การเปรียบเทียบแบบรูปของโปรตีนที่ตอบสนองต่อภาวะแล้งของข้าวพันธุ์เหลืองประทิว 123 *Oryza sativa* L.cv. Leung Pratew123 และข้าวสายพันธุ์กลายทนแล้ง

นางสาวณัฐวดี จินตโกวิท

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COMPARISON OF DROUGHT RESPONSIVE PROTEIN PATTERNS IN 'Leung Pratew123' RICE *Oryza sativa* L.cv. Leung Pratew123 AND ITS DROUGHT RESISTANT MUTANT LINE

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Thesis Title	COMPARISON OF DROUGHT RESPONSIVE PROTEIN PATTERNS IN 'Leung Pratew123' RICE *Oryza sativa* L.cv. Leung Pratew123 AND ITS DROUGHT RESISTANT MUTANT LINE
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ณัฐวดี จินตโกวิท : การเปรียบเทียบแบบรูปของโปรตีนที่ตอบสนองต่อภาวะแล้งของข้าวพันธุ์เหลืองประทิว 123 *Oryza sativa* L.cv. Leung Pratew123 และข้าวสายพันธุ์กลายทนแล้ง (COMPARISON OF DROUGHT RESPONSIVE PROTEIN PATTERNS IN 'Leung Pratew123' RICE *Oryza sativa* L.cv. Leung Pratew123 AND ITS DROUGHT RESISTANT MUTANT LINE) อ.ที่ปรึกษาวิทยานิพนธ์หลัก: รศ. ดร. ศุภจิตรา ชัชวาลย์, อ.ที่ปรึกษาวิทยานิพนธ์ร่วม: สิทธิรักษ์ รอยตระกูล, 180 หน้า.

การศึกษาระดับโปรติโอมของข้าวที่อ่อนแอต่อภาวะแล้งข้าวพันธุ์เหลืองประทิว 123 (SS) และสายพันธุ์กลายทนแล้งเหลืองประทิว 123-TC171 (SR) มีวัตถุประสงค์เพื่อระบุกลไกการทนแล้งในข้าวสายพันธุ์ทนแล้ง จากผลการศึกษาข้อมูลโปรตีนทั้งหมด ได้คัดเลือกโปรตีน GT-2 LIKE 1 (GTL1) และ Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) ซึ่งเกี่ยวข้องกับการควบคุมจำนวนปากใบและการส่งอิเล็กตรอนในวัฏจักรคัลวินตามลำดับ สำหรับการศึกษาในขั้นต่อไป จากการศึกษาพบว่าข้าว SR มีจำนวนปากใบต่อพื้นที่น้อยกว่าข้าว SS เมื่อปลูกในภาวะแล้ง ซึ่งสอดคล้องกับระดับโปรตีน GTL1 ที่น้อยลงในข้าว SR ในภาวะแล้ง ยิ่งไปกว่านั้นข้าว SR แสดงปริมาณน้ำสัมพัทธ์ในใบสูงกว่าข้าว SS หลังจากได้รับภาวะแล้ง นอกจากนี้ได้วัดตัวแปรต่างๆของกระบวนการแลกเปลี่ยนแก๊สของใบแก่และใบอ่อนในข้าว SS และ SR หลังจากได้รับภาวะแล้งด้วย 12.5% PEG เป็นเวลา 3 วัน พบว่า ใบอ่อนของข้าว SR มีอัตราการสังเคราะห์แสงสุทธิและประสิทธิภาพการใช้น้ำสูงกว่าข้าว SS ในทำนองเดียวกันค่าประสิทธิภาพการทำงานของระบบแสงสองภายใต้สภาพที่มีแสง (ΦPSII) และค่าอัตราส่งผ่านอิเล็กตรอนสูงขึ้นในข้าว SR มากกว่าข้าว SS สอดคล้องกับระดับโปรตีน GAPDH ที่สูงขึ้นในภาวะแล้งในข้าว SR ยิ่งไปกว่านั้นอัตราการคายน้ำในใบอ่อนของข้าว SR มีค่าต่ำกว่าอย่างมีนัยสำคัญ ทั้งนี้ข้าว SR จัดการต่อภาวะแล้งผ่าน *GTL1* ซึ่งควบคุมจำนวนปากใบในใบที่พัฒนาขึ้นใหม่ทำให้สูญเสียน้ำน้อยลง และ *GAPDH* ที่ช่วยปกป้องระบบการสังเคราะห์ด้วยแสงในใบเก่าเมื่ออยู่ภายใต้ภาวะแล้ง

