การประเมินการตอบสนองต่อภาวะแล้งของข้าว *Oryza sativa* L. ที่มีพื้นฐานทางพันธุกรรมของข้าว
ขาวดอกมะลิ105 และมียีนทนแล้งบนโครโมโซมที่ 4

นางสาวเมวิกา สำลีปั้น

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรมหาบัณฑิต
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EVALUATION OF DROUGHT STRESS RESPONSES OF RICE *Oryza sativa* L. WITH GENETIC

BACKGROUND OF 'KDML105' RICE AND DROUGHT TOLERANCE GENES ON

CHROMOSOME 4

Miss Mawika Samleepan

A Thesis Submitted in Partial Fulfillment of the Requirements

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Thesis Title	EVALUATION OF DROUGHT STRESS RESPONSES OF RICE *Oryza sativa* L. WITH GENETIC BACKGROUND OF 'KDML105' RICE AND DROUGHT TOLERANCE GENES ON CHROMOSOME 4
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เมวิกา สำลีปั้น : การประเมินการตอบสนองต่อภาวะแล้งของข้าว *Oryza sativa* L. ที่มีพื้นฐานทางพันธุกรรมของข้าวขาวดอกมะลิ105 และมียีนทนแล้งบนโครโมโซมที่ 4 (EVALUATION OF DROUGHT STRESS RESPONSES OF RICE *Oryza sativa* L. WITH GENETIC BACKGROUND OF 'KDML105' RICE AND DROUGHT TOLERANCE GENES ON CHROMOSOME 4) อ.ที่ปรึกษาวิทยานิพนธ์หลัก: อ. ดร. วราลักษณ์ เกษตรานันท์, อ.ที่ปรึกษาวิทยานิพนธ์ร่วม: รศ. ดร. ศุภจิตรา ชัชวาลย์, 126 หน้า.

ข้าวเป็นหนึ่งในอาหารที่สำคัญที่สุดของผู้คนทั่วโลกโดยเฉพาะอย่างยิ่ง 'ขาวดอกมะลิ 105' ซึ่งเป็นที่รู้จักกันอย่างแพร่หลายซึ่งมีรสสัมผัสที่ดีและมีกลิ่นหอม ความเครียดจากภาวะแล้งเป็นปัญหาสำคัญที่ส่งผลต่อการผลิตข้าว การพัฒนาสายพันธุ์ทนแล้งเป็นหนึ่งในกลยุทธ์ที่ใช้ในการแก้ปัญหานี้ โดยใช้สายพันธุ์ที่มีชิ้นส่วนของยีนทนแล้งบนโครโมโซมที่ 4 และมีพื้นฐานทางพันธุกรรมของข้าวขาวดอกมะลิ105 จำนวน 4 สายพันธุ์และสายพันธุ์พ่อแม่ คือ DH212 และ 'ขาวดอกมะลิ105' ในการศึกษา เพื่อการประเมินลักษณะการตอบสนองต่อภาวะแล้งในระยะต้นกล้าข้าว การทดลองดำเนินการมีการควบคุมระดับน้ำ 3 ระดับ ประกอบด้วย 100%, 75% และ 50% ของความจุสนามของดิน ผลการศึกษาพบว่า ค่าคลอโรฟิลล์ฟลูออเรสเซนต์และค่าการคงความเขียวเป็นค่าที่เหมาะสมสำหรับการคัดเลือกสายพันธุ์ทนแล้ง นอกจากนี้การศึกษาครั้งนี้ยังยืนยันว่าความแตกต่างในความสามารถในการทนแล้งเป็นผลมาจากความหลากหลายทางพันธุกรรม

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MAWIKA SAMLEEPAN: EVALUATION OF DROUGHT STRESS RESPONSES OF RICE *Oryza sativa* L. WITH GENETIC BACKGROUND OF 'KDML105' RICE AND DROUGHT TOLERANCE GENES ON CHROMOSOME 4. ADVISOR: WARALUK KASETTRANAN, Ph.D., CO-ADVISOR: ASSOC. PROF. SUPACHITRA CHADCHAWAN, Ph.D., 126 pp.

Rice (*Oryza sativa* L.) is one of the most essential food of people around the world, especially 'KDML105' which is widely known for its great mouthfeel and aroma. Drought stress has long been a major problem affecting rice productivity. However, drought-tolerant cultivars has been developed to overcome this problem. In this study, four chromosome substitution lines (CSSLs) from back-crossing of 'KDML105' and DH212 were used to evaluation of proper drought responsive traits in rice seedlings. The experiment was carried out with three water levels consist of 100%, 75%, and 50% field capacity. Results showed that chlorophyll fluorescence (F_v/F_m ratio) and stay green score were suitable parameters for the selection of drought-tolerant lines. Moreover, this study also showed that the difference in drought tolerance capability was due to genetic variation.

Department: Botany	Student's Signature	
Field of Study: Botany	Advisor's Signature	
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Chapter I

Introduction

Rice (*Oryza sativa* L.) has been cultivated as a primary food crop for more than seven thousand years (Izawa and Shimamoto, 1996). It is a staple food that directly supplies more than fifty percent of the world's population, especially those living in Asia where rice is the main diet (Hadiarto and Tran, 2011). Most of the world's paddy fields are located in Asia and due to the immense demand for rice, the number of paddy fields tends to have an increasing trend (IRRI, 2015).

Rice is the most important economic crop in Thailand, especially 'Khao Dawk Mali 105' ('KDML105') rice. It is a famous Thai rice cultivar which has good cooking and eating qualities, but most of the areas for 'KDML105' rice production is the rainfed lowland in the northeastern part of Thailand such as Surin, Roi-Et and Yasothon (Thai rice exporters association, 2015). In this area, seasonal rainfall is highly varied that can cause the development of drought stress during the growing season (Office of Agricultural Economics, 2015).

Drought is a phenomenon which water is deficient over an extended period of time. This doesn't only cause lackage of water, but is a major environmental stress in rice, especially in the rainfed lowland and upland areas (Fukai and Cooper, 1995). Drought stress affects plant growth, development, and also reduces crop production. Many studies have shown that rice production is highly affected by drought stress. Usman et al. (2013) studied the effect of drought stress on rice, which they found that drought stress reduced photosynthesis rate. Furthermore, it can also reduce shoot and root fresh weight and photosynthetic pigments. Data from Pieters and El Souki (2005) have shown how drought stress affects photosystem II activity at the grain filling stage.

Various studies have been made on determination of gene loci of drought-resistant rice crops by employing the quantitative trait loci (QTL) assay. Recent studies from the Rice Gene Discovery Laboratory, Faculty of Agriculture, Kasetsart University discovered that the genes involved in drought-resistance are located on chromosomes 1, 3, 4, 8 and 9. The characteristic trait is that drought-resistant plants still give high productivity under drought. Therefore, if the genes involved can be transferred into 'KDML105' rice, it should result in high yields under drought condition. To generate the rice population with 'KDML105' rice background, the double haploid lines, generated from pollens of F_1 from CT9993 and IR62266 cross, were developed and screened for drought tolerant lines. The putative drought tolerant gene containing regions were transferred to 'KDML105' rice by crossing. The 'KDML105' rice genetic background was increased by backcrossing to 'KDML105' rice for 5 generations (BC_5). The homogeneity of the population was created by self-fertilization for 4 generations (BC_5F_4) (Toojinda et al., 2011).

Drought causes a major economic loss to the agricultural industry, especially to rice since the seedling stage. However, the plants tend to survive under drought by altering its morphology, cellular biochemical reactions, and physiological responses, all of which are regulated of genetic level. Moreover, drought was found to also be involved in lipid degradation of the cell membrane (Gigon et al., 2004). Therefore, drought-resistant rice crops will be able to retain cell membrane stability (CMS) by measuring electrical conductivity of the solution. If electrical conductivity is low, then the ions inside the cells hardly leak, providing evidence of its high capability in drought resistance (Venkateswarlu and Ramesh, 1993). As mentioned above, drought induces morphological alterations in plants leaves such as accumulation of cutin on the leaves, coiling (Li et al., 2006; Chen et al., 2007) sunlight accessibility, leaf temperature and hydration rate; which cause problems to photosynthesis and development (O'Toole and Cruz, 1980). As reported by Blum and Ebercon (1981), the Leaf Rolling Score (LRS) is a method used to select drought-resistant plants because leaves with coiled leaves can reduce hydration, thus sustaining water inside the leaves. The relative water content (RWC) inside the plant

tissue can be used to observe the hydration status, in which the intracellular water is crucial for various cellular activities. If water is loss in great amount, it will affect plant physiology. Furthermore, if plant undergoes extended lackage of water, it will die. Even though plants may have high hydration rates, but it is crucial for plants to keep water for growth (Yamasaki and Dillenburg, 1999), inferring that plants that can keep the relative water content at constant level might be able to tolerate drought.

Another plant drought response criteria is chlorophyll fluorescence (F_v/F_m) which is caused by reactive oxygen species (ROS). The more drought, the higher the ROS are, but the lower the F_v/F_m ratio is. This causes the cell membrane to rupture which affects photosynthesis because proteins involved in photosystems I and II will be damaged, lowering the food production in plant. Moreover, the F_v/F_m ratio is also proportional to the relative water content. Also, the F_v/F_m can also be used to determine the chlorophyll level in plant leaves by using a chlorophyll meter (SPAD). The amount of chlorophyll present in the leaves of rice can determine the amount of nitrogen in the leaves. As a result, the SPAD value can determine the amount of nitrogen required for plant growth at different stages (Khurana et al., 2007; Huang et al., 2008). The stay green value indicates the amount of chlorophyll present in plants which can be determined by overall color scoring. The lower the score, the better in the drought-resistant characteristics are (Jinwen et al., 2009).

Previous studies have shown that genetic information and physiological features of drought-resistant rice are not enough to be classified efficiently, especially information on QTL. This information collected for QTL analysis is usually collected when plants give high productivity during drought. But those drought-resistant plants are influenced by many factors, including primary traits (root depth, root thickness, root distribution), secondary traits (cell membrane stability, stay green), including other integrative traits (hydration rate, color (stay green)) (Kamoshita et al., 2008). When productivity is used as the main selection criteria, some plants that contain drought-resistant genes might be missed out.

Lanceras et al. (2004) reported that QTL that is responsible for drought-resistance is located on chromosomes 1, 3, 4, 8 and 9 by studying the population from of CT9993 crossed with IR62266. In addition, the information given above correlates with the studies of crossing other rice species (Price et al., 2002; Jiang et al., 2004; Kamoshita et al., 2008). Also, Reddy (2004) has reported that leaf morphology and productivity under drought are located on chromosomes 1 and 4, respectively.

There are many factors for rice to maintain its productivity to avoid drought such as physiological responses (Taiz and Zeiger, 2006), drought tolerance (Jiang et al., 2004), membrane stabilization (Tripathy et al., 2000), and maintaining photosynthesis rate. However, the traits mentioned above are used for development of drought-resistant rice crops (Price et al., 2002; Jiang et al., 2004)

From the knowledge that physiological characteristics and high yield components under drought are located on chromosome 4 (Reddy, 2004), the objective of this study is to evaluate the physiological features of rice in the seedling stage under drought by assessing various parameters such as stay green score, cell membrane stability, leaf drying score, leaf rolling score, relative water content, including fresh and dry weight of the shoot and root. 'KDML105' rice phenotypes will be compared with CSSL phenotypes. The knowledge obtained from this project will be further used to develop drought-resistant rice.

The objective of this thesis:

To evaluate the drought stress response of some CSSL rice (*Oryza sativa* L.) containing genetic background of 'KDML105' rice.

Chapter II

Literature review

2.1. History of rice in Thailand

Rice has been embedded in the history of Thailand for more than 5,500 years, as evidenced by the earthenware made from rice hull in Ban-chiang; the rice used to make the earthenware was proposed to be the oldest rice in the country. Three Japanese scientists from the University of Tottori in collaboration with Thailand's Ministry of Agriculture and Forestry had done research on Thai rice by studying the rice hull inside the ancient relics from 108 historic sites from 39 provinces. This proved that rice cultivation was established since 600 A.D. in which the first type of rice cultivated was sticky rice. Then, cultivation of sticky rice decreased while rice increased. This study showed that during 1100 - 2000 A.D., three types of rice were cultivated which were Javanica (upland rice), Japonica (lowland rice) and Indica (The Agricultural Research Development Agency, 2015).

Currently, only Japonica rice is regularly cultivated in the north and northeast whereas Indica is commonly cultivated in the south and central region, where the land is fertile. The northeast has the highest yield which accounts for 45% of the nation's rice production. The most popular cultivated rice is the Khao Dawk Mali 105 which is the best strain in the world. In addition, this strain is mostly cultivated in the northeast, central, and north, respectively (Thai rice exporters association, 2015).

Rice is the main industrial crop of Thailand which is consumed both domestic and abroad. Due to the high demand, a great amount of Thai rice is exported globally each year. As mentioned earlier, the main cultivation area is in the northeastern part of Thailand, especially in the "Tung-Kula-Rong-Hai" area which has an approximate area of more than 7.6 million acres. The major producers are Surin, Buriram, Srisaket, Nakon Ratchasima, Ubonratchathani and Roi-Et province because the weather is suitable for growing rice. Moreover, the area is an upland and the humidity in November is quite low due to the cool wind from China making it an optimal period for harvesting, ultimately leading to high quality rice (Thai rice exporters association, 2015).

2.2. Rice

There are more than 120,000 rice lines, but only two species are generally grown, which are *Oryza Savita,* popularly grown in Asia and *Oryza glaberrina*, popularly grown in Africa. However, most of the rice grown and traded in the markets are mostly from Asia. Rice can be divided into three groups according to the tribe and area as mentioned below (The Agricultural Research Development Agency, 2015)

1) Indica

Indica rice was firstly discovered in India. It is slender in shape and is popular cultivated in Asia, especially China, Vietnam, the Philippines, Thailand, Indonesia, India, and Sri Lanka. In Thailand, the area used to cultivate this crop are the lowlands of the Chaopraya River. Due to the increase in Indica rice cultivation and the popularity amongst Thais, its name was shortened to just "rice" until today.

2) Japonica

Rice seed is a short and oval. The origin of japonica was in the north of Asia. It has distributed along the river for more than 20 centuries. Then, this rice cultivation is popular in temperate zone such as Japan, Korea, Russia, and USA.

3) Javanica

Rice seed is big and large oval. It was hybrid from Indica and Japonica. The most cultivation area was in Indonesia, Philippine, Taiwan, and Japan, but it is not popular because it has low yield component.

2.3. History of Khao Dawk Mali 105 (KDML105)

In 1945, Mr. Charoon Tuntawutto brought KDML rice from Lam Pradoo, Chonburi. He divided grains to Bang Kla, Chacheongsao. Then in 1950, Mr. Soontorn Sihanen, who was the head of Rice Office in Bang Kla, gathered a variety of KDML from farmers. After that all rice panicles were sent to Rice Experiment Station in Koksamrong, Lopburi for selected pure line. Finally, the row of 105th panicles were selected by Mr. Opas Polsilp and Mr. Mangkorn Joomthong (Joomthong, 1955; Sri-aun, 2005; Bureau of Rice Research and Development, 2010).

In 1957, Rice Experiment Station was proceeded comparative rice cultivar. Then in 1969, KDML 4-2-105 was developed (4 meaning the place that was Bang Kla, 2 meaning the plot of experiment, and 105 meaning the 105th row from 199 row). After that the name 'Khao Dawk Mali 105 or KDML105' was use to multiple which has characteristics as aromatic of pandan scent, long grains, and clear. Named 'Khao Dawk Mali' meaning white as a jasmine flower (Bureau of Rice Research and Development, 2010).

2.4. Morphological characters of KDML105

Khao Dawk Mali 105 or KDML105 is a sensitive to photoperiod and KDML105 is a non-glutinous rice variety which is highly sensitive to photoperiod and grows well in wet season. Plant height is 140 centimeters with 33 centimeters panicle length. The culm erected with leaves and had light yellow internodes. The leaf is pubescent and droopy. The ligules shape is acute and had two white clefts. The color of both auricle and collar are light green. At the flowering stage, the color of the apiculus, short sterile lemma, and stigma are white. Moreover, at grain filling stage, the apiculus and lemma of grain will turn into straw color and stem is stand with some lodging. The panicle is long and compact with secondary branching. Leaves below flag leaf are senescence (Smith and Dilday, 2003; Bureau of Rice Research and Development, 2010).

Jasmine rice or Hom Mali rice is widely popular consumed. The jasmine rice is classified as non-glutinous type with slender shape and long grains. The grains is transparent or clear and few chalky kernel. The rice has soft texture and present a fragrant smell like pandan (Bureau of Rice Research and Development, 2010).

2.5. Development of CSSLs Lines

2.5.1. Quantitative Trait Locus (QTL) Analysis

Quantitative trait locus or QTL is a statistical method that includes two types of information, which are phenotypic data and genotypic data (usually molecular markers), to explain the basic genetic of traits. QTL links complex traits to specific regions of chromosome. QTL analysis is genetically used to differentiate whether

phenotypic difference are caused by few loci with a large effect, or many loci with a minute effect (Miles and Wayne, 2008).

In 2011, Vaiphot Kanjoo and team studied co-location of QTL for salinity and drought tolerance in rice. It was found that QTL of drought tolerance (DT-QTL) located on chromosome 1, 3, 4, 8, and 9. They used DT-QTL for evaluated QTL of salinity tolerance (ST-QTL). Based on their research four of ST-QTL were located in same position as DT-QTL, previously reported. Furthermore, they showed good performance under drought and salinity stresses (Kanjoo et al., 2011).

2.5.2. Development of QTL of KDML105 rice for drought tolerance

Dr. Theerayut Toojinda and team from Rice Gene Discovery Unit were developed single QTL of KDML105 by used the doubled haploid lines that derived from a cross between CT9993 rice, with good root system, and IR62266 rice having high osmotic adjustment. The specific lines were selected based on good performances, consisting of physiological and agronomical traits. The doubled haploids lines are DH103, DH126, and DH212 that used as donors of drought tolerance genes to develop CSSLs (Siangliw et al., 2007; Toojinda et al., 2011).

The backcross method was used to generate CSSLs lines. Firstly, each doubled haploid lines was crossed to 'KDML105' rice. In addition, every backcrossing was selected by using markers specific for the interesting QTLs. After that, DT-QTLs were backcrossed with 'KDML105' rice for 5 times, so called BC_5F_1. The BC_5F_1 had mostly genetic background similar to 'KDML105' rice but had only one drought tolerance segment from one chromosome of donor line. Then, BC_5F_2 plants were generated by self-pollination to increase the number of plants containing the putative drought tolerance segment (Fig. 1). After that, CSSLs were investigated the percentage of 'KDML105' rice genetic background by genome scan. They found that the genome of CSSLs was similar to 'KDML105' rice around 96.3%. Finally, the

phenotypic data and genotypic data were collected in order to link complex traits to specific regions on each chromosome (Toojinda et al., 2011).
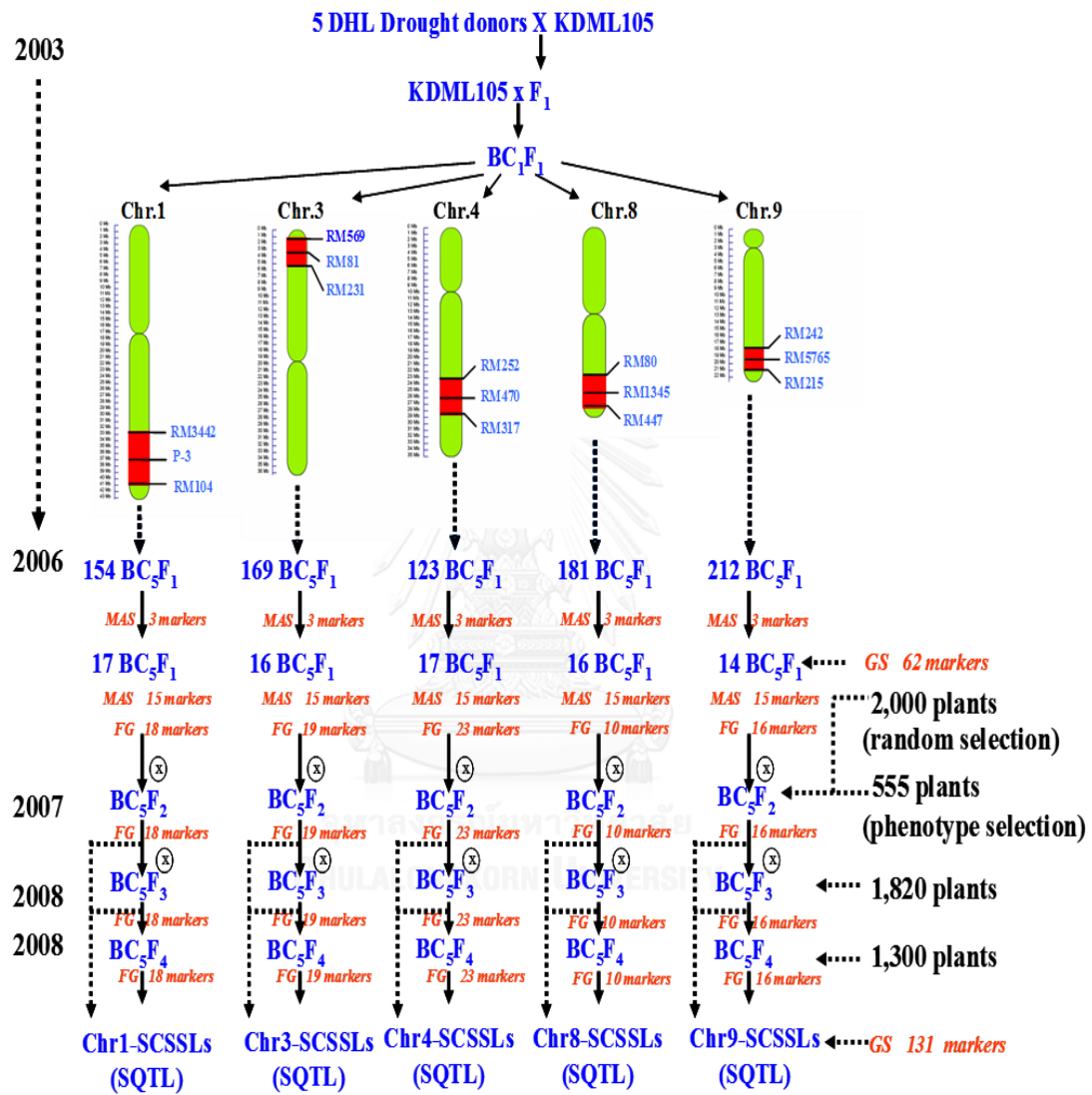
Figure 1 Development of KDML105 backcross introgression lines using marker assisted selection (Toojinda et al., 2011)

2.6. Stress

Abiotic stresses are the environmental factors that cause the damage to physical and chemical characteristics of living things. The changed of water potential and cell expansion were often the first thing that change to responses the environmental stress. If the stress caused during the short period of time, the responses may occur at the level of biochemical processes or the formation and decomposition substances. Moreover, if the stress is prolonged, the responses may occur with morphological characteristics or anatomical characteristics of the plant to reduce stress to keep the plant survive. If plants cannot adapt, plant may die (Taiz and Zeiger, 2006).

