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GERMINATION, SEEDLING GROWTH AND ANATOMICAL RESPONSES OF Cucumis sativus cv. MTi2 IN DIFFERENT SALTS AND DEVELOPMENT OF GERMINATION ENHANCER

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By

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Chairman : Associate Professor Rosimah Nulit, PhD
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Salinity continues to be one of the most serious environmental problems. One of the strategies in dealing with salinity is producing salt tolerant plants and understanding the effects of salinity on crops. Seed germination is an important stage in the life cycle of plants and understanding of tolerance to salinity during the germination stage is crucial for the establishment and management of plant in saline soils. Many studies had been conducted using the effect of NaCl on seed germination, and although there are other types of salt found in soil, until to date, studies about these salts are still limited. Therefore, this study aimed to compare the effects of NaCl, KCl, $MgCl_2$, $MgSO_4$ and $CaCl_2$ on the germination of MTi2 seed, early seedling growth and anatomical of seedling cotyledon leaves. This study also aimed to develop the liquid enhancer in order to improve the germination of salt stressed MTi2 seed. Five types of salts (NaCl, KCl, $MgCl_2$, $MgSO_4$ and $CaCl_2$) at different concentration (50,100,150, and 200 mM) and deionized water as a control were used with nine replicates for each treatment. A 10 sterilized MTi2 seeds were placed in petri dishes containing 5 ml of deionized water or each salinity solution and placed in a completely randomized design in the growth room at 25 ± 1°C. The number of germinated seeds was recorded daily until day 8. On day 8, the length of the hypocotyl, radicle and the biomass of seedlings was measured and the cotyledon leaves were fixed for anatomical study. Germination percentage, germination rate, seed vigor, relative salt injury rate, salt tolerance was calculated. To develop liquid enhancer, sterilized MTi2 seeds were primed with 300mM NaCl for 72 hours and then treated with Salicylic acid alone (SA) (0.25, 0.5, 0.75, 1mM) and KCl alone (10, 20, 30, 40, 50 mM) and deionized water as a control. Germination parameters were calculated as mentioned before. Following this, the ideal concentration of the SA and KCl was mixed together and was used to study its effectiveness as a germination enhancer on the salt-stressed MTi2 seeds. Data were analyzed using SPSS windows version 22. Data are subjected two way ANOVA at confidence level, p≤0. 05 to determine the significant difference between treatment and followed by Tukey at p≤0. 05 for means comparison. The Study found that the germination response of MTi2 seed on NaCl, KCl, $MgCl_2$, $MgSO_4$ and $CaCl_2$ is significantly different. MTi2 seeds are able to germinate in KCl and NaCl until the high concentration (200 mM) which is % GP more than 50%. In addition, MTi2 seeds able to germinate in medium and low concentration of $CaCl_2$ (50-100 mM) and $MgSO_4$ (50 mM) but unable to germinate in any concentration of $MgCl_2$. The germination rates and the vigor of seed found higher in KCl and NaCl but lower in $CaCl_2$ and $MgSO_4$. Results also found Relatives Salts Injury Rate of MTi2 germinating seed are very low in KCl and NaCl but higher in $CaCl_2$ and $MgSO_4$. The tolerance level of MTi2 seed on different types of salts can be concluded as follows, KCl > NaCl > $CaCl_2$ > $MgSO_4$ > $MgCl_2$. This study also found that 50 mM KCl increased the germination percentage, germination rate and the vigor of MTi2 seeds. Histological studies revealed that changes of MTi2 cotyledon leaves as a response to NaCl, KCl, $MgCl_2$, $MgSO_4$ and $CaCl_2$ are different. The degree histological changes of MTi2 cotyledon leaves increased as the concentration of salts increased. Another type of salts significantly changes the structure and arrangement of the upper epidermis cells, mesophyll tissue and lower epidermis cells, also reduce of intracellular space among the mesophyll cells. Results also found that no anatomical changes of MTi2 cotyledon leaves in any concentration of KCl. Results showed that 0.5-0.75 mM SA and 20-30 mM KCl are the ideal concentration that increased significantly the germination and the early growth of MTi2 seedling compared with control treatment. Moreover, the combination of the ideal concentration of SA (0.5 -0.75 mM) and KCl (20-30 mM) increased the germination and the early growth of MTi2 seedlings more than 1x higher than SA and KCl individually. Salt-stressed MTi2 seeds treated with salicylic acid (SA) and low level of (KCl) can contribute in mitigation of the deleterious effects of salinity stress and improve seed germination percentage, germination rate, seed vigor, seedling length and seedling biomass. It can be concluded that the tolerance level of MTi2 seed on different types of salts can be summarized as follows, KCl > NaCl > $CaCl_2$ > $MgSO_4$ > $MgCl_2$. Low concentration of a mixture of KCl and SA can be used as an enhancer to increase germination of salt stressed MTi2 seeds.