ภาควิชา พฤกษศาสตร์	ลายมือชื่อนิสิต .....
สาขาวิชา พฤกษศาสตร์	ลายมือชื่อ อ.ที่ปรึกษาหลัก .....
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NUTWADEE CHINTAKOVID: COMPARISON OF DROUGHT RESPONSIVE PROTEIN PATTERNS IN 'Leung Pratew123' RICE *Oryza sativa* L.cv. Leung Pratew123 AND ITS DROUGHT RESISTANT MUTANT LINE. ADVISOR: ASSOC. PROF. SUPACHITRA CHADCHAWAN, Ph.D., CO-ADVISOR: SITTIRUK ROYTRAKUL, Ph.D., 180 pp.

The proteome-level study of the stress-susceptible (SS) *Oryza sativa* L. cv. Leung Pratew123 and its stress-resistant (SR) mutant line, Leung Pratew123-TC171 were conducted to identify a drought response mechanism in SR line. Based on the proteomics data, two proteins; GT-2 LIKE 1 (GTL1) and Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) which are involved in stomata density reduction and e^- transfer in Calvin Cycle, respectively were selected for further study. It was found that the SR rice had the lower stomatal density than SS rice when grown under drought stress. This is consistent with the lower level of GTL1 protein found in SR during drought stress. In addition, SR showed higher relative water content than SS after drought treatment. Besides, measurement of the leaf gas exchange parameters was conducted in the old and the young leaves in both SS and SR. After 3 days of drought stress (12.5% PEG), old leaf of SR had significant higher net photosynthetic rate and water use efficiency than SS. Likewise, effective quantum yield of PSII photochemistry (ΦPSII) and electron transport rate were also higher in SR than SS line. Similarly, higher GAPDH level under drought stress was found in SR line. Moreover, transpiration rate in the young leaf was significantly lower in SR line. Overall, SR rice mediates drought stress through *GTL1* which regulates stomatal density leading to less water loss in the newly developed leaves, while in the old leaves the adaptation in *GAPDH* helps protecting photosystem under drought stress.

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

INTRODUCTION

Rice (*Oryza sativa* L.) is a staple food for more than one third of the people around the world. The demand for rice is expected to increasing because the world's population growth rate is rising 1.5-fold by 2025 (Sasaki, 2002). Rice is a semi-aquatic plant which required a lot of water during plantation. Therefore, water limiting during the growing period can causes physiological changes and lead to yield loss (Farooq et al., 2009).

Nowadays, drought stress occurs more often and it is the most significant constraint environmental that limits growth, development, and rice productivity (Bhushan et al., 2011). Plant responses to water loss involves in several strategies such as stomatal closure, osmotic adjustment or reduction of the photosynthetic activity (Farooq et al., 2009). To deal with stress, several genes are induced rapidly (Yamaguchi-Shinozaki and Shinozaki, 2006). These gene products function in stress resistance and also regulate signal transduction and other gene expression which can alter plant protein profiles. However, mRNAs of expressed genes are not always translate to a functional proteins (Urano et al., 2010). Therefore, to clarify plant responses to environmental stimuli, a proteome-level investigation can be a better option for revealing cellular adaptations (Kosová et al., 2015).

Proteomics is a molecular tool for protein profile analysis on plant stress responses. With the complete genome sequencing project, proteomics can be defined as the systematic analysis of proteome. This technology allows us to study a changes

