UNIVERSITI PUTRA MALAYSIA

SEED PRIMING AGENT FOR YIELD IMPROVEMENT AND ALLEVIATION OF MOISTURE STRESS IN RICE (Oryza sativa L.)

KAREEM ISIAKA

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SEED PRIMING AGENT FOR YIELD IMPROVEMENT AND ALLEVIATION OF MOISTURE STRESS IN RICE (*Oryza sativa* L.)

By

KAREEM ISIAKA

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

June 2016

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DEDICATION

I dedicate this work to Allah, the custodian of knowledge and understanding.

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

SEED PRIMING AGENT FOR YIELD IMPROVEMENT AND ALLEVIATION OF MOISTURE STRESS IN RICE (*Oryza sativa* L.)

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June 2016

Chairman : Professor Mohd Razi Ismail, PhD
Institute : Tropical Agriculture

Yield improvement and alleviation of moisture stress are very important to achieve self-sufficiency in rice production. Therefore, this research was conducted to determine the efficacy of seed priming in yield improvement and moisture stress alleviation in MR219 rice. The first experiment was for determination of potential priming concentrations for MR219 production. It was found that calcium chloride, polyethyl glycol and kinetin priming were 73.91, 69.57 and 95.65% respectively better than the control. This was followed by the second experiment which focused on selecting the best priming durations for the selected priming agents for MR219 production under normal and moisture stressed conditions. From this experiment, for overall performance under both normal and stress conditions, calcium chloride, polyethyl glycol and kinetin priming were 33.74, 26.86 and 23.69% respectively better than their respective alternate priming duration in yield production. The efficacy of the selected priming agents was tested against pre-germination under normal and moisture stress conditions. It was found that 100 ppm kinetin priming was 12% better than pre-germination under normal condition in grain yield. When stress was imposed at tillering and post-anthesis stages, the grain yield from 100 ppm kinetin priming was 12.39 and 30.10% respectively better than pre-germination. However, none of the priming treatments could improve the yield of MR219 rice when moisture stress was imposed at pre-anthesis stage. Based on this result, a 24-hour priming with 100ppm kinetin was selected for detailed physiological and biochemical studies under moisture stress. It was found that the betterment of 100 ppm kinetin over pre-germination was found that the betterment of 100 ppm kinetin over pre-germination was 91.84% in primary chlorophyll fluorescence, 36.84% in maximum quantum yield of photosystem II, 6.84% in intercellular carbon dioxide, 16.67% in transpiration rate, 6.54% in cell membrane thermo-stability, 17.88% in relative injury, 43.03% in water use efficiency, 31.88% relative water content, 36.35% in superoxide dismutase, 28.78% in anthocyanin, 22.17% in proline, 14.67% in total phenol, 5.88% polyphenol, 16.04% in peroxidase activity, 17.28% in phenylalanine ammonia lyase activity, 27.88% in ascorbate oxidase activity, 5.16% in ascorbate peroxidase activity,

11.40% in hydrogen peroxide, 21.38% in lipid peroxidation, 34.61% in number of tillers,10.24% in plant height, 33.33% in productive tillers and 12.39% in yield as compared with the control. All these results explain the vegetative stability of the plants when they were stressed.

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

AGEN PRIMING BIJI BENIH BAGI PENAMBAHBAIKAN HASIL DAN MENGURANGKAN TEGASAN AIR DALAM TANAMAN PADI (*Oryzasativa* L.)