2.6.1. Drought stress

Drought stress is an important problem that limit plant growth and development and leads to the decrease in crop productivity (Hadiarto and Tran, 2011). Drought stress caused by many factors such as low water in soil, high transpiration rate, hot and dry weather, low precipitation, and salinity soil etc. Drought stress can classified into three groups by timing and stage that plant had stress (Chang et al., 1979; Fukai and Cooper, 1995).

2.6.1.1. Early stress

Early stress started from seedling to tillering stage with in the first rain period. Rice seedling may be affected by drought, while mature plant may have sufficient time recover from stress (Maurya and O'Toole, 1986).

2.6.1.2. Mild or intermittent stress

Mild or intermittent stress started from tillering to flowering stage with in the high rainfall. This stage was very sensitive to drought stress because plant most developed in this stage. If plant had stress, it may reduce yield component (Boonjung, 1993).

2.6.1.3. Late stress

Late stress during the grain filling stage and this period affected by drought from dry season. It was effected to plant less than mild stress.

2.6.2. Drought resistance

Plants have many mechanisms which used to survive from drought stress, which are drought escape, drought avoidance, drought tolerance, and drought recovery (Arrandeau, 1989; Fukai and Cooper, 1995). Plants respond to drought stress differently, depending on genetic composition, which leads to the action and interaction of morphological, physiological, and biochemical characters (Mitra, 2001).

2.6.2.1. Drought escape

Drought escape was a mechanism that plant used minimal time for growth and development to complete life cycle before water deficits. Drought stress reduces grain yield of rice because of the decrease in pollen development and increase in infertility. Boonjung (1993) showed the results that drought stress can reduced 2% of grain yield per day. In eastern India and Bangladesh found that drought escape was the important mechanism for rice to prevented drought stress and produced grain yield (Khush, 2001; Bernier et al., 2008).

2.6.2.2. Drought avoidance

Drought avoidance is a mechanism that plants maintain water in plant tissue by improving the water uptake, and reducing water loss. Some mechanisms that plants use to improve the water uptake are having deep roots and high branching ability. These plants have root/shoot ratio during drought stress. Some mechanisms that plants use to reduce water loss are the phenotypes of containing hairs or thick cuticles on leaves, early stomata closure, and elastic leaf rolling (Wang et al., 2006; Singh et al., 2012).

2.6.2.3. Drought tolerance

Drought tolerance is a mechanism that plants maintain metabolism at water deficits for live, growth, and development. Drought tolerance is a complex trait that is controlled by polygenes and is responded by complex morpho-physiological mechanisms (Li and Xu, 2007). Common mechanisms are osmotic adjustment and antioxidant capacity (Sanchez et al., 2002). Osmotic adjustment is the ability of plant to maintain turgor pressure by accumulation of compatible solutes such as sugar, amino acids, and ion that difference by species (Morgan, 1984). Antioxidant capacity is the ability of plants to eliminated or detoxify reactive oxygen species that damaged cell membrane (McKersie and Leshem, 1995).

2.6.2.4. Drought recovery

Drought recovery is a mechanism that plants retain green leaves during timing after rewatering and it is an important mechanism in early plant development (Lilley and Fukai, 1994). Some genotypes were able to produce more spikelets and more tillers (Malabuyoc et al., 1985; Fukai and Cooper, 1995).

2.7. Physiological traits

2.7.1. Leaf Rolling Score (LRS)

Leaf rolling is the important agronomic trait in breeding (Xiang et al., 2012). It is the first symptom of drought stress that is easy to see. Leaf rolling is drought avoidance mechanism that plants use to prevent stress (O'Toole and Cruz, 1980). Leaf rolling score was efficient method to screen drought resistance lines (Courtois et al., 2000; Salunkhe et al., 2011). Leaf rolling increases avoidance capacity by reduced leaf area and transpiration (Kadioglu et al., 2012).

Leaf rolling is controlled by the turgor pressure in bulliform cells, which are large cells with thin membrane located on epidermis layer (Fig. 2). These cells reduce water loss by rolling the leaves when plants face to stress and the plasmolysis occurs (Fig. 3). On the other hand, if bulliform cells receive water they are deplasmoplysis (Price et al., 1997). O'Toole and Cruz (1980) divided leaf rolling score into five levels (Fig. 4).
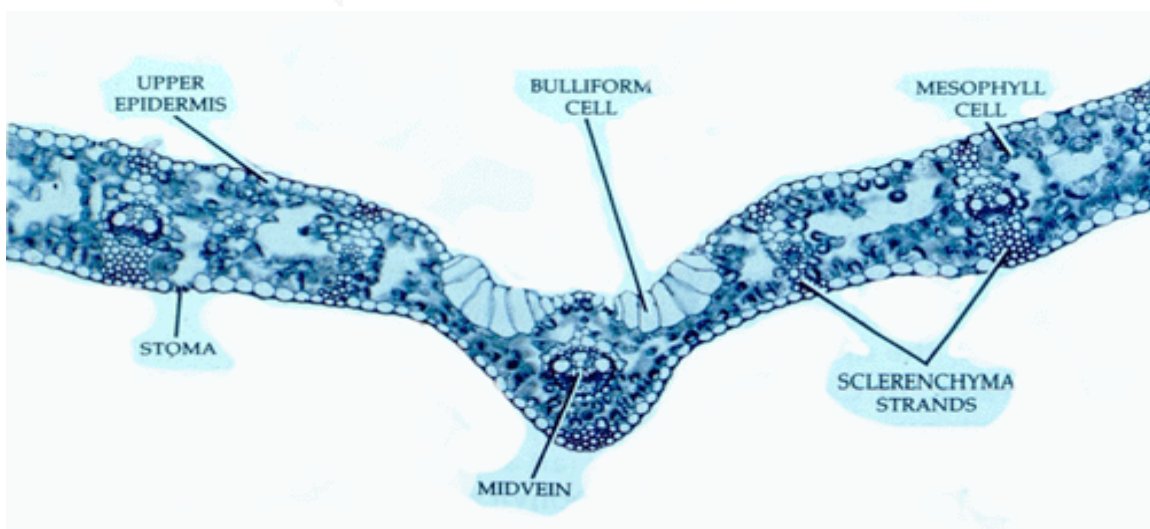
Figure 2 Bulliform cell in normal condition (Sampow, 2013)

Figure 3 Bulliform cell in drought stress condition (Sampow, 2013)
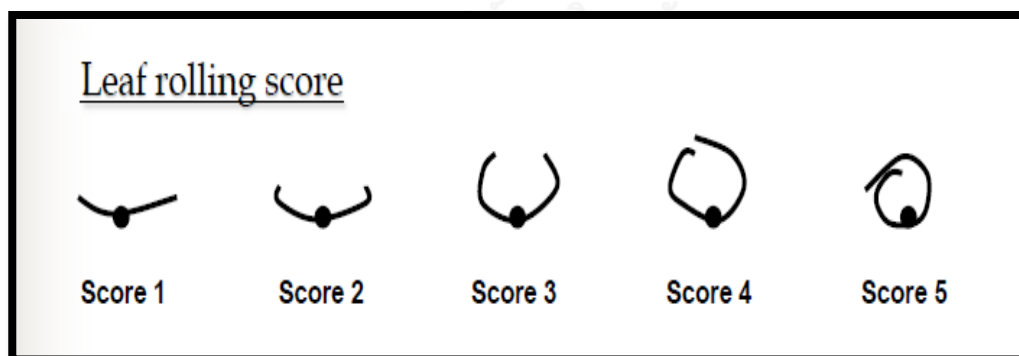
Figure 4 Leaf rolling score (O'Toole and Cruz, 1980)

2.7.2. Leaf Drying Score (LDS)

The symptom of senescence is the loss of green color of leaves causing by the loss of chlorophyll due to the chloroplasts degradation (Smart et al., 1995; He et al., 2005). Drought stress induces leaf drying. There were many researches that studied about leaf performance under drought stress. Monneveux et al. (2008) reported that leaf senescence could be used to screen drought susceptible, but it was less efficient for drought tolerance screen. The reported was similar to Abd Allah (2006) and Gana (2011) that studied in rice to screen drought resistance cultivars. They classified the response to drought stress of rice cultivars by the leaf drying.

2.7.3. Cell Membrane Stability (CMS)

2.7.3.1. Cell membrane

Cell membrane is also known as the plasma membrane or cytoplasmic membrane. It is a biological membrane that separated cells from outside environment. The cell membrane is selectively permeable to ions and organic molecules and controls the movement of substances in and out of cells. The basic function of the cell membrane is to protect cells from surroundings. It consists of the phospholipid bilayer with embedded proteins. Cell membrane is involved in many cellular processes such as cell adhesion, ion conductivity, cell signaling and serve as attachment surface for extracellular structures, including the cell wall, glycocalyx, and intracellular cytoskeleton (Garrett and Grisham, 1999).

2.7.3.2. Cell membrane structure

The cell membrane structure consists of protein 60% and lipid 40%. The most proteins that are embedded in the phospholipid bilayer are glycoproteins and mucoproteins. On the other hand, the lipid content consists of two types,

phospholipid and cholesterol. The arrangement of proteins and lipids are complex and it called the fluid mosaic model (Fig. 5) as described by Singer and Nicolson in 1972 (Garrett and Grisham, 1999).
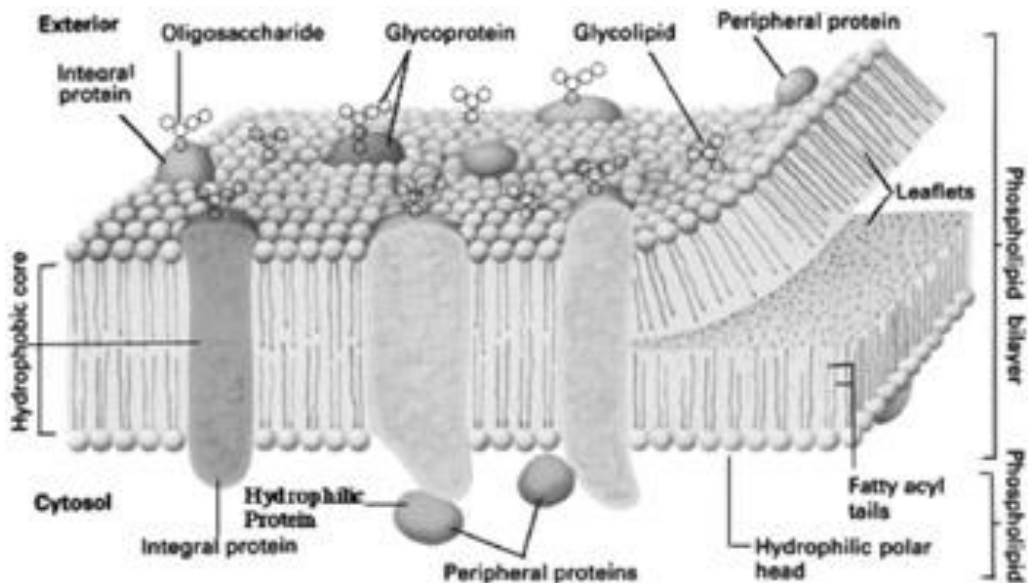
Figure 5 Fluid mosaic model of cell membrane (Nair, 2008)

2.7.3.3. Cell membrane stability

Cell membrane stability is an important mechanism of plants for adaption to drought stress. By maintaining cell membrane stability, it has been found to decrease cell damage severity. In order to measure cell membrane stability, electrolytes can be measured by an electrical conductivity meter. The cell membrane is composed of two major components, fats and proteins, which controls the entrance and exit of solutes or ions. When the cell membrane is injured by stress or high temperature, the membrane loses its retention substances and resulting in leakage of electrolytes such as amino acids, sugar, and organic compounds. Gigon et al. (2004) studied the effect of stress on membrane lipids in *Arabidopsis thaliana* (ecotype Columbia), which they found that lipid content in leaves decreased after exposing to drought stress. The results correlated to Da Silva et al. (1974) who reported the effects of drought stress on cell membrane stability,

which includes cell membrane damage, disturbed cell membrane stability, and reduced lipid contents.

The drought stress effected cell membrane, thus result in the leakage of from cell and directly affects the growth of plants. There were many reported about effect of drought to electrolyte leakage such as Dionisio-Sese and Tobita (1998) found that stress induced the membrane injury that leak electrolyte from cell. Geravandi et al. (2011) found that cell membrane stability can use as an indicator to screen drought tolerance in wheat (*Triticum aestivum* L.)

2.7.4. Relative Water Content (RWC)

Leaf water content is related to several physiological features such as leaf turgor, stomatal conductance, transpiration rate, photosynthesis rate and respiration (Kramer and Boyer, 1995). The water potential and water content have been widely used to evaluate water status in tissues. The relative water content is a desirable indicator of the relative amount of water present in plant tissues, while water potential measures the energetic status of water inside the leaf cells (Slater-Hammel, 1960). Measuring relative water content is the most appropriate approach to measure the water status of plants. Measurements of relative water content expressed on tissue based on the maximum water that tissues can hold (Barrs, 1968). Drought stress was found to reduce relative water content in plant leaves. The qualities of leaves with good water status are leaves with high relative water content and low leaf water loss (El-Tayeb, 2006; Gunes et al., 2008).

2.7.5. Chlorophyll fluorescence (f_v/f_m)

Photosynthesis is the main processes that determines plant crops and it is directly affected by drought stress. Drought stress can decrease photosynthesis rate which includes four events. Firstly, early stomatal closure (Cornic and Massacci,

1996) Secondly, reductions in numbers and activities of key enzymes such as Rubisco (Parry et al., 2002) and decrease of RuBP regeneration caused by low ATP synthesis (Tezara et al., 1999). Thirdly, changes in sugar content and composition in leaves due to altered carbon metabolism and changes in numbers and activities of key enzymes involved in carbohydrate metabolism. Finally, leaves have lower quantum yield, higher non-photochemical quenching of photosystem II, and decreased function of reaction centre in PSII (Masojidek et al., 1991; Giardi et al., 1996).

Photosynthesis is an essential process to maintain plants growth and development, and it is well known that in higher plants the photosynthetic systems are more sensitive to drought stress. Drought stress is an abiotic stress that affects chlorophyll fluorescence (f_v/f_m) by decreasing the maximum quantum yield of photosystem II. They found that PS II of drought tolerant genotypes could be less damaged by drought stress than drought sensitive genotypes in barley *Hordeurn vulgare* L. (Li et al., 2006).

2.7.6. SPAD Index

Leaf senescence is the loss of greenness due to the loss of chlorophyll (He et al., 2005). The SPAD index recorded with a portable chlorophyll meter will provide an information of the total chlorophyll content and can indicate senescence. Xu et al. (2000) were found highly correlations between SPAD index and total chlorophyll are a relation of SPAD index and stay-green score in sorghum. They reported that stay-green score was an indication of leaves senescence and provided as the tool that breeders can use to evaluate drought tolerance.

2.7.7. Stay Green Score (SGS)

Plant responses to drought stress was clearly affected by time and level of stress (Ludlow and Muchow, 1990). Stay green score is a classic method of

quantitative trait that showed variation of plants under drought stress (Crasta et al., 1999). Stay green is one of a kind of senescence that is generally genetic process which induced by environment such as drought, salinity, and low nitrogen at the genetic level (Masclaux et al., 2001; Borrás et al., 2003). An early symptom of senescence was loss of greenness which is caused by chlorophyll degradation.

Leaf senescence has a major effect on yield of many crops while this relationship was not clear that delay or reduction in leaf senescence is the cause of drought tolerance in maize (Borrell et al., 2000). In sorghum, plant breeders focus on improving drought tolerance cultivars for decreasing yield loss and increasing productivity under water deficiency. Leaf and plant senescence were used to characterize drought sensitive cultivars (Kassahun et al., 2010). Thomas and Howarth (2000) were reported that leaf greenness is related to the chlorophyll content. If chlorophyll content is reduced, plant greenness was decreased too. Stay green is a drought resistance mechanism that made sorghum resistance to water deficits. It was an ability to tolerate during the post-flowering stage to delay leaf senescence and plant death (Subudhi et al., 2000).

2.7.8. Fresh Weight (FW) and Dry Weight (DW)

Drought stress affects many physiological, morphological, anatomical, and biochemical aspects in plants such as photosynthesis, plant respiration, water and ion uptake, nutrient metabolism, and plant growth due to decrease in cell enlargement and cell division (Farooq et al., 2008). In addition, drought also affects the turgor pressure which resulted in reduced cell growth and expansion (Shao et al., 2008). In general drought stress reduced fresh and dry weight of plant and decreased photosynthetic pigments (Usman et al., 2013).

Root development is the main factor involved in plant adaptation under drought stress (Passioura, 1982). It mostly affects fresh weight of plant especially in

rice. Water is vital for plants to survive. There was many research that reported about water stress on plant weight and roots were primary sensors to detect water deficiency in the soil cause by study in physiological and biochemical (Pirzad et al., 2011). However, drought stress shown to reduce the amount of sugar beet weight as same as Lafitte et al. (2007).

Chapter III

Materials and methods

3.1. Plant Materials

3.1.1 Chromosome substitution lines (CSSLs)

RGD05131-4-MAS39

RGD05131-6-MAS5

RGD0128-10-MAS12

RGD05128-4-MAS40-MAS11

3.1.2 Parental strains

DH212

KDML105

3.2. Investigation of field capacity

Field capacity was adapted from Angkinand and Chadchawan (2000). Firstly, the soil is crushed and poured into a 100 ml cylinder. Then, 10 ml of water is added and is covered with aluminum foil. Wait until the soil fully absorbs the water and then weigh both the fresh and dry weight from cylinder. Finally, calculate the percentage of field capacity from the equation below.

% Field capacity = [(FW -DW) / DW] x100

FW = Fresh weight

DW = Dry weight

3.3. Optimization of timing for drought treatment

One CSSL was used to compare its drought-tolerant ability with KDML105 and DH212. To test this capability, triplicates of Completely Randomized Design (CRD) were used in this experiment to find the optimal time and level of drought treatment. First, the rice were germinated in plastic cup for 3 days and the rice seedling were transferred to a pot with 900 g of soil in which water and fertilizer was added for twenty-eight days. Then, the level of drought treatment was maintained at three different field capacities: 100%, 50% and 25% field capacity. Leaf rolling score (LRS) was estimated by the visual score and was given a score from one to five according to the method of and LRS was collected daily until the leaf score reaches five according to the method of de Datta et al. (1988), modified from O'Toole and Cruz (1980). Finally, LRS was analyzed by using SPSS (Statistics Package for the Social Sciences) Statistic. Also, ANOVA and Duncan's Multiple Range Test are used for comparison of mean between lines.

