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GERMINATION CAPABILITY OF *AMARANTHUS* spp. IN DIFFERENT SALTS, SALTS STRESS ALLEVIATION AND HYDROTIME MODEL ANALYSIS

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By

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Chairman : Prof. Madya Rosimah binti Nulit
Faculty : Science

Salinity continues to be one of the abiotic stresses which effect plant especially at the germination and early seedling growth. Extensive amount of lands in the world is affected by salinity and is increasing day by day due to secondary salinization. The continuous crop cultivation on same land, improper irrigation technique as well as overuse of fertilizers intensify the soil salinity problems in Malaysia. Despite most of the salinity studies were on sodium chloride as saline condition, it is hypothesized that other salts may affect the plant growth. However, study on the effect of other salts on seed germination and early seedling growth remains insufficient. The effects of sodium chloride (NaCl), potassium chloride (KCl), magnesium chloride ($MgCl_2$) and magnesium sulphate ($MgSO_4$) on the germination and early seedling growth of *Amaranthus tricolor* L. and the stress alleviation by inorganic compounds was carried out for this study. This study also aimed to evaluate the germination capability for four different species of *Amaranthus* namely *Amaranthus hybridus* L., *Amaranthus viridis* L., *Amaranthus tricolor* L. and *Amaranthus giganticus* L. using hydrotime model analysis. Ten sterilized seeds of *A. tricolor* were germinated in sterilized petri dishes containing 5 ml of deionized water (control), 50mM, 100mM, 150mM, 200mM, 250mM and 300mM of NaCl, KCl, $MgCl_2$ and $MgSO_4$ solution separately and placed in completely randomized design (CRD) in laboratory condition for seven days. Germination percentage, germination rate, mean germination time, relative injury rate, seed vigor and early seedling growth were measured. For the stress alleviation study, *A. tricolor* seeds which initially primed in 300mM NaCl for 72 hours to induce stress condition were germinated in solution of KCl (5, 10, 20, 30 and 50 mM), potassium nitrate (KNO_3) (5, 10, 25, 50, 75 mM) and thiourea (TU) (1.0, 2.0, 3.5, 7.0 and 10.0 mM). Following this, the ideal concentration of KCl, KNO_3 and TU was mixed together to study the effectiveness in alleviating salt stress on *A. tricolor*. One-way ANOVA at confidence level (p=0.05) was used to determine the significant difference between treatment followed by Tukey test

for mean comparison. For the study on the germination capability using hydrotime model analysis, 25 seeds from each four different *Amaranthus* species were germinated in petri dishes at salinity condition with 0 (control), -0.3, -0.6, -0.9 and -1.2 MPa. Repeated probit regression analysis was used to evaluate the salinity sensitivity of each *Amaranthus* species. The data for this study was evaluated using SPSS windows version 24. Results showed that *A. tricolor* more tolerance to NaCl compared to KCl, $MgCl_2$ and $MgSO_4$ during germination. However, greater reduction was pronounced at early seedling growth of *A. tricolor* in NaCl compared to KCl. Salinity stress also decreased the seed vigor and increased relative injury rate. 50mM KCl was found to enhance the hypocotyl length of *A. tricolor* seedlings. However, $MgCl_2$ and $MgSO_4$ suggested to have inhibitory effects on the growth since abnormal seedlings were observed at all concentrations. Results found that 5mM and 10mM of KCl and 5mM, 10mM and 25mM of KNO_3 showed positive effect on hypocotyl and early seedling growth of salt stressed *A. tricolor* while 1.0mM TU enhance the root growth. The combination of KNO_3, KCl and TU solution at the optimum concentration increased the seedling growth compared to control with combination of KCl (5mM), KNO_3 (10 mM) and TU (1.0 mM) give the highest seedling growth. Apart from that, in the hydrotime analysis of *Amaranthus* species, it showed that *A. giganticus* showed better tolerance towards salinity than *A. hybridus*, *A. viridis* and *A. tricolor* with more negative value of $\Psi_{b(50)}$ compared to the other species. In conclusion, the presence of different salt components as well as increased in salt concentrations affects the germination and early seedling growth of *Amaranthus* sp. to a certain degree. Nevertheless, the availability of inorganic compounds helps to mitigate the salinity effects on *Amaranthus* sp. seedling growth. Variation in the degree of tolerance among different *Amaranthus* species to salt stress was observed with *A. giganticus* showed more tolerance.