of proteome in various tissues and physiological states of cells triggered by environmental stimuli (Park, 2004). Identification of drought-responsive proteins and genes by proteomics technique has been reported in many crops, including rice (Ali and Komatsu, 2006; Chamnanmanoontham et al., 2015; Ji et al., 2012), cotton (Deeba et al., 2012), grapevine (Lovisolo et al., 2010), soybean (Deshmukh et al., 2014; Oh and Komatsu, 2015), wheat (Alvarez et al., 2014; Ford et al., 2011), and watermelon (Akashi et al., 2011). The study of proteome changes can be performed by using two-dimensional polyacrylamide gel electrophoresis (2D-PAGE) or one-dimensional polyacrylamide gel electrophoresis (SDS-PAGE) coupled with protein identification by mass spectrometry (MS) (Salekdeh et al., 2002 and Ali and Komatsu, 2006). Phloem and xylem sap from rice were investigated using a SDS-PAGE connects to a nano LC-MS device (1D-LC). Eighty different proteins were identified. However, 2D-PAGE connects to a nano LC-MS device (2D-LC) provided 53 different proteins identification. It shows that 1D-LC can detect proteins with a very low level of expression that are undetectable by gel staining (Aki et al., 2008). Gammulla et al. (2010) used 1D-LC to investigate the proteomic responses of rice cell suspension cultures to sudden temperature changes. Forty novel stress–response proteins that involve in the classical and the alternative pathways of sucrose metabolism respond to extremes of temperature.

Therefore, the better understanding of drought-responsive genes/proteins will contribute to drought-resistant rice line development in future. The aims of this study were to compare drought-induced protein profile in two rice lines, *O. sativa* L. cv. Leung Pratew123 (LPT123) and its drought resistant mutate line, LPT123-TC171 which have contrasting drought-tolerant ability and gel-based liquid chromatography–

tandem mass spectrometry (GeLC-MS/MS) were performed. After that identification of drought-responsive genes in rice were elucidated and the selected genes were further study according to gene function. These findings may contribute to better understanding of drought-responsive mechanisms in drought-tolerant rice.

The objectives of this study are:

1. To investigate leaf protein profiles of Leung Pratew 123 (*Oryza sativa* L. cv. Leung Pratew123) and its drought resistant mutated line responding to the drought stress.
2. To determine the appropriate data analysis methods for the whole rice proteins after drought stress.
3. To identify and characterize the drought responsive gene(s) selected from the gene/protein expression patterns.

CHAPTER II

LITERATURE REVIEW

1. Rice (*Oryza sativa* L.)

Rice (*Oryza sativa* L. *spp. indica*) is an important cereal crop and it is a staple food for more than half of the people around the world especially in Asia (Kumar et al., 2014; Salekdeh et al., 2002). Rice has a small genome size compared with other cereal crops and its genome is completely sequenced (Goff et al., 2002). It is widely used as a monocot model for plant molecular biology.

1.1. LPT123 rice and its drought tolerant mutant line, LPT123-TC171

'Leung Pratew123' (LPT123; SS) rice is a Thai indica rice originated from Phetchaburi province. LPT123 is a photo-sensitive variety so it can flower only in short-day. It has average height of 150 centimeters, long and wide leaf and long inflorescence. LPT123 has long, yellow seeds (Bureau of Rice Research and Development (http://www.brrd.in.th/rvdb/)).

Vajrabhaya and Vajrabhaya (1991) developed salt-tolerance line from LPT123. Leung Pratew123-TC171 (LPT123-TC171; SR) contains a somaclonal variation of LPT123 which was selected under high salt stress condition (2% NaCl). It showed the best survival rate (94.3%) under 0.5% NaCl treatment, when grown in natural condition. SR rice have been studied in their physiological and molecular changes due to salt and drought stresses compared to SS (Pongprayoon et al., 2013; Sripinyowanich et al., 2013; Thikart et al., 2005; Udomchalothorn et al., 2009; Udomchalothorn et al., 2014; Vajrabhaya and Vajrabhaya, 1991b)

A comparison of exome sequencing in SS and SR indicated that the selection of salt-tolerant rice in vitro causes a telomere shorten in SR rice. This study revealed that there are point mutations spread all over the genome. This lead to the different phenotype of SS and SR under salt and drought condition due to changes in salt-and/or drought-responsive genes (Udomchalothorn et al., 2014). Thikart et al. (2005) showed that SR rice are more tolerate to drought stress than SS. A higher shoot fresh weight, shoot dry weight, root fresh weight, root dry weight and plant height under drought stress were found in SR. An application of chitosan to SS and SR during drought stress showed that chitosan enhanced shoot growth and maintain photosynthetic pigments in SS but had no effect in SR (Pongprayoon et al., 2013). Moreover, SS reduced fresh and dry weight after 9 days of salt stress but this phenomenon was not found in SR (Udomchalothorn et al., 2009).