Leaf rolling score (LRS)

Table 1 Leaf Rolling Score and description

Leaf Rolling Score	Symptoms	Reaction
1	No rolling	Resistant
2	Slight rolling	Moderately resistant
3	V-shape or U-shape roll	Intermediate resistant
4	U-shape rolling or leaf margin touching each other	Susceptible
5	Tube-like rolling	Highly susceptible

3.4. Optimization time for chlorophyll fluorescence (F_v/F_m) collection

One CSSL line was used to compare with KDML105 and DH212. Completely Randomized Design (CRD) experiment was done in triplicates to find the optimal time for chlorophyll fluorescence (F_v/F_m) collection. Firstly, germinate rice in plastic cup for 3 days and transfer rice seedling to a pot containing 900 g of soil. Afterwards, water and add fertilizer for twenty-eight days. Then, control the level of drought treatment by use three different field capacities such as 100 %, 75 % and 50 % and chlorophyll fluorescence was estimated by using a Pocket PEA (PAR-FluorPen FP 100-MAX-LM-D (PSI, Czech Republic)) from the youngest fully expanded leaf. Adapted from Li et al. (2006), the plant was stored in the dark for 30 minutes before measuring the chlorophyll fluorescence. Data was collected every three hours starting from 6 a.m. to 6 p.m. for two days. Finally, data were analyzed by using SPSS Statistic. ANOVA and Duncan's Multiple Range Test are using to compare mean between lines.

3.5. Methods for experiment

Four CSSLs that are RGD05131-4-MAS39, RGD05131-6-MAS5, RGD0128-10-MAS12 and RGD05128-4-MAS40-MAS11 were used to compare with KDML105 and DH212. Randomize Completely Block Design (RCBD) with 4 replications were used in this experiment. First, germinate rice in plastic dip for 3 days and transfer rice seedling to the pot that cover the bottom of the pot with plastic bag and has 900 g. of soil. After that, watering and add fertilizer to pot plant for twenty-eight days. Then, control the level of drought treatment by use three difference %field capacity such as 100%, 75% and 50 % field capacity. Later, collect the physiological data following the timing from previous studies and analyzed by using SPSS Statistic.

Generalized Linear Models and Duncan's Multiple Range Test are using to compare mean between lines.

3.5.1. Leaf rolling score (LRS)

Leaf rolling score was collected every day by using standard evaluation system for rice that reported by O'Toole and Cruz (1980)

3.5.2. Leaf drying score (LDS)

Leaf drying score was collected every day from the youngest fully expanded leaf by using standard evaluation system for rice that reported by Abd Allah (2006) and was given a score from 0 to 9.

Table 2 Leaf drying score and description

Leaf Drying Score	Symptoms	Reaction
0	No symptoms	Highly resistant
1	Slight leaf tip drying	Resistant
3	Leaf tip drying extended to one-fourth of leaf length	Moderately resistant
5	Leaf drying one-fourth extended to half of leaf length	Intermediate resistant
7	Leaf drying more than two-third of leaf length	Susceptible
9	Most of leaf dry or plant dead	Highly susceptible

3.5.3. Cell membrane stability (CMS)

Cell membrane stability was determined by modified protocol of Sullivan and Ross (1979). Samples collected from the youngest fully expanded leaf were cut into 0.5 cm in length and weighed 0.05 g per sample. Then the sample was submerged in 5 ml of deionized water in test tube for 2 hours and electro conductivity (EC) was measured before and after autoclaving at 121℃ for 20 min. Moreover, data was collected at every four hours and the percent Cell Membrane Stability was calculated by according to the equation of Blum and Ebercon (1981).

$$\% \text{ cell membrane stability} = 100 - [(EC_1 / EC_2) \times 100$$

EC1 = electric conductivity after dipped in deionized water for 2 hours

EC2 = electric conductivity after autoclaved for 20 minutes

3.5.4. Relative water content (RWC)

Relative water content used in this study was determined by modifying the Protocol of Barrs (1968). On day twenty-eight, the youngest fully expanded leaf was cut into two pieces and the fresh weight was weighed. Then, transfer the small pieces into a plastic dip with 10 ml of deionized water and samples were kept in the dark for 24 hours. After that, the turgid weight of samples were recorded. Next, samples were dried inside hot air oven at 60℃ for 3 days in order to make the recorded dry weight constant. Data was collected for four times a day and the Relative Water Content was calculated from the following equation.

$$\% RWC = [(FW-DW) / (TW-DW)] \times 100$$

FW = Fresh weight

TW = Turgid weight

DW = Dry weight

3.5.5. Chlorophyll fluorescence (F_v/F_m)

At twenty-eight days after germination, chlorophyll fluorescence was measured from youngest fully expanded leaf at 11 a.m. to 1 p.m. All plants were moved under the shade and dark adaptation period was 30 minutes Li et al. (2006).

3.5.6. SPAD index

Chlorophyll content was determined by using chlorophyll meter (SPAD-502) according to the methods of Li et al. (2006) from 8 to 9 a.m. The youngest fully expanded leaf from three different locations were measured for chlorophyll content. Moreover, the data was collected four timing on day 0, 3, 6, and 9.

3.5.7. Stay green score (SGS)

Stay green score was recorded at twenty-eight days after germination that start from zero to day nine after treat drought stress. Stay Green Score was collected every day from the whole plants according to Gana (2011) and was given a score from 0 to 5 (Table 3).

Table 3 Stay green score and description

Stay Green Score	Symptoms	Reaction
1	No symptoms	Resistant
2	A slight yellow, less than 40%	Moderately resistant
3	Yellow covered between 40-60% and leaf tip drying	Intermediate resistant
4	Yellowish, more than 60% and leaf drying	Susceptible
5	All plant yellow and dried	Highly susceptible

3.5.8. Fresh weight (FW) and dry weight (DW)

Fresh weight and dry weight were collected every three day. Plant shoots and roots were collected separately and fresh weight was recorded after washing the sample. Next, samples were dried in hot air oven at 60 degree Celsius for 3 days to make constant weight and dry weight were recorded.

Chapter IV

Results

To identify drought tolerant lines, various criterias were used to evaluate drought tolerant lines under different drought conditions, the parameters used for drought responses evaluation are leaf rolling score (LRS), leaf drying score (LDS), cell membrane stability (CMS), relative water content (RWC), chlorophyll fluorescence (F_v/F_m), SPAD, stay green score (SGS), and growth parameters.

4.1. Preliminary data

4.1.1 Investigation of field capacity I

Table 4 Percent field capacity of soil for preliminary study

Sample	Fresh Weight (g)	Dry Weight(g)	%Field Capacity
1	15.28	13.15	16.21
2	16.41	14.20	15.56
3	17.73	15.29	16.00
Average			15.93

Firstly, the soil used in the experiment must be analyzed for field capacity to optimize timing under different drought conditions. As shown in table 4, the average field capacity of the triplicates was 15.93%. For the preliminary study was used 220 g. of soil per pot was used and the level of water was controlled by used percent field capacity. Therefore, 35.05 ml of water was added to a 100% field capacity pot, 17.52 ml of water was added to a 50% field capacity pot, and 8.76 ml of water was added to a 25% field capacity pot.

4.1.2. Optimization of timing under different drought conditions
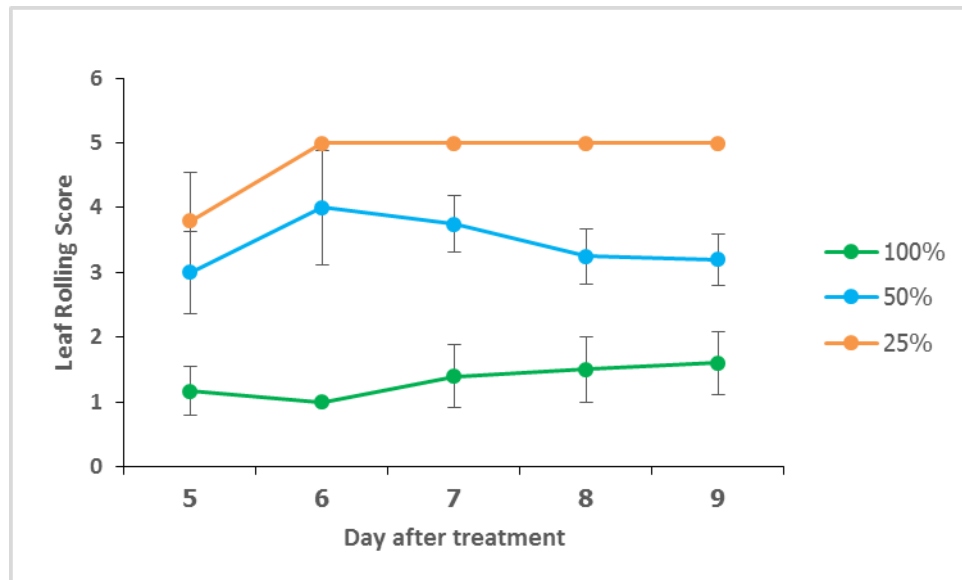
Figure 6 Time course of changes in the leaf rolling score of rice to different percent field capacity (mean±sd).

Figure 6 shows the leaf rolling score of plants under three drought conditions during day five to nine post-treatment. A considerable difference between the three drought conditions (25%, 50%, and 100% field capacity) was observed. However, there is a correlation between the leaf rolling score and field capacity of plants under all conditions. Plants in 100% field capacity showed the least leaf rolling score of approximately 1 (day 5), with a slight increase on days 7 to 9. In contrast, plants under 25% field capacity scored the highest among the three conditions at just below 4 (day 5). Moreover, the score raised up to five after one day and remained stable until day 9. For plants from 50% field capacity, the leaf rolling score was 3 at day 5 post-treatment. The score raised to 4 after a day and decreased until the last day of data collection. The leaf rolling score correlated with the level of drought. That is, the higher the drought intensity, the higher the leaf rolling score.

To sum up, 100% and 50% field capacity were good condition that can be used to control drought condition for the experimental study.

4.1.3 Investigation of field capacity II

Table 5 Percent Field capacity of soil for experiment study

Sample	Fresh Weight (g)	Dry Weight(g)	%Field Capacity
1	16.00	13.99	14.37
2	13.77	12.25	12.39
3	19.59	17.44	12.28
4	19.42	17.09	13.64
Average			13.17

For experimental study, the soil used in the experiment was analyzed for field capacity again. The average field capacity was 13.17% as shown in table 5. For this experiment, 900 g of soil was used per pot. 88.9 ml of water was added to a 75% field capacity pot and 59.25 ml of water was added to another port with 50% field capacity. Also, the water level of all pots was maintained throughout the experiment.

4.1.4. Optimization time for chlorophyll fluorescence (F_v/F_m) collection

The optimization timing to collect data for chlorophyll fluorescence was determined. Figures 7, 8 and 9 shows the response in terms of chlorophyll fluorescence of three rice species of three different drought conditions (50%, 75%, and 100% field capacity). The chlorophyll fluorescence data was collected between 7 a.m. and 6 p.m. of day zero and day five post-treatment.

From day zero after treatment, at 7 a.m. two lines (RGD05131-4-MAS39 and DH212) did not show any significant change (Fig. 7 A and C). The chlorophyll

fluorescence of the two lines stayed constant at 0.8 for the rest of the day, whereas at 3 p.m., RGD05131-4-MAS39 rice showed a drop of chlorophyll fluorescence at 100% field capacity. KDML105 also showed a significant difference at 9 p.m., but did not show much difference between different drought conditions (Fig. 7 B).

From graph of three lines consisting of RGD05131-4-MAS39, KDML105, and DH212 five days after treatment. However, the only significant change was observed at 12 p.m. of RGD05131-4-MAS39 (Fig. 8 A). At 100% and 75%, the chlorophyll fluorescence level was maintained in all of the 6 lines at 0.8 (Fig. 8 B and C).
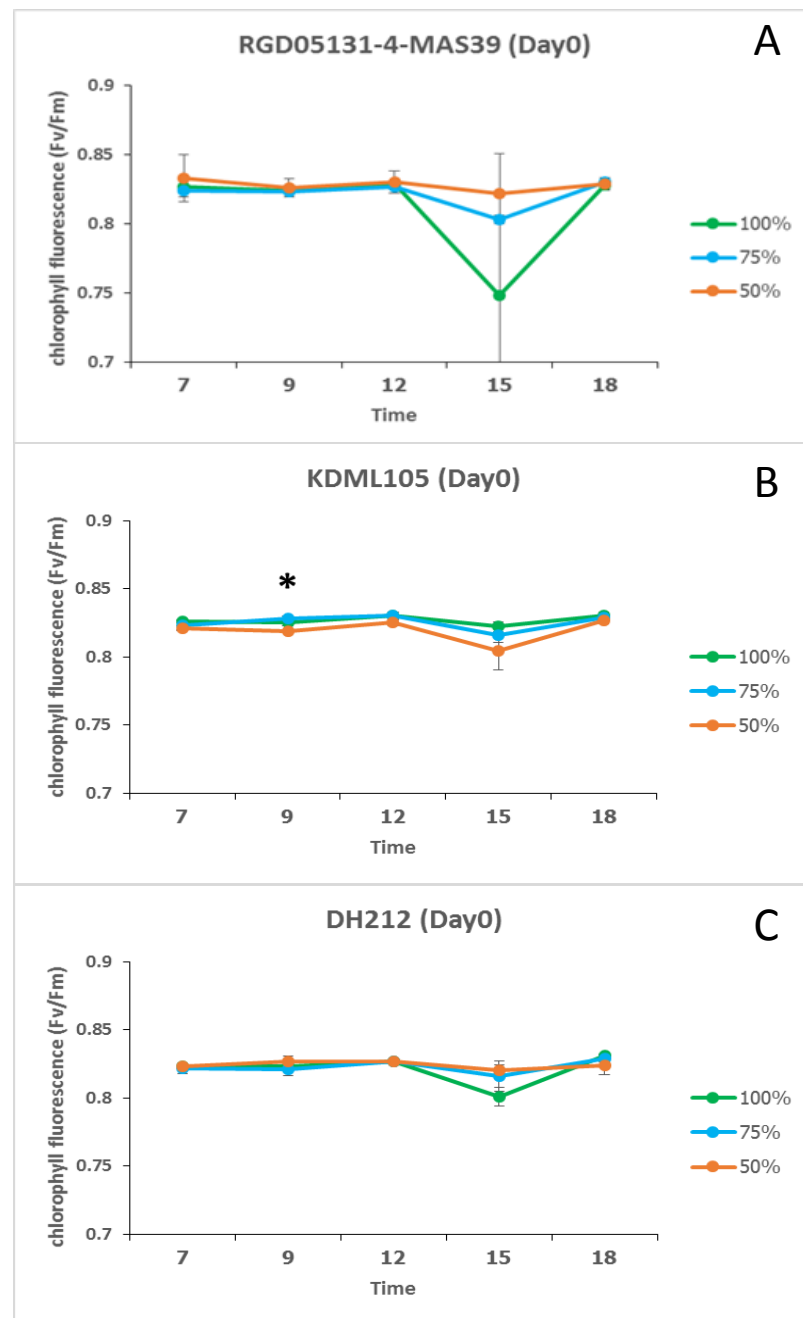
Figure 7 Changes in the chlorophyll fluorescence of (A) RGD05131-4-MAS39
(B) KDML105 and (C) DH212 to different percent field capacity between 7 am. and 6
pm on day 0. (mean±sd)
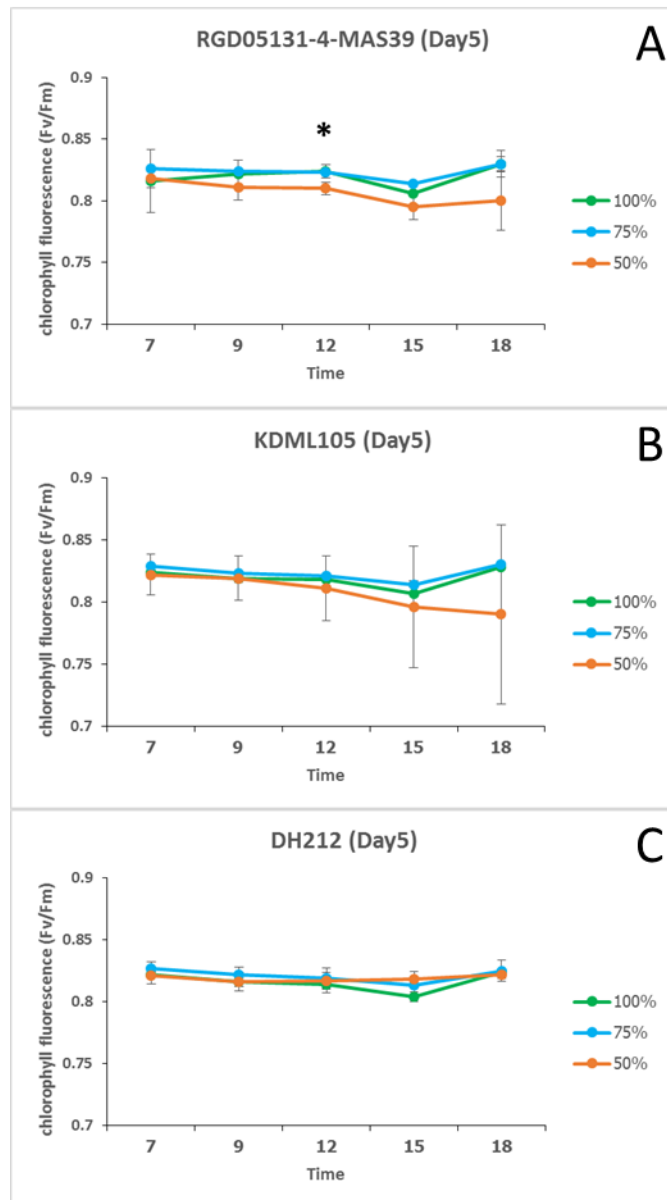
Figure 8 Changes in the chlorophyll fluorescence of (A) RGD05131-4-MAS39
(B) KDML105 and (C) DH212 to different percent field capacity between 7 am. and 6
pm on day 5. (mean±sd)

4.2. Experiments

The condition of greenhouse between 9 am to 4 pm included temperature, relative humidity, and photosynthetically active radiation (PAR) are presented in figures 9.
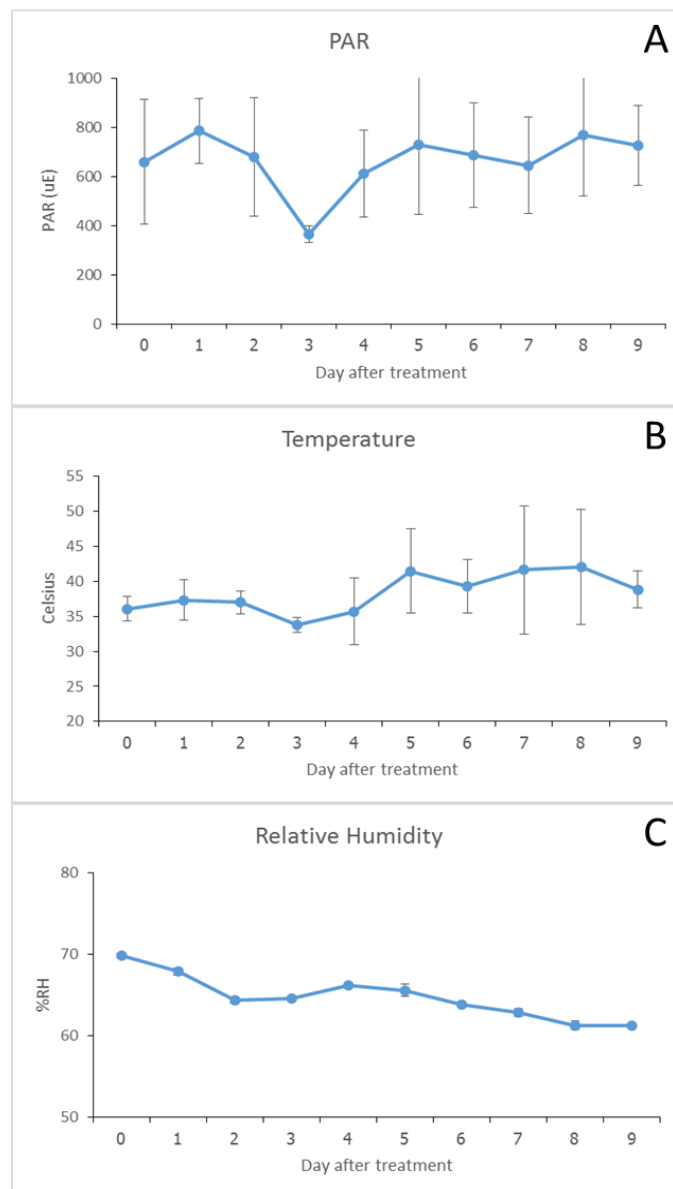
Figure 9 The condition of greenhouse (A) photosynthetically active radiation (B) temperature and (C) relative humidity between June 27th and July 6th, 2014 (mean±sd)

4.2.1. Leaf rolling score (LRS)

Graphs show the leaf rolling score of six lines that were treated with three different conditions, 100%, 75% and 50% field capacity. It can clearly be seen that there was correlation between leaf rolling score and field capacity, and there was no significant differences between the lines during the ten days of treatment even though the leaf rolling score fluctuated between score 1 and 2.

