UNIVERSITI PUTRA MALAYSIA

AGRO-MORPHOLOGICAL, PHYSIOLOGICAL AND BIOCHEMICAL CHARACTERISTICS OF RICE SUBJECTED TO CYCLIC WATER STRESS AND POTASSIUM FERTILIZERS

NURUL AMALINA BT MOHD ZAIN

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By

NURUL AMALINA BT MOHD ZAIN

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

August 2015

DEDICATION

For my beloved father, Mohd Zain and my dearest mother, Doyah, thank you for all hardship to guide me from child until now, from primary until my PhD. To My grandfather, Abdul Wahab and my late grandmother, Rokiah, who raised me since child, they are my idol and my backbone in my life. And for my beloved husband, Mohd Hafiz bin Ibrahim, thank you for always be with me, believed in me, guiding me along the journey. Without you there would be no excuses for me to stand still and work hard to achieve my dreams. And to my daughter, Nurul Alisha Fathia, you are my precious diamond and always my forever supporter. My heartfelt gratitude for all love, encouragement and support through the years of my quest for knowledge. May this achievement shall be our stepping stone towards living our dreams and ambitions……………..

The vegetation of a good land comes forth (easily) by the Permission of its Lord; and that which is bad, brings forth nothing but (a little) with difficulty. Thus do We explain variously the Ayât (proofs, evidences, verses, lessons, signs, revelations, etc.) for a people who give thanks".

"[Al-A'râf 7 : 58]

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

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August 2015

Chairman : Professor Mohd Razi Ismail, PhD

Institute : Institute Tropical Agriculture

Water deficit is a major problem in rice production due to increase scarcity of water resource. To solve this problem, the present study was conducted to determine how the use of cyclic water stress and potassium fertilizer could be used to alleviate water stress in rice. In the first experiment, a series of cyclic water stress by days [CW5, CW10, CW15, CW20, CW25 and CW30 including Control flooded (CF), control saturated (CS)] were used to investigate the effect of cyclic water stress on yield, growth, physiological and biochemical response of rice. It was found that higher duration of cyclic water stress (CW15, CW20, CW25 and CW30) had reduced grain yield, total biomass, filled spikelet, 1000 grains weight, total panicle hill^{-1}, plant height and total tillers hill^{-1} and increase oxidative stress and tiller mortality. Grain yield was the highest both in CF and CS treatments and followed by CW5 and CW10 with a 14.3% difference. The application of CW10 was found to have higher yield and water use efficiency under reduction of water. In the second experiment, three rates of potassium [80 kg K_2O/ha (control), 120 kg K_2O/ha and 160 kg K_2O/ha] and three levels of cyclic water stress (CW5, CW10 and CW15) was used to characterize the role of potassium in alleviating water stress. It was observed that rice yield, harvest index, leaf gas exchange, total chlorophyll content and relative water content was influenced by interaction effects between cyclic water stress and potassium rates. The 1000 grains weight, total biomass and water productivity was influenced by potassium rates. It was found that cyclic water stress 10 days (CW10) with potassium fertilization at 120 kg K_2O/ha was the best practices in achieving higher yield with less water, maximum efficiency of photosystem II (F_V/F_M), 1000 grains weight, total biomass production as well as uptake of major nutrient elements (N, P, K, Ca, Mg and Fe) in rice. In the third experiment, four levels of potassium rates [Control, 80 kg K_2O/ha, 120 kg K_2O/ha and 160 kg K_2O/ha) and two types potassium sources (KCl and K_2SO_4) were used to investigate the influence of potassium fertilization in minimizing the effect of cyclic water stress in rice production. It was observed that panicle dry weight hill^{-1}, root dry weight, rice yield, Catalase activity (CAT), proline, maliondialdehyde (MDA) and harvest index was influenced by potassium rates. The leaves numbers, total tillers and 1000 grains weight were influenced by potassium types. Interaction effects (potassium rate x potassium types) was observed in shoot dry weight, leaf area, total spikelet panicle^{-1}, net assimilation rate, transpiration rate and water use efficiency. From the study, the application either KCl or K_2SO_4 at 120 kg K_2O/ha was efficient in minimizing yield reduction under water stress. In the fourth experiment, five treatments including (1) standard local grower's practice (control, 80CF = 80 kg K_2O/ha + control flooded); (2) 120CW15 = 120 kg K_2O/ha + cyclic water stress 15 days; (3) 120DS15V = 120 kg K_2O/ha + drought stress for 15 days during the vegetative stage; (4) 120DS25V = 120 kg K_2O/ha + drought stress for 25 days and (5) 120DS15R = 120 kg K_2O/ha + drought stress for 15 days during the reproductive stage, were evaluated to assess the effects of different water stress combined with potassium fertilization on growth, yield, leaf gas exchanges and biochemical changes in rice. It was found that rice under 120CW15 treatment showed tolerance to drought stress by having high water use efficiency, peroxidase (POX), catalase (CAT), proline, maximum efficiency of photosystem II (F_V/F_M) and lower minimal fluorescence (F_O), compared to other treatments. Based on the result , the sub experiment was conducted to identify *LEA* gene relation with water stress and potassium input on rice. Three treatments were evaluated i.e. Control (control flooded + 80 kg K_2O/ha), Water stress 25 days + 80 kg K_2O/ha and Water stress 25 days + 120 K_2O/ha to detect the expression of Late Embryogenesis Abundant (*LEA*) genes under water stress conditions. The result showed that imposition of 25 day water stress with 120 kg K_2O/ha (WSK) was proven to reduce *LEA* gene expression and achieve high plant growth and yield. Meanwhile, the 25 day water stress with 80 kg K_2O/ha (WS) was shown to have the highest induction of the *LEA* gene and lower rice yield and plant growth. From this project, it can be concluded that application of cyclic water stress and potassium fertilizer was able to mitigate water stress and maximize water use efficiency in rice.