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

PERCAMBAHAN, PERTUMBUHAN ANAK BENIH, GERAKBALAS ANATOMI *Cucumis sativus* cv. MTi2 TERHADAP BERLAINAN GARAM-GARAM DAN PEMBANGUNAN PENGGALAK PERCAMBAHAN BAGI BIJI BENIH TIMUN YG DIBERIKAN TEGASAN GARAM

Oleh

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April 2017

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Kemasinan terus menjadi salah satu masalah alam sekitar yang serius. Salah satu strategi dalam menanganinya dengan menghasilkan tumbuhan yang toleran dengan kemasinan dan memahami kesannya kepada tanaman. Percambahan benih adalah satu peringkat yang penting dalam kitaran hidup tumbuhan dan kefahaman toleransi kepada kemasinan pada peringkat ini adalah penting bagi pertumbuhan pokok. Banyak kajian telah dijalankan menggunakan kesan NaCl pada percambahan benih, dan walau kandungan tanah terdapat garam yang lain, kajian mengenai garam-garam ini masih terhad. Oleh itu, kajian ini dijalankan bertujuan untuk membandingkan kesan NaCl, KCl, $MgCl_2$, $MgSO_4$ dan $CaCl_2$ pada percambahan biji benih timun MTi2, pertumbuhan awal anak benih dan anatomi daun kotiledon anak benih. Kajian ini juga bertujuan untuk membuat satu larutan formulasi yang dapat meningkatkan percambahan garam benih MTi2 yang diberikan tegasan garam. Lima jenis garam (NaCl, KCl, $MgCl_2$, $MgSO_4$ dan$CaCl_2$) pada kepekatan yang berbeza (50, 100,150, dan 200 mM) dan air ternyahion sebagai kawalan digunakan dengan sembilan replikat pada setiap rawatan. Sebanyak 10 bijibeih MTi2 disterilkan diletakkan di dalam piring petri yang diberi 5 ml air ternyahion atau larutan garam dan diatur secara rawak di ruang pertumbuhan pada suhu 25±1°C. Bilangan benih bercambah dicatatkan setiap hari sehingga hari ke 8. Pada hari ke 8, panjang hipokotil, radikel dan biomas benih diukur. Daun kotiledon diambil dan diawetkan untuk kajian anatomi. Peratusan percambahan, kadar percambahan, vigor benih, kadar kecederaan relatif, toleransi terhadap garam dikira. Untuk menyediakan formulasi, bijibenih MTi2 telah direndam dengan 300mm NaCl selama 72 jam untuk memberikan tegasan garam kepada bijibenih MTi2 dan kemudian dirawat secara berasingan dengan asid salisilik sahaja (SA) (0.25, 0.5, 0.75, 1mM) dan KCl (10, 20, 30, 40, 50 mM) dan air ternyahion sebagai kawalan.