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

KEUPAYAAN PERCAMBAHAN BENIH *AMARANTHUS* spp. DI DALAM GARAM BERBEZA, PENGURANGAN KESAN TEGASAN GARAM DAN ANALISIS MENGGUNAKAN HYDROTIME MODEL

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Kemasinan menjadi salah satu daripada komponen tegasan abiotik yang merencatkan pertumbuhan pokok terutama pada peringkat percambahan biji benih dan pertumbuhan awal anak benih. Keluasan kemasinan tanah semakin bertambah dari hari ke hari disebabkan kemasinan sekunder. Di Malaysia, aktiviti pertanian yang berterusan di tanah yang sama, teknik pengairan yang tidak efisien serta penggunaan baja yang terlalu banyak adalah faktor yang meningkatkan lagi masalah kemasinan tanah. Penyelidikan terdahulu kebanyakannya menggunakan NaCl tetapi kajian kesan garam yang lain masih terlalu sedikit. Kesan natrium klorida (NaCl), kalium klorida (KCl), magnesium klorida ($MgCl_2$) dan magnesium sulfat ($MgSO_4$) terhadap percambahan dan pertumbuhan awal anak benih bayam (*Amaranthus tricolor* L.) dan pengurangan kesan tekanan garam menggunakan sebatian bukan organik dijalankan dalam kajian ini. Kajian ini turut membabitkan penilaian keupayaan percambahan empat jenis bayam berbeza iaitu *Amaranthus hybridus* L., *Amaranthus viridis* L., *Amaranthus tricolor* L. dan *Amaranthus giganticus* L. yang diberi tegasan kemasinan menggunakan analisis 'hydrotime' model. Benih *A. tricolor* dicambahkan di dalam piring petri yang mengandungi 5 ml air ternyahion sebagai kawalan dan NaCl, KCl, $MgCl_2$ dan $MgSO_4$ dengan julat kepekatan: 50 mM, 100 mM, 150 mM, 200 mM, 250 mM dan 300 mM. Setiap piring petri diletakkan secara rawak di dalam makmal selama 7 hari. Peratusan percambahan, kadar percambahan, purata masa percambahan, kadar kecederaan relatif, vigor benih dan pertumbuhan awal benih diukur. Manakala bagi eksperimen untuk meningkatkan percambahan dan pertumbuhan benih yang diberi tegasan NaCl, benih *A. tricolor* diberi tegasan garam selama 3 hari pada 300 mM NaCl, kemudian dicambah di dalam larutan KCl (5, 10, 20, 30 dan 50 mM), KNO_3 (5, 10, 25, 50 dan 75 mM) dan thiourea (TU) (1.0, 2.0, 3.5, 7.0 dan 10.0 mM) untuk mendapatkan kepekatan ideal bagi setiap sebatian inorganik. Seterusnya, kepekatan ideal bagi KCl, KNO_3 dan TU digabungkan bersama dan potensinya sebagai penggalak terhadap percambahan dan pertumbuhan awal benih *A. tricolor* yang telah diberi tegasan garam. 'One-way' ANOVA dan ujian Tukey pada aras keertian (p=0.05) digunakan untuk menentukan perbezaan di antara rawatan. Bagi analisa 'hydrotime', 25 benih dari setiap empat jenis bayam berbeza dicambahkan di dalam piring petri yang mengandungi larutan garam dengan 0, -0.3, -0.6, -0.9 dan -1.2 MPa. Analisis Probit Regresi digunakan untuk menganalisa sensitiviti kemasinan bagi setiap jenis bayam (*Amaranthus* spp.). Analisa data dengan menggunakan SPSS versi windows 24. Kajian mendapati *A. tricolor* menunjukkan tahap toleransi lebih tinggi terhadap NaCl dari KCl, $MgCl_2$ dan $MgSO_4$ dari sudut percambahan. Peningkatan tegasan kemasinan menurunkan vigor benih dan meningkatan kecederaan relatif. Walaubagimanapun, kajian mendapati 50 mM KCl meningkatkan panjang hipokotil bagi *A. tricolor*. Sebaliknya, $MgCl_2$ dan $MgSO_4$ merencat percambahan dan pertumbuhan awal benih di mana anak benih didapati tidak normal pada setiap kepekatan yang digunakan. Kajian menunjukkan 5mM dan 10mM KCl dan 5mM, 10mM dan 25mM KNO_3 meningkatkan pemanjangan hipokotil dan pertumbuhan awal pokok *A. tricolor* di samping itu, 1.0 mM TU menggalakan pertumbuhan akar. Kombinasi KCl (5mM), KNO_3 (10 mM) dan TU (1.0 mM) pada kepekatan optimum meningkatkan pertumbuhan awal benih *A. tricolor* yang diberi tegasan NaCl. Analisis 'hydrotime' mendapati *A. giganticus* lebih toleransi terhadap kemasinan berbanding *A. hybridus*, *A. viridis* dan *A. tricolor* dengan nilai $\Psi_{b(50)}$ yang lebih negatif berbanding jenis bayam lain. Kesimpulannya, garam yang berbeza dan semakin bertambah tegasan garam memberi kesan terhadap percambahan dan pertumbuhan awal benih *Amaranthus* sp. Namun begitu, kajian menunjukkan sebatian bukan organik mampu membantu dalam mengurangkan kesan tegasan garam terhadap pertumbuhan *Amaranthus* sp. Hasil kajian juga mendapati *A. giganticus* lebih toleransi terhadap kemasinan dalam kalangan *Amaranthus* spp. yang dikaji.