Abstrak tesis yang dikemukakan kepada Senat Univerisiti Putra Malaysia sebagai memenuhi keperluan untuk Ijazah Doktor Falsafah

AGRO-MORFOLOGI, FISIOLOGI DAN CIRI-CIRI BIOKIMIA PADI BERDASARKAN KEPADA TEGASAN AIR BERKITAR DAN BAJA KALIUM

Oleh

NURUL AMALINA BT MOHD ZAIN

Ogos 2015

Pengerusi : Profesor Mohd Razi Ismail, PhD

Institusi : Institut Pertanian Tropika

Defisit air adalah masalah utama bagi produksi padi kerana sumber air semakin sukar didapati. Untuk menyelesaikan masalah ini, kajian ini telah dijalankan untuk menentukan bagaimana pengunaan yaring penggunaan tegasan air berkitar dan pbaja kalium pula boleh digunakan untuk mengurangkan kesan tegasan air pada padi. Dalam eksperimen pertama, beberapa siri tegasan air berkitar berdasarkan hari [CW5, CW10, CW15, CW20, CW25 dan CW30 termasuk kawalan banjir (CF), kawalan tepu (CS)] telah dilaksanakan untuk mengkaji kesan tegasan air berkitar pada hasil, pertumbuhan, tindak balas fisiologi dan biokimia. Kajian mendapati, lebih lama tempoh tegasan air berkitar (CW15, CW20, CW25, CW30) telah mengurangkan hasil bijirin, jumlah biomass, bijirin padi berisi, berat 1000 bijirin padi, jumlah tangkai padi per rumpun, ketidaksuburan tangkai padi, ketinggian pokok , jumlah tangkai padi per rumpun dan meningkatkan tekanan oksidatif. Hasil bijirin adalah paling tinggi dalam kedua-dua CF dan CS dan dikuti oleh CW5 and CW10 dengan perbezaan 14.3%. Pengunaan CW10 didapati dapat memberikan hasil dan kecekapan penggunaan air (WUE) tinggi dalam kekurangan air. Dalam eksperimen kedua, tiga kadar kalium [80 kg K_2O/ha, 120 kg K_2O/ha dan 160 kg K_2O/ha] dan tiga tegasan air berkitar (CW5, CW10 dan CW15) telah digunakan untuk mengkaji kesan kalium dalam mngurangkan kesan tegasan air. Pemerhatian menunjukkan hasil bijirin, indeks tuaian, pertukaran gas daun, jumlah kandungan klorofil dan kandungan air relatif telah dipengaruhi oleh interaksi antara tegasan air berkitar dan kadar kalium. Berat 1000 bijirin , jumlah biomass, produksi air telah dipengaruhi oleh kadar kalium. Penemuan menunjukkan tegasan air berkitar 10 hari dengan pembajaan kalium pada 120 kg K_2O/ha (CW10) adalah praktis terbaik dalam mencapai hasil tuaian lebih tinggi hasil dengan pengurangan air, tinggi kecekapan maksimum Fotosistem II (Fv/Fm), berat 1000 bijirin, jumlah pengeluaran biomass dan juga pengambilan nutrien utama (N, P,K, Ca, Mg and Fe) dalam padi. Dalam eksperimen ketiga, empat tahap kadar kalium [Kawalan, 80 kg K_2O/ha, 120 kg