Furthermore the physiological changes under drought and salt stress were observed and some of the molecular mechanisms have been reported in these rice lines. *OsNUC1* transcript expressed differently between SS and SR. The resistant line showed the higher expression under salt stress condition. The overexpression of *OsNUC1* in rice exhibited the higher shoot fresh weight after salt stress for 3 days. A study in transgenic Arabidopsis showed that the overexpression line had smaller reduction of root length under salt stress than wild type. Therefore, the function of *OsNUC1* was proposed to regulate root (Sripinyowanich et al., 2013). Moreover, salinity stress induced leaf sucrose and reduced the ratio of carbon assimilated to starch in both SS and SR. However, SR had more significant changed than SS. The transcript of *F6P2K/F26BPase* which regulates cellular level of fructose-2,6-bisphosphate (F26BP) could be detected in SR but not in SS under normal condition. Salinity stress for 72

hours induced *F6P2K/F26BPase* was higher in SR than SS. The susceptible line enhanced both *F6P2K* and *F26BPase* while the resistance only induced the *F26BPase* activity, resulting in significant reduction of the *F6P2K/F26BPase* activity ratio after 9 days of salt treatment. Therefore, this suggested that the regulation of sucrose level and a partition of carbon to sucrose may contribute to salt-tolerance in rice (Udomchalothorn et al., 2009).

1.2. Effect of drought stress on rice production in Thailand

Most of rice farm is located in Asia and Asian people is the major group consuming rice in daily life. Rice is a semi-aquatic plant which mainly cultivated under flooding system. The rice can be categorized according to cultivated methods, which are the rainfed lowland rice (in Africa and Madagascar), upland rice (in high land or mountains), and the deep water or flood-prone rice (in Bangladesh and in the Mekong, Chao Phraya). However, the irrigated rice is commonly found in Asia. Rice is very sensitive to water limiting than other cereals (e.g. wheat and maize) which can be grown with less water (Gnanamanickam, 2009; Kumar et al., 2014). Therefore, rice cultivation in Asia depend on water supply.

A study of rice yields in Notheastern region of Thailand showed a yield loss because of the drought stress. Actual rice yield was 700 to 1000 kg per hectare in many villages. However, the attainable yield should be more than 1200 kg per hectare if there is no drought stress. It means that approximately 40% yield reduction is due to drought stress (Polthanee et al., 2014).

Two reports (Jongdee, 2003; Prapertchop et al., 2005) showed that more than 50 % rice yield loss was caused by the drought stress. Thai rice farm in Northeastern

experienced the drought stress at planting stage, tillering stage and at any growing stage accounting for 19%, 40% and 23%, respectively (Gypmantasiri et al., 2003).

2. Drought stress

Drought is a major stress occurring throughout the world. Since water is essential for plant growth, the water limitation will threaten agriculture industry (Somerville and Briscoe, 2001). Drought alters physiological and biochemical functions of plants which affect in both cellular and molecular levels. The responses involve in stomatal closure, growth reduction, changes in photosynthetic rate, accumulation of osmolytes and proteins, specifically the proteins involving in stress tolerance. Several drought traits have been used as indicators to evaluate a drought resistance such as root/ leaf traits, capability of osmotic adjustment, water potential value, ABA content and stability of the cell membrane (Fang and Xiong, 2015; Shinozaki and Yamaguchi-Shinozaki, 2007).

1.1. Drought resistance mechanism

Drought resistance is a plant ability to grow normally under disfavor condition. The mechanisms of drought resistance have been divided in 3 alternative strategies, drought avoidance, drought tolerance, and drought escape.

Drought avoidance is a mechanism which plant maintains basic physiological processes to avoid the negative result from mild or moderate drought stress. Drought avoidance is a process that plant reduces water loss (e.g. stomatal closure), maximizes

water uptake (e.g. increase root depth) and accelerates or decelerates the conversion from vegetative stage to reproductive stage (Fang and Xiong, 2015).

Drought tolerance refers to plant ability to withstand dehydration by maintaining their physiology activities and reducing the damage from the stress *via* gene regulation and metabolic pathways. The tolerance ability commonly involves with osmotic adjustment to maintain turgor pressure and adjusting the level of reactive oxygen species (ROS) by reducing the accumulation (Fang and Xiong, 2015).

Drought escape is usually referred to plant adjustment by completing their life cycle before subjected to drought period, for example; earlier flowering time, rapid growth and reproducing before the onset of drought (Araus et al., 2002; Fang and Xiong, 2015; Kooyers, 2015).