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This thesis was submitted to the Senate of the 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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ANOVA	Analysis of Variance
$CaCl_2$	Calcium chloride
CAT	Catalase
dSm^{-1}	deciSiemens per meter
EC	Electrical Conductivity
ESP	Exchangeable Sodium Percentage
FAMA	Federal Agricultural Marketing Authority
FAO	Food and Agriculture Organization
HSD	Honestly Significance Difference
ISTA	International Seed Testing Association
KCl	Potassium chloride
KNO_3	Potassium nitrate
$MgCl_2$	Magnesium chloride
$MgSO_4$	Magnesium sulphate
Na_2SO_4	Sodium sulphate
NaCl	Sodium chloride
$NaNO_3$	Sodium nitrate
POD	Peroxidase
R^2	Coefficients of determination
ROS	Reactive Oxygen Species
SOD	Superoxide dismutase
SPSS	Statistical Package for the Social Sciences
SSL	Self Sufficiency Level
TU	Thiourea
UN	United Nation
USDA	United States Department of Agriculture
θ_H	Hydrotime constant
$\Psi_{b(50)}$	Base water potential for 50% germination
$\Psi_{b(g)}$	Base water potential
Ψ_w	Water potential

CHAPTER 1

INTRODUCTION

1.1 Background of study

Global population is estimated to reach 9.7 billion in 2050 and 11.2 billion in 2100 (UN, 2015). The projected increased in world population demands the increase in food supply however agriculture nowadays are threatened by environmental constraints in the form of abiotic stresses which adversely influence plant growth and development causing crop failure and decreasing plant yields (Ahmad et al., 2015; Porcel, Aroca, & Ruiz-Lozano, 2012). Predominant abiotic stresses such as drought, heat, cold and high salinity reportedly generated up to 70% loss in plant survival, biomass production and yield (Ahmad et al., 2012, 2010; Chellamma & Pillai, 2012; Mantri et al., 2012; Thakur et al., 2010). Among the abiotic stresses, salinity is a major threat to agriculture. The damage caused by salinity stress on plant growth and development is aggravated by the global environmental changes due to rising temperature and sea water level. Besides, the usage of improper irrigation methods, poor quality water and deforestation has increased the salinization problem.