K_2O/ha dan 160 kg K_2O/ha) dan dua jenis kalium (KCl dan K_2SO_4) telah digunakan pada padi untuk mengkaji pengaruh pembajaan kalium dalam mengurangkan kesan tegasan air berkitar dalam pengeluaran padi. Diperhatikan bahawa berat kering tangkai per rumpun, berat kering akar, hasil bijirin, aktiviti katalase (CAT), prolin, maliondialdehida (MDA) dan indeks tuaian telah dipengaruhi oleh kadar kalium. Bilangan dedaun, jumlah tiller padi dan berat 1000 bijirin telah dipengaruhi oleh jenis kalium. Kesan interaksi (kadar kalium x jenis kalium) diperhatikan pada berat kering bahagian pokok selain akar, luas daun, jumlah spikelet padi tangkai^{-1}, kadar asimilasi bersih, kadar transpirasi dan kecekapan penggunaan air. Daripada kajian, aplikasi sama ada KCl atau K_2SO_4 pada 120 kg K_2O/ha adalah efisien dalam mengurangkan pengurangan hasil ketika defisit air. Dalam eksperimen keempat, lima rawatan termasuk (1) standard praktis penanam tempatan (kawalan, 80CF = 80 kg K_2O/ha + kawalan banjir); (2) 120CW15 = 120 kg K_2O/ha + tegasan air kitaran 15 hari; (3) 120DS15V = 120 kg K_2O/ha + tegasan kemarau untuk 15 hari ketika peringkat vegetatif; (4) 120DS25V = 120 kg K_2O/ha + tegasan kemarau untuk 25 hari dalam peringkat vegetatif dan (5) 120DS15R = 120 kg K_2O/ha + tegasan kemarau untuk 15 hari dalam peringkat reproduktif, telah dikaji dalam eksperimen ini untuk menilai kesan-kesan kepelbagaian tegasan air berkombinasi dengan sistem pembajaan kalium pada pertumbuhan, hasil, pertukaran gas daun, pertukaran biokimia dalam pertumbuhan padi. Didapati bahawa padi di bawah rawatan 120CW15 mempunyai toleransi kepada tegasan kemarau melalui peningkatan kecekapan penggunaan air, peroksidase (POX), katalase (CAT), prolin, kecekapan maksimum fotosistem II (F_V/F_M) dan fluorescence minimum yang lebih rendah (F_O) berbanding dengan rawatan lain. Berdasarkan keputusan eksperimen keempat, sub eksperimen telah dijalankan untuk mengenalpasti kaitan gen LEA dengan tegasan air dan input kalium pada padi. Tiga rawatan telah dijalankan i.e. kawalan (kawalan banjir + 80 kg K_2O/ha), tegasan air 25 hari + 80 kg K_2O/ha dan tegasan air 25 hari + 120 K_2O/ha untuk mengesan ekpresi gen Late Embryogenesis Abundant (*LEA*) dalam keadaan tegasan air. Perlaksanaan ketegasan air 25 hari dengan 120 K_2O/ha terbukti mengurangkan ekspresi gen *LEA* dan mencapai pertumbuhan pokok dan hasil yang tinggi. Sementara, ketegasan air 25 hari dengan 80 K_2O/ha telah menunjukkan paling tinggi induksi gen *LEA* dan hasil padi dan pertumbuhan pokok yang rendah. Daripada projek ini, dapat disimpulkan bahawa penggunaan tegasan air berkitar dan baja kalium pada padi boleh mengurangkan tegasan air dan memaksimumkan kecekapan penggunaan air di dalam padi.