From Figure 10 A, the leaf rolling score of the six lines that were treated with 100% field capacity remained stable with score of one throughout seven days after treatment, but all lines slightly shifted on day 8. At days eight and nine, two lines, RGD0128-10-MAS12 and RGD05128-4-MAS40-MAS11, raised up to score two. In contrast, at 75% field capacity and 50% field capacity (Fig 10 B and C), the graphs showed similar trends were the leaf rolling score fluctuated between scores 1 and 2. In all figures, RGD05131-6-MAS5 and DH212 remained stable at all conditions.

Figure 10 Changes in the leaf rolling score of rice to (A) 100 percent field capacity (B) 75 percent field capacity and (C) 50 percent field capacity at day 0 to day 9. (mean±sd)

4.2.2. Leaf drying score (LDS)

The graphs of leaf drying score of six lines at three condition show similar trends and six lines of rice responded similarly at 100% field capacity and 75% field capacity (Fig. 11 A and B). They had small increase. Differentiation between lines became apparent at high drought stress, 50% field capacity, when leaf showed high leaf drying score.

Six lines of rice have the same LDS in three drought conditions prior to treatment. At 100% field capacity and 75% field capacity, LDS fluctuated around score one to three and at 100% field capacity the score remained constant when compared to 75% field capacity. Whereas at 50% field capacity an increasing trend was observed. The graph showed significant difference between lines at day seven after maintaining the water level at 50% field capacity (Fig. 11 C). From day seven, leaf drying score of DH212 was the least and other CSSLs responded of leaf drying score in a similar manner to KDML105 (Figure 19).

Figure 11 Changes in the leaf drying score of rice to (A) 100 percent field capacity (B) 75 percent field capacity and (C) 50 percent field capacity at day 0 to day 9. (mean±sd)

4.2.3. Cell membrane stability (CMS)

Cell membrane stability was non-significantly different between lines when exposed to different stress conditions. At 75% field capacity, all lines responded to drought similar to 100% field capacity, while 50% field capacity showed some difference.

From figure 12 A and B, it showed that there was not much difference in cell membrane stability between the six lines at 100% and 75% field capacity. This showed that cell membrane stability could be maintained under drought stress in all lines. Cell membrane stability remains unchanged above 80% after 9 day of treatment. At 50% field capacity, the response to drought was the same at day 0 and 3. They were stable around 90% cell membrane stability. However, they showed some difference at day 6 and 9. They drop to approximately 60% cell membrane stability. It can be concluded that irrigation level has an effect on cell membrane stability under drought stress as shown in Figure 12 C. KDML105 and RGD05131-6-MAS5 were the group that showed sharp reduction in cell membrane stability since day 6. In day 9, cell membrane stability in all lines decreased for more than 40%, in which RGD0128-10-MAS12 and RGD05128-4-MAS40-MAS11 were the only two lines that had higher cell membrane stability than KDML105 but not significantly different from KDML105.

Figure 12 Changes in the cell membrane stability of rice to (A) 100 percent field capacity (B) 75 percent field capacity and (C) 50 percent field capacity at day 0 to day 9. (mean±sd)

4.2.4. Relative water content (RWC)

The graphs compare the relative water content of six lines by the water level that is maintained by field capacity. It can be clearly seen that relative water content did not correlate with the level of field capacity. The greatest decrease in relative water content was found in plants with 50% field capacity that reduced the water status in plants throughout of the time period. However, different trends can be seen for the response of each line towards water level.

At 100% field capacity, the differences in relative water content between lines were not significant. At 100% and 75% field capacity, the relative water content were all above 80% (Fig. 13 A and B) and remained constant throughout nine days of treatment. On the other hand, lines in 50% field capacity showed significant reduction in relative water content after exposure to drought treatment (Fig. 13 C). Six days after treatment, 50% of the relative water content was lost in five of the six lines. In contrast, DH212 was only line that can retain the water level. In addition, RGD05128-4-MAS40-MAS11 line was the only CSSLs line that has more percent relative water content than KDML105 and DH212 nine days after treatment.

Figure 13 Changes in the relative water content of rice to (A) 100 percent field capacity (B) 75 percent field capacity and (C) 50 percent field capacity at day 0 to day 9. (mean±sd)

4.2.5. Chlorophyll fluorescence (F_v/F_m)

The graph compares chlorophyll fluorescence (F_v/F_m) in plants from different field capacities and the data was collected daily for ten days after treatment. It can be clearly seen that in all plants, chlorophyll fluorescence declined when they received a much higher level of drought stress and longer exposure.

In 100% and 75% field capacity plants (Fig. 14 A and B), all lines had chlorophyll fluorescence of 0.8. However, at 50% field capacity most lines remained stable with chlorophyll fluorescence of 0.8, but significantly declined between day 4 and day 6 (Fig. 14 C). In addition, RGD05128-4-MAS40-MAS11 was the only line that showed a steep drop on day 4 and stay constant at 0 after day 4.

At day four and five of 50% field capacity, the chlorophyll fluorescence of RGD05128-4-MAS40-MAS11 maintained the same level which was 0 whereas other lines had chlorophyll fluorescence between 0.6 and 0.8. However, on day 6, RGD05128-4-MAS40-MAS11 remained stable at 0. Moreover RGD05131-4-MAS39 and KDML105 also showed great decrease in chlorophyll fluorescence below 0.2 (Fig. 14 C). According to the results, all lines could be divided into two groups. The first group consist of lines that have high level of chlorophyll fluorescence. There were RGD05131-6-MAS5, RGD0128-10-MAS12, and DH212 in the high chlorophyll fluorescence group. Another group was the group of lines with high reduction in chlorophyll fluorescence which include RGD05131-4-MAS39, RGD05128-4-MAS40-MAS11, and KDML105.
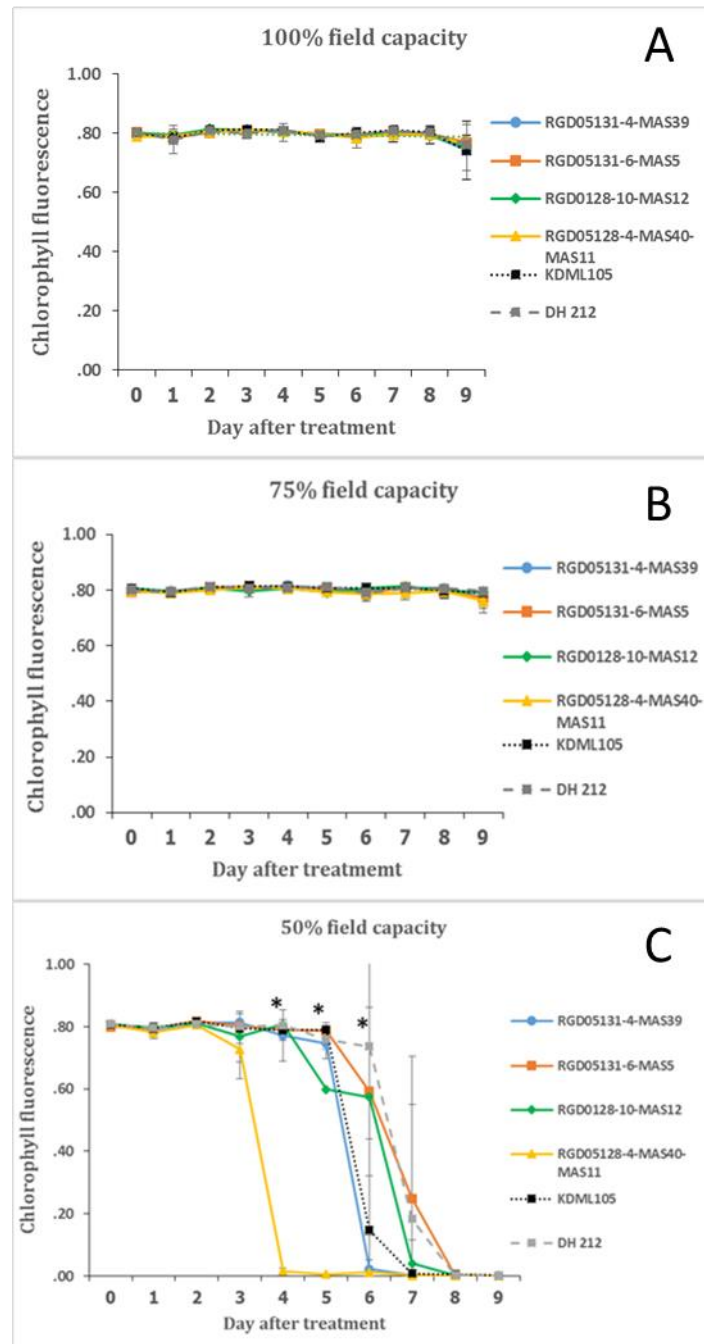
Figure 14 Changes in the chlorophyll fluorescence of rice to (A) 100 percent field capacity (B) 75 percent field capacity and (C) 50 percent field capacity at day 0 to day 9. (mean±sd)

4.2.6. SPAD index

For SPAD, there was no any significant difference between genotypes under three different conditions, 100%, 75%, and 50% field capacity.

At 100% and 75% field capacity, six lines maintained SPAD index around 30 (Fig. 15 A and B). Moreover, at 50% field capacity SPAD index of all lines stay slightly above 30 in day 0 and day 3. Then, SPAD index moderately decreased after day 3 (Fig. 15 C). Three lines, RGD05131-6-MAS5, RGD05128-4-MAS40-MAS11, and KDML105, were reduced more than other lines. Their SPAD data were lower than 20 but other lines such as RGD05131-4-MAS39, RGD0128-10-MAS12, and DH212 were more than 20. From figure 33 RGD05128-4-MAS40-MAS11 had lower SPAD index than other lines in every condition.

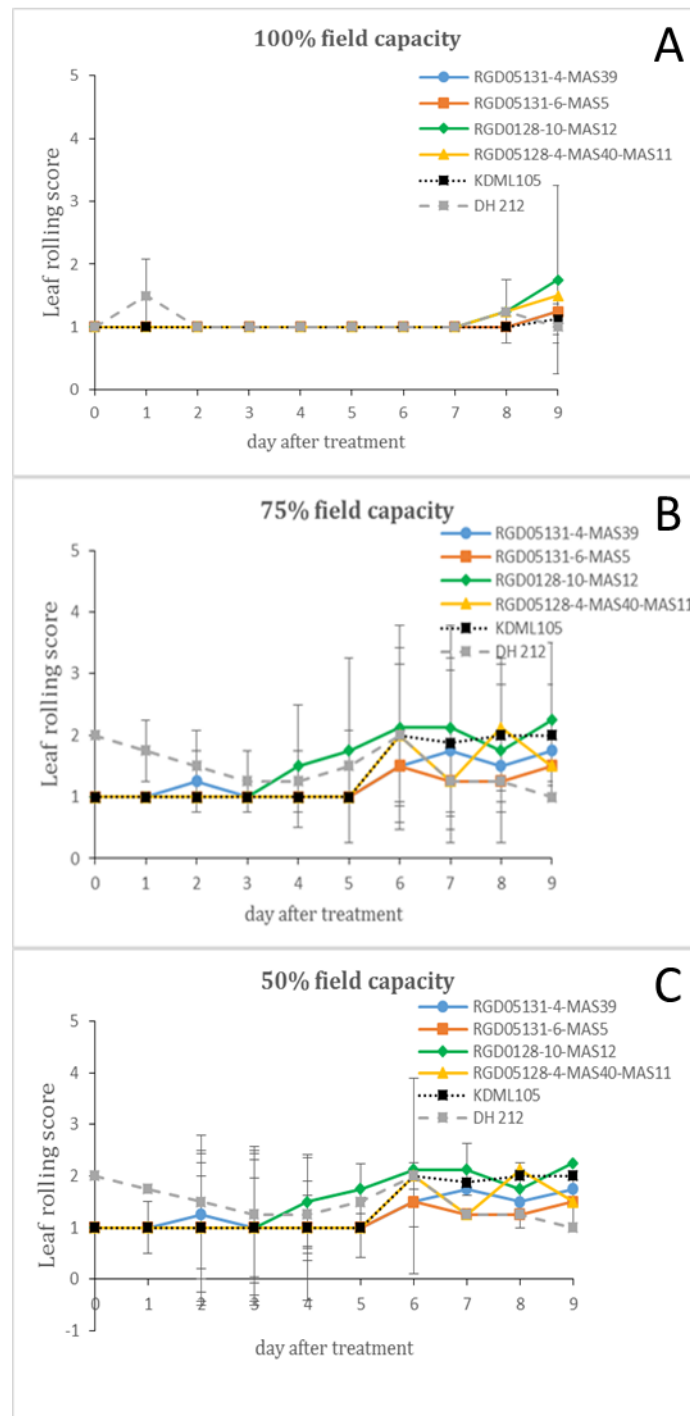**Figure 15** Changes in the SPAD index of rice to (A) 100 percent field capacity (B) 75 percent field capacity and (C) 50 percent field capacity at day 0 to day 9. (mean±sd)

4.2.7. Stay green score (SGS)

The panel below shows the effect of field capacity on stay green score of lines after ten days of drought exposure. Results showed that drought affected all lines in 50% field capacity, but did not have much impact on 100% and 75% field capacity.

Stay green score started off at score one and fluctuated around score two. At 100% and 75% field capacity, slight increase was observed, but at 75% field capacity showed higher increase than 100% field capacity (Fig.16 A and B). Moreover, at 50% field capacity all lines gradually increased and there was significant difference in response between lines in day 7 after drought treatment (Fig. 16 C). According to the results, the lines could be divided into three groups. The first group, which consists of KDML105 and RGD05131-4-MAS39 had the highest score indicating that these lines were susceptible to drought stress. The second group, RGD0128-10-MAS12, and RGD05128-4-MAS40-MAS11 were in the intermediate resistant group. Lastly, the third group, which comprises of DH212 and RGD05131-6-MAS5, were the line with the lowest stay green score was in the moderate resistant group.
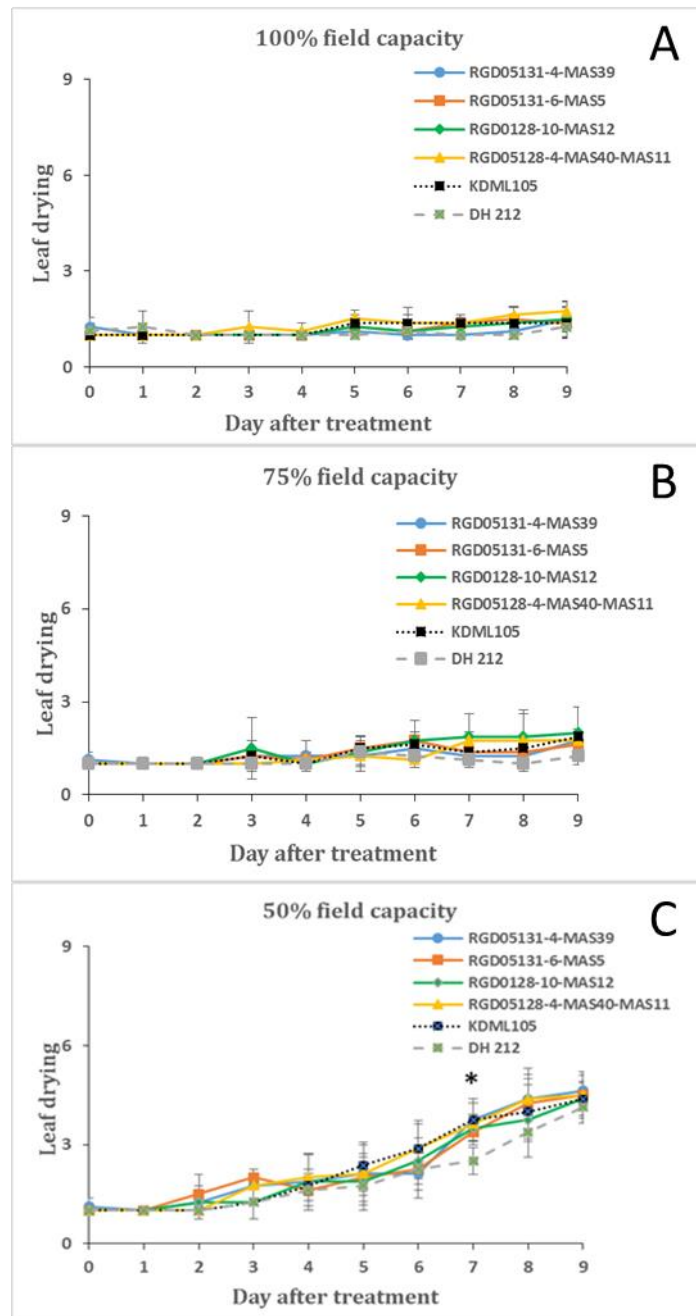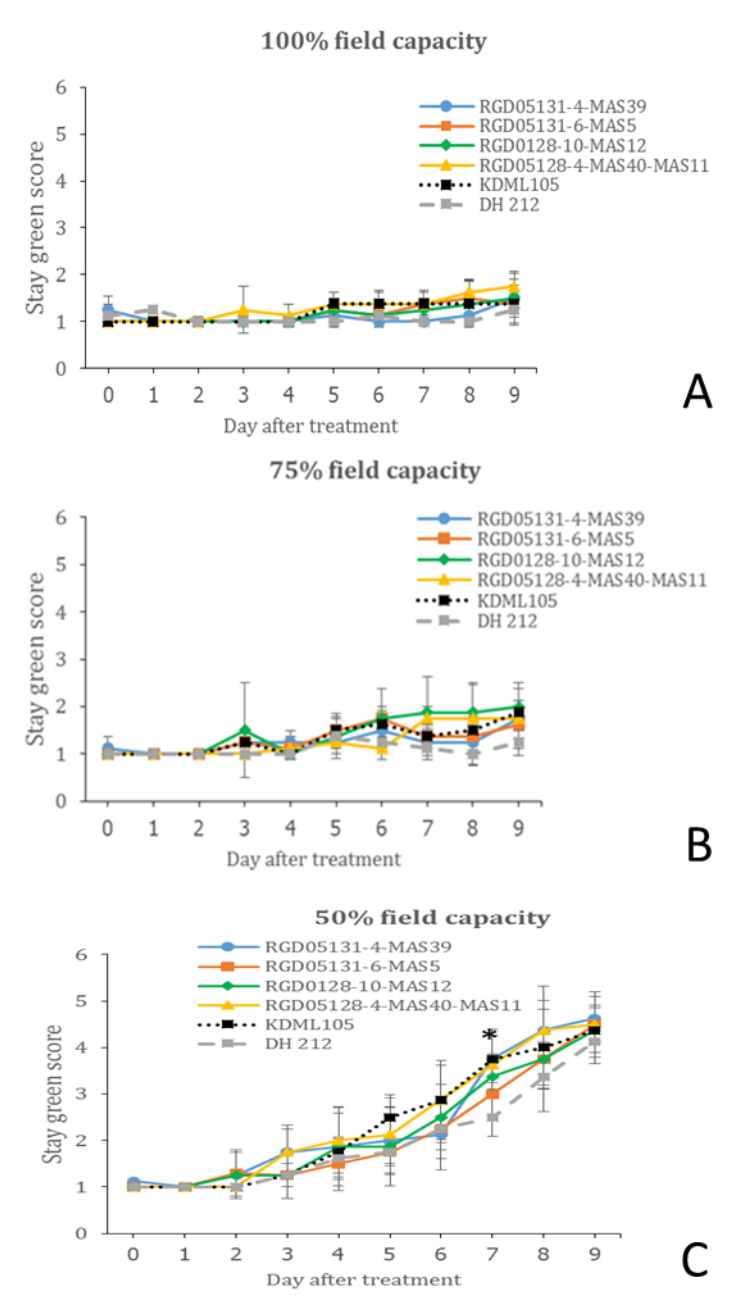
Figure 16 Changes in the stay green score of rice to (A) 100 percent field capacity
(B) 75 percent field capacity and (C) 50 percent field capacity at day 0 to day 9.
(mean±sd)

4.2.8. Growth

4.2.8.1. Fresh weight (FW)

To determine whether fresh weight changes under drought stress, the lines used in this study were weighed before and after exposure to drought stress. To investigate the fresh weight, a graph was plotted between fresh weight and three drought conditions. Results showed significant differences in both 100% and 50% field capacity, but there were no significant differences in 75% field capacity (Fig. 17).

