DROUGHT STRESS MEMORY OF PRIMED SEEDS AND THE DYNAMICS AND RESILIENT OF ANTHESIS QUALITY TRAITS IN DROUGHT STRESS RICE (*Oryza sativa* L.)

BY

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ABSTRACT

Seed priming and breeding for drought tolerant rice could be promoted as a potentially sustainable alternative in alleviating drought stress challenges in rice cultivation. The present study was aimed to: 1) investigate the physiological mechanism and durability of drought stress memory in primed seeds of rice, 2) propose a new term called anthesis quality traits for all interrelated traits involved in the flowering process of rice, 3) determine the effects of re-watering on the dynamics of anthesis quality traits in drought stress rice, 4) study the genetics and physiological basis of resilient anthesis quality traits in drought stress rice, 5) screen for potentially tolerant genotype having resilient anthesis quality traits under severe drought at the heading stage, and 6) ascertain the presence of *qDTY2.2* and *qDTY12.1* in the identified drought tolerant genotype with resilient anthesis quality traits. To date, scientific information on the physiological mechanism and durability of drought stress memory in primed seeds are scarcely available. Laboratory and pot-trial experiment for two seasons were conducted to fulfil those gaps. Results of the present study indicated the involvement of proline in the recruitment of drought stress memory in primed seeds. The acquired stress memory however was temporary and only effective during germination and early seedling growth stages. Therefore, study towards breeding drought tolerant rice was then conducted. The present study hypothesizes that anthesis quality traits is strongly associated with harvestable grain yield under drought and could be potentially used as a selection criterion in breeding drought tolerant rice. Pot-trial experiments using randomized complete block design (RCBD) were conducted to verify this hypothesis. Briefly, spikelet moisture content at above 80% was required to maintain optimum anthesis process in rice for higher spikelet fertility and grain yield under drought. Two novel genotypes of local landraces namely Jarom Mas and Merah Wangi (1) have been identified having resilient anthesis quality traits under severe drought stress at the heading stage. Those two genotypes can retain their spikelet moisture content at above 80% under severe stress condition. The rate of lipid peroxidation level in those genotypes was significantly lower as compared to susceptible control, IR64 suggesting that they were not under stress condition. The genetics and traits association analysis indicated that anthesis quality traits were highly associated with harvestable grain yield under drought and could be used as a selection criterion due to high inheritance values and potential genetic advance. However, assessing anthesis quality traits during the selection process under field condition could be extremely difficult. Therefore, novel phenotyping assessment based on spikelet moisture content and drying score index has been proposed as a more sensible selection criterion. Moreover, the drought grain yield quantitative trait loci (*QTLs*) of *qDTY12.1* was more superior with significantly lower grain yield reduction under severe stress as compared to *qDTY2.2*. However, those two *qDTYs* were not detected in the novel genotypes Jarom Mas and Merah Wangi (1) suggesting that other *qDTYs/QTLs* might be associated with resilient anthesis quality traits under drought in rice. Further study on the association mapping of *qDTYs/QTLs* with spikelet moisture content may be conducted to identify closely linked major-effect *QTLs* for future use in marker-assisted breeding of drought tolerant rice.