Ini bertujuan untuk mendapatkan kepekatan yang ideal. Parameter percambahan dikira seperti yang dinyatakan sebelum ini. Kepekatan SA dan KCl yang telah dicampurkan bersama-sama dan digunakan untuk mengkaji keberkesanannya sebagai penggalak untuk percambahan bijibenih MTi2 yang telah diberikan tegasan garam. Data dianalisis dengan menggunakan SPSS versi window 22. Analisis data meggunakan ‘two way “ ANOVA pada tahap aras keertian, p=0.05 untuk menentukan perbezaan signifikan di antara rawatan-rawatan dan diikuti oleh DMRT pada p=0.05 untuk perbandingan antara purata rawatan. Kajian mendapati bahawa tindakbalas percambahan benih MTi2 pada NaCl, KCl, $MgCl_2$, $MgSO_4$ dan $CaCl_2$ adalah sangat berbeza. Benih MTi2 didapati bercambah dalam KCl dan NaCl sehingga kepekatan yang tinggi (200 mM) dimana peratus percambahan (% GP) lebih daripada 50%. Bijibenih MTi2 hanya boleh bercambah dalam kepekatan $CaCl_2$ yang sederhana (50-100 mM) dan pada kepekatan yang rendah (50 mM) dalam $MgSO_4$ tetapi tidak bercambah dalam mana-mana kepekatan $MgCl_2$. Kadar percambahan dan vigor benih didapati lebih tinggi dalam rawatan KCl dan NaCl berbanding dalam rawatan $CaCl_2$ dan $MgSO_4$. Keputusan juga menunjukkan kadar kecederaan relatif benih MTi2 sangat rendah dalam KCl dan NaCl tetapi lebih tinggi dalam $CaCl_2$ dan $MgSO_4$. Oleh itu, tahap toleransi benih MTi2 terhadap garam dapat disimpulkan seperti berikut, KCl> NaCl> $CaCl_2$> $MgSO_4$> $MgCl_2$. Kajian ini juga mendapati bahawa 50 mM KCl meningkat peratusan percambahan, indeks percambahan dan vigor benih MTi2. Kajian histologi menunjukkan bahawa perubahan histologi MTi2 daun kotiledon sebagai tindak balas kepada garam-garam adalah berbeza. Tahap perubahan histologi MTi2 daun kotiledon meningkat seiring dengan kepekatan garam. Rawatan benih MTi2 dengan garam didapati mengubah struktur dan susunan sel-sel epidermis atas, tisu-tisu mesofil dan sel-sel epidermis. Ruang intraselular antara sel-sel mesophyll juga semakin mengecil disebabkan oleh kesan tegasan larutan garam. Keputusan juga mendapati bahawa tiada perubahan anatomi MTi2 daun kotiledon dalam mana-mana kepekatan KCl. Hasil kajian menunjukkan bahawa 0.5-0.75 mM SA dan 20-30 mM KCl adalah kepekatan yang ideal yang dapat meningkat dengan kadar percambahan dan pertumbuhan awal MTi2 anak benih berbanding dengan rawatan kawalan. Selain itu, kombinasi campuran SA (0.5 -0.75 mM) dan KCl (20-30 mM) meningkatkan percambahan dan pertumbuhan awal anak benih MTi2 1x lebih tinggi daripada rawatan SA dan KCl secara berasingan. Sebagai kesimpulan, tahap toleransi benih MTi2 terhadap pelbagai jenis garam boleh diringkaskan seperti berikut, KCl> NaCl> $CaCl_2$> $MgSO_4$> $MgCl_2$. Kombinasi campuran SA dan KCl boleh digunakan sebagai penggalak untuk meningkatkan percambahan benih MTi2 yang diberikan tegasan garam.

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I certify that a Thesis Examination Committee has met on 21 April 2017 to conduct the final examination of Samar Jasim Mohammed on her thesis entitled "Germination, Seedling Growth, and Anatomical Responses of *Cucumis sativus* cv. MTi2 in Different Salts and Development of Germination Enhancer" 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 the Universiti Putra Malaysia and has been accepted as fulfillment of the requirement for the degree of Master of Science. The members of the Supervisory Committee were as follows:

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pH	Potential of hydrogen
Na^{+}	Sodium ion
Ca^{2+}	Calcium ion
Mg^{2+}	Magnesium ion
Cl^{-1}	Chloride ion
SO_4^{-2}	Sulfate ion
HCO_3^{-1}	Bicarbonate ion
NO_3^{-2}	Nitrate ion
CO_3^{-2}	Carbonate ion
K^{+}	Potassium ion
EC	Electrical conductivity
mm/l	Millimole/liter
mM	Millimole
SA	Salicylic acid
cm	Centimeter
DSm^{-1}	DeciSemens per meter
NaCl	Sodium chloride
KCl	Potassium chloride
$MgSO_4$	Magnesium sulfate
$MgCl_2$	Magnesium chloride
$CaCl_2$	Calcium chloride
GP	Germination percentage
GR	Germination rate