However, some researchers also consider drought recovery as one of drought resistance mechanisms. Drought recovery is an ability to resume growth and gain yield after severe drought stress (Luo, 2010).

2.2. Morphological, physiological, biochemical and molecular changes due to drought stress

Mechanism of plant for dealing with the drought stress are an adaptation in morphological, physiological, biochemical and molecular levels.

2.2.1. Morphological and anatomical changes due to drought stress

Morphological changes due to drought stress has been reported in many researches. Diminish of cell elongation and enlargement are a consequence of turgor pressure loss during drought stress (Jaleel et al., 2009). In addition, water stress limits

expansion of leaf area and leaf number (Ghanbari et al., 2013). Shoot and root dry weight are also reduced by drought stress in many studies (Ji et al., 2012; Pongprayoon et al., 2013; Wang et al., 2009). Stomatal density is the anatomical adaptation due to drought stress. Reducing stomatal density enhance drought tolerant ability in Arabidopsis (Yoo et al., 2010), *Medicago Truncatula* (Xie et al., 2012) and rice (Liu et al., 2011).

2.2.2. Physiological changes due to drought stress

Drought stress affects plant physiology in many aspect such as reduction of photosynthesis rate (Allahverdiyev, 2016; Hu et al., 2010; Souza et al., 2004), decreased in chlorophyll content (Nikolaeva et al., 2010) and accumulation of proline (De Ronde et al., 2004) .

Photosynthesis adaptation is one of physiological responses due to drought stress. Photosynthesis is a fundamental process which contributes to plant growth and development. Water deficit causes stomatal closure which lead to decrease of stomatal conductance (g_s). The stomata closure is the most effective way to minimize water loss and affects the CO_2 diffusion resulting in reduction of Ci. Thus, the photosynthetic rate reduction during drought stress is commonly found (Ashraf and Harris, 2013; Cornic, 2000). Photosynthesis rate, stomatal conductance and transpiration rate are reduced after drought in wheat flag leaf (Allahverdiyev, 2016), C_3 perennial grass species (Hu et al., 2010) and cowpea (Souza et al., 2004). Ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) is the main enzyme that can alter the photosynthesis rate. The shrinkage of chloroplast is an effect of drought stress which lead to conformation changes in Rubisco (Jia et al., 2008). Severe drought stress (30% PEG)

significantly decreases the Rubisco activity in rice and also results in stomatal conductance and net photosynthesis reduction (Zhou et al., 2007). In addition, the proteomic study of two rice cultivars with different drought tolerance ability show reduction of Rubisco after drought stress in flag leaves (Ji et al., 2012). Chlorophyll content is also an important parameter that directly affect photosynthesis process. The reduction of chlorophyll content was found in wheat after 7 days of drought stress (Nikolaeva et al., 2010). In the leaves of 11-day-old barley, the pigment contents were also reduced by water deficit (Pshibytko et al., 2004).

Osmotic adjustment is one of the plant adaptation mechanism to survive the stress. An accumulation of osmolytes (proline, ABA, LEA protein, glycine betaine and sugar) has been found in many plant species during drought stress (Farooq et al., 2012). This lowers the osmotic potential of cell, so the plant can uptake water normally and maintain cell turgor pressure. The accumulation of proline was found particularly in young leaf of lemon under drought stress (Pérez-Pérez et al., 2009). Similar result was also found in transgenic soybean. The level of proline was significant higher in drought-tolerant transgenic soybean than wild type (De Ronde et al., 2004).

2.2.3. Biochemical changes due to drought stress

Oxidative burst is one of the early event for plant protection, a biochemical response. Reactive oxygen species (ROS) in a proper amount have been reported as a signalling molecule which triggers other molecule downstream. The balancing of ROS homeostasis is important for reducing their toxicity and providing a signalling to downstram event. Cell is damaged when the activity of ROS is over the effectiveness of antioxidant response. The antioxidants include glutathione reductase (GR)

superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (Apx), peroxide (POD), and monodehydroascorbate reductase (MDAR) (Anjum et al., 2011; Pongprayoon et al., 2013; Ray et al., 2012).