خلاصة البحث

إن تنشيط واستيلاد البذور لانتاج أرز مقاوم للجفاف بامكانه أن يكون بديلا مستديما للتخفيف من التحديات الناتجة عن الجفاف في زراعة الأرز. هدفت هذه الدراسة إلى: 1) التحقيق في الآلية الفسيولوجية ومتانة ذاكرة الإجهاد الناتج عن الجفاف في بذور الأرز المنشَّطة، 2) اقتراح مصطلح بديل باسم سمات جودة عملية الإزهار لجميع السمات المترابطة والمشاركة في عملية إزهار الأرز، 3) تحديد آثار إعادة الري على ديناميكيات سمات جودة الإزهار في الأرز المعرض للجفاف، 4) دراسة الأسس الوراثية والفسيولوجية لسمات جودة الإزهار المرنة في الأرز المعرض للجفاف، 5) البحث عن نمط جيني قادر على تحمل الاجهاد ويتميز بسمات جودة الإزهار المرنة تحت ظروف الجفاف الشديد في مرحلة بروز السنبلة، و 6) التأكد من وجود qDTY2.2 و qDTY12.1 في النمط الجيني المتحمل للجفاف مع سمات جودة الإزهار المرنة. حتى الآن هناك القليل من المعلومات العلمية حول الآلية الفسيولوجية ومتانة ذاكرة إجهاد الجفاف في البذور المنشطة. أجريت التجارب المختبرية وتجارب الأوعية لمدة موسمين زراعيين لملء هذه الثغرات. أوضحت نتائج هذه الدراسة مشاركة البرولين في توظيف ذاكرة الإجهاد الناتج عن الجفاف في البذور المنشطة. كانت ذاكرة الإجهاد المكتسبة مؤقتة وفعالة فقط خلال الإنبات والمراحل المبكرة لنمو الشتلات. ولذلك أجريت هذه دراسة لاستزراع أرز مقاوم للجفاف. افترضت الدراسة الحالية أن سمات جودة التوليف ارتبطت ارتباطًا وثيقًا بإنتاج الحبوب القابلة للحصاد تحت الجفاف وبالامكان استخدامها كمعيار انتقائي في زراعة أرز مقاوم للجفاف. أجريت تجارب الأوعية باستخدام تصميم الكتلة الكاملة العشوائية للتحقق من هذه الفرضية. كان محتوى رطوبة السنبلة أعلى من 80٪ مطلوبًا للحفاظ على عملية التخصيب المثالية في الأرز وذلك لزيادة خصوبة السنبلة وإنتاج الحبوب تحت الجفاف. تم تحديد نوعين وراثيين جديدين من السلالات المحلية وهما جاروم ماس وميره وانجي (1) حيث أن لهما صفات جودة إزهار مرنة تحت الجفاف الشديد في مرحلة إزالة الأزهار. بامكان هذين النوعين الجينيين الحفاظ على محتواهما الرطوبي في السنبلة بنسبة أعلى من 80٪ تحت ظروف إجهاد شديدة. كان معدل مستوى أكسدة الدهون في هذه الأنماط الجينية أقل بكثير مقارنة بالعينة الضابطة الحساسة IR64، مما يشير إلى أنها لم تكن تحت ظروف إجهادية. أوضح تحليل الروابط الوراثية والصفات أن سمات جودة الإزهار ارتبطت ارتباطًا وثيقًا بإنتاج الحبوب القابلة للحصاد تحت الجفاف وبالامكان استخدامها كمعيار للاختيار نظرا لارتفاع قيمة التوريث والتقدم الجيني المحتمل. ومع ذلك فإنه قد يكون من الصعب جدا تقييم سمات جودة الإزهار أثناء عملية الاختيار في ظل الظروف الحقلية. ولذلك تم اقتراح تقييم جديد للأنماط الظاهرية بالاعتماد على محتوى رطوبة السنبلة ومؤشر درجات التجفيف كمعيار اختيار معقول. بالإضافة إلى ذلك فإن مواضع السمات الكمية لمحصول الجفاف لـ qDTY12.1 كان أكثر تفوقًا لمحصول الحبوب تحت الإجهاد الشديد، مقارنة بـ qDTY2.2. ومع ذلك لم يتم الكشف عن هذين النوعين من الـ qDTY في الأنماط الجينية الجديدة جاروم ماس وميره وانجي (1) مما يشير إلى أن الـ qDTY/QTL الأخرى قد ترتبط بسمات جودة الإزهار المرنة تحت الجفاف في الأرز. بالإمكان لاحقا إجراء المزيد من الدراسات حول رسم خرائط الارتباط لـ qDTY/QTL مع محتوى رطوبة السنبلات لتحديد روابط الـ QTL ذات التأثير الكبير المرتبطة ارتباطًا وثيقًا للاستخدام المستقبلي في زراعة أرز مقاوم للجفاف بمساعدة المؤشرات.

APPROVAL PAGE

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DECLARATION

I hereby declare that this thesis is the result of my own investigations, except where otherwise stated. I also declare that it has not been previously or concurrently submitted as a whole for any other degrees at IIUM or other institutions.