Oleh

KAREEM ISIAKA

Jun 2016

Pengerusi : Profesor Mohd Razi Ismail, PhD
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Penambah baikan hasil dan mengurangkan kesan tegasan air adalah sangat penting dalam mencapai tahap sara-diri dalam pengeluaran tanaman padi. Oleh itu, penyelidikan ini telah dijalankan untuk menentukan keberkesanan priming biji benih danpengurangan tegasan kelembapan di dalam padi MR219. Kajian yang pertama adalah untuk mengenalpasti kepekatan priming yang berpotensi bagi pengeluaran MR219. Kajian tersebut mendapati bahawa priming daripada kalsium klorida, *polyethyl glycol* dan kinetin adalah 73.91, 69.57 dan 95.65% masing – masing adalah lebih baik berbanding kawalan. Ini diikuti dengan kajian kedua yang memfokuskan pemilihan tempoh priming yang terbaik untuk agen priming terpilih bagi pengeluaran MR219 di bawah keadaan biasa dan tegasan air. Dari eksperimen ini, prestasi keseluruhan bagi kedua –dua jenis keadaan normal dan di bawah tegasan, priming oleh kalsium klorida, *polyethyl glycol* an kinetin masing-masing 33.74, 26.86 dan 23.69% lebih baik daripada tempoh priming bergilir masing-masing dalam pengeluaran hasil. Keberkesanan agen priming terpilih telah diuji terhadap pra-percambahan bawah keadaan normal dantegasan air. Didapati bahawa priming menggunakan 100 ppm kinetin adalah 12% lebih baik daripada sebelum percambahan di bawah keadaan normal bagi hasil bijirin. Apabila tegasan air berlaku pada fasa pertumbuhan aktif (*tillering*) dan peringkat pasca-antesis), hasil bijirin dari priming 100 ppm kinetin adalah masing-masing 12.39 dan 30.1% lebih baik daripada sebelum percambahan. Walau bagaimanapun, tiada rawatan priming yang boleh meningkatkan hasil padi MR219 apabila tegasan kelembapan dikenakan pada peringkat pra-antesis.. Berdasarkan keputusan ini, priming dengan 100 ppm kinetin selama 24 jam telah dipilih untuk kajian fisiologi dan biokimia terperinci di bawah tegasan air. Kajian telah mendapati bahawa kebaikan rawatan priming 100 ppm kinetin terhadap pra – percambahan adalah 91.84% *fluorescence* klorofil primer, 36.84 % hasil kuantum maksima fotosistem II, 6.84 % karbon dioksida intersel, 16.67% kadar transpirasi, 6.54% kestabilan termo sel membran, 17.88% kecederaan relatif, 43.03% kecekapan peggunaan air, 31.88 kandungan air relatif, 36.35% *superoxide dismutase*, 28.78% antosianin, 22.17% proline, 14.67% jumlah fenol, 5.88% polifenol, 16.04%

aktiviti *peroxidase*, 17.28% aktiviti *phenylalanine ammonia lyase*, 27.88% aktiviti *oxidase* askorbat, 5.16% aktiviti peroxidase askorbat, 11.40 % hydrogen peroksida, 21.38% *lipid peroxidation*, 36.84% bilangan tangkai, 10.24% ketinggian tanaman, 33.33% tangkai produktif dan 12.39 % penghasilan dibandingkan dengan kawalan. Kesemua keputusan ini menunjukkan kestabilan vegetative tanaman apabila berada dalam keadaan tegasan air.

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I certify that a Thesis Examination Committee has met on 15th June, 2016 to conduct the final examination of Kareem Isiaka on his thesis entitled "seed priming agent for yield improvement and alleviation of moisture stress in rice" in accordance with the Universities and University Colleges Act 1971 and the Constitution of Universiti Putra Malaysia [P.U. (A) 106] 15 March 1998. The committee recommends that the student be awarded the degree of Doctor of Philosophy.

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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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LIST OF ABBREVIATIONS

NAR	Net assimilation rate
CGR	Crop growth rate
RGR	Relative growth rate
LAI	Leaf area index
$CaCl_2$	Calcium chloride dihydrate
PEG	Polyethyl glycol 6000
PAL	Phenylalanine ammonia lyase
SAS	Statistical Analysis System
MDA	Malondialdehyde
SOD	Superoxide dismutase
CAT	Catalase
P_n	Net photosynthesis
SC	Stomatal conductance
CO_2	Carbon dioxide
AGR	Absolute growth rate
LSD	Least significant difference
mM	Millimolar
ml	milliliter
RDW	Root dry weight
SDW	Shoot dry weight
RFW	Root fresh weight
RDW	Root dry weight
FW	Fresh weight
DW	Dry weight
ABA	Abscisic acid
w/v	Weight per volume
v/v	Volume per volume
GAE	Gallic acid equivalent
mol	Mole
µmol	Micro mole
ANOVA	Analysis of variance
APX	Ascorbate peroxidase
BSA	Bovine Serum Albumin

CHAPTER ONE

INTRODUCTION

1.1 Background

Rice (*Oryza sativa*) provides food for more than half of the world's population. The problem of low yield has led Malaysia to rice importation to meet the demands of her growing population. This problem needs sound solution to make the target of having higher yield and feeding the nation achievable for the farmers. The solution should bea simple and adoptable technique. From physiological perspective, the solution is seed treatment called seed priming. This might solve the problem of low yield and moisture stress.

Another bane to achievement of higher yield in rice production is the problem of drought resulting from climate change. Drought becomes an important issue now because it prevents rice from exhibiting its full potential. Rice inevitably requires higher level of water supply as a necessity for survival because it is a semi aquatic plant. Therefore, every possible alternative which can alleviate the problem of water inadequacy in the plants should be sought to keep rice production on a balanced wheel. In the search of a reliable alternative, farmers and researchers are now tending towards upland system of rice production with supplementary irrigation like other cereals with the ultimate aim of getting higher yield (Huaqi et al., 2002). However, the major setbacks of upland system of rice production are high weed infestation and poor seedling establishment (Farooq et al., 2006). It has now been discovered that seed priming could improve yield of lowland and upland rice as well as solve the problems of transplanting in rice production (Farooq et al., 2007).

