UNIVERSITI PUTRA MALAYSIA

OPTIMIZATION OF SEED PRIMING TO ENHANCE GERMINATION AND EARLY SEEDLINGS GROWTH OF RICE (Oryza sativa L.) VAR. FARO44 UNDER DROUGHT STRESS

LAWAN GANA ALI

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By

LAWAN GANA ALI

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirements for the Degree of Doctor of Philosophy

April 2021

DEDICATION

In the name of Allah, the all-high and all-knowing.
This thesis is dedicated to my parents and family.

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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Chairperson : Associate Professor Rosimah binti Nulit, PhD
Faculty : Science

Priming of seed is a simple physiological approach that enhances germination, seedling growth and imparts drought stress tolerance to germinating seeds and seedlings. Rice is an important staple crop produced and consumed by more than 50% of people worldwide. However, poor germination and irregular seedling emergence in direct-seeded rice production caused by drought in a changing climate are the major causes of low yield in the tropical countries. In this study, rice seeds var. FARO44 was primed with different chemicals to enhance germination, seedling growth and drought tolerance. The objectives of the study include: (i) evaluate the suitability of potassium nitrate (KNO_3), silicon dioxide (SiO_2) and salicylic acid (SA) as priming agents for FARO44 rice germination and early seedling growth, (ii) evaluate the effects of seed priming on germination, early seedling growth and biochemical characteristics of rice seedlings under drought conditions, (iii) compare the activities of protective enzymes superoxide dismutase (SOD), catalase (CAT) and ascorbate peroxidase (APX) of rice seedlings under drought conditions and (iv) evaluate the effects of seed priming on drought tolerance of FARO44 rice seedlings under drought conditions. In experiment one, rice seeds were primed each with KNO_3 (2.5%, 5%, 10%, 15% and 20%), SiO_2 (2.5%, 3%, 3.5%, 4% and 4.5%) and SA (0.007%, 0.014%, 0.021%, 0.028% and 0.035%). Primed rice seeds were then germinated in Petri dishes for two weeks. The experiments were arranged in a completely randomized design with each treatment replicated five times. Thereafter, germination percentage (GP), mean germination time (MGT), germination index (GI) and seedling growth were evaluated. In experiment two, rice seeds were primed with optimal concentrations of KNO_3 (2.5% and 5% w/v), SiO_2 (3% and 3.5%) and SA (0.014% and 0.035%) and their combinations (5% KNO_3+3% SiO_2; 2.5% KNO_3+3.5% SiO_2; 0.014% SA+5% KNO_3; 0.035% SA+2.5% KNO_3; 3% SiO_2+0.035% SA; 3.5% SiO_2+0.014% SA; 5% KNO_3+3% SiO_2+0.035% SA and 2.5% KNO_3+3.5% KNO_3+0.014% SA). Primed rice seeds were then germinated and grown under three osmotic stress conditions of low (-0.3 Mpa), moderate (-0.6 Mpa) and severe (-0.9 Mpa) induced by polyethylene glycol for two weeks. Thereafter, GP, MGT, GI, seedling growth performances, activities of SOD, CAT and APX, total soluble protein, proline,