ACKNOWLEDGEMENTS

I would like to express my sincere appreciation and gratitude to Prof. Dr. Mohd Razi Ismail, chairman of my supervisory committee, for his attentive supervision, unfailing guidance and consistent encouragement during the course of this study. I am also indebted to Prof. Dr. Maziah Mahmood and Prof. Madya Dr. Adam Puteh, for their helpful supervision, guidance and discussions during my study. Special thanks are also extended with gratitude for Professor Asraffuzaman and Dr Robioul Islam, ITA's Post Doctorial fellows and ITA's research officers, Mr Zulkarami Berahim, Mrs Azrin Ariffin for their time, encouragement, idea and expert assistance during my study. And I am also grateful to get SLAB scholarship from MOHE and University Malaya during my study.

My incomparable to all my family members, I thank you for giving me the comfort and support to pursue my dream. Thanks to all staff in the Institute Tropical Agriculture and Crop Physiology Department UPM especially Mr. Hj Khoiri, Mr. Mazlan, Mrs. Farah Wahida, Mrs. Siti Samsiah, Mrs. Norafidah and Mr. Adzan for their help and co-operation during laboratory analysis and field work. I will not forget the sacrifice of my parent and my friends (Mrs Afifah bt Abdul Razak, Mr Fauzihan B Karim, Ms Nurul Idayu, Ms Khatijah) help for their frequent communication, moral support and constant encouragement, which made my life easy throughout my study. A special thanks to my beloved husband for his support and assistance during my study.

This study was supported by Universiti Putra Malaysia and Long Term Research Grant Scheme (LRGS) in Food Security - Enhance Sustainability Rice Production under The Ministry of Higher Education (MOHE), Malaysia.

I certify that a Thesis Examination Committee has met on 21 August 2015 to conduct the final examination of Nurul Amalina binti Mohd Zain on her Doctor of Philosophy thesis entitled "AGRO-MORPHOLOGICAL, PHYSIOLOGICAL AND BIOCHEMICAL CHARACTERISTICS OF RICE SUBJECTED TO CYCLIC WATER STRESS AND POTASSIUM FERTILIZERS", in accordance with the Universities and University Colleges Act 1971 and the Constitution of the University Putra Malaysia [P.U.(A) 106] 15 March 1998. The Committee recommends that the candidate be awarded the Doctor of Philosophy.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS AND SYMBOLS

%	percent
*	significant at 0.05 probability level
**	significant at 0.01 probability level
1000gw	1000 grains weight
18sRNA	18S ribosomal RNA
$\mu g\ g^{-1}$	micro gram per gram
$\mu mol\ m^{-2}s^{-1}$	micro mole per meter square per second
$\mu mol\ m^{-1}$	micro mole carbon dioxide per mole air
µA	micro ampere
µL	micro litre
^oC	degree-celcius
L	litre
3PGA	3-Phosphoglicolate Acid
A	net photosynthesis
AA	ascorbic acid
ABA	abscisic acid
AtHK1	Arabidopsis Histidine Kinase
A_{max}	maximum net photosynthesis/net assimilation rate
ANOVA	analysis of variance
AOX	alternative oxidase
APX	ascorbate peroxidases
ATP	adenosine triphosphate
AVP	average vapor pressure
CF	Control flooded
cm	centimeter
cm^2	centimeter square
C.V	coefficient variation
C_3	carbon 3 species
C_4	carbon 4 species
Ca	calcium
CAT	catalase
CAM	crassulacean acid metabolism
cDNA	Complementary Deoxyribonucleic Acid
Chl a	Chlorophyll a
Chl b	Chlorophyll b
Ci	intercellular carbon dioxide concentration
CO_2	carbon dioxide
C/N	carbon to nitrogen ratio
DAS	day after sowing
DAT	day after transplanting
DREB	dehydration-responsive transcription factors
DMRT	Duncan Multiple Range Test
$dS\ m^{-1}$	electrical conductivity (EC) formula
DW	dry weight
E	transpiration rate
e.g	example