(%)	Percentage
(g)	Gram
HSD	(Honestly significant difference)
FAO	Food and Agriculture Organization

CHAPTER 1

INTRODUCTION

1.1 Background of the study

Plant exposes to abiotic and biotic stresses since decades ago that cause severe effects on metabolism, growth, development, and productivity (Vorasoot *et al.*, 2003; Kaur *et al.*, 2008; Thakur *et al.*, 2010; Doupis *et al.*, 2011). One of worldwide problem stress is salinity and caused about 323 million hectares worldwide land salinized (Brinkman, 1980) is estimated to exceed 400 million hectares by 2025 (Hakim *et al.,* 2014; Flowers & Muscolo, 2015).

Salinity changes soil properties, both physically and chemically, this eventually change the osmotic pressure (Rowell, 1988; Hossain *et al*, 2015). According to Zhao *et al.*, (2007), soil salinity causes substantial reduction in crop yields, particularly in arid and semi-arid regions with naturally high soil salt and low rainfall that inhibits leaching. The pH and availability of nutrients of soil such as phosphorus, iron and manganese affected by salinity (Hassan *et al*., 1970).

Adaptation of plants to salinity during germination and early seedling stages is critical for the plant stand to be established. The common cations in soil are Na^{+}, Ca^{2+}, Mg^{2+}, meanwhile Cl^{-}, SO_4^{2-}, and HCO^{3-} are the common anions (Flower *et al*., 1997; Hasegawa *et al*., 2000; Ali, 2010). Current studies found that different salts cause different effects on the germination and the growth of plants (Ghanad, 2016), this is due to the osmotic and ionic stress in each type of salts had different degrees of influence on germination and development (Panuccio, 2014). Studies demonstrate that the germination of plant is strongly affected by the nature of the ions in the salt solutions and their interactions (Sosa *et al.*, 2005). Seeds have different response to levels of salt during germination (Hussain *et al*., 2013). Variety or cultivar of plant species vary in various ontogenetic stages to salt tolerance, which give scope for choice of genotype for salt tolerance, indicating the differential response of genotypes in plant species to salinity (Ashagre *et al.*, 2013).

1.2 Problem Statement, Justification and Objectives of the Study

Germination stage in the life cycle of the plant is important and understanding the tolerance level of seed at this stage is needed. Many studies have been conducted extensively on the effect of NaCl on seed germination, but until to date, the study of the different type of salt on the germination of seed still limited.

The impact of salinity depends on its type, level and the genotype of plant species (Adolf, 2012; Ghanad, 2016). This study was conducted on the Malaysia cucumber cv. MTi2 which is the best known and most popular cucumber cultivars among the locals. Cucumber (*Cucumis sativus*) belongs to the gourd, family Cucurbitaceae. It is a widely cultivated, creeping vine that bears cylindrical, fruits that are used as culinary vegetables (Grubben *et al.*, 2004). The cucumber originated from South Asia, but is currently found on most continents. This plant is an important greenhouse crop in semi- arid areas with saline ground water. Therefore, it is important more study to be done on the impact of salinity on germination of this plant (Sato *et al.*, 2006). Cucumber plant was classified as glycophytes and moderately sensitive to salinity. In addition, previous studies focused on other cucumber cultivars to reveal the tolerance to salinity, however, tolerance study of Malaysian cucumber cv. MTi2 is still not done yet.

Therefore, the objectives of the present study are:

i. To study the effects of different concentration of NaCl, KCl, $MgCl_2$, $MgSO_4$, and $CaCl_2$ on the germination, early seedling growth and the anatomical changes of *C. sativus* cv. MTi2.
ii. To develop the liquid enhancer in order to improve the germination of salt stressed *C. sativus* cv. MTi2 seed.

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