carbohydrate, total chlorophyll, total soluble sugar and malondialdehyde content, and membrane stability were evaluated. In experiment three and four, primed rice seeds with KNO_3 (2.5% and 5%), SiO_2 (3% and 3.5%) and SA (0.014% and 0.035%) were grown in the net house in plastic pots under well-watered and low, moderate and severe drought conditions for six weeks. Plastic pots were arranged in CRD and replicated five times. Emergence characteristics, seedling growth performances, activities of CAT, APX, SOD, total soluble protein, proline, carbohydrate, total soluble sugar, total chlorophyll and malondialdehyde content, and membrane stability of seedlings were evaluated. In the laboratory studies under well-watered conditions, the results revealed that KNO_3, SiO_2 and SA priming significantly ($P \leq 0.05$) enhanced germination index, shortened germination time and improved seedling growth, seedling biomass and seedling vigour indices of rice seedlings. However, germination percentage was not improved by seed priming. Seed priming with 2.5% and 5% KNO_3, 3% and 3.5% SiO_2, 0.014% SA and 0.035% SA showed more effects in enhancing germination and seedling growth of FARO44 rice than other concentrations of these chemicals. Likewise, under osmotic stress conditions, 2.5% and 5% KNO_3, 3% and 3.5% SiO_2, 0.014% SA and 0.035% SA priming significantly ($P \leq 0.05$) improved GP, GI, MGT and seedling growth of FARO44 rice more than combined priming with KNO_3+SiO_2, SiO_2+SA, SA+KNO_3 and KNO_3+SiO_2+SA. The results also showed that 2.5% and 5% KNO_3, 3% and 3.5% SiO_2, 0.014% SA and 0.035% SA priming significantly ($P \leq 0.05$) enhanced CAT, APX and SOD activities of rice seedlings by more than 2 folds compared to control. Likewise, seed priming significantly ($P \leq 0.05$) improved total soluble protein, total chlorophyll, carbohydrate, soluble sugar and proline content, membrane stability and decreased lipid peroxidation by more than 5-folds in rice seedlings under osmotic stress conditions. Combined priming with KNO_3+SiO_2, SiO_2+SA, SA+KNO_3 and KNO_3+SiO_2+SA showed less effects in enhancing protective activities of CAT, APX and SOD, and total soluble protein, total chlorophyll, carbohydrate, soluble sugar and proline contents of rice seedling under osmotic stress conditions than priming with these chemicals separately. Under both well-watered and drought conditions in the net house, 2.5% and 5% KNO_3, 3% and 3.5% SiO_2, 0.014% SA and 0.035% SA priming significantly ($P \leq 0.05$) enhanced emergence attributes, seedling growth and drought tolerance of FARO44 rice seedlings. However, 2.5% and 5% KNO_3, 3% and 3.5% SiO_2 priming showed more effects than SA priming. Similarly, the results found that seed priming significantly ($P \leq 0.05$) improved total soluble protein content, activities of CAT, APX and SOD, carbohydrate, total soluble protein, soluble sugar, total chlorophyll and proline content and membrane stability, while MDA content decreased by more than 4-folds than control in rice seedlings grown in net house under drought conditions. Thus, the findings of this study established that KNO_3 (2.5% and 5%) and SiO_2 (3% and 3.5%) priming are effective for fast germination, seedling establishment, seedling growth and for imparting drought tolerance to FARO44 rice grown in tropical ecosystems.

Keywords: catalase; ascorbate peroxidase; germination; drought; seedling vigour

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Doktor Falsafah

PENGOPTIMAAN PERANGSANGSEDIAAN BIJI BENIH BAGI MEMPERTINGKATKAN PERCAMBAHAN DAN PERTUMBUHAN AWAL ANAK BENIH PADI (*Oryza sativa* L.) VAR. FARO44 DI BAWAH TEKANAN KEMARAU

