UNIVERSITI PUTRA MALAYSIA

DEVELOPMENT OF LIQUID ENHANCER FOR GERMINATION AND EARLY GROWTH OF RICE (Oryza sativa L.) UNDER DROUGHT STRESS

SITI NURATIQAH MAHADI

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By

SITI NURATIQAH MAHADI

Thesis submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the requirement for the degree of Doctor of Philosophy

November 2019

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Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Doctor of Philosophy

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November 2019

Chair : Rosimah Nulit, PhD
Faculty : Science

Drought affect growth and development of main crops over the world. It has severely affect the yield and quality of rice plant production, including in Malaysia. Recently, rice plantation in Kelantan, Sabah and Terengganu were reported to be in high risk of water scarcity due to the dry and hot weather caused by El Nino phenomena. One of the strategy to alleviate drought stress in plant growth and development is by the application of fertilizer. Therefore, this study aimed to develop liquid enhancer for germination and early growth of rice under drought stress. The first objective was conducted to screen and select low drought tolerant Malaysia Indica rice cultivars. Five rice cultivars produced by MARDI (MR219, MR220, MR263, MR269 and MR284) were germinated in a petri dishes containing 8 ml of distilled water (0 MPa), -0.3 MPa, -0.6 MPa and -1.2 MPa PEG 6000 solution at ± 26°C for ten days. Number of germinated seed, shoot length, root length, fresh weight and dry weight were measured to analyse the germination and early growth performance. The results found that PEG 6000 solution significantly reduced the germination performance and early growth of all rice cultivars in low osmotic potential. The tolerance level of the rice cultivars towards drought can be concluded as follow, MR219 > MR269 > MR263 > MR284 > MR220. MR220 and MR284 were found to be the most sensitive cultivars. The second objective was followed to determine ideal concentration of KCl, TU, Kin, GA_3, and SA for germination and early growth of MR220 and MR284 under drought stress. Seed priming and medium supplementation methods were applied. In seed priming method, MR220 and MR284 seeds were primed in KCl (10, 20, 30, 40 mM), TU (10, 20, 30, 40 mM), Kin (0.25, 0.5, 0.75, 1.0 mM), and SA (0.25, 0.5, 0.75, 1.0 mM) for ±16H and germinated in petri dishes containing 8ml of -1.2 Mpa PEG 6000 solution at ± 26°C for ten days. The petri dishes were arranged in complete

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randomized design (CRD). The findings showed that 10 mM of KCl, 30 mM of TU, 0.25 mM of Kin and 1.0 mM of SA were the ideal concentration for MR220. Meanwhile, 20 mM of KCl, 30 mM of TU, 0.5 mM of Kin and 1.0 mM of SA were the ideal concentration for MR284 for germination and early growth under drought stress. In medium supplementation method, MR220 and MR284 seeds were stressed in -1.2 MPa PEG 6000 (severe drought level) solution for three days and germinated in petri dishes containing KCl (0.5, 1.0, 1.5, 2.0, 10, 20, 30, 40 mM), TU (0.5, 1.0, 1.5, 2.0), GA_3 (0.06, 0.12, 0.18, 0.24 mM), and SA (0.25, 0.5, 0.75, 1.0 mM) at ± 26°C for ten days. Based on the results, 1.0 mM of KCl, 2.0 mM of TU, 0.06 mM of GA_3 and 1.0 mM of SA were the ideal concentration for drought-stressed MR220. Meanwhile, the ideal concentration for drought-stressed MR284 were shown by 30 mM of KCl, 2.0 mM of TU, 0.12 mM of GA_3 and 0.75 mM of SA. The third objective was progressed to determine the best combination treatments of the ideal concentration acquired from the objective two for liquid enhancer development. Similar germination procedure with different liquid enhancer formula was carried out. In seed priming method, the results showed that the best combination treatments for MR220 were 10 mM KCl + 30 mM TU, 10 mM KCl + 1.0 mM SA, and 30 mM TU + 1.0 mM SA. Meanwhile, the best combination treatments for MR284 were 20 mM KCl + 30 mM TU + 0.5 mM Kin + 1.0 mM SA, 20 mM KCl + 30 mM TU + 0.5 mM Kin, and 30 mM TU + 1.0 mM SA. In medium supplementation method, the results showed that the best combination treatments for drought-stressed MR220 were 1.0 mM KCl + 0.06 mM GA_3, 0.06 mM GA_3 + 1.0 mM SA, and 1.0 mM KCl + 2.0 mM TU + 0.06 mM GA_3. Meanwhile, the best combination treatments for drought-stressed MR284 were 30 mM KCl + 0.12 mM GA_3, 0.12 mM GA_3 + 0.75 mM SA, and 30 mM KCl + 2.0 mM TU + 0.12 mM GA_3. The last objective was carried out to evaluate the effectiveness of liquid enhancer in soil medium. This objective was proceeded with medium supplementation method. Drought-stressed MR220 and MR284 were germinated in soil medium for 30 days and supplemented with the liquid enhancer at day 0 and day 15, with 50 ml watered daily. PEG 6000 (-1.2 MPa) was given at 30 ml in alternate day before liquid enhancer treatment to induce drought stress. The best three combination treatments from objective three were used as liquid enhancer treatments. The plant height, root length, biomass, root to shoot ratio, chlorophyll, protein and proline content were measured to analyse the effectiveness of the liquid enhancer on the early growth performance of drought-stressed MR220 and MR284 seedlings. Among the combination treatments tested, combination treatment consist of 1.0 mM KCl + 2.0 mM TU + 0.06 mM of GA_3 and 30 mM KCl + 0.12 mM GA_3 showed the most effective in improving early growth performance for drought-stressed MR220 and MR284 seedlings respectively. The combination treatments showed 2-fold higher plant height (29.8 cm and 34.3 cm) than control (16.3 cm and 15.7 cm) of drought-stressed MR220 and MR284 respectively. These two combination treatments were found to be the suitable liquid enhancer that able to enhance germination performance and early growth of drought-stressed MR220 and MR284.