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LIST OF ABBREVIATION

ADST	After Drought Stress Treatment
ANOVA	Analysis of Variance
BIL	Backcross Inbred Line
CGR	Crop Growth Rate
CRD	Completely Randomized Design
DAS	Day After Sowing
DNA	Deoxyribonucleic Acid
DMRT	Duncan New Multiple Range Test
DSB	Drought Stress at Booting
DSH	Drought Stress at Heading
FW	Fresh Weight
GI	Germination Index
GP	Germination Percentage
GY	Grain Yield
HI	Harvest Index
IKI	Iodine Potassium Iodide
IIUM	International Islamic University Malaysia
IRRI	International Rice Research Institute
kPa	Kilo Pascal
LAI	Leaf Area Index
LRWC	Leaf Relative Water Content
MARDI	Malaysian Agricultural Research and Development Institute
MAB	Marker-Assisted Breeding
MDA	Malondialdehyde
mPa	Mili Pascal
NAR	Net Assimilation Rate
NIL	Near Isogenic Line
PCR	Polymerase Chain Reaction
PEG	Polyethylene Glycol
Pro	Proline
QTL	Quantitative Trait Loci
qDTY	Drought Grain Yield QTL
RCBD	Randomized Complete Block Design
ROS	Reactive Oxygen Species
SAS	Statistical Analysis Software
SDGs	Sustainable Development Goals
SF	Spikelet Fertility
SMC	Spikelet Moisture Content
SN	Number of Seed
SSR	Simple Sequence Repeat
SVI	Seedling Vigour Index
UKM	Universiti Kebangsaan Malaysia
UPGMA	Unweighted Pair Group Method with Arithmetic Mean
WW	Well-watered

CHAPTER ONE

INTRODUCTION

1.1 BACKGROUND

Globally, all of the United Nations (UN) Member States have adopted the agenda for sustainable development in 2030 as a commonly shared blueprint for peace and prosperity for people and the planet. The main pillar of this global aspiration is the 'Sustainable Development Goals (SDGs)' (UNDP, 2019). Among the seventeen listed SDGs, two of the most significant goals related to agricultural productivity are the 'No. 2: Zero hunger' and 'No. 13: Climate action'. Great impediment in the risk of climate change and disaster may become a hindrance in achieving the zero hunger goal. Increasing global temperature, seawater level and areas of arid land along with unpredictable weather and climate disaster may restrain food and agricultural production. The sustainable approach in mitigating climate change impacts thus being highlighted and promoted globally.

Among various climatic change challenges, drought has been perceived as one of the most severe climate-related risks for rice production especially in the lowland rainfed areas (Shamsudin et al., 2016a). This could be due to the high susceptibility of modern high yielding semi-dwarf rice cultivars to drought stress (Vikram et al., 2011). Liu et al. (2006) reported that reproductive stage drought stress at heading significantly reduced pollen quality, anther dehiscence, spikelet fertility and harvestable grain yield of modern high-yielding rice cultivar IR64. Besides, Puteh et al. (2013) also reported that pre-anthesis drought stress significantly reduced harvestable grain yield of our local rice cultivar MR219 due to lower rate of pollen viability and spikelet fertility.

Various approaches could be used in mitigating drought stress challenges in rice cultivation. According to Gana et al. (2020), the approaches could be genetic in nature such as breeding for drought tolerant cultivar or physiological strategies such as seed priming. Breeding for drought tolerant rice is however more complex, time-consuming and expensive as compared to seed priming approach which is a simple, practical and cost-effective alternative (Gana et al., 2020).

Seed priming could be described as a pre-sowing seed treatment through control or restricted imbibition in various types of solution to initiate important germination processes without the protrusion of radicle, then re-dried to close to initial weight before sowing (Chen and Arora, 2013; Farooq et al., 2019; Gana et al., 2020). Previous studies had reported positive effects of seed priming on enhancing seed germination and seedling growth performance of various crops under drought stress condition (Chen and Arora, 2011; Chen et al., 2012; Mouradi et al., 2016). Kalhori et al. (2018) reported that primed seeds recorded higher germination percentage and germination index than non-primed seeds under drought. Farooq et al. (2019) stated that seed priming could improve germination performance, stand establishment, crop growth and yield under optimal and sub-optimal conditions. Advance metabolic processes in primed seeds promote rapid germination, vigorous seedlings with uniform crop stands and later translated into higher harvestable yield due to efficient remobilisation of nutrients from the source to the sink (Farooq et al., 2019; Gana et al., 2020). However, some previous studies reported contradictory findings that seed priming did not significantly improve grain yield both under the glasshouse and field conditions (Giri and Schillinger, 2003; Subedi and Ma, 2005). Lutts et al. (2016) also stated that improvement in primed seeds normally obvious during germination and early seedling growth but progressively disappears at the adult stage.