Oleh

LAWAN GANA ALI

April 2021

Pengerusi : Profesor Madya Rosimah binti Nulit, PhD
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Perangsangsediaan biji benih adalah pendekatan fisiologi ringkas yang meningkatkan percambahan, pertumbuhan anak benih dan memberikan toleransi terhadap kemarau kepada percambahan biji benih dan anak pokok. Padi adalah tanaman ruji utama yang dimakan oleh majoriti manusia di seluruh dunia. Namun, kadar percambahan yang rendah dan kemunculan anak benih yang tidak sekata dalam pengeluaran padi secara tabur terus yang disebabkan oleh kemarau akibat perubahan iklim menjadi punca utama penghasilan yang rendah di timur laut Nigeria yang panas dan kering. Dalam kajian ini, benih padi varieti FARO44 dirangsangsedia dengan menggunakan beberapa bahan kimia untuk meningkatkan percambahan, pertumbuhan anak benih dan toleransi terhadap kemarau. Objektif kajian merangkumi: (i) menilai kesesuaian KNO_3, SiO_2 dan SA sebagai agen perangsangsedia untuk percambahan padi FARO44 dan pertumbuhan awal anak benih, (ii) menilai kesan perangsangsediaan biji benih terhadap percambahan, pertumbuhan awal benih dan ciri biokimia anak benih padi dalam keadaan kemarau, (iii) membandingkan aktiviti enzim-enzim pelindung seperti superoksida dismutase (SOD), katalase (CAT) dan askorbat peroksidase (APX) di dalam anak benih padi dalam keadaan kemarau dan (iv) menilai kesan perangsangsediaan biji benih terhadap toleransi kemarau bagi benih padi FARO44 dalam keadaan kemarau. Dalam eksperimen pertama, biji benih padi dirangsangsedia secara berasingan dengan KNO_3 (2.5%, 5%, 10%, 15% dan 20%), SiO_2 (2.5%, 3%, 3.5%, 4% dan 4.5%) dan SA (0.007%, 0.014%, 0.021%, 0.028% dan 0.035%). Kemudian dicambah dalam piring petri selama dua minggu dan disusun dalam reka bentuk blok lengkap terawak dengan lima replikasi untuk setiap rawatan. Peratusan percambahan (GP), masa percambahan purata (MGT), indeks percambahan (GI) dan pertumbuhan anak benih dinilai. Dalam eksperimen kedua, biji benih padi dirangsangsedia menggunakan kepekatan optimum daripada eksperimen pertama iaitu KNO_3 (2.5% dan 5% w/v), SiO_2 (3% dan 3.5%) dan SA (0.014% dan 0.035%) dan gabungannya (5% KNO_3+3% SiO_2; 2.5% KNO_3+3.5% SiO_2; 0.014% SA+5% KNO_3; 0.035% SA+2.5% KNO_3; 3% SiO_2+0.035% SA; 3.5% SiO_2+0.014% SA; 5% KNO_3+ 3% SiO_2+0.035% SA dan 2.5% KNO_3+3.5% KNO_3+0.014% SA). Benih padi yang telah dirangsangsedia kemudian dicambah dan ditanam di dalam tiga tahap tekanan osmotik iaitu rendah (-0,3 Mpa), sederhana (-0.6 Mpa) dan tinggi (-0.9 Mpa) menggunakan polietilena glikol selama dua minggu. Selepas itu, penilaian dilakukan terhadap GP,