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Keywords: Drought stress, liquid enhancer, MR220 and MR284, germination enhancer, early growth enhancer

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PENGHASILAN LARUTAN PENGGALAK UNTUK MENINGKATKAN PERCAMBAHAN DAN PERTUMBUHAN AWAL PADI (*Oryza sativa* L.) DI BAWAH TEGASAN KEMARAU

Oleh

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November 2019

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Kemarau menjejaskan pertumbuhan dan perkembangan tanaman utama seluruh dunia. Kemarau menjejaskan hasil dan kualiti pengeluaran padi, termasuk di Malaysia. Terkini, sawah padi di Kelantan, Sabah dan Terengganu dilaporkan berisiko tinggi kekurangan air akibat cuaca kering dan panas berikutan fenomena El Nino. Antara strategi mengurangkan tegasan kemarau dalam perkembangan tumbuhan ialah dengan penggunaan baja. Justeru, kajian ini bertujuan untuk menghasilkan larutan penggalak bagi percambahan dan pertumbuhan awal anak benih padi di bawah tegasan kemarau. Objektif pertama dilakukan untuk menyaring dan memilih kultivar padi Malaysia yang mempunyai toleransi kemarau yang rendah. Lima kultivar padi yang dihasilkan MARDI (MR219, MR220, MR263, MR269 dan MR284) telah dicambahkan dalam piring petri yang mengandungi 8 ml air suling (0 MPa), -0.3 MPa, -0.6 MPa, -0.9 MPa dan -1.2 MPa larutan PEG 6000 pada suhu ± 26°C selama sepuluh hari. Bilangan benih bercambah, panjang daun, panjang akar, berat basah dan kering telah diukur. Hasil mendapati larutan PEG 6000 jelas mengurangkan prestasi percambahan dan pertumbuhan awal anak benih semua kultivar padi dalam potensi osmotik rendah. Tahap toleransi kultivar padi terhadap kemarau adalah seperti berikut, MR219 > MR269 > MR263 > MR284 > MR220. MR220 dan MR284 didapati adalah kultivar yang paling sensitif. Objektif kedua adalah untuk menentukan kepekatan ideal KCl, TU, Kin, GA_3 dan SA untuk percambahan dan pertumbuhan awal anak benih MR220 dan MR284 di bawah tegasan kemarau. Kaedah perendaman benih dan suplemen media telah diaplikasi. Dalam kaedah perendaman benih, benih MR220 dan MR284 direndam dalam larutan KCl (10, 20, 30, 40 mM), TU (10, 20, 30, 40 mM), Kin (0.25, 0.5, 0.75, 1.0 mM) dan SA (0.25, 0.5, 0.75, 1.0 mM) selama ± 16 jam dan dicambah dalam piring petri yang mengandungi 8 ml larutan -