FAO	Food and Agricultural Organization of the United Nations
Fe	Iron
F_M	maximal fluorescence
F_O	minimal fluorescence
FS	filled spikelet
F_V	variable fluorescence
F_V/F_O	indication of maximum quantum yield of photochemical and non-photochemical processes in photosystem II and correlates with leaf photosynthetic capacity
F_V/F_M	maximum quantum efficiency of PSII system
FW	fresh weight
g	gram
GA3	gibberellin
GAPDH	Glyceraldehyde 3-phosphate dehydrogenase
GR	glutathione reductase
gs	stomata conductance
GLM	General Linear Model
Gly Bet	glycinebetaine
h	hour
ha	hectare
H_2O	water
H_2O_2	hydrogen peroxide
H_2SO_4	sulphuric acid
HCI	hydrochloric acid
HI	harvest index
Hsps	heat-shock proteins
IAA	indole-3-acetic acid
i.e	that is
IRRI	International Rice Research Institute
WUE	water use efficiency
K	potassium
K^+	Ion potassium
KCl	potassium chloride
K_2SO_4	potassium sulphate
kg	kilogram
kg N/ha	kilogram nitrogen per hectare
kg P_20_5/ha	kilogram phosphorus pentoxide per hectare
kg K_2O/ha	kilogram potassium oxide per hectare
Kj	kilo joule
km^3	cubic kilometre
kPa	kilo pascal
LAR	leaf area ratio
LEA	Late Embryogenesis-Abundant
LSD	least significant difference
$LSD_{0.05}$	least significant difference at 5 % level
Ibs	pound
m	metre
m^3	cubic metre (volume)

MDA	Malondialdehyde
MDAR	Monodehydroascorbate Reductase
Mg	magnesium
mg/g	mili gram per gram
mg/kg	mili gram per kilo gram
mm	milimeter
$mmol/m^2/s$	mili mole per meter square per second
MOA	Ministry of Agriculture
$mol\ m^{-2}s^{-1}$	mole per meter square per second
MOP	Muriate of Potash
$m\ s^{-1}$	meter per second
mRNA	messenger ribonucleic acid
N	Nitrogen
nm	nano meter
n.s.	not significant
NADPH	nicotinamide adenine dinucleotide phosphate
NaOH	sodium hydroxide
$NaNO_3$	sodium nitrate
NAR	net assimilation rate
NCED3	9-cis-epoxycarotenoid dioxygenase
NTC	No Template Control
O_2	oxygen
PAL	Phenyl alanine ammonia lyase
pH	power of hydrogen
Phe	Phenylalanine
p	probability
P	Phosphorus
PDW	panicle dry weight
Pi	inorganic phosphorus
P_N	Net photosynthesis
PNUE	photosynthesis nitrogen use efficiency
pKa	acidity
PAR	photosynthetically active radiation
Pc	photosynthesis carboxylation
PSII	photosystem ii
PEPCase	pep carboxylase
POD	peroxidases
qRT-PCR	Quantitative Reverse-Transcriptase Polymerase Chain Reaction
RCBD	Randomized Complete Block Design
RDW	root dry weight
RGR	relative growth rate
RH	relative humidity
ROS	reactive oxygen species
rpm	rotations per minute
R_d	dark respiration rate
Rubisco	ribulose biphosphate carboxylase/oxygenase
RuBP	ribulose biphosphate
RWC	relative water content

r^2	coefficient of determination or r square
s	second
SAS	Statistical Analysis System
SDW	shoot dry weight
SEM	standard error of difference between means
SLA	specific leaf area
SOD	superoxide dismutase
S/R	shoot to root ratio
SVP	saturation vapor pressure
SPS	sucrose phosphate synthase
SPSS	Statistical Product and Service Solutions
t	time
TB	total biomass
TBA	thiobarbituric acid
TCC	total chlorophyll content
TNC	total non structural carbohydrate
tonnes/ha	metric tonnes per hectare or 1,000 kilogram per hectare
TSP	Triple Super Phosphate
TSS	total soluble sugar
TW	turgid weight
UV	ultra violet ray
VPD	vapor pressure deficit
Var.	variety
V_{cmax}	RuBP carboxylation efficiency of PSII
WP	water productivity
WUE	water use efficiency

CHAPTER 1

INTRODUCTION

1.1 Overview

Rice (*Oryza sativa L.*) is a major staple crop for about 75% of the world population. It is a good source of energy that contains nutrients, vitamins and minerals. Rice is also a major export crop for several Asian countries. In Malaysia, it is cultivated in eight granary areas along Peninsular Malaysia with an area of approximately 389, 544 ha (Jabatan Pertanian, 2012). In Sabah and Sarawak, hill rice is the major rice found suitable for that environment. Rice comes under the Gramineae Family and there are only two species generally used as food for humans, i.e. *Oryza sativa* and *Oryza glaberrima* (IRRI, 2012). It is a unique crop that can resist and tolerate submergence conditions.