MGT, GI, prestasi pertumbuhan anak benih, aktiviti SOD, CAT dan APX, jumlah protein terlarut, prolina, karbohidrat, jumlah klorofil, jumlah kandungan gula terlarut dan kandungan malondialdehid, dan kestabilan membran. Dalam eksperimen ketiga dan keempat, biji benih padi yang telah dirangsangsedia dengan KNO_3 (2.5% dan 5%), SiO_2 (3% dan 3.5%) dan SA (0.014% dan 0.035%) ditanam didalam pot plastik di rumah naungan dalam keadaan cukup air dan kemarau selama enam minggu. Pot plastik disusun secara CRD dan diulang sebanyak lima replika. Ciri-ciri kemunculan, prestasi pertumbuhan anak benih, aktiviti CAT, APX, SOD, jumlah protein terlarut, prolina, karbohidrat, jumlah gula terlarut, jumlah kandungan klorofil dan malondialdehid, dan kestabilan membran anak benih dinilai. Kajian makmal dalam keadaan cukup air menunjukkan bahawa perangsangsediaan menggunakan KNO_3, SiO_2 dan SA meningkatkan indeks percambahan, memendekkan masa percambahan dan meningkatkan pertumbuhan anak benih, biojisim benih dan indeks kesuburan anak benih padi secara signifikan ($P \leq 0.05$). Walau bagaimanapun, peratusan percambahan tidak meningkat dengan perangsangsediaan biji benih. Perangsangsediaan biji benih dengan menggunakan 2.5% dan 5% KNO_3, 3% dan 3.5% SiO_2, 0.014% SA dan 0.035% SA menunjukkan lebih banyak kesan dalam meningkatkan percambahan dan pertumbuhan benih padi FARO44 berbanding kepekatan bahan kimia lain. Begitu juga dalam keadaan tekanan osmotik, perangsangsediaan menggunakan 2.5% dan 5% KNO_3, 3% dan 3.5% SiO_2, 0.014% dan 0.035% SA menunjukkan peningkatan ketara ($P \leq 0,05$) dalam nilai GP, GI, MGT dan pertumbuhan anak benih bagi padi FARO44, lebih banyak berbanding perangsangsediaan gabungan KNO_3+SiO_2, SiO_2+SA, SA+KNO_3 dan KNO_3+SiO_2+SA. Hasil kajian juga menunjukkan bahawa perangsangsediaan menggunakan 2.5% dan 5% KNO_3, 3% dan 3.5% SiO_2, 0.014% dan 0.035% SA meningkatkan aktiviti CAT, APX dan SOD anak benih padi secara signifikan ($P \leq 0.05$) lebih dari 2 kali ganda berbanding dengan kawalan. Perangsangsediaan biji benih dengan ketara ($P \leq 0,05$) meningkatkan jumlah protein terlarut, jumlah klorofil, karbohidrat, gula terlarut dan kandungan prolina, kestabilan membran dan menurunkan peroksidasi lipid sebanyak 5 kali ganda pada anak benih padi dalam keadaan tekanan osmotik. Perangsangsediaan gabungan dengan KNO_3+SiO_2, SiO_2+SA, SA+KNO_3 dan KNO_3+SiO_2+SA menunjukkan kurang kesan dalam meningkatkan aktiviti perlindungan oleh CAT, APX dan SOD, jumlah protein terlarut, jumlah klorofil, karbohidrat, gula terlarut dan kandungan prolina anak benih padi dalam keadaan tekanan osmotik daripada perangsangsediaan dengan bahan kimia ini secara berasingan. Di dalam kedua-dua keadaan cukup air dan kemarau di rumah naungan, 2.5% dan 5% KNO_3, 3% dan 3.5% SiO_2, 0.014% SA dan 0.035% SA meningkat dengan ketara ($P \leq 0.05$) ciri-ciri percambahan, pertumbuhan anak benih dan toleransi kemarau anak benih padi FARO44. Walau bagaimanapun, perangsangsediaan menggunakan 2.5% dan 5% KNO_3, 3% dan 3.5% SiO_2 menunjukkan lebih banyak kesan daripada perangsangsediaan menggunakan SA. Begitu juga, keputusan menunjukkan bahawa perangsangsediaan biji benih meningkatkan secara signifikan ($P \leq 0,05$) jumlah protein larut, aktiviti enzim iaitu CAT, APX dan SOD, karbohidrat, gula terlarut, jumlah klorofil dan kandungan prolina dan kestabilan membran, sementara kandungan MDA menurun lebih dari 4 kali ganda berbandin anak benih padi kawalan yang ditanam di rumah naungan dalam keadaan kemarau. Oleh itu, hasil kajian ini membuktikan bahawa perangsangsediaan menggunakan KNO_3 (2.5% dan 5%) dan SiO_2 (3% dan 3.5%) berkesan untuk mempercepatkan percambahan, penyediaan anak benih, pertumbuhan anak benih dan memberi toleransi kemarau kepada padi FARO44 yang ditanam dalam ekosistem tropika.