1.2 MPa PEG 6000 pada suhu ± 26°C selama sepuluh hari. Hasil kajian menunjukkan 10 mM KCl, 30 mM TU, 0.25 mM Kin dan 1.0 mM SA adalah kepekatan ideal MR220. Manakala, 20 mM KCl, 30 mM TU, 0.5 mM Kin dan 1.0 mM SA adalah kepekatan ideal MR284. Dalam kaedah suplemen media, benih MR220 dan MR284 direndam dalam larutan -1.2 MPa PEG 6000 (kemarau tahap tinggi) selama tiga hari untuk induksi tegasan kemarau dan dicambah dalam piring petri yang mengandungi larutan KCl (0.5, 1.0, 1.5, 2.0, 10, 20, 30, 40 mM), TU (0.5, 1.0, 1.5, 2.0 mM), GA_3 (0.25, 0.5, 0.75, 1.0 mM) dan SA (0.25, 0.5, 0.75, 1.0 mM) pada suhu ± 26°C selama sepuluh hari. Hasil menunjukkan 1.0 mM KCl, 2.0 mM TU, 0.06 mM GA_3 dan 1.0 mM SA adalah kepekatan ideal MR220 bertegasan kemarau. Kepekatan ideal bagi MR284 bertegasan kemarau ialah 30 mM KCl, 2.0 mM TU, 0.12 mM GA_3 dan 0.75 mM SA. Objektif ketiga dilanjutkan untuk menentukan rawatan kombinasi yang terbaik dari kepekatan ideal yang diperoleh dari objektif kedua untuk menghasilkan larutan penggalak. Prosedur percambahan yang sama dengan larutan rawatan yang berbeza dari objektif kedua telah dijalankan. Dalam kaedah perendaman benih, hasil menunjukkan rawatan kombinasi terbaik untuk MR220 bertegasan kemarau ialah 10 mM KCl + 30 mM TU, 10 mM KCl + 1.0 mM SA dan 30 mM TU + 1.0 mM SA. Rawatan kombinasi terbaik untuk MR284 bertegasan kemarau adalah 20 mM KCl + 30 mM TU + 0.5 mM Kin + 1.0 mM SA, 20 mM KCl + 30 mM TU + 0.5 mM Kin dan 30 mM TU + 1.0 mM SA. Dalam keadah suplemen media, hasil menunjukkan bahawa rawatan kombinasi terbaik untuk MR220 bertegasan kemarau ialah 1.0 mM KCl + 0.06 mM GA_3, 0.06 mM GA_3 + 1.0 mM SA dan 1.0 mM KCl + 2.0 mM TU + 0.06 mM GA_3. Rawatan kombinasi terbaik untuk MR284 bertegasan kemarau ialah 30 mM KCl + 0.12 mM GA_3, 0.12 mM GA_3 + 0.75 mM SA dan 30 mM KCl + 2.0 mM TU + 0.12 mM GA_3. Objektif terakhir dijalankan untuk menilai keberkesanan larutan penggalak dalam media tanah dengan keadah suplemen media. MR220 dan MR284 bertegasan kemarau dicambah dalam tanah selama 30 hari dan ditambah larutan penggalak pada hari 0 dan hari 15, dan disiram 50 ml air setiap hari. Larutan PEG 6000 (-1.2 MPa) diberikan selang sehari sebelum rawatan larutan penggalak. Tiga rawatan kombinasi yang terbaik dari objektif ketiga digunakan sebagai rawatan larutan penggalak. Ketinggian pokok, panjang akar, biojisim, nisbah akar kepada daun, kandungan klorofil, protin dan prolina diukur untuk menganalisis keberkesanan larutan penggalak pada pertumbuhan awal MR220 dan MR284 bertegasan kemarau. Rawatan kombinasi yang mengandungi 1.0 mM KCl + 2.0 mM TU + 0.06 mM GA_3 dan 30 mM KCl + 0.12 mM GA_3 menunjukkan rawatan yang paling berkesan untuk meningkatkan prestasi pertumbuhan awal bagi MR220 dan MR284 bertegasan kemarau. Rawatan kombinasi ini telah meningkatkan 2 kali ketinggian pokok (29.8 sm and 34.3 sm) MR220 dan MR284 masing-masing berbanding rawatan kawalan (16.3 sm and 15.7 sm). Kedua-dua rawatan kombinasi ini didapati sebagai larutan penggalak yang sesuai untuk meningkatkan prestasi percambahan dan pertumbuhan awal MR220 dan MR284 bertegasan kemarau.