From the Malaysian perspective, seed priming strategy is currently not being practice by our local rice farmers. Instead, our rice farmers practice direct sowing of the pre-germinated rice seeds (pre-soaked seeds in clean water for overnight, rinse for about 48 to 72 hours under the shade, then finally broadcasted onto puddled soil). However, the pre-germinated seeds with protruded radicle and plumule are susceptible to damage during sowing (Harris et al., 2002). Primed seeds, in contrast, behave as dry seeds and will not germinate unless sown under moist-soil environment. This mainly because primed seeds were surface dried after pre-soaked at a definite time depending on types of crop and cultivar before sowing (Harris et al., 2002).

However, due to surface drying procedure, which is more labour intensive as compared to the pre-germination procedure, the seed priming practice is not yet adopted by our local rice farmer. Nonetheless, if seed priming could be proven to significantly improve harvestable grain yield under reproductive stage drought, it could be potentially promoted to our local rice farmers for adoption. However, if it is found to be not effective, another approach which is genetic in nature such as breeding for drought tolerant rice should be emphasized. The present study hence was conducted as an attempt to verify the potential of seed priming in improving harvestable grain yield under reproductive stage drought in rice.

1.2 PROBLEM STATEMENT

Chen and Arora (2013) suggest that seed priming might induce the recruitment of drought stress memory in the primed seeds. However, physiological mechanisms in the induction and recruitment of drought stress memory in the primed seeds of rice are still unclear. Besides, Lutts et al. (2016) raised an argument that although seed priming may undoubtedly improve germination performance and crop establishment under adverse condition, its impact on crop production and harvestable yield is still uncertain. Therefore, it was strongly urged that more studies need to be conducted focusing on adult plants issued from primed seeds, especially at the reproductive stage, to assess the long-term impact of priming on cultivated plants throughout the plant life cycle (Lutts et al., 2016).

Theoretically, seed priming treatment will induce and promote the recruitment of drought stress memory which will lead to the improvement in harvestable grain yield under reproductive stage drought stress. Nevertheless, this postulation needs to be proven by a reliable scientific experiment.

In light of this argument, if the drought stress memory of primed seeds could be prolonged until reproductive growth stages and significantly reduce grain yield loses under reproductive stage drought stress, it would be an economical approach for farmer's adoption especially during the prolonged drought season. However, if it is proven otherwise, another sustainable approach mainly breeding drought tolerant rice need to be taken into action.

Breeding drought tolerant rice however is regarded as a challenging task due to involvement of complex quantitative traits along with various tolerance mechanisms to match drought stress patterns in nature. Initiation of trait development program prior to official breeding program establishment may aid in simplifying such complexness. The trait development program would provide; 1) basic information about the genetics and physiological basis of the most significant trait to be improved, 2) potentially tolerant donor having the targeted traits, and 3) recommendation on the appropriate breeding method and selection criterion.

1.3 RESEARCH HYPOTHESIS

1- Proline is involves in the induction and recruitment of drought stress memory in primed seeds.

2- Drought stress memory in primed seeds is durable, starting from the germination stage until the reproductive growth stages.

3- All interrelated traits involved in the flowering process of rice could be collectively termed as the anthesis quality traits.

4- The re-watering activity affects the dynamics of anthesis quality traits in drought stress rice.

5- Anthesis quality traits are strongly associated with the harvestable grain yield under drought and potentially be used as selection criterion in breeding for drought tolerant rice.

6- Drought tolerant genotype possess resilient anthesis quality traits.

7- The *qDTY2.2* and *qDTY12.1* are present in the potentially drought tolerant genotype with resilient anthesis quality traits.

1.4 RESEARCH OBJECTIVES

1- To investigate the physiological mechanism and durability of drought stress memory in primed seeds of rice.

2- To propose a new term called anthesis quality traits for all interrelated traits involved in the flowering process of rice.

3- To determine the effects of re-watering on the dynamics of anthesis quality traits in drought stress rice.

4- To study the genetics and physiological basis of resilient anthesis quality traits in drought stress rice.

5- To screen for potentially tolerant genotype having resilient anthesis quality traits under severe drought at the heading stage.

6- To ascertain the presence of *qDTY2.2* and *qDTY12.1* in the identified drought tolerant genotype with resilient anthesis quality traits.

1.5 SIGNIFICANCE OF STUDY

The present study demonstrate the involvement of proline in the induction and recruitment of drought stress memory in primed seeds and verify potential performance of primed seeds in improving the agronomic performance of rice under reproductive stage drought. The original contribution of the present study towards breeding drought tolerant rice would be the identification of potentially tolerant donor having resilient anthesis quality traits and the development of novel phenotyping selection criterion based on the spikelet moisture content and drying score index.