In 100% field capacity, there was a significant increase in day 3 after treatment. RGD05128-4-MAS40-MAS11, RGD05131-4-MAS39, and RGD0128-10-MAS12 had more fresh weight than KDML105 (Fig. 17 A). Fresh weight was significantly different between lines under 50% field capacity in day 9 after treatment. As for day 9 of 50% field capacity (Fig. 17 C), RGD05131-4-MAS39 was the only line that had more fresh weight than KDML105 and RGD0128-10-MAS12 was a line that has similar fresh weight to KDML105.
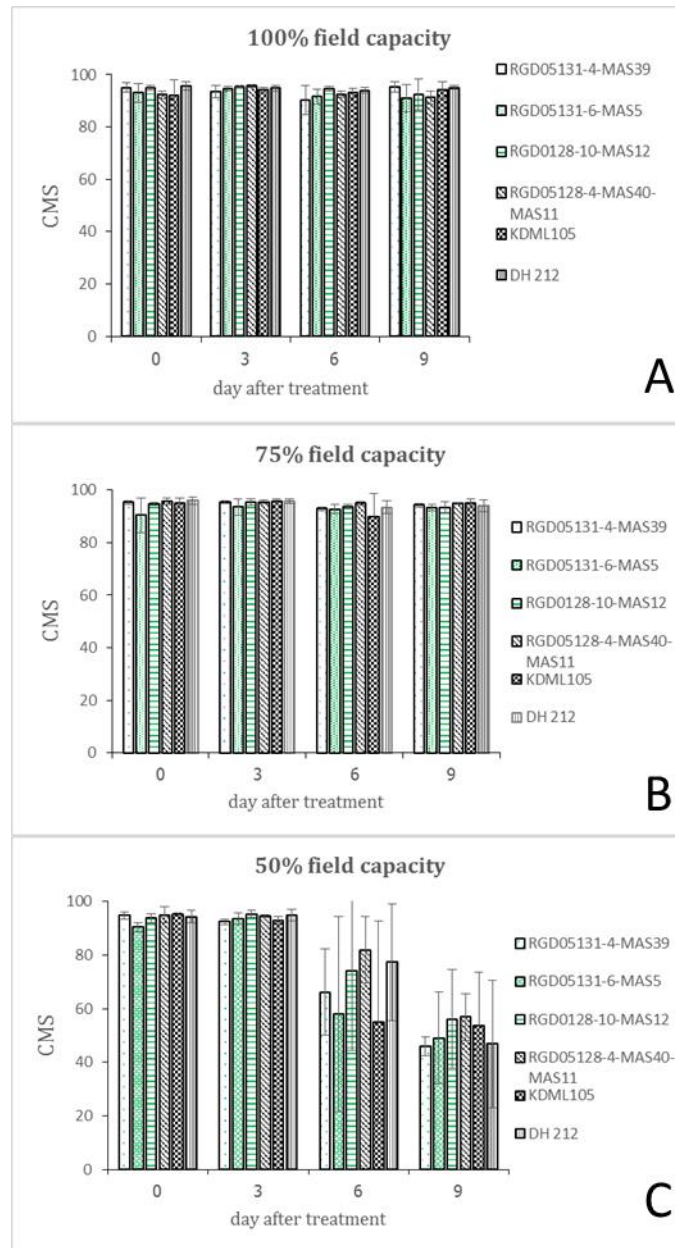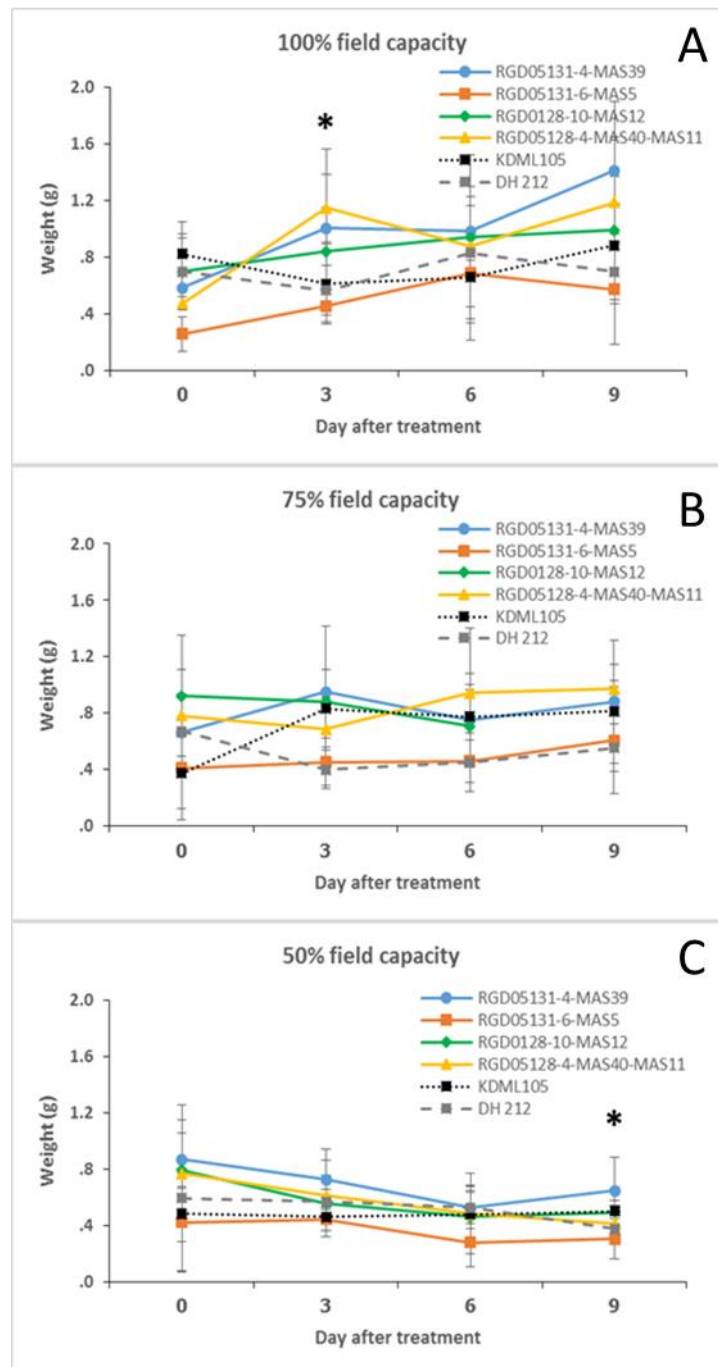
Figure 17 Changes in the fresh weight of rice to (A) 100 percent field capacity (B) 75 percent field capacity and (C) 50 percent field capacity at day 0 to day 9. (mean±sd)

4.2.8.2. Dry weight (DW)

To study the effect of drought exposure on dry weight, dry weight was measured after exposure to drought conditions every three days for nine days such as day 0, 3, 6, and 9. Three conditions, 100%, 75%, and 50% field capacity were used to study. There were significant difference between lines under three conditions.

On day six, there was significant difference in both 100% and 75% field capacity (Fig. 18 A and B). RGD05131-4-MAS39, RGD0128-10-MAS12, and RGD05128-4-MAS40-MAS11 were high dry weight than KDML105 under 100% field capacity (Fig. 18 A), whereas in 75% field capacity there was only RGD05131-4-MAS39 that had more dry weight than KDML105 (Fig. 18 B). The effect of drought treatment, 50% field capacity, on the dry weight revealed that dry weight was significant on day nine (Fig. 18 C). As for day nine, a similar trend under 75% field capacity reviewed that RGD05131-4-MAS39 has more dry weight than KDML105, whereas RGD05131-6-MAS5 had the lowest dry weight (Fig. 18).
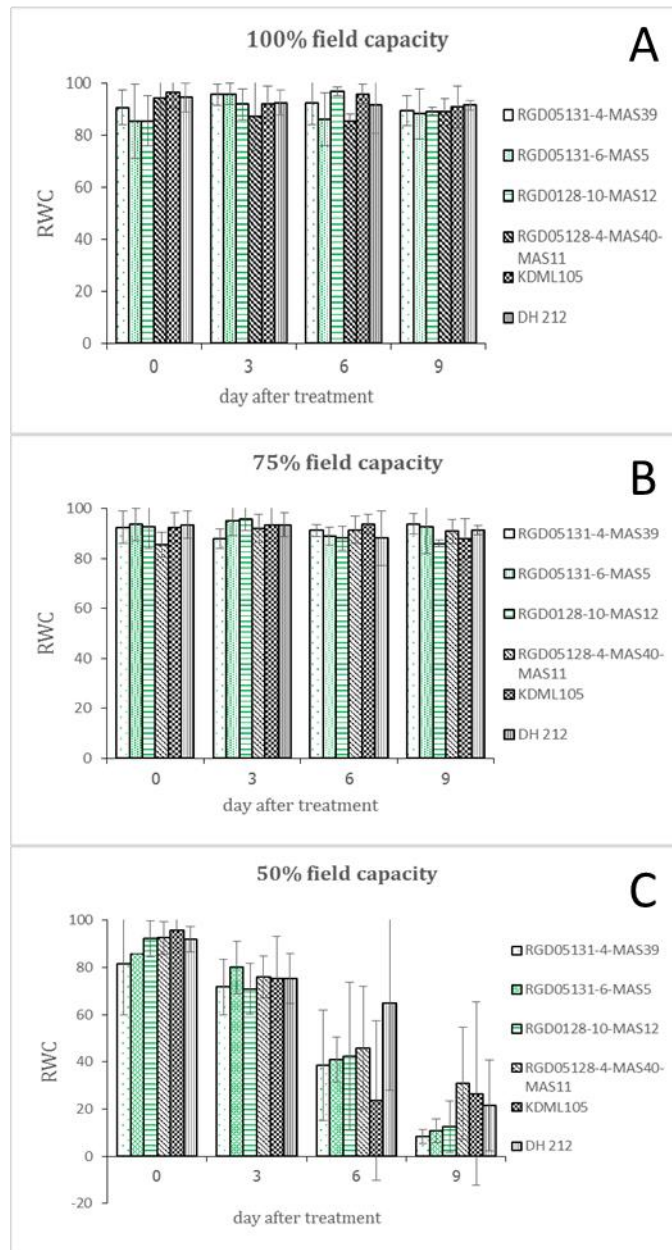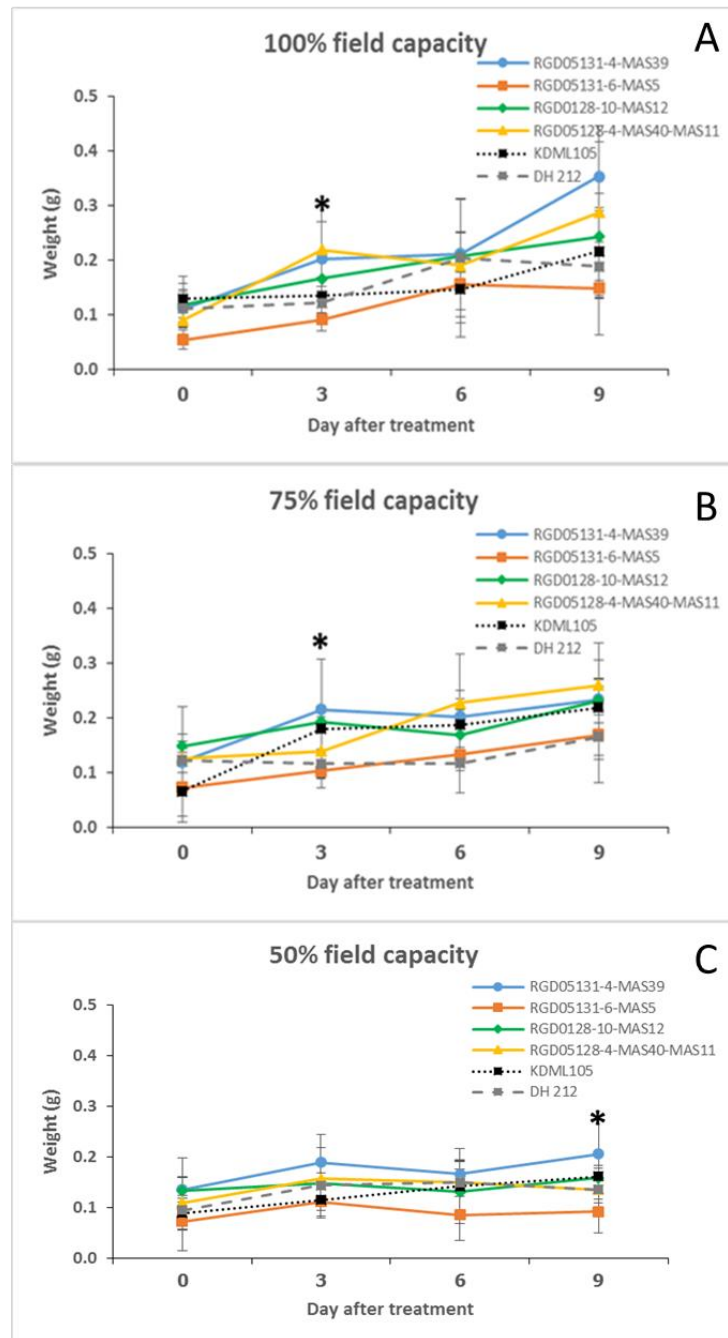
Figure 18 Changes in the dry weight of rice to (A) 100 percent field capacity (B) 75 percent field capacity and (C) 50 percent field capacity at day 0 to day 9. (mean±sd)

4.2.8.3. Relative growth rate

The panel below shows the effect of field capacity on relative growth rate of lines after ten days of drought exposure. Results showed that drought affected in 50% field capacity, but did not have much impact on 100% and 75% field capacity.

From relative growth rate of rice shoot, there are not significantly different between lines at 100% and 75% field capacity that show in figures 19 A and B. Whereas at 50% field capacity, relative growth rate of shoot shows a significantly different in 3 and 6 day after treated. RGD05131-6-MAS5 and KDML105 show increasing trend but another lines have reducing trend of relative growth rate (Fig. 19 C). In addition, relative growth rate of rice root is significantly different between lines at day 3 of 100% field capacity which are RGD05131-6-MAS5 and RGD05128-4-MAS40-MAS11 have reduced trend. While, another lines have stable relative growth rate of root (Fig. 20 A). Moreover, RGD05131-6-MAS5 also show decreased trend at 75% field capacity but not significant (Fig. 20 B). On the other hand, when RGD05131-6-MAS5 and KDML105 have a drought stress at 50% field capacity, they show an increase of relative growth rate of root between day 3 and day 6 differ from another lines that have decrease trend (Fig. 20 C).
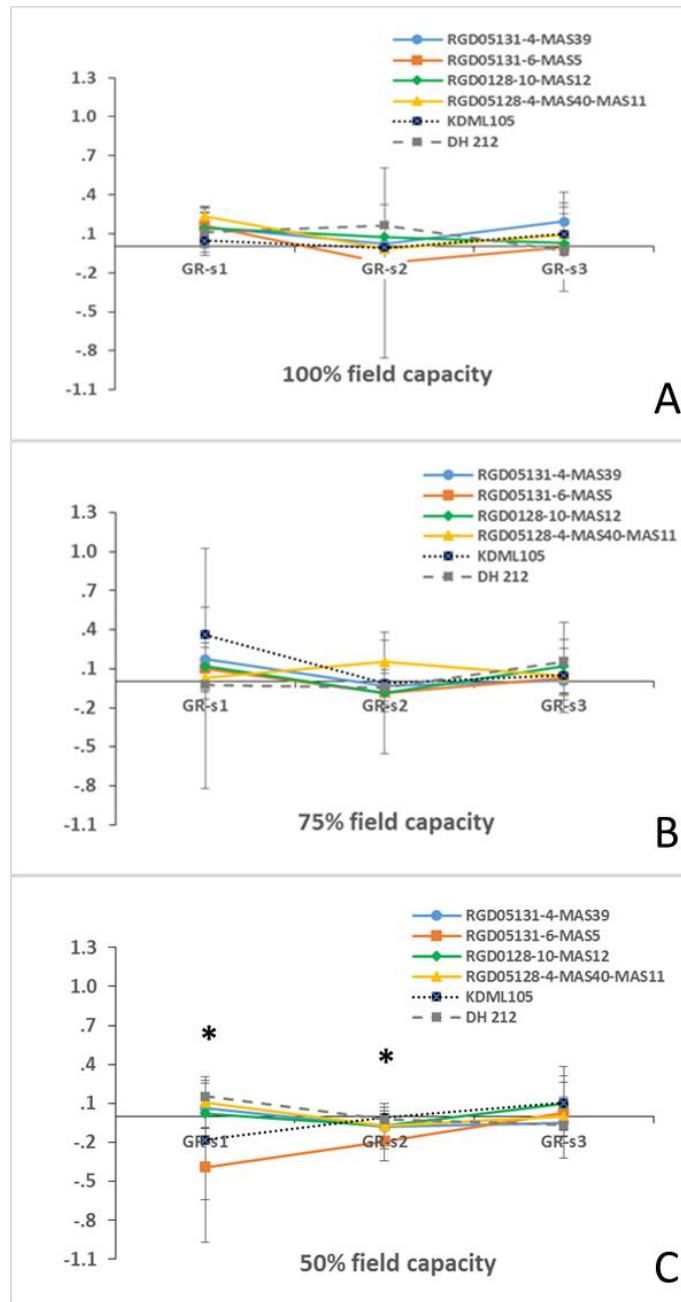
Figure 19 Changes in relative growth rate of rice shoot to (A) 100 percent field capacity (B) 75 percent field capacity and (C) 50 percent field capacity at day 0 to day 9. (mean±sd)
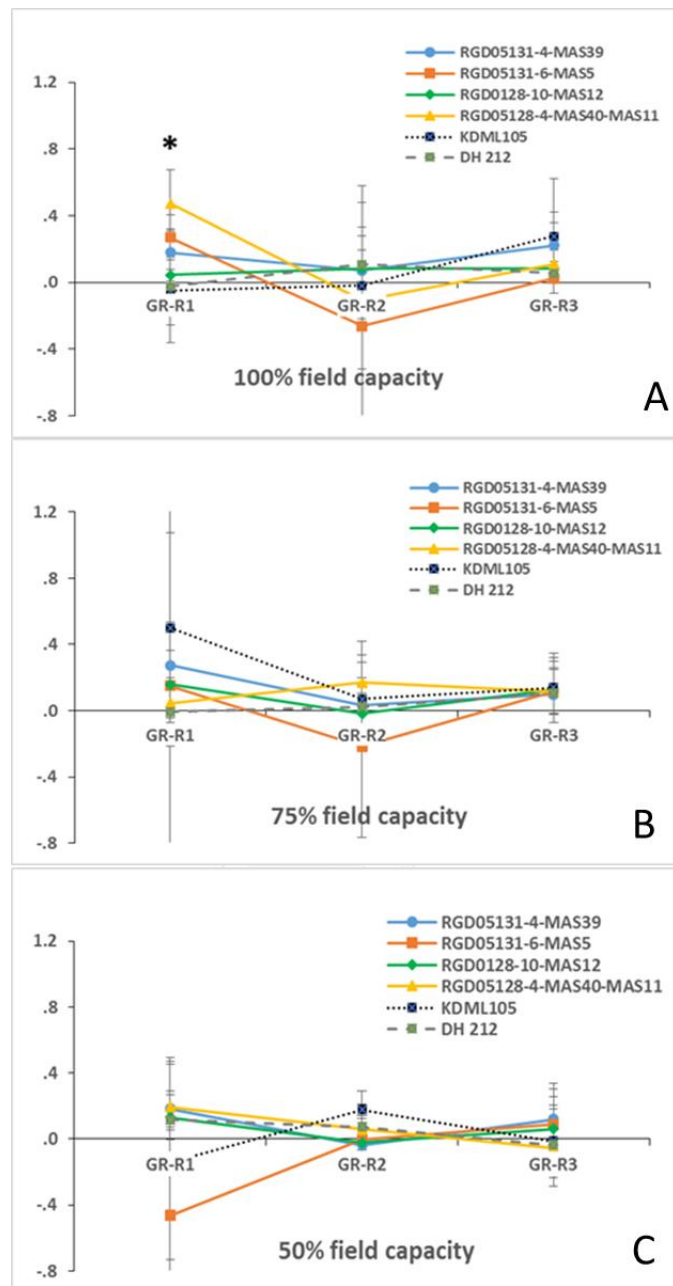
Figure 20 Changes in relative growth rate of rice root to (A) 100 percent field capacity
(B) 75 percent field capacity and (C) 50 percent field capacity at day 0 to day 9.
(mean±sd)

4.2.8.4. Root/shoot ratio

Graphs show the root/shoot ratio of six lines that were treated with three different conditions, 100%, 75% and 50% field capacity. The root/shoot ratio was non-significantly different between lines when exposed to different stress conditions. RGD05128-4-MAS40-MAS11 was a higher root/shoot ratio under normal condition (Fig. 21 A). On the other hand, six lines of rice responded similarly at 75% field capacity and 50% field capacity (Fig. 21 B and C). RGD05131-6-MAS5 and KDML105 had higher root/shoot ratio than other lines, but at 75% field capacity KDML105 had 50% higher root/shoot ratio than RGD05131-6-MAS5.

4.2.9. Correlation

From the results found the correlation among the physiological parameters as showed in table 6. Relative water content, cell membrane stability, SPAD index, chlorophyll fluorescence and fresh weight are correlated. Chlorophyll fluorescence is correlated with fresh weight and root/shoot ratio. In addition, fresh weight also correlated with dry weight and root/shoot ratio.