Katakunci: Tegasan kemarau, larutan penggalak, MR220 dan MR284, penggalak percambahan, penggalak pertumbuhan awal

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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 Doctor of Philosophy. The members of the Supervisory Committee were as follows:

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TABLE OF CONTENTS

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LIST OF TABLES

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LIST OF FIGURES

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LIST OF ABBREVIATIONS

PEG	Polyethylene glycol
KCl	Potassium Chloride
TU	Thiourea
Kin	Kinetin
GA	Gibberellin
GA_3	Gibberellic Acid
SA	Salicylic Acid
PGR	Plant Growth Regulator
mM	Milimolar
MPa	Megapascal
g	Gram
mg	Milligram
ml	Milliliter
cm	Centimeter
°C	Celcius
CRD	Complete randomized design
RCBD	Random complete block design
GP	Germination Percentage
GI	Germination Index
SV	Seed Vigor
SHR	Shoot Height Reduction
SL	Shoot length
RL	Root length
SDL	Seedling Length

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%	Percentage
MARDI	Malaysian Agriculture Research and Development Institute
SPEI	Standard Precipitation Evapotranspiration Index
SPI	Standard Precipitation Index
ANOVA	Analysis of Variance

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CHAPTER 1

INTRODUCTION

1.1 Background of study

The main idea of this study is to develop a liquid enhancer containing chemical and plant growth regulator (PGR) for the germination and early growth of rice seed under drought stress. Drought is one of the abiotic stresses that affected the growth and development of many plant species. It is a water stress condition that is related to high degree of temperature that disturb the biological process in plant where it reduces turgor pressure and tissue water content (Shariatmadari, Parsa, Nezami and Kafi, 2017). At this point, it can cause the plant to slow down their growth. Normally, when drought happened, there will be a great loss in the crop production of the affected area, including rice. Rice is the staple food in Malaysia and many other countries worldwide. Rice is one of the plant that requires a lot of water to complete its life cycle. In Peninsular Malaysia, rice is planted in paddy field area via flooded technique. Thus, drought effect has really give negative impact on the rice growth and production, worldwide. It has significantly reduced the number of tillers and the plant height, caused leaf rolling and leaf death and also increased the accumulation of proline content (Bunnag and Phongthai, 2013).

To date, Malaysia is still unsuccessful to fully supply the Malaysian rice demands. According to Ismail Mohamad Yusof, Managing Director of Bernas, Malaysia has fulfil only 60% of the total rice demands (Zulkipli, 2019). There are a number of factors that lead to this problem, such as decrease paddy field area, ageing farmer, pest attacks and also natural disaster such as flood and drought. In order to solve these problems, the government and private sectors have taken action by providing improvised rice cultivars, educating the farmers and encouraging younger generation to involve in agriculture. However, there is less concern on how to fight drought in rice production in Malaysia.

Early in this year, rice production in Kelantan, Sabah and Terengganu were reported to be severely affected by drought due to El Nino Modoki (Ismail, 2019; Fredolin, 2019). Furthermore, drought occurrence had severely affected Malaysia as reported in early 2014. As reported by Chillymanjaro (2014), there were several rice production area in Peninsular Malaysia that are affected with extreme drought in year 2014, which are Kedah, Perlis, Perak, Pulau Pinang, Negeri Sembilan, Melaka, Johor and Pahang. The consequences have prolonged until early of 2016. This shows the severity of the impact of drought in rice production in Malaysia. Therefore, strategies

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need to be developed to fight this problem in order to sustain the rice production.

1.2 Justification and objectives of study

In life cycle of rice, there are few stages which are germination, vegetative, maturation and reproductive stage. This study is focusing in the germination and vegetative stage of the life cycle of rice. Germination is the most vital stage in the plant life cycle. Seed germination process requires water to initiate the plant growth and development. Scarcity of water source disrupt the germination process to occur. This is where seed priming technique becomes a benefactor for a successful germination. Seed priming is a seed soaking method before planting and is very simple, low cost but very effective for early seed growth.

