research was conducted because seed germination is a critical stage in the life cycle of plants and salt tolerance during germination is crucial for the establishment of plants that grow in saline soils. In addition, study on the effects of NaCl on the germination and antioxidant activity has never been conducted of tomato MT1. Salt stress study also contributes to productivity of agriculture and ways to overcome the problem of salinity in agriculture. This is important to increase food production and fulfill food demand worldwide. The objectives of study are:

1. To study the effects of different concentrations of NaCl on the germination and early seedling growth of tomato.
2. To study the effects of different concentrations of NaCl on the histological leaves of tomato seedlings
3. To study the effects of different concentrations of NaCl on anti-oxidative profiling of tomato plant

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