This comes through seed soaking in aerated water or solution of low osmotic potential to give partial hydration of the seeds so that germination processes will begin until *germination sensu stricto* stage is reached without radicle emergence (Farooq et al., 2010). After draining the priming solution, the seeds are thoroughly washed with water and dried back to their original moisture levels to give room for storage until the time of sowing (McDonald, 2000). Increased germination, seedling vigour improvement and ability to grow under diverse environmental conditions which finally result in yield and quality improvements are some of the advantages of seed priming. It also has the ability to improve low temperature tolerance (Sasaki et al., 2005) and reduce plasma membrane permeability as well as the level of reactive oxygen species (Fashui, 2002) in rice. Another added advantage of seed priming is the improvement of drought and salinity tolerance in the resulting plants. Though the benefits of seed priming have been proven and established, it becomes harmful to the seeds on occasions. Consequently, germination and the resulting plants (seedlings) will be adversely affected. Finally, the major advantages of seed priming are much more pronounced in plants under adverse environmental conditions whether biotic or abiotic.

Seed priming is counted among seed vigour improvement strategies and can be achieved through the use of different solutions or water. The cheapest and most abundant means of priming is water which may be ordinary, de-ionized or distilled. On the other hand, the use of osmotic salts like polyethyl glycol (PEG) and sodium chloride as well as growth regulators like gibberellic acid and salicylic acid or any other seed-friendly chemicals like fertilizer which can lower water potential are more effective than the use of ordinary water. These osmotic salts are added to water and the solutions are used for soaking seeds for a particular duration before sowing. The duration of soaking ranges from hours to days as long as it does not exceed the safe limit (the maximum duration for priming the seeds of a specific cultivar). If the safe limit is exceeded, it may result in pre-mature germination or damage of seeds or seedlings due to toxicity (Harris et al., 2002). Furthermore, retardation of germination could be an eventuality.

It is now clear that priming effectiveness is duration-dependenteven when powerful osmotic salts or phyto-hormones are used. This is because imbibition depends on the duration of soaking. So, the longer the treatment duration: the more the imbibition. Variance occurs on this aspect in different plants. Some plants are favoured by long duration while short duration is the favourite of others. This should be determined to save the seeds from exceeding the safe limit which might predispose the seeds to chemical toxicity or seed damage which are both detrimental to germination and final seedling establishment.

The most important aspects of rice production are germination and sound seedling establishment. These determine the plant population which determines the final yield because it is one of the yield determinants in rice production. Since better germination and building of stress tolerance are gained from priming, the challenge of unexpected stoppage in rainfall could be successfully tackled with a view to preventing total yield loss that is popular with drought stress.

The present research used MR 219 rice because it is the most cultivated variety in Malaysia. Its height is between 83.0 and 87.0 cm. It matures between 105 and 112 days. Its panicle length is 24.5 cm. The length of its grain is 10.04 mm, its width is 2.27mm while its 1000 grain weight is 27.10 g. It has milling recovery of 65%, head rice of 72% while its amylose content is 20.10%.It is moderately resistant to leaf blast, bacteria leaf blight, sheath blight, Tungro (PMW) and brown plant hopper while it is tolerant to panicle blast (Hassan, 2012).

1.2 Problem Statement

Low yield, drought stress and inability to produce round the year have led to insufficiency in production of rice for the teaming population of Malaysia for years. Drought alone leads to loss of millions of ringgit between 2003 and 2012 in Malaysia. The loss estimates were RM 10.6 to RM 16.3 million and RM4 to RM6 million for the main and off-seasons respectively in Muda Agricultural Development Authority (MADA). For Kemubu Agricultural Development Authority (KADA), the loss estimates were RM 5.5 to RM 8.1 million and RM5.5 to RM8.2 million for the main and off-seasons respectively (DOA, 2013). The problems have also forced the country to resort to importation of rice leading to high leakage which could cripple the economy and render the farmers (producers) perpetually poor for not having solutions to their problems. Therefore, there is dire need for improving rice yield and alleviating drought problem. This comes through the use of seed priming which is simple in its process and easy in its adoption. This will allow year-round productions which will greatly increase yield as expected for feeding the nation.

1.3 Hypothesis

Seed priming improves germination, seedling establishment, yield and alleviates drought stress in direct seeded rice (MR219 variety).

1.4 Objectives

- To determine the effects of priming agent concentrations on performance or rice.
- To determine the effects of priming agents and soaking durations on performance of rice under different irrigation regimes.
- To improve growth and yield of rice under normal and moisture stress conditions with seed priming.
- To establish physiological and biochemical bases of moisture stress tolerance induced by seed priming in rice.

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