Katakunci: katalase; askorbat peroksidase; percambahan; kemarau; kesuburan anak benih

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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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°C	Degree Celsius
ANOVA	Analysis of variance
APX	Ascorbate peroxidase
CAT	Catalase
cm	Centimetre
DMRT	Duncan's multiple range test
DNA	Deoxyribonucleic acid
dS/m	decisiemens per meter
EI	Emergence index
EL	Electrolyte leakage
GI	Germination index
GP	Germination percentage
H_2O_2	Hydrogen peroxide
L	Litre
LEL	Leaf electrolyte leakage
MDA	Malondialdehyde
MET	Mean emergence time
Mg/l	Milligram per litre
Mg/ml	Milligram per millilitre
MGT	Mean germination time
min	Minute
ml	Millilitre
mm	Millimetre
P	Probability

PEG	Polyethylene glycol
pH	Negative logarithm of hydrogen ion concentration
REL	Root electrolyte leakage
RL	Root length
RNA	Ribonucleic acid
ROS	Reactive oxygen species
SDB	Seedling dry biomass
SE	Standard error
SFB	Seedling fresh biomass
ShL	Shoot length
SL	Seedling length
SOD	Superoxide dismutase
SV	Seedling vigour
SVI I	Seedling vigour index I
SVI II	Seedling vigour index II
U/mg^{-1} protein	Unit per milligram protein
U/mg^{-1}FW	Unit per milligram fresh weight
µmolmg^{-1}FW	Micro molar per milligram fresh weight

CHAPTER 1

INTRODUCTION

1.1 Background of the Study

Rice (*Oryza sativa*) is an essential cereal cultivated on the earth surface. It is a major staple food being consumed by more than 50% of the global population, it is produced on irrigation fields and under rain-fed agriculture (Esmaeili & Heidarzade, 2012; Dien et al., 2019). The world's production of rice has been estimated at around 650 million tonnes and the land under its cultivation has been estimated at around 156 million hectares (FAO, 2016). Asia is the major producer of rice accounting for about 90% of the global production (Hussain et al., 2015).

Rice contributes to the essential dietary energy requirement of the human body (Rohman et al., 2014). It consists of about 80% carbohydrate, 7% protein, 2% water and 3.5% fat (Oko et al., 2012). Rice also consists of essential vitamins such as vitamin B1 (thiamine), vitamin B2 (riboflavin), tocopherol, B3 (niacin), some appreciable quantities of iron, phosphorus and calcium (Deepak & Shukla, 2011; Rohman et al., 2014).

The ever teeming world population necessitated the increased production of rice around the world to meet the higher demand for food and ensure its security. However, drought, declining precipitation, salinization of water resources, frequent storms and extreme temperatures driven by a changing climate threatened rice production (Zheng et al., 2016; Dien et al., 2019). Greater than a half of global land under rice plantations are affected by drought driven by a changing climate (Sheteiwy et al., 2018). Increasing drought episodes associated with climate change have caused tremendous yield declines in rice, corn, maize and wheat productivity in Asian and African countries that have lower adaptation capabilities (Fei et al., 2020). Poor germination and emergence of seedlings under water deficit stress are reported in rice, millet and peas (Marthandan et al., 2020). Investigations reported that rice-growing major coastal areas of the world are susceptible to be affected by climate change due to increased severe storms, wetland loss and heightened salinity (Fahad et al., 2019).

Many approaches for enhancing germination and drought resistance of rice such as breeding and selection, mutation breeding, polyploidy breeding and production of transgenic lines were practiced. However, these approaches are very expensive, labour-intensive and beyond the reach of low-skilled and low-income farmers in Asian and African countries (Jisha et al., 2013; Zheng et al., 2016). Physiological enhancement approach of seed priming has been practiced by farmers and was proven effective to enhance germination, seedling emergence, growth and drought resistance of rice (Hussain et al., 2017; Javed et al., 2020; Wang et al., 2016; Zheng et al., 2016). Seed priming entails soaking seeds in water, inorganic salts, hormones and nutrients to initiates essential germination processes before sowing. It is an easy, cheap and less

harmful physiological technique (Hussain et al., 2015; Zheng et al., 2016; Parveen et al., 2019).