CHAPTER TWO

LITERATURE REVIEW

2.1 THE BIOLOGY OF RICE

2.1.1 Taxonomic Classification

Rice is classified under family Gramineae or Poaceae and the genus *Oryza* L. as shown in Figure 2.1. Although there are more than 23 species of *Oryza* that have been identified, only two species are currently domesticated mainly *Oryza sativa* in Asia and *Oryza glaberrima* in Africa (Elzebroek, 2008). The *Oryza sativa* L. has two major subspecies known as the indica (widely grown in tropics) and the japonica (usually grown in temperate). Commercially, there are two types of rice grain available in the market mainly the white rice and glutinous rice, respectively.

KINGDOM	Plantae
DIVISION	Magnoliophyta
CLASS	Liliopsida
ORDER	Cyperales
FAMILY	Poaceae
GENUS	*Oryza* L.

Figure 2.1 Taxonomic Classification of Rice (Source: USDA, 2020)

2.1.2 Growth Stages and Development

The growth and development of rice could be divided into three phases mainly the vegetative, reproductive and ripening or maturation stages as illustrated in Figure 2.2 and described in Table 2.1. According to Dunand and Saichuck (2014), the growth stages are a series of continuous process rather than a distinct event. The duration of each stage, however, varies according to variety and ecosystem or environment. The vegetative stage includes seed germination, seedling, tillering, and internode elongation. The reproductive stage begins with panicle initiation and ended with the anthesis. The focus of the present study was on the flowering process which consists of the booting stage (upward extension of panicle causes flag leaf sheath to protrude), the heading stage (emergence of panicle tip from the flag leaf), and the anthesis stage (pollination and fertilization). The ripening phase includes grain filling and maturity.

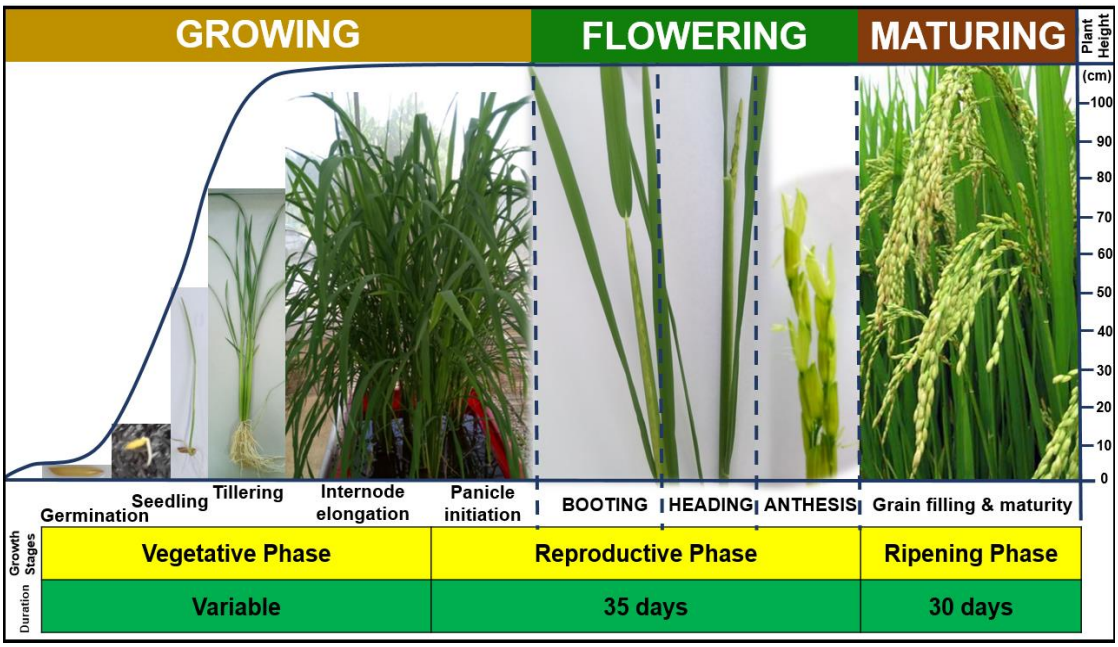

Figure 2.2 Growth and Development Stages of Rice