Seed priming technique has been proved in several previous studies that primed seeds showed better performances in seed germination and also increase some of biochemical components in the plant of interest, under certain abiotic stresses (Sheykhbaglou, Rahimzadeh, Ansari and Sedghi, 2014; Sneideris, Gavassi, Campos, D'Amico-Damia and Carvalho, 2014; Kaur, Chawla and Pathok, 2015; Toklu, Baloch, Karakoy and Ozkan, 2015). In another condition, a drought-stressed seed is one of the problem of seed dormancy. This will delay the germination process to occur. In this study, the seeds were stress-induced by using polyethylene glycol (PEG) 6000 solution which is the common drought stress inducer used in previous studies of rice varieties (Swapna and Shylaraj, 2017; Islam, Kayesh, Zaman, Urmi and Haque, 2018). The seeds were grew in a less amount of water condition compared to normal paddy field condition. Here, the developed liquid enhancer will be a benefactor to the rice seed to germinate and grow better in a limited water resource condition. Thus, this study proposed that the application of this liquid enhancer can enhance the germination and early growth development of rice seed in drought stress.

There are a few strategies that had been done to overcome drought stress in crop production. Selection of drought tolerant cultivars, water irrigation management and also application of fertilizer. However, production of drought tolerant cultivars and water irrigation management had been proven as high cost treatments compared to the application of chemical. Furthermore, development of drought tolerance cultivars takes longer time. Application of liquid enhancer is seen to be more practical in order to increase the production of rice in paddy field. The application of this liquid enhancer as seed priming solution and as medium supplementation during germination stage and watering technique during vegetative stage is easier to be practiced by farmers. This can be another solution instead of producing new breed of rice cultivar that is resistance to drought condition and is more practical, cheaper and efficient. Therefore, in this study the application of

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chemical formulation and PGR is proposed as the strategy to alleviate drought stress in the rice seed germination.

Liquid enhancer that can increase germination and early growth of rice under drought stress is the main product of this study. It is a combination of selected chemical and PGR. In this study, potassium chloride (KCl), thiourea (TU), kinetin (Kin), salicylic acid (SA) and gibberellic acid (GA_3) were selected in order to develop the liquid enhancer. Potassium is one of the macronutrient that is very essential for the plant growth and development. KCl is commonly used as the potassium source in seed germination and plant growth until now (Mohammed and Abdul Elrahman, 2015). Next, TU has been widely used to enhance plant growth under drought stress. Previous study, Sharma, Asarey and Verma, (2015) had reported that application of TU is effective for enhancing wheat productivity under environmental stress. The use of PGR exogenously in plant growth development has been reported and discussed in many studies. Kin has an important roles in shoot meristem formation and activity, nutrient mobilization and response to stresses (Azizi *et al.*, 2014). Gibberellin has been discussed for promoting seed germination in several plant such as *Oryza sativa* (Vieira, Viera, Fraga, Oliveira and Santos, 2002) and *Moringa oleifera* (Eghobor, Umar, Munir, Abubakar and Collins, 2015). According to Sheykhbaglou *et al.,* (2014), SA and GA had increased germination rate and germination index in sorghum under drought stress. Previous studies proved that these chemicals and PGR able to enhance plant growth in stress condition. Hence, the liquid enhancer containing combination of KCl, TU, Kin, GA_3 and SA is expected to have a high potential to promote germination and early growth of rice under drought stress.

This liquid enhancer designed to increase germination performance and also to enhance the early growth of rice seedlings under drought stress. The application of this liquid enhancer can be one of the alternative ways to maintain the rice production and reduce the loss during drought season. With respect to this issue, the aim of this study was to develop a liquid enhancer containing KCl, TU, Kin, GA_3 and SA for germination and as growth enhancer for MR220 and MR284 under drought condition. Hence, in order to achieve this aim, there are four objectives of this study.

1. To screen and select low drought tolerant Malaysia Indica rice cultivars.

2. To determine the ideal concentration of each KCl, TU, Kin, SA and GA_3 that enhance the germination performance and early growth of the selected low drought tolerant rice cultivars under drought stress.

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3. To select the best combination treatments from the ideal concentration of KCl, TU, Kin, SA and GA_3 in order to develop liquid enhancer for germination and early growth of MR220 and MR284 under drought stress.

4. To evaluate the effectiveness of the liquid enhancer on the early growth and physiology of MR220 and MR284 seedlings planted in soil medium under drought stress.

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