1.2 Statement of the Problem and Justification of Study

Sufficient production of food is one of the main challenges confronting many developing nations. These challenges are due to climate change, population growth and many other ecological pressures. Larger quantity of rice is being produced in Nigeria on vast fertile land estimated at 4.6-4.9 million hectares. However, drought and extreme temperature driven by a changing climate affect germination and establishment of rice seedling that cause yield decline (Liu et al., 2014). Climate change effects manifesting in insufficient rainfall and increasing temperature annually negatively affect germination, seedling emergence and agricultural productivity of rain-fed and irrigated rice in Nigeria (Kim et al., 2017).

FARO44 rice is one of the varieties farmers mainly produced in Nigeria because of its long grain, pest rersistance and higher productivity. However, its production under a changing climate has been affected by drought which causes poor germination, emergence and colossal yield decline (Akinwale et al., 2012). Many survival approaches have been evolved for decades by FARO44 rice farmers to adapt to the adverse impacts of climate variability on rice production; these include mixed cropping, used of improved drought resistant crops as well as diversification of farming activities (Kim et al., 2017). However, little success has been achieved.

Several rice-growing areas in northern Nigeria practice direct seed broadcasting system under rain-fed agriculture because this system has less input cost, resource use effective and produce higher yield. However, drought due to limited rainfall annually affect germination, emergence and establishment of FARO44 rice variety which is produced mainly by farmers. Poor germination and establishment of seedlings lead to decline in the yield of FARO44 rice (Emoghene et al., 2015).

There were several seed priming studies carried out to enhance germination, establishment of seedlings and drought tolerance of many rice varieties. However, to date, no seed priming study has been conducted using potassium nitrate (KNO_3), silicon dioxide (SiO_2) and salicylic acid (SA) on FARO44 rice to address poor germination, seedling emergence and enhance its drought tolerance. Thus, the novelty of this study being the first on this rice variety cannot be overemphasised. The choice of FARO44 rice as a study subject premised on its long grain, high yield and its adaptability to different ecological zones in the tropics (Akinwale et al., 2012; Oluwaseyi et al., 2016).

Priming of seeds with KNO_3, SiO_2 and SA is a reliable, pragmatic, cheap and an effective technique for improving germination, seedling emergence, seedling growth and drought resistance of crops such as rice (Hussain et al., 2015; Parveen et al., 2019; Wang et al., 2016; Javed et al., 2020). Pre-germination metabolic processes in KNO_3, SiO_2 and SA primed seeds include increased water take up, repair and synthesis of DNA, RNA and

protein, activation of reserve mobilising enzymes like acid phosphatase, dehydrogenase, α-amylase, β-amylase and antioxidants for embryonic growth and development (Wojytila et al., 2016; Zheng et al., 2016).

It is expected that seed priming study with KNO_3, SiO_2 and SA afford Nigerian farmers with a simple and easy technique to address poor germination and seedling emergence, and manage drought effects on FARO44 rice thereby boost its yield. Nigeria is the most populous country in West Africa that spends about ten billions of US dollars on the importation of rice from different countries around the world despite the vast fertile and arable land the nation has (Kim et al., 2017). In 2015 and 2017, the country has imported about 20,000 metric tonnes of rice from India, Vietnam, Thailand and China (FAO, 2016). Thus, seed priming study with KNO_3, SiO_2 and SA helps Nigerian farmers to boost local production of FARO44 rice and reduces spending on importation of expensive rice.

1.3 Objectives of the Study

The objectives of the study are:

i. To evaluate the suitability of potassium nitrate (KNO_3), salicylic acid (SA) and silicon oxide (SiO_2) as priming agents for FARO44 rice germination and early seedling growth.
ii. To investigate the effects of seed priming on germination, early seedling growth and biochemical characteristics of FARO44 rice seedlings under drought conditions.
iii. To compare the activities of protective enzymes superoxide dismutase (SOD), catalase (CAT) and ascorbate peroxidase (APX) of FARO44 rice seedlings under drought conditions.
iv. To evaluate the effects of seed priming on membrane stability and drought tolerance of FARO44 rice seedlings under drought conditions.

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