2.2.4. Molecular changes due to drought stress

During the stress response, plant can protect themselves in a level of molecular defense. Molecular responses can be classified into 3 categories, transcriptional regulation, post-transcriptional RNA and osmoprotectant metabolism (Yang et al., 2010)

Transcription factors (TFs) act as molecular switches for gene expression responding to environmental factors. One of the well-known transcription factors is MYB families. MYB transcription factor is a big family that currently, over 100 MYB TFs have been found in Arabidopsis, rice (*Oryza sativa*), and other plant species (Baldoni et al., 2015; Gao et al., 2014). Several of them were reported as stress-induced proteins/genes such as *MYB2* (Abe et al., 2003; Yang et al., 2012), *MYB10* (Villalobos et al., 2004), *MYB15* (Ding et al., 2009), and *AtMYB20* (Gao et al., 2014). *OsMYB2* expression was induced by salinity, low temperature and osmotic stress (20% PEG). Moreover, overexpression of *MYB2* in rice and Arabidopsis enhanced drought tolerance (Abe et al., 2003; Yang et al., 2012). In addition, an overexpression of *MYB10* from *Craterostigma plantagineum* increased the drought and salt tolerance ability and led to ABA hypersensitivity (Villalobos et al., 2004). *AtMYB15* overexpression line improved the survival and reduced water loss less than wild type under water deficiency conditions and *MYB15* promoter is active in guard cells of stomata (Ding et al., 2009). Loss of function mutant plant (*myb20*) resisted to desiccation stress, whereas the

overexpression of *AtMYB20* resulted in the higher sensitivity to stress (Gao et al., 2014). The other transcription factors families have been characterized including APETALA2 (AP2), bZIP, NAC, WRKY, SBP (Squamosa-promoter binding protein) and zinc-finger which play a crucial role in stress response (He et al., 2016; Yang et al., 2010).

Some TFs will be activated after protein phosphorylation by protein kinase. Mitogen-activated protein kinases (MAPKs) have been studied in plant response to environmental stimuli. MAPK cascades function in many signal transduction pathways, responding to dehydration, cold, and high salt conditions (Yang et al., 2010). For example, the overexpression of a *Nicotiana tobacum* MAPKKK protein kinase (*NPK1*) triggered an oxidative stress-response signalling cascade and led to freezing, heat and salt stress tolerance (Kovtun et al., 2000). The transgenic maize with constitutively expressed *NPK1* also showed a drought tolerance ability with higher photosynthetic rate (Shou et al., 2004). The other protein kinases are calcium-dependent protein kinases (CDPKs) and CBL (calcineurin B-like) interacting protein kinase (CIPK/sucrose non-fermenting protein (SNF1)-related kinase 2 (SnRK3) and SNF1-related kinase 2 (SnRK2) (Yang et al., 2010). CDPKs induce Ca^{2+} fluxes after sensing the environmental changes. Salt and cold stresses induced *OsCDPK7* transcript in rice roots and shoots. The constitutive *OsCDPK7* overexpression exhibited drought, salt and cold tolerance of rice seedlings (Saijo et al., 2000). (Xiang et al., 2007) characterized stress-responsive *CIPK* genes in rice. Several *OsCIPKs* were induced by drought (e.g. *OsCIPK01, 02, 05, 12,* and *15*), salt (e.g. *OsCIPK07, 08, 11,* and *15*), cold (e.g. *OsCIPK01, 03,* and *09*) and ABA treatment (e.g. *OsCIPK01, 02, 09, 11,* and *15*). The overexpression of *OsCIPK12* and *OsCIPK15* improved drought and salt tolerance,

respectively. Farnesylation is another post-translational protein modification that has a potential role in protein farnesylation during drought stress (Yang et al., 2010).

Under drought stress, an accumulation of osmotic compounds was found. This led to decreasing the osmotic potential and water loss (Chaves et al., 2003). Several genes that encode enzymes involving in osmoprotectant biosynthetic pathway have been studied such as proline, ABA, LEA protein, glycine betaine and sugar (Yang et al., 2010). Proline is a compatible solutes that is highly accumulated in stressed plant under drought and salinity stress (Delauney and Verma, 1993). *OsP5CS* gene involved with proline biosynthesis was up-regulated after dehydration. The constitutively expressed of *P5CS* in rice (Zhu et al., 1998) and the overexpression of *P5CR* in soybean (De Ronde et al., 2004) increased proline content after treated with drought stress and led to the higher relative water content and growth. ABA accumulation is one of the fastest responses of plants to drought stress which activates ABA-inducible gene expression (Himmelbach et al., 2002; Shinozaki and Yamaguchi-Shinozaki, 2007) and lead to stomatal closure, which prevents water loss (Schroeder et al., 2001). The overexpression of *AtMYB2* enhanced drought tolerance because of an ABA-hypersensitive phenotype. This phenomenal was also found in the overexpression of *AtMYC2* (Abe et al., 2003). The rice mutant (*dss1*) had higher drought tolerant ability because an accumulation of ABA was found (Tamiru et al., 2015). The overexpression of *ABI1* revealed ABA-insensitive phenotype and made the Arabidopsis more sensitive to drought stress (Himmelbach et al., 2002).