The most important stages in the life cycle of plant are germination and early growth where firm plant establishment from these two stages will determine the plant density, consistency and management options (Hubbard, Germida, & Vujanovic, 2012) but abiotic stress hampered these processes which then negatively affected the plant growth and development. In the heterogenous environment of crop planting, the ability of a seed to germinate and continued seedling growth is crucial to ensure succession in plant propagation (Ma et al., 2016). According to Schwabe, Iddo, and Knapp (2006), salinity is one of the most brutal environmental constraints that resulted in extensive crop yield losses all over the world with its threat is intensify day by day. All around the world, salinization has become a main environmental problem with about 8.31 x 10^6 km^2 of soil jeopardized by salinization accounting for about 7% of the world's land (Ghassemi, Jakeman, & Nix, 1995) and further increased by 1-1.5 x 10^4 km^2 annually (Li et al., 2014). By 2050, it is expected that the arable land affected by direct and indirect salinization will reach an alarming level (Joshi, Saxena, & Arora 2011).

Plant adaptation to salinity during germination and seedling stages is very important as crop establishment depends on successful germination and seedling emergence. It is reported that saline soil contains several different component of soluble salts in which each posses a different effect on the initial growth of plant at germination and seedling stages as well as the influx and outflux of cations in the seedlings (Abou-Shady, 2016; Tobe, Li, & Omasa, 2002; Ungar, 1996). Depending on the type of salts, Panuccio et al. (2014) found that there is existence of both ionic and osmotic effects on the seed germination.

1.2 Problem statement, justification and objectives of study

Seed germination and early seedling growth stages are very important in the development of plant. Many factors could influence the seed germination capability such as temperature, light, pH, and soil moisture as well as water potential and salinity (An & Zhou, 2017; Laghmouchi et al., 2017; Wang et al., 2016).

Salinity is one of the most serious environmental problems which could influence the crop growth and production (Lopez, Takahashi, & Yamazaki, 2002). Salinity could cause alteration in morphology, anatomy and metabolism during plant growth. Salinization and sodification was a 3rd main threat to Malaysia soil condition (Montanarella et al., 2016). In Cameron Highland which located in central Malaysia, the extensive vegetable cultivation and flower cropping as well as the usage of high amount of fertilizer and manure intensify the salinity problem in that area (Wong & Jaafar, 1993). Soils comprises of many soluble salts components. Despite that, many studies conducted extensively on the effect of sodium chloride (NaCl) on seed germination (Zhao, Lu, & He, 2014), but until to date, the study of the different type of salt on the seed germination are still limited. Depending upon the type of salt, plant species and developmental stage, the impact of salinity might be different (Bueno et al., 2017; Ahmad, Azooz, & Prasad, 2013).

This study was conducted on amaranth plant (*Amaranthus tricolor* L.) which is known to have high nutritive value and adaptable in diverse conditions (Sleugh et al., 2001). The study by Rahman, Lund, and Bryceson (2011) reported that the number of cultivated species of winter vegetables in Bangladesh area which includes the *Amaranthus* species have declined from 1975 to 2006 which is due to the elevated soil salinity. Despite that, the study on the tolerance of amaranth to salinity stress as well as in different salt types especially at germination stage are still lacking (Omami, Hammes, & Robbertse, 2006).

Therefore, the objectives of the present study are:

i. To determine the germination capability of *Amaranthus tricolor* L. in different salt types (NaCl, KCl, $MgSO_4$ and $MgCl_2$) at different concentrations.
ii. To determine the ideal concentration of KCl, KNO_3, TU and combination as enhancer on the germination of salt stressed *Amaranthus tricolor* L. seeds.
iii. To evaluate the germination capability of different species of *Amaranthus* spp. (*Amaranthus hybridus* L., *Amaranthus viridis* L., *Amaranthus tricolor* L. and *Amaranthus giganticus* L.) under salinity condition using hydrotime model analysis.

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