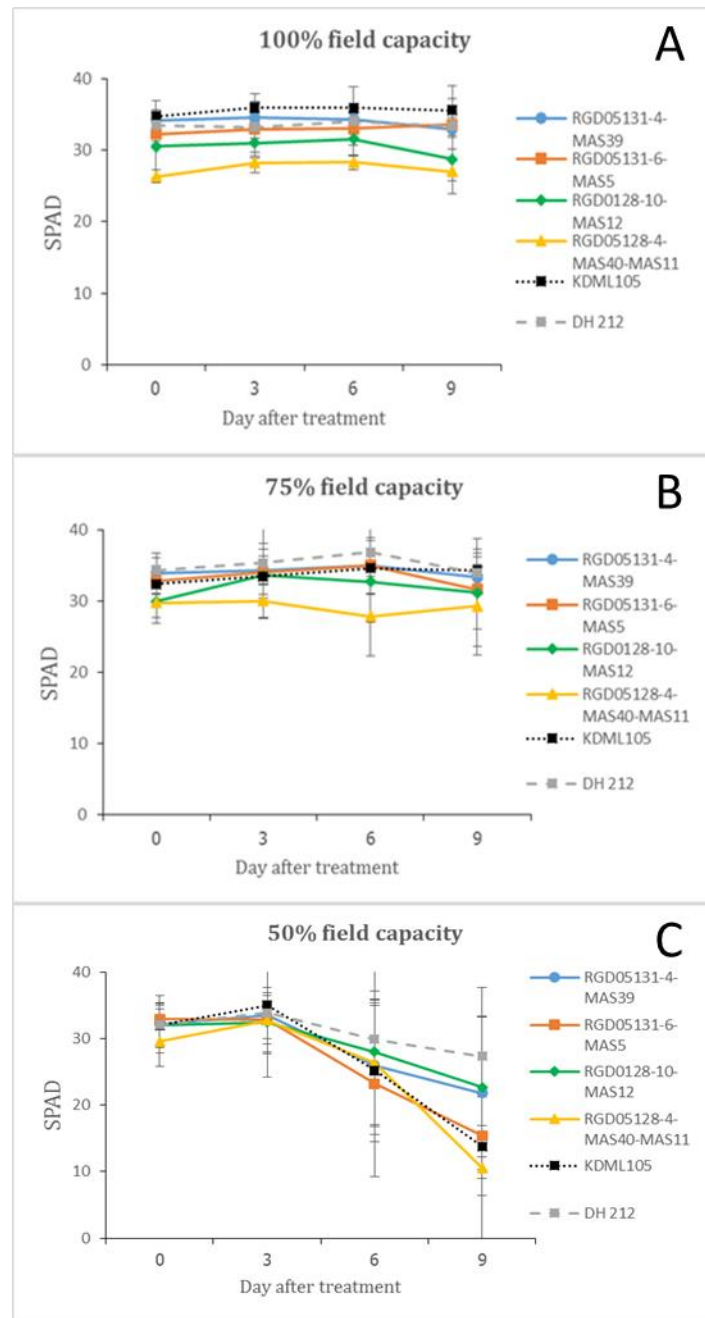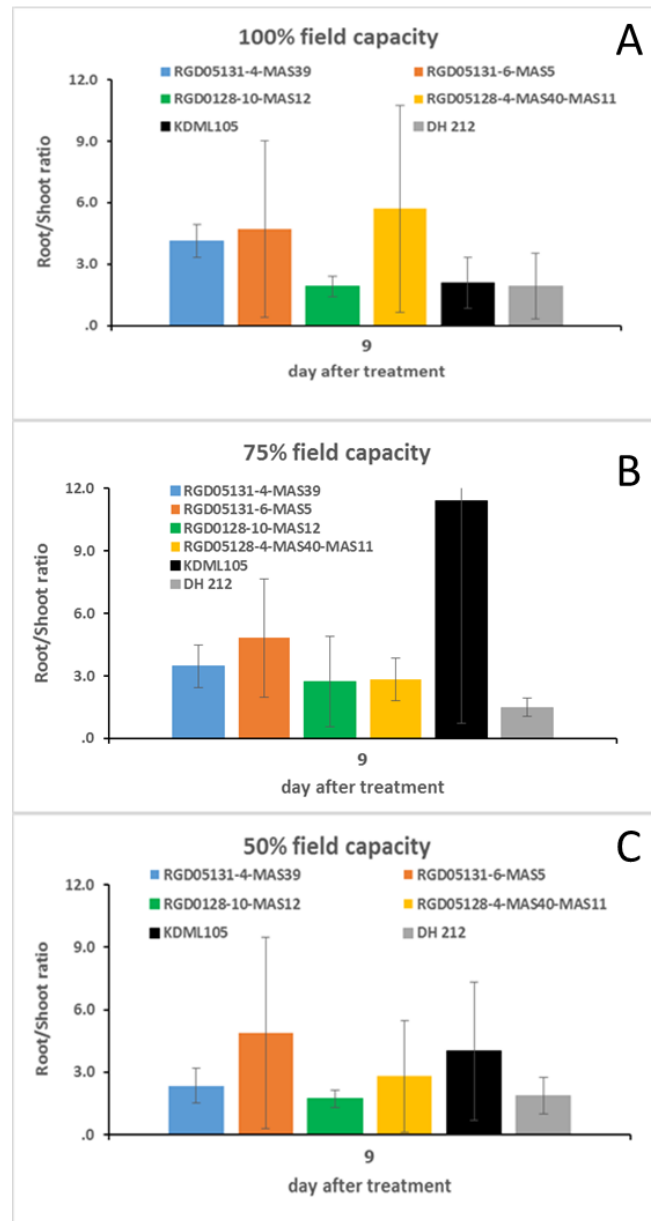**Figure 21** Root/shoot ratio of rice to (A) 100 percent field capacity (B) 75 percent field capacity and (C) 50 percent field capacity at day 9. (mean±sd)

Table 6 Correlation of SPAD index, relative water content, cell membrane stability, chlorophyll fluorescence, fresh weight, dry weight and root/shoot ratio

* Correlation is significant at the 0.05 level (2-tailed).

** Correlation is significant at the 0.01 level (2-tailed).

		SPAD	RWC	CMS	F_v/F_m	FW	DW	Root/ Shoot ratio
SPAD	Pearson Correlation Sig. (2-tailed)	1						
RWC	Pearson Correlation Sig. (2-tailed)	.514** .000	1					
CMS	Pearson Correlation Sig. (2-tailed)	.526** .000	.861** .000	1				
$f_v f_m$	Pearson Correlation Sig. (2-tailed)	.595** .000	.803** .000	.796** .000	1			

Table 6 Correlation of SPAD index, relative water content, cell membrane stability, chlorophyll fluorescence, fresh weight, dry weight and root/shoot ratio

* Correlation is significant at the 0.05 level (2-tailed).

** Correlation is significant at the 0.01 level (2-tailed).

		SPAD	RWC	CMS	f_v/f_m	FW	DW	Root/ Shoot ratio
FW	Pearson Correlation	.136[*]	.272[**]	.259[**]	.229[**]	1		
	Sig. (2-tailed)	.022	.000	.000	.000			
DW	Pearson Correlation	.032	.064	.063	.017	.884[**]	1	
	Sig. (2-tailed)	.595	.289	.297	.782	.000		
Root/ Shoot ratio	Pearson Correlation	-.084	-.078	-.117	-.138[*]	.164[**]	.303[**]	1
	Sig. (2-tailed)	.165	.197	.055	.022	.006	.000	

Chapter V

Discussion

5.1. Leaf Rolling Score (LRS)

Change in leaf form or shape, especially the leaf rolling, is an adaptive responses to water deficits. Leaf rolling is a hydro nastic mechanism which decrease transpiration rate, leaf drying, and light interception (Kadioglu and Terzi, 2007). Kadioglu et al. (2012) described the advantage of leaf rolling response such as reduced leaf area and decrease transpiration rate. Thus, it is an evidenced of drought avoidance mechanisms under water deficits. Leaf rolling was used to classify symptom of drought stress in rice by Abd Allah (2006) and Gana (2011). The research showed that rice which had high leaf rolling score appeared to be drought susceptible cultivar.

The data for leaf rolling score of 100% field capacity showed low level of rolling. However, at 75% and 50% field capacity the score was increased but no significant. They mean all plant had a leaf rolling for decreased effect of water stress by avoided from drought. Although, there were many reports that used leaf rolling score to screen drought resistant cultivar but from this greenhouse experiment it was found that leaf rolling score was not suitable to screen drought tolerance lines because there were external factors such as high temperature and low relative humidity that combined with drought stress. This combination of stress led to rice increases transpiration rate by decreases the leaf rolling to reduce leaf temperature.

5.2. Leaf drying score (LDS)

Leaf drying score was evaluated as plant leaf deterioration under drought stress. Leaf drying score was easy to recognizable in drought tolerance study. Leaf drying was often used as a selective tool of drought resistance cultivar (de Datta et al., 1988). At seedling stage, leaf drying score was related to leaf water potential (Chang et al., 1979) that leaf could maintain greenness, delay deterioration, and had low leaf drying score when it can maintain high leaf water potential (Henderson et al., 1993; Fukai and Cooper, 1995).

In this experiment, line that can protect leaf under drought stress was only DH212. Leaf drying score classified rice in to five groups from high tolerant to highly susceptible (Abd Allah, 2006). DH212 was a drought resistance line because it had low symptom of stress and had low score. On the other hand, KDML105 and CSSLs were in the same group that were moderately resistant to drought stress. These results were estimated that the ability to retain green leaf from a donor did not transfer to CSSLs and had leaf drying similar to KDML105. It will confirmed by further study.

5.3. Cell membrane stability (CMS)

Stress affect cell membrane and it is one of the most common targets of destruction (Levitt, 1980). Cell membrane was damage by many stresses such as drought, salt, heat, and freezing. Drought stress induced membrane injury and severed metabolic dysfunction caused by the deterioration leading (Buttrose and

Swift, 1975). There was some reports on drought stress and membrane damage such as Bewley (1979) and described that drought tolerance depend on that abilities of membrane which can reduced membrane injury. Plant use many ways to limit membrane damage which include increase activity of membrane bound and regain membrane integrity. The cell membrane stability was used as the indicator to screen drought tolerant cultivars.

There were many researches who use cell membrane stability as an indicator in selecting drought tolerance in many crops such as maize, wheat, and sorghum (Premachandra and Shimada, 1988). In rice, there were some studies about cell membrane stability to screen tolerant cultivars in many stress including heat stress (Kumar et al., 2012) and salt stress (Siringam et al., 2011). However, there are some reports that described cell membrane stability that was not a good methods to study tolerance cultivar alone (Blum et al., 2001). It has to be supported by many studies the relationship of membrane, biological and other physiological traits: PremachandrA et al. (1991) reported that cell membrane damage showed relationship with leaf surface wax content and leaf thickness underwater deficits in maize and another study had been reported that cell membrane stability is related with osmotic adjustment through the accumulation of solute such as potassium, sugar, and amino acids (Premachandra et al., 1991).

The results from experiment found that high drought stress induced the reduction of membrane stability due to increase in membrane injury. Moreover, the results showed non-significant difference between CSSLs and parents lines. CSSLs had percent cell membrane stability similar to KDML105 and DH212. While the physiological and biochemical properties of CSSLs and parents were not clear to cause genetic differences in cell membrane stability. Thus, cell membrane stability cannot be used as a screening method of drought tolerant for CSSLs.

5.4. Relative water content (RWC)

Relative water content is used as a tool to screen drought resistance in cereal because it indicates plant water status at a specific time point (Teulat et al., 2003; Manickavelu et al., 2006). Kramer (1969) suggested that 70% Relative water content was a real physiological stress in rice. Relative water content is related to cell volume and it has close relation with balance of transpiration rate and water supply to leaf (Sinclair and Ludlow, 1985). Percent relative water content was used to evaluate tolerant plant under drought stress for plant breeders (Schonfeld et al., 1988)

This study found that the percent relative water content of rice leaf at 100% and 75% field capacity had higher than 70% relative water content, which mean that plants did not experience water stress and physiological stress. In contrast, at 50% field capacity plants drought stress after day 3 and had percent relative water content lower than 70 and result to physiological responses to observed drought stress. There had no significant difference between lines in three conditions. To sum up, from this experiment relative water content was not a good indicator to screen drought tolerant line in rice, but it was a good parameter to evaluate water status in rice.

5.5. Chlorophyll fluorescence (F_v/F_m)

Drought stress decreased photosystem II activity in rice leaves and it is evident on the quantum yield of PS II, but it depends on the ability of rice variety (Pieters and El Souki, 2005). Drought stress promoted photoinhibition due to temporary or permanent limitation in plant sink. Limited plant sink resulted to

increased carotenoids and xanthophyll cycle (Demmig-Adams et al., 1999). This report was support by Demmig-Adams et al. (1999) research. Indirect photosynthesis functions such as light reaction, level of pigments, thylakoid electron transport, and dark enzymatic stoma, were studied by means of chlorophyll fluorescence. It was useful to study reduction of photosynthesis under environmental stress such as drought, heat, cold, nutrients deficiency and pathogen infections (Araus et al., 1998).

There were several reports indicating that chlorophyll fluorescence was used to detect and quantify tolerant plant and evaluate integrity of photosynthetic process in leaf (Krause and Weis, 1991; Clark et al., 2000). Response of PS II activity under water deficit in rice can be used to select tolerant cultivars by chlorophyll fluorescence. In addition, values of chlorophyll fluorescence in drought sensitive cultivar was lower than in drought tolerance cultivar and it can used as an indicator for drought tolerance screening in barley (Li et al., 2006). Moreover, Lu et al. (2013) described that drought stress in turf grass was effected on photosynthesis and plant growth than changed of chlorophyll fluorescence. A report by Faraloni et al. (2011) indicated that chlorophyll fluorescence could use as drought tolerant criteria in olive cultivars.

In this study, chlorophyll fluorescence at 100% and 75% field capacity showed no significant difference among rice tested, but it was significant in day 4, 5, and 6 at 50% field capacity. These results classified six lines into two groups the drought tolerance (RGD05131-6-MAS5, RGD0128-10-MAS12, and DH212) and drought susceptible group (RGD05131-4-MAS39, RGD05128-4-MAS40-MAS11, and KDML105). They showed that chlorophyll fluorescence can be used to select drought tolerance line. This is similar to the result of Araus et al. (1998) who studied chlorophyll

fluorescence in wheat. Therefore, chlorophyll fluorescence could be a good parameter for drought tolerance screening in rice.

5.6. SPAD index

Leaf chlorophyll content could be evaluated by *in situ* SPAD (Soil Plant Analysis Development) reading. Level of nitrogen which is an important element of chlorophyll affects chlorophyll content of plants (Huang et al., 2008; Jinwen et al., 2009). There were many reports that suggest SPAD index had a correlation with nitrogen in leaf in rice (Turner and Jund, 1991; Turner and Jund, 1994), corn (Dwyer et al., 1995), barley (Peltonen et al., 1995), and wheat (Reeves, 1993; Shukla et al., 2004). SPAD index significantly related to rice cultivar, stage of plant growth and development, position of leaf, and SPAD point on leaf (Hoel and Solhaug, 1998; Esfahani et al., 2008; Lin et al., 2010).

Chlorophyll content was decreased at high drought stress than at well water. Results in the effects of drought stress on chlorophyll content had been showed in peach, eggplants (Kirnak et al., 2001), and rice (Chen et al., 1991). SPAD is a simple and portable tool for measuring nitrogen in leaf.

From this experiment, there was no significant difference between lines. The results showed decreasing trends of SPAD index under drought stress particularly high drought stress (50% field capacity). There are many reasons that SPAD index not suitable for indicate drought tolerance in rice: Firstly, nitrogen which is an important element of leaf could be translocated from old leaf to young leaf. Therefore, the average of SPAD at young leaf was higher than old leaf and did not change at 100%

and 75% field capacity. On the other hand, drought stress affected to the translocation of nitrogen in rice leaf due to the reduced chlorophyll content detected by SPAD at 50% field capacity. From the results, it was hard to selected drought tolerant lines because they showed similar trend. Secondly, it was a good idea to mark the measurement point for SPAD. The measurement point of SPAD was very important because different point showed difference in SPAD index. To sum up, it was difficult to evaluate chlorophyll content and nitrogen status in rice leaf by using only SPAD index because it was depends on rice cultivar.

5.7. Stay green score (SGS)

Genotypes tolerant to drought stress were characterized by premature and plant senescence. Stay green was suggested by many researches as a suitable parameter in selecting drought tolerance in many crops species. Houman et al. (2011) reported in maize that leaf chlorophyll and stay green was used to indicate drought tolerant lines. In addition, stay green score was one of drought resistance mechanism that made sorghum tolerant to water stress (Xu et al., 2000; Sanchez et al., 2002). Similar to Semenov et al. (2014) who reported on the ability to stay green to evaluate tolerant line in wheat.

In this study, stay green score showed significant difference between lines at 50% field capacity in day 7 after treatment and found that DH212 and RGD05131-6-MAS5 had high ability to resist to drought stress. Moreover, RGD0128-10-MAS12, and RGD05128-4-MAS40-MAS11 were moderately resistant to drought stress. Stay green was a balance between nitrogen demand and supply because nitrogen is linked to chlorophyll and leaf senescence (Thomas and Rogers, 1990; Borrell et al., 2014).

During senescence, the yellowish characteristic increased due to breaking down of chlorophyll-protein complex (Kassahun et al., 2010). Delayed plant senescence had been linked to sorghum yield and increase drought tolerance will prolong plant greenness resulting in increase of yield component. Stay green score is a good criteria to evaluate drought tolerant lines in rice because it can show the different responses between tolerance and susceptible lines.

5.8. Growth

Drought stress reduced plant root and shoot weight in both fresh and dry weight because it decreased many physiological and morphological features of plant such as decrease in photosynthetic rate (Farooq et al., 2009), reduced cell expansion (Shao et al., 2008), and reduce nutrient and growth hormones (Usman et al., 2013).

From this study, fresh and dry weight of rice under water deficits were lower than in well water. The significant difference found in the experiment came from a difference of plant RGD05131-4-MAS39 which had higher weight than RGD05131-6-MAS5 and DH212. On the other hand, under stress condition RGD05131-6-MAS5 and KDML105 increase their growth rate by increase root growth. RGD05131-6-MAS5 has a good chlorophyll fluorescence lead to it increase relative growth rate but the mechanism that help KDML105 increase growth is not known. It can confirm by future study. Drought stress showed an effect to rice by reducing plant growth. Although, the evaluation of root weight in this study was not suitable because pot limited root growth. Thus, measurement weight in rice in future study need careful design plant cultivation that does not limit growth to study root and shoot response under drought stress.

5.9. Correlation

Drought stress reduce water status in plant leads to the reduction of relative water content. Lower relative water content than 70% can cause the changing of other physiological responses such as chlorophyll content, stay green and cell membrane stability. The reduction of chlorophyll content and stay-green result in the decrease of chlorophyll fluorescence and leaf drying. Moreover, reduction of cell membrane stability bring about reduce chlorophyll fluorescence too. All of the physiological responses have an effect on plant growth.

Chapter VI

Conclusion

Under drought stress, plants use many drought resistance mechanisms to survive which include drought escape, drought avoidance, and drought tolerance. Drought tolerance traits that changed physiological, morphological, and biochemical properties of the plant that are suitable for selection tolerant genotypes under water deficits which mean decrease the effect of stress in breeding program. Drought stress affect rice and induced drought stress responses. Drought stress resulted to the reduction of seedling cell membrane stability, relative water content, chlorophyll fluorescence, SPAD index, and whole plant fresh and dry weight. On the other hand, it increased leaf rolling score, leaf drying score, and stay green score. Rice has many mechanisms which used to survive from drought stress. Firstly, rice prevents water loss by drought avoidance mechanism which is leaf rolling and root adaptation. After that some rice lines have developed drought tolerant mechanisms, which are cell membrane stability, chlorophyll fluorescence and stay green, to survive under drought stress.

From the results, chlorophyll fluorescence and stay green score are good parameters to evaluate the ability to resist drought as they could differentiate the characteristics among lines under drought stress condition. Our investigation also showed that these CSSLs studied had different genotypes as they responded to drought stress differently.