Crop cultivation in Malaysia uses large quantities of water. It is estimated that total water withdrawal for agriculture was 4.520 km^3 (34%), while 3.902 km^3 (30%) was for use by municipalities and 4.788 km^3 (36%) was used by industries (Frenken, 2012). Double cropping of rice cultivation started on the 1960s and by early 1970 created a high demand for irrigation development and efficient water management. Moreover, current water scarcity due to the rapidly increasing world population, progressive global warming, competition of water consumption from domestic and urban sectors and the lack of fresh water sources has resulted in a dextrous problem for crop cultivation. From the irrigation management perpesctive, drought has become a scary and dextrous environmental factor limiting rice productivity and farmers income (Chai *et al*., 2006; Yang and Zhang, 2006; Sarvestani *et al*., 2008).

1.2 Problem Statement /Significance of The Study

Water stress occurs when water uptake by plants decreases over water transpiration and in severe conditions, it could stop physical plant growth and disrupt chemical balance in plants. As a further impact, water stress increases yield reduction and retards crop growth. For tuber crops like potato and radish, the depletion of vegetative storage organs occurs, while in cereal crops aborted tillers, unfilled spikelets and damaged grains frequently occur, and in fruit crops the size and content of the fruits are reduced due to water stress. In facing water scarcity risks, knowledge and technology needs to be rapidly developed to mediate the damaging effects of water scarcity, not only in the urban sector, but also in the agriculture and domestic sectors. In agriculture, research is important to determine new ways to decrease water stress impact on agriculture, in relation to changes in the physiology, breeding, chemistry or molecular approaches (Frenken, 2012).

Therefore, it is important to find the means to reduce water usage and increase the yield of rice. One possible way is to study rice water usage by applying cyclic water stress to the plants. Izanloo *et al*. (2008) have shown that wheat cultivars that are adapted to cyclic water stress gained highest grain number per spike and reduced aborted tillers. The usage of potassium fertilization can minimize the water stress effects on rice. Potassium is a pre-requisite for normal functioning of all plant biochemical and physiological systems. It has been shown to reduce the effects of water stress in many plants (Bajehbaj *et al*., 2009). Previous studies had reported that potassium was able to mediate adverse effects of water stress on mung bean (Fooladivanda *et al*., 2014), canola (Rose *et al*., 2008), and beans (Nasri and Khalatbary, 2011).

No information is available on the water requirements of Malaysian rice varieties and the effect of fertilization with potassium to minimize the effects of water stress on rice productivity. Research on the use of potassium fertilization in Malaysia rice is also scarce. Hence, it is pertinent to establish the water requirements of Malaysian rice by using cyclic water stress technique and the effects of potassium fertilization to minimize the water stress effects on rice yields. This information would be beneficial for efficient use of water by Malaysian rice farmers.

1.3 Objectives of the Study

1. To characterize plant growth, physiology and gas exchange responses of rice under different cyclic water stress.

2. To investigate the usage of potassium rates and cyclic water rates to remedy the effect of cyclic water stress on growth, physiology and biochemistry of rice.

3. To determine biochemical regulation of primary metabolites and antioxidative enzymes of rice subjected to cyclic water stress under different potassium rates and sources.

4. To assess the effects of different cyclic water stress combined with potassium fertilization regimes on growth, yield, leaf gas exchanges and biochemical changes in rice grown in pots compared with standard local rice growing.

5. To characterize gene activities during recovery by potassium remediation.

It was hypothesized that cyclic water stress can increase plant resistance to water stress, leaf gas exchange and enhance root growth. This promotes flowering and leaf senescence. It was also hypothesized that addition of potassium to rice can increase plant water status and osmotic adjustments under cyclic water stress.

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