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APPENDIX

Table A. *1 Condition of Greenhouse between June 27th and July 6th, 2014*

Day of experiment	Date	Temperature (Celsius)	Relative Humidity (%)	Light Intensity (uE)
Day 0	June 27th, 2014	36.09	69.85	659.38
Day 1	June 28th, 2014	37.32	67.89	786.43
Day 2	June 29th, 2014	37.01	64.39	680.75
Day 3	June 30th, 2014	33.77	64.63	365.06
Day 4	July 1st, 2014	35.70	66.25	611.65
Day 5	July 2nd, 2914	41.48	65.61	730.06
Day 6	July 3rd, 2014	39.33	63.88	688.25
Day 7	July 4th, 2014	41.62	62.90	645.52
Day 8	July 5th, 2014	42.04	61.31	770.06
Day 9	July 6th, 2014	38.85	61.31	726.43

Table A. 2 Changes in the stay green score of rice to 100 percent field capacity at day 0 to day 9. (mean±sd)

Day after treatment	stay green score (100% field capacity)					
	RGD05131-4-MAS39	RGD05131-6-MAS5	RGD0128-10-MAS12	RGD05128-4-MAS40-MAS11	KDML105	DH212
0	1.25±0.29	1.00±0.00	1.00±0.00	1.00±0.00	1.00±0.00	1.13±0.25
1	1.00±0.00	1.00±0.00	1.00±0.00	1.00±0.00	1.00±0.00	1.25±0.00
2	1.00±0.00	1.00±0.00	1.00±0.00	1.00±0.00	1.00±0.00	1.00±0.00
3	1.00±0.00	1.00±0.00	1.00±0.00	1.25±0.50	1.00±0.00	1.00±0.00
4	1.00±0.00	1.00±0.00	1.00±0.00	1.13±0.25	1.00±0.00	1.00±0.00
5	1.13±0.25	1.25±0.25	1.25±0.25	1.38±0.25	1.38±0.25	1.00±0.00
6	1.00±0.00	1.13±0.25	1.13±0.25	1.38±0.25	1.38±0.29	1.13±0.00
7	1.00±0.00	1.38±0.29	1.25±0.25	1.38±0.00	1.38±0.25	1.00±0.00
8	1.13±0.25	1.50±0.41	1.38±0.48	1.63±0.25	1.38±0.25	1.00±0.00
9	1.50±0.41	1.38±0.41	1.50±0.58	1.75±0.29	1.38±0.00	1.25±0.29

Table A 3 Changes in the stay green score of rice to 75 percent field capacity at day 0 to day 9. (mean±sd)

Day after treatment	stay green score (75% field capacity)					
	RGD05131-4-MAS39	RGD05131-6-MAS5	RGD0128-10-MAS12	RGD05128-4-MAS40-MAS11	KDML105	DH212
0	1.13±0.25	1.00±0.00	1.00±0.00	1.00±0.00	1.00±0.00	1.00±0.00
1	1.00±0.00	1.00±0.00	1.00±0.00	1.00±0.00	1.00±0.00	1.00±0.00
2	1.00±0.00	1.00±0.00	1.00±0.00	1.00±0.00	1.00±0.00	1.00±0.00
3	1.25±0.00	1.25±0.00	1.50±1.00	1.00±0.00	1.25±0.00	1.00±0.00
4	1.25±0.25	1.13±0.25	1.00±0.00	1.13±0.00	1.00±0.00	1.00±0.00
5	1.25±0.25	1.50±0.25	1.38±0.48	1.25±0.25	1.50±0.29	1.38±0.25
6	1.50±0.29	1.75±0.25	1.75±0.63	1.13±0.25	1.63±0.29	1.25±0.25
7	1.25±0.29	1.38±0.25	1.88±0.75	1.75±0.25	1.38±0.25	1.13±0.25
8	1.25±0.48	1.38±0.41	1.88±0.63	1.75±0.71	1.50±0.41	1.00±0.25
9	1.75±0.25	1.63±0.29	2.00±0.50	1.75±0.63	1.88±0.25	1.25±0.29

Table A 4 36 Changes in the stay green score of rice to 50 percent field capacity at day 0 to day 9. (mean±sd)

Day after treatment	stay green score (50% field capacity)					
	RGD05131-4-MAS39	RGD05131-6-MAS5	RGD0128-10-MAS12	RGD05128-4-MAS40-MAS11	KDML105	DH212
0	1.13±0.00	1.00±0.00	1.00±0.00	1.00±0.00	1.00±0.00	1.00±0.00
1	1.00±0.00	1.00±0.00	1.00±0.00	1.00±0.00	1.00±0.00	1.00±0.00
2	1.25±0.50	1.30±0.50	1.25±0.50	1.00±0.00	1.00±0.00	1.00±0.00
3	1.75±0.58	1.25±0.50	1.25±0.50	1.75±0.50	1.25±0.50	1.25±0.25
4	1.875±0.71	1.50±0.58	1.88±0.85	2.00±0.71	1.75±0.29	1.63±0.41
5	2.00±0.71	1.75±0.29	1.88±0.85	2.13±0.85	2.50±0.41	1.75±0.25
6	2.125±0.75	2.25±0.65	2.50±0.71	2.88±0.85	2.88±0.75	2.25±0.29
7*	$3.750±0.50^{c}$	$3.00±0.00^{ad}$	$3.38±0.49^{bc}$	$3.63±0.63^{bc}$	$3.75±0.65^{c}$	$2.50±0.41^{ab}$
8	4.375±0.95	3.75±0.63	3.75±0.65	4.38±0.63	4.00±0.82	3.38±0.75
9	4.625±0.48	4.50±0.71	4.38±0.25	4.50±0.41	4.38±0.48	4.13±0.48

* significant difference at the 0.05 level (DMRT)

Table A 5 Changes in the SPAD index of rice to 100 percent field capacity at day 0 to day 9. (mean±sd)

Day after treatment	SPAD index (100% field capacity)					
	RGD05131-4-MAS39	RGD05131-6-MAS5	RGD0128-10-MAS12	RGD05128-4-MAS40-MAS11	KDML105	DH212
0	34.15±0.40	32.23±1.42	30.50±4.44	26.33±0.86	34.70±2.21	33.45±2.16
3	34.53±2.25	32.93±3.72	31.00±2.09	28.20±1.45	35.93±1.91	33.23±1.55
6	34.33±1.76	33.03±2.30	31.53±2.30	28.28±1.08	35.93±2.98	34.05±1.00
9	32.90±6.09	33.53±1.07	28.73±3.01	27.00±3.10	35.58±1.63	33.33±1.19

Table A 6 Changes in the SPAD index of rice to 75 percent field capacity at day 0 to day 9. (mean±sd)

Day after treatment	SPAD index (75% field capacity)					
	RGD05131-4-MAS39	RGD05131-6-MAS5	RGD0128-10-MAS12	RGD05128-4-MAS40-MAS11	KDML105	DH212
0	33.98±2.83	32.83±3.34	29.98±3.00	29.78±2.11	32.43±1.50	34.38±2.44
3	34.28±3.84	34.08±6.51	33.63±2.72	30.03±2.36	33.50±1.14	35.40±1.90
6	35.00±3.90	35.08±2.38	32.78±5.75	27.88±5.61	34.63±2.03	36.85±5.82
9	33.35±1.64	31.68±5.57	31.23±7.52	29.33±6.94	34.33±2.39	33.98±1.11

Table A 7 Changes in the SPAD index of rice to 50 percent field capacity at day 0 to day 9. (mean±sd)

Day after treatment	SPAD index (50% field capacity)					
	RGD05131-4-MAS39	RGD05131-6-MAS5	RGD0128-10-MAS12	RGD05128-4-MAS40-MAS11	KDML105	DH212
0	32.15±4.26	32.90±1.49	32.05±3.20	29.53±3.73	31.98±2.92	32.03±3.33
3	33.43±3.40	32.78±3.63	32.43±4.45	32.68±5.01	34.98±10.84	33.88±1.24
6	26.00±9.00	23.20±13.90	28.00±12.43	26.28±9.46	25.23±10.69	29.90±5.47
9	21.83±11.57	15.43±6.44	22.70±10.54	10.53±11.13	13.80±7.36	27.30±10.37

Table A 8 Changes in the chlorophyll fluorescence of rice to 100 percent field capacity at day 0 to day 9. (mean±sd)

Day after treatment	chlorophyll fluorescence (F$_v$/F$_m$) (100% field capacity)					
	RGD05131-4-MAS39	RGD05131-6-MAS5	RGD0128-10-MAS12	RGD05128-4-MAS40-MAS11	KDML105	DH212
DH212	0.802±0.003	0.805±0.007	0.801±0.013	0.790±0.014	0.802±0.005	0.803±0.011
1	0.785±0.007	0.787±0.022	0.794±0.019	0.793±0.010	0.787±0.007	0.779±0.047
2	0.808±0.007	0.804±0.013	0.815±0.010	0.806±0.008	0.812±0.004	0.809±0.010
3	0.812±0.007	0.810±0.005	0.811±0.006	0.811±0.010	0.813±0.013	0.800±0.017
4	0.810±0.014	0.807±0.010	0.804±0.030	0.807±0.015	0.810±0.011	0.811±0.007
5	0.795±0.012	0.797±0.012	0.797±0.012	0.798±0.008	0.788±0.017	0.792±0.021
6	0.792±0.018	0.7960.018	0.793±0.019	0.785±0.035	0.802±0.009	0.795±0.006
7	0.795±0.024	0.807±0.009	0.806±0.025	0.798±0.025	0.812±0.009	0.808±0.006
8	0.794±0.029	0.798±0.018	0.796±0.028	0.796±0.006	0.805±0.067	0.802±0.012
9	0.743±0.100	0.768±0.024	0.753±0.078	0.763±0.097	0.743±0.097	0.762±0.020

Table A. 9 Changes in the chlorophyll fluorescence of rice to 75 percent field capacity at day 0 to day 9. (mean±sd)

Day after treatment	chlorophyll fluorescence (F_v/F_m) (75% field capacity)					
	RGD05131-4-MAS39	RGD05131-6-MAS5	RGD0128-10-MAS12	RGD05128-4-MAS40-MAS11	KDML105	DH212
DH212	0.802±0.012	0.794±0.019	0.805±0.009	0.797±0.015	0.806±0.026	0.800±0.012
1	0.795±0.011	0.793±0.010	0.794±0.010	0.790±0.013	0.793±0.013	0.793±0.017
2	0.808±0.006	0.808±0.011	0.805±0.007	0.801±0.005	0.810±0.009	0.813±0.006
3	0.809±0.006	0.809±0.009	0.797±0.023	0.811±0.006	0.814±0.004	0.805±0.007
4	0.814±0.007	0.808±0.013	0.806±0.016	0.806±0.011	0.812±0.008	0.809±0.006
5	0.808±0.008	0.804±0.014	0.791±0.013	0.794±0.019	0.808±0.004	0.812±0.010
6	0.804±0.010	0.790±0.031	0.805±0.010	0.786±0.020	0.808±0.004	0.790±0.018
7	0.807±0.011	0.809±0.008	0.812±0.009	0.790±0.025	0.807±0.004	0.808±0.005
8	0.805±0.008	0.796±0.016	0.795±0.025	0.795±0.025	0.799±0.014	0.805±0.010
9	0.772±0.039	0.774±0.01235	0.793±0.044	0.762±0.044	0.790±0.017	0.795±0.011

Table A. 10 Changes in the chlorophyll fluorescence of rice to 50 percent field capacity at day 0 to day 9. (mean±sd)

Day after treatment	chlorophyll fluorescence (F_v/F_m) (50% field capacity)					
	RGD05131-4-MAS39	RGD05131-6-MAS5	RGD0128-10-MAS12	RGD05128-4-MAS40-MAS11	KDML105	DH212
0	0.807±0.008	0.799±.0.166	0.808±0.011	0.806±.002	0.807±0.010	0.809±0.009
1	0.791±0.011	0.794±0.018	0.796±0.015	0.781±0.020	0.794±0.004	0.795±0.016
2	0.812±0.010	0.817±0.008	0.810±0.008	0.805±0.012	0.817±0.008	0.808±0.014
3	0.814±0.003	0.802±0.017	0.768±0.082	0.727±.093	0.794±0.045	0.801±0.014
4*	0.772±0.082[a]	0.790±0.011[a]	0.806±0.016[a]	0.015±0.010[b]	0.789±0.033[a]	0.804±0.007[a]
5*	0.746±0.049[a]	0.786±0.014[a]	0.597±0.015[b]	0.005±0.0005[c]	0.790±0.022[a]	0.759±0.030[a]
6*	0.022±0.030[b]	0.592±0.270[a]	0.574±0.443[a]	0.012±0.014[b]	0.146±0.029[b]	0.736±0.045[a]
7	0.003±0.004	0.290±0.455	0.040±0.077	0.0018±0.009	0.008±0.008	0.183±0.366
8	0.000	0.002±0.005	0.002±0.004	0.000	0.005±0.006	0.0025±0.005
9	0.000	0.000	0.000	0.000	0.002±0.004	0.000

* significant difference at the 0.05 level (DMRT)

Table A 11 Changes in the cell membrane stability of rice to 100 percent field capacity at day 0 to day 9. (mean±sd)

Day after treatment	cell membrane stability (CMS) (100% field capacity)					
	RGD05131-4-MAS39	RGD05131-6-MAS5	RGD0128-10-MAS12	RGD05128-4-MAS40-MAS11	KDML105	DH212
0	95.1±1.78	93.08±3.58	94.90±0.85	92.36±1.50	92.09±6.05	95.58±1.62
3	93.49±2.47	94.61±1.03	95.38±0.41	95.60±0.55	94.13±1.15	94.80±0.96
6	90.13±5.57	91.68±2.88	94.60±0.98	92.60±1.25	93.08±1.73	93.95±1.10
9	95.27±2.06	91.00±5.21	92.52±5.89	91.27±2.61	94.14±3.29	95.01±0.75

Table A 12 Changes in the cell membrane stability of rice to 75 percent field capacity at day 0 to day 9. (mean±sd)

Day after treatment	cell membrane stability (CMS) (75% field capacity)					
	RGD05131-4-MAS39	RGD05131-6-MAS5	RGD0128-10-MAS12	RGD05128-4-MAS40-MAS11	KDML105	DH212
0	95.32±1.15	90.47±6.59	94.76±0.40	95.85±1.25	95.19±1.79	96.02±1.359
3	95.46±1.69	93.60±3.08	95.41±1.30	95.57±0.65	95.97±0.60	95.94±0.89
6	92.90±1.60	92.76±1.78	93.69±0.98	94.96±0.82	89.98±8.92	93.54±2.52
9	94.27±0.43	93.28±1.82	93.54±1.93	94.94±0.39	95.07±1.46	94.14±2.23

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Table A 13 Changes in the cell membrane stability of rice to 50 percent field capacity at day 0 to day 9. (mean±sd)

Day after treatment	cell membrane stability (CMS) (50% field capacity)					
	RGD05131-4-MAS39	RGD05131-6-MAS5	RGD0128-10-MAS12	RGD05128-4-MAS40-MAS11	KDML105	DH212
0	94.77±1.43	90.51±1.68	93.77±1.65	95.08±2.95	95.12±0.72	94.43±2.22
3	92.53±0.97	93.54±2.23	95.42±1.47	94.53±0.59	92.91±1.64	95.05±2.17
6	66.25±16.1	58.08±36.3	74.21±29.9	81.90±12.4	55.11±37.7	77.44±21.8
9	45.96±3.68	49.13±17.1	56.19±18.6	56.87±8.63	53.69±19.9	46.85±23.8

Table A 14 Changes in the relative water content of rice to 100 percent field capacity at day 0 to day 9. (mean±sd)

Day after treatment	relative water content (RWC) (100% field capacity)					
	RGD05131-4-MAS39	RGD05131-6-MAS5	RGD0128-10-MAS12	RGD05128-4-MAS40-MAS11	KDML105	DH212
0	90.85±6.81	85.60±14.32	85.63±9.69	94.48±8.46	96.63±4.42	94.59±6.22
3	95.70±4.20	96.04±3.39	92.07±5.99	87.46±13.12	92.33±4.96	92.72±4.20
6	92.65±8.46	86.28±10.24	97.02±1.61	85.32±3.11	96.05±4.61	91.83±7.27
9	89.61±5.07	88.38±9.68	89.26±1.44	89.27±4.98	91.06±2.95	91.70±3.03

Table A 15 Changes in the relative water content of rice to 75 percent field capacity at day 0 to day 9. (mean±sd)

Day after treatment	relative water content (RWC) (75% field capacity)					
	RGD05131-4-MAS39	RGD05131-6-MAS5	RGD0128-10-MAS12	RGD05128-4-MAS40-MAS11	KDML105	DH212
0	92.53±6.47	93.63±6.50	92.76±8.80	85.55±4.95	92.41±6.12	93.57±5.53
3	87.85±3.97	95.26±6.12	95.96±4.74	91.89±5.61	93.56±6.88	93.5±4.73
6	91.27±2.39	89.06±3.60	88.11±4.94	91.45±5.40	93.80±3.83	88.22±10.98
9	93.87±4.08	92.67±10.69	86.01±1.41	90.90±4.83	87.93±8.16	91.33±1.78

Table A 16 Changes in the relative water content of rice to 50 percent field capacity at day 0 to day 9. (mean±sd)

Day after treatment	relative water content (RWC) (50% field capacity)					
	RGD05131-4-MAS39	RGD05131-6-MAS5	RGD0128-10-MAS12	RGD05128-4-MAS40-MAS11	KDML105	DH212
0	81.45±21.68	85.63±0.20	92.17±7.07	92.52±6.92	95.62±8.82	91.88±5.46
3	71.76±11.76	80.07±11.07	70.91±10.87	75.90±8.86	75.21±17.92	75.31±10.63
6	38.61±23.38	40.93±9.65	42.26±31.47	45.79±26.25	23.59±33.79	64.76±36.95
9	8.32±3.04	10.84±4.98	12.64±10.94	±23.7430.78	26.54±38.91	21.58±19.14

Table A 17 Changes in the leaf rolling score of rice to 100 percent field capacity at day 0 to day 9. (mean±sd)

Day after treatment	leaf rolling score (100% field capacity)					
	RGD05131-4-MAS39	RGD05131-6-MAS5	RGD0128-10-MAS12	RGD05128-4-MAS40-MAS11	KDML105	DH212
0	1.00±0.00	1.00±0.00	1.00±0.00	1.00±0.001	1.00±0.00	1.00±0.00
1	1.00±0.00	1.00±0.00	1.00±0.00	1.00±0.00	1.00±0.00	1.50±0.58
2	1.00±0.00	1.00±0.00	1.00±0.00	1.00±0.00	1.00±0.00	1.00±0.00
3	1.00±0.00	1.00±0.00	1.00±0.00	1.00±0.00	1.00±0.00	1.00±0.00
4	1.00±0.00	1.00±0.00	1.00±0.00	1.00±0.00	1.00±0.00	1.00±0.00
5	1.00±0.00	1.00±0.00	1.00±0.00	1.00±0.00	1.00±0.00	1.00±0.00
6	1.00±0.00	1.00±0.00	1.00±0.00	1.00±0.00	1.00±0.00	1.00±0.00
7	1.00±0.00	1.00±0.00	1.00±0.00	1.00±0.00	1.00±0.00	1.00±0.00
8	1.00±0.00	1.00±0.00	1.25±0.00	1.25±0.50	1.00±0.00	1.25±0.50
9	1.25±0.50	1.25±0.50	1.75±1.50	1.50±0.58	1.13±0.25	1.00±0.00

Table A 18 Changes in the leaf rolling score of rice to 75 percent field capacity at day 0 to day 9. (mean±sd)

Day after treatment	leaf rolling score (75% field capacity)					
	RGD05131-4-MAS39	RGD05131-6-MAS5	RGD0128-10-MAS12	RGD05128-4-MAS40-MAS11	KDML105	DH212
0	1.00±0.00	1.00±0.00	1.00±0.00	1.00±0.00	1.00±0.00	2.00±0.00
1	1.00±0.00	1.00±0.00	1.00±0.00	1.00±0.00	1.00±0.00	1.75±0.50
2	1.25±0.50	1.00±0.00	1.00±0.00	1.00±0.00	1.00±0.00	1.50±0.58
3	1.00±0.00	1.00±0.00	1.00±0.00	1.00±0.00	1.00±0.00	1.25±0.50
4	1.00±0.00	1.00±0.00	1.50±1.00	1.00±0.00	1.00±0.00	1.25±0.50
5	1.00±0.00	1.00±0.00	1.75±1.50	1.00±0.00	1.00±0.00	1.50±0.58
6	1.50±0.58	1.50±0.58	2.13±1.65	2.00±1.15	2.00±1.15	2.00±1.41
7	1.75±1.50	1.25±0.50	2.13±1.65	1.25±0.50	1.88±1.18	1.25±0.50
8	1.50±0.58	1.25±0.50	1.75±1.50	2.13±1.03	2.00±0.82	1.25±0.50
9	1.75±0.50	1.50±0.58	2.25±1.26	1.50±0.58	2.00±0.82	1.00±0.00

Table A 19 Changes in the leaf rolling score of rice to 50 percent field capacity at day 0 to day 9. (mean±sd)

Day after treatment	leaf rolling score (50% field capacity)					
	RGD05131-4-MAS39	RGD05131-6-MAS5	RGD0128-10-MAS12	RGD05128-4-MAS40-MAS11	KDML105	DH212
0	1.00±0.00	1.00±0.00	1.00±0.00	1.00±0.00	1.00±0.00	2.00±0.00
1	1.00±0.50	1.00±0.50	1.00±0.50	1.00±0.00	1.00±0.50	1.75±0.00
2	1.25±0.00	1.00±1.00	1.00±1.26	1.00±1.44	1.00±1.5	1.50±1.29
3	1.00±0.96	1.00±1.50	1.00±1.44	1.00±1.50	1.00±1.31	1.25±1.31
4	1.00±0.50	1.00±1.41	1.50±0.85	1.00±0.63	1.00±1.41	1.25±0.65
5	1.00±0.58	1.00±0.58	1.75±0.48	1.00±0.58	1.00±0.00	1.50±0.00
6	1.50±0.478	1.50±0.00	2.13±0.00	2.00±0.25	2.00±0.00	2.00±0.89
7	1.75±0.00	1.25±0.00	2.13±0.50	1.25±0.00	1.88±0.00	1.25±0.00
8	1.50±0.50	1.25±0.00	1.75±0.50	2.13±0.00	2.00±0.00	1.25±0.00
9	1.75±0.00	1.50±.050	2.25±0.00	1.50±0.50	2.00±0.00	1.00±0.00

Table A 20 Changes in the leaf drying score of rice to 100 percent field capacity at day 0 to day 9. (mean±sd)

Day after treatment	leaf drying score (100% field capacity)					
	RGD05131-4-MAS39	RGD05131-6-MAS5	RGD0128-10-MAS12	RGD05128-4-MAS40-MAS11	KDML105	DH212
0	1.25±0.28	1±0.00	1±0.00	1±0.00	1±0.00	1.13±0.25
1	1.00±0.00	1.00±0.00	1.00±0.00	1.00±0.00	1.00±0.00	1.25±0.50
2	1.00±0.00	1.00±0.00	1.00±0.00	1.00±0.00	1.00±0.00	1.00±0.00
3	1.00±0.00	1.00±0.00	1.00±0.00	1.25±0.50	1.00±0.00	1.00±0.00
4	1.00±0.00	1.00±0.00	1.00±0.00	1.13±0.25	1.00±0.00	1.00±0.00
5	1.13±0.25	1.25±0.29	1.25±0.29	1.53±0.25	1.38±0.25	1.00±0.00
6	1.00±0.00	1.13±0.25	1.13±0.25	1.38±0.49	1.38±0.25	1.30±0.25
7	1.00±0.00	1.38±0.25	1.25±0.29	1.38±0.25	1.38±0.25	1.00±0.00
8	1.13±0.25	1.50±0.41	1.38±0.49	1.63±0.25	1.38±0.25	1.00±0.00
9	1.50±0.41	1.38±0.48	1.50±0.58	1.75±0.29	1.38±0.25	1.25±0.29

Table A 21 Changes in the leaf drying score of rice to 75 percent field capacity at day 0 to day 9. (mean±sd)

Day after treatment	leaf drying score (75% field capacity)					
	RGD05131-4-MAS39	RGD05131-6-MAS5	RGD0128-10-MAS12	RGD05128-4-MAS40-MAS11	KDML105	DH212
0	1.13±0.25	1.00±0.00	1.00±0.00	1.00±0.00	1.00±0.00	1.00±0.00
1	1.00±0.00	1.00±0.00	1.00±0.00	1.00±0.00	1.00±0.00	1.00±0.00
2	1.00±0.00	1.00±0.00	1.00±0.00	1.00±0.00	1.00±0.00	1.00±0.00
3	1.25±0.50	1.25±0.50	1.50±1.00	1.00±0.00	1.25±0.50	1.00±0.00
4	1.25±0.50	1.19±0.25	1.00±0.00	1.13±0.25	1.00±0.00	1.00±0.00
5	1.25±0.50	1.50±0.41	1.38±0.48	1.25±0.29	1.50±0.41	1.38±0.48
6	1.50±0.00	1.75±0.29	1.75±0.65	1.13±0.25	1.63±0.25	1.25±0.29
7	1.25±0.29	1.38±0.25	1.88±0.75	1.75±0.28	1.38±0.25	1.13±0.25
8	1.25±0.50	1.38±0.49	1.88±0.85	1.75±0.87	1.50±0.41	1.00±0.00
9	1.75±0.29	1.69±0.25	2.00±0.82	1.75±0.29	1.88±0.25	1.25±0.29

Table A 22 Changes in the leaf drying score of rice to 50 percent field capacity at day 0 to day 9. (mean±sd)

Day after treatment	leaf drying score (50% field capacity)					
	RGD05131-4-MAS39	RGD05131-6-MAS5	RGD0128-10-MAS12	RGD05128-4-MAS40-MAS11	KDML105	DH212
0	1.13±0.25	1.00±0.00	1.00±0.00	.00±0.00	1.00±0.00	1.00±0.00
1	1.00±0.00	1.00±0.00	1.00±0.00	1.00±0.00	1.00±0.00	1.00±0.00
2	1.25±0.50	1.50±0.58	1.25±0.50	1.00±0.00	1.00±0.00	1.00±0.00
3	1.75±0.50	2.00±0.00	1.25±0.50	1.75±0.50	1.25±0.50	1.25±0.50
4	1.88±0.85	1.63±0.48	1.88±0.85	2.00±0.71	1.75±0.29	1.63±0.63
5	2.13±0.95	2.00±0.41	1.88±0.85	2.13±0.85	2.38±0.25	1.75±0.29
6	2.13±0.75	2.25±0.65	2.50±0.71	2.88±0.85	2.88±0.75	2.25±0.29
7*	3.75±0.50[b]	3.387±0.25[b]	3.50±0.41[b]	3.63±0.63[b]	3.75±0.65[b]	0±0.412.5[a]
8	4.38±0.95	4.25±0.87	3.75±0.65	4.38±0.63	4.00±0.82	3.38±0.75
9	4.63±0.48	4.50±0.71	4.38±0.25	4.50±0.41	4.38±0.48	4.13±0.48

* significant difference at the 0.05 level (DMRT)

Table A 23 Changes in the fresh weight of rice to 100 percent field capacity at day 0 to day 9. (mean±sd)

Day after treatment	fresh weight (g) (100% field capacity)					
	RGD05131-4-MAS39	RGD05131-6-MAS5	RGD0128-10-MAS12	RGD05128-4-MAS40-MAS11	KDML105	DH212
0	0.58±0.14	0.26±0.12	0.70±0.27	0.47±0.05	0.82±0.23	0.70±0.24
3	1.00±0.56	0.45±0.11	0.84±0.31	1.15±0.24	0.61±0.28	0.57±0.17
6	0.98±0.54	0.69±0.47	0.94±0.29	0.87±0.10	0.66±0.33	0.83±0.47
9	1.41±0.24	0.57±0.39	0.99±0.38	1.19±0.71	0.88±0.29	0.70±0.20

Table A 24 Changes in the fresh weight of rice to 75 percent field capacity at day 0 to day 9. (mean±sd)

Day after treatment	fresh weight (g) (75% field capacity)					
	RGD05131-4-MAS39	RGD05131-6-MAS5	RGD0128-10-MAS12	RGD05128-4-MAS40-MAS11	KDML105	DH212
0	0.67±0.28	0.41±0.36	0.92±0.43	0.78±0.33	0.38±0.25	0.67±0.26
3	0.95±0.47	0.46±0.17	0.88±0.07	0.69±0.23	0.84±0.27	0.40±0.13
6	0.75±0.25	0.46±0.15	0.71±0.26	0.95±0.46	0.77±0.30	0.45±0.21
9	0.88±0.44	0.61±0.38	0.87±0.17	0.97±0.17	0.82±0.22	0.56±0.17

Table A 25 Changes in the fresh weight of rice to 50 percent field capacity at day 0 to day 9. (mean±sd)

Day after treatment	fresh weight (g) (50% field capacity)					
	RGD05131-4-MAS39	RGD05131-6-MAS5	RGD0128-10-MAS12	RGD05128-4-MAS40-MAS11	KDML105	DH212
0	0.87±0.19	0.42±0.35	0.79±0.36	0.77±0.49	0.48±0.40	0.59±0.07
3	0.73±0.22	0.44±0.08	0.56±0.20	0.61±0.25	0.46±0.14	0.57±0.09
6	0.52±0.11	0.28±0.17	0.46±0.22	0.48±0.28	0.48±0.17	0.52±0.15
9	0.65±0.24	0.30±0.14	0.49±0.08	0.41±0.13	0.50±0.16	0.37±0.10

Table A 26 Changes in the dry weight of rice to 100 percent field capacity at day 0 to day 9. (mean±sd)

Day after treatment	dry weight (g) (100% field capacity)					
	RGD05131-4-MAS39	RGD05131-6-MAS5	RGD0128-10-MAS12	RGD05128-4-MAS40-MAS11	KDML105	DH212
0	0.11±0.03	0.06±0.02	0.10±0.04	0.09±0.00	0.13±0.04	0.11±0.04
3	0.20±0.11	0.09±0.02	0.17±0.43	0.22±0.05	0.14±0.03	0.12±0.03
6	0.21±0.10	0.16±0.10	0.21±0.04	0.19±0.01	0.15±0.06	0.20±0.11
9	0.35±0.06	0.15±0.09	0.24±0.08	0.29±0.16	0.22±0.08	0.19±0.06

Table A 27 Changes in the dry weight of rice to 75 percent field capacity at day 0 to day 9. (mean±sd)

Day after treatment	dry weight (g) (75% field capacity)					
	RGD05131-4-MAS39	RGD05131-6-MAS5	RGD0128-10-MAS12	RGD05128-4-MAS40-MAS11	KDML105	DH212
0	0.12±0.04	0.07±0.06	0.15±0.07	0.13±0.04	0.07±0.05	0.12±0.02
3	0.22±0.09	0.10±0.03	0.19±0.01	0.14±0.05	0.18±0.04	0.12±0.03
6	0.20±0.01	0.13±0.01	0.17±0.07	0.23±0.09	0.19±0.06	0.12±0.06
9	0.23±0.10	0.17±0.09	0.23±0.04	0.26±0.05	0.22±0.05	0.17±0.04

Table A 28 Changes in the dry weight of rice to 50 percent field capacity at day 0 to day 9. (mean±sd)

Day after treatment	dry weight (g) (50% field capacity)					
	RGD05131-4-MAS39	RGD05131-6-MAS5	RGD0128-10-MAS12	RGD05128-4-MAS40-MAS11	KDML105	DH212
0	0.13±0.02	0.07±0.06	0.13±0.06	0.11±0.05	0.09±0.03	0.09±0.02
3	0.19±0.05	0.11±0.03	0.15±0.04	0.16±0.06	0.12±0.03	0.14±0.03
6	0.16±0.03	0.08±0.05	0.13±0.06	0.15±0.06	0.14±0.05	0.15±0.03
9	0.21±0.07	0.09±0.04	0.16±0.02	0.13±0.03	0.16±0.05	0.14±0.04

Table A 29 Root/shoot ratio of rice to 100 percent field capacity at day 9. (mean±sd)

Day after treatment	Root/shoot ratio (100% field capacity)					
	RGD05131-4-MAS39	RGD05131-6-MAS5	RGD0128-10-MAS12	RGD05128-4-MAS40-MAS11	KDML105	DH212
0	0.20±0.11	0.18±0.07	0.28±0.09	0.13±0.07	0.35±0.06	0.49±0.35
3	0.31±0.12	0.17±0.13	0.25±0.08	0.48±0.12	0.35±0.19	0.25±0.12
6	0.23±0.14	0.45±0.31	0.34±0.23	0.30±0.18	0.38±0.29	0.39±0.23
9	4.15±0.80	4.70±4.43	1.91±0.51	5.70±5.04	2.09±1.25	1.92±1.59

Table A 30 Root/shoot ratio of rice to 75 percent field capacity at day 9. (mean±sd)

Day after treatment	Root/shoot ratio (75% field capacity)					
	RGD05131-4-MAS39	RGD05131-6-MAS5	RGD0128-10-MAS12	RGD05128-4-MAS40-MAS11	KDML105	DH212
0	0.16±0.13	0.32±0.32	0.28±0.18	0.36±0.20	0.13±0.12	0.47±0.19
3	0.34±0.15	0.24±0.20	0.53±0.23	0.28±0.21	0.43±0.25	0.52±0.16
6	0.41±0.27	0.37±0.12	0.38±0.24	0.35±0.13	0.40±0.09	0.38±0.22
9	3.47±1.02	4.81±2.84	2.71±2.17	2.83±1.04	11.39±10.68	1.49±0.43

Table A 31 Root/shoot ratio of rice to 50 percent field capacity at day 9. (mean±sd)

Day after treatment	Root/shoot ratio (50% field capacity)					
	RGD05131-4-MAS39	RGD05131-6-MAS5	RGD0128-10-MAS12	RGD05128-4-MAS40-MAS11	KDML105	DH212
0	0.30±0.08	0.23±0.21	0.33±0.18	0.27±0.16	0.41±0.31	0.36±0.07
3	0.62±0.33	0.69±0.18	0.57±0.19	0.58±0.30	0.52±0.21	0.57±0.15
6	0.51±0.15	0.84±0.46	0.43±0.21	0.67±0.39	0.68±0.34	0.88±0.28
9	2.34±0.84	4.88±4.59	1.72±0.42	2.80±2.69	4.01±3.32	1.86±0.87

Table A 32 Changes in relative growth rate of rice shoot to 100 percent field capacity at day 0 to day 9. (mean±sd)

Day after treatment	relative growth rate of shoot (100% field capacity)					
	RGD05131-4-MAS39	RGD05131-6-MAS5	RGD0128-10-MAS12	RGD05128-4-MAS40-MAS11	KDML105	DH212
1	0.15±0.16	0.16±0.13	0.14±0.12	0.24±0.07	0.05±0.12	0.11±0.15
2	0.02±0.15	-0.12±0.73	0.07±0.08	-0.02±0.08	-0.01±0.16	0.16±0.16
3	0.19±0.11	-0.00±0.33	0.03±0.15	0.09±0.16	0.10±0.32	-0.03±0.14

Table A 33 Changes in relative growth rate of rice shoot to 75 percent field capacity at day 0 to day 9. (mean±sd)

Day after treatment	relative growth rate of shoot (75% field capacity)					
	RGD05131-4-MAS39	RGD05131-6-MAS5	RGD0128-10-MAS12	RGD05128-4-MAS40-MAS11	KDML105	DH212
1	0.17±0.09	0.10±0.92	0.12±0.18	0.03±0.11	0.36±0.21	-0.02±0.11
2	-0.03±0.12	-0.08±0.47	-0.09±0.15	0.15±0.16	-0.01±0.16	-0.04±0.16
3	0.01±0.24	0.03±0.14	0.12±0.20	0.04±0.13	0.05±0.06	0.16±0.30

Table A 34 Changes in relative growth rate of rice shoot to 50 percent field capacity at day 0 to day 9. (mean±sd)

Day after treatment	relative growth rate of shoot (50% field capacity)					
	RGD05131-4-MAS39	RGD05131-6-MAS5	RGD0128-10-MAS12	RGD05128-4-MAS40-MAS11	KDML105	DH212
1	0.07±0.03	-0.39±0.58	0.02±0.11	0.11±0.20	-0.18±0.46	0.15±0.10
2	`-0.08±0.10	-0.19±0.15	-0.07±0.11	-0.07±0.17	-0.01±0.08	-0.03±0.07
3	`-0.05±0.11	0.03±0.35	0.10±0.17	-0.00±0.15	0.10±0.21	-0.07±0.14

Table A 35 Changes in relative growth rate of rice root to 100 percent field capacity at day 0 to day 9. (mean±sd)

Day after treatment	relative growth rate of root (100% field capacity)					
	RGD05131-4-MAS39	RGD05131-6-MAS5	RGD0128-10-MAS12	RGD05128-4-MAS40-MAS11	KDML105	DH212
1	0.18±0.14	0.27±0.14	0.04±0.03	0.47±0.20	-0.05±0.20	-0.03±0.33
2	0.07±0.21	-0.26±0.84	0.0±0.11	-0.11±0.11	-0.02±0.50	0.11±0.22
3	0.22±0.20	0.02±0.10	0.08±0.15	0.11±0.25	0.28±0.35	0.05±0.14

Table A 36 Changes in relative growth rate of rice root to 75 percent field capacity at day 0 to day 9. (mean±sd)

Day after treatment	relative growth rate of root (75% field capacity)					
	RGD05131-4-MAS39	RGD05131-6-MAS5	RGD0128-10-MAS12	RGD05128-4-MAS40-MAS11	KDML105	DH212
1	0.27±0.26	0.15±1.15	0.16±0.20	0.04±0.24	0.50±0.57	-0.01±0.21
2	0.03±0.16	-0.22±0.55	-0.02±0.12	0.17±0.25	0.07±0.22	0.02±0.13
3	0.10±0.25	0.12±0.13	0.12±0.19	0.12±0.14	0.14±0.02	0.10±0.19

Table A 37 Changes in relative growth rate of rice root to 75 percent field capacity at day 0 to day 9. (mean±sd)

Day after treatment	relative growth rate of root (50% field capacity)					
	RGD05131-4-MAS39	RGD05131-6-MAS5	RGD0128-10-MAS12	RGD05128-4-MAS40-MAS11	KDML105	DH212
1	0.18±0.11	-0.46±0.93	0.13±0.14	0.20±0.29	-0.14±0.59	0.12±0.06
2	-0.04±0.16	-0.00±0.14	-0.02±0.08	0.06±0.23	0.18±0.11	0.07±0.13
3	0.12±0.18	0.09±0.25	0.06±0.19	-0.05±0.23	-0.01±0.21	-0.04±0.13

Figure A. 1 *Rice at 100% field capacity on day 0*

Figure A. 2 *Rice at 75% field capacity on day 0*
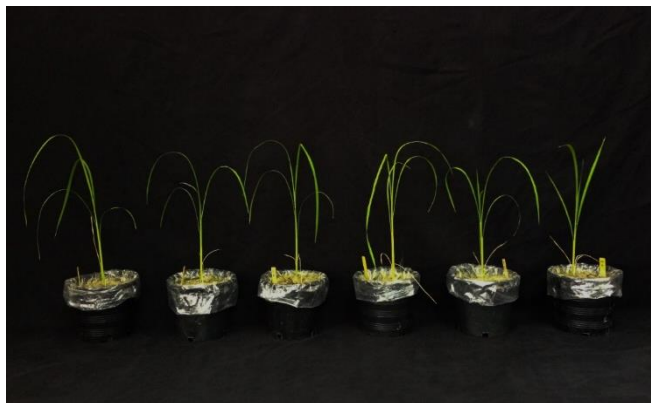

Figure A. 3 *Rice at 50% field capacity on day 0*
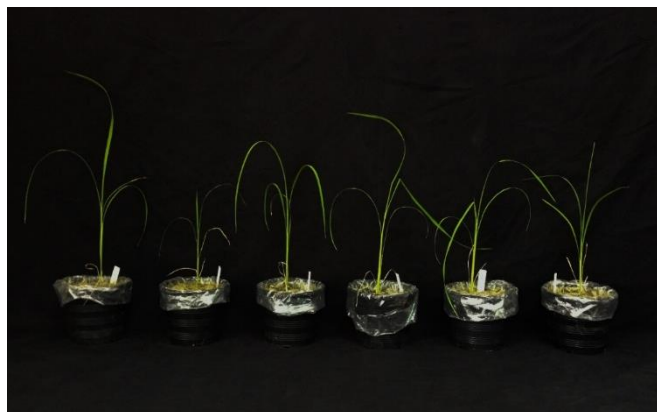
Figure A. 4 *Rice at 100% field capacity on day 3*
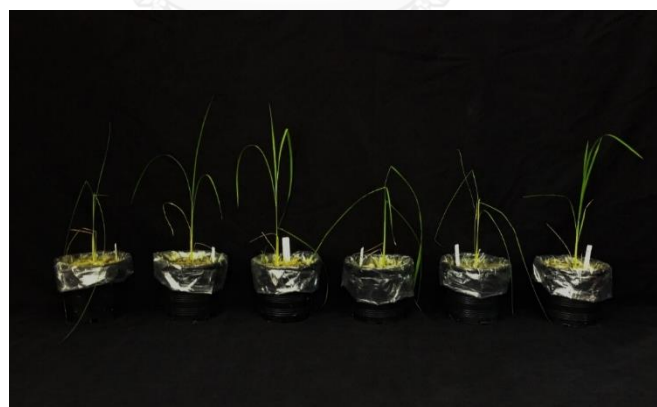

Figure A. 5 *Rice at 75% field capacity on day 3*

Figure A. 6 *Rice at 50% field capacity on day 3*

Figure A. 7 *Rice at 100% field capacity on day 6*

Figure A. 8 *Rice at 75% field capacity on day 6*

Figure A. 9 *Rice at 50% field capacity on day 6*

Figure A. 10 *Rice at 100% field capacity on day 9*

Figure A. 11 *Rice at 75% field capacity on day 9*

Figure A. 12 *Rice at 50% field capacity on day 9*

VITA

        Miss Mawika Samleepan was born on July 24th, 1990 in Bangkok. After she finished high school in 2008 from Triam Udom Suksa School of the North, Pitsanulok, she was enrolled in Department of Botany, Faculty of Science, Chulalongkorn University. She was graduated with degree of Bachelor of Science. In 2013, she continued her Master of Science in Botany at Department of Botany, Faculty of Science, Chulalongkorn University. Since 2013, she has been supported by Thailand Graduate Institute of Science and Technology (TGIST) scholarships: TG-CPMO 01-56-005, National Science and Technology Development Agency (NSTDA).