3. Proteomics

Protein is a final product from translated genome of plant which has transcription of mRNA as an intermediate step. To understand their functions, a study of the proteins is one of the approaches. A studying of global protein expression and their functional mechanisms is known as proteomics. Proteomics includes a study of whole proteins in several aspects include a study of protein interaction, protein function, proteins structure and proteins sequences (Wilkins et al., 1996). Therefore, proteomics allow us to understand the change of protein due to environmental stress (Twyman, 2004).

3.1. Proteomics in contrasting drought-tolerant background

Many studies have been conducted by using wheat (Bowne et al., 2012; Faghani et al., 2015; Ford et al., 2011), rice (Ali and Komatsu, 2006; Maksup et al., 2014; Salekdeh et al., 2002) and tobacco (Gharechahi et al., 2015) that have contrasting stress tolerant ability because it can elucidate drought-responsive mechanism and improve drought-tolerant plant (Basu et al., 2016). Nipponbare; a drought sensitive rice, and Zhonghua 8; drought tolerant rice, were used in a study of drought-responsive proteins in rice leaf sheath. It was found that the accumulation level of actin depolymerizing factor, light harvesting complex chain II, PSII oxygen evolving complex protein and oxygen evolving enhancer protein 2 in ‘Zhanghua 8’ rice were higher than ‘Nipponbare’ rice (Ali and Komatsu, 2006). An analysis of mass spectrometry in two contrasting genotypes, IR62266-42-6-2 (lowland indica rice) and CT9993-5-10-1-M (upland japonica rice) during drought stress and recovery period were conducted. The proteomics revealed that an S-like RNase homologue, an actin depolymerizing factor

and RuBisCO activase were up-regulated under drought stress while an isoflavone reductase-like protein was down-regulated (Salekdeh et al., 2002). A studied of drought- responsive proteins in Khao Dawk Mali105 (KDML105) rice, and two check cultivars, drought tolerant cultivar (NSG19) and drought sensitive cultivar (IR20), showed the different expression groups of proteins. A protein involving with stomatal closure, coronatine-insensitive 1 protein was found in NSG19. This correlates with rapid stomatal closing and highest stability of photosystem II in NSG19 phenotype. In IR20, an increasing of WD-40 repeat protein was found while H-protein promoter binding factor-2a extremely increased in KDML105 (Maksup et al., 2014). A transgenic plant was also used to study the protein changes when treated with PEG. The TERF1-overexpresed transgenic sugarcane which has drought-tolerant ability and the wild-type plant were used to study a tolerance mechanism at molecular level. The proteomics was performed by using two-dimensional gel electrophoresis technique, then coupled with tandem mass spectrometry (MS/MS) analyses. The comparison of the wild-type and the transgenic sugarcane under PEG stress showed a majority of proteins involving with metabolism, energy, protein synthesis, and disease/defense. Under the stress, pentatricopeptide repeat (PPR) containing protein and peptidyl prolyl cis-trans isomerase (PPIase) were decreased, but the RuBisCO large subunit, PEP carboxylase, ferredoxin, glyceraldehyde 3-phosphate dehydrogenase, elongation factor Tu, several small heat shock proteins, and peroxidases were increased (Rahman et al., 2014).

CHAPTER III

MATERIALS AND METHODS

I. Materials

1. Plant materials

Two rice lines were used in this study. The first one is rice (*Oryza sativa* L.) cultivar ‘Leung Pratew123’ (LPT123; SS) which is obtained from Department of Rice, Ministry of Agriculture and Cooperative, Thailand. The other is rice (*Oryza sativa* L.) line ‘Leung Pratew123-TC171’which was generated from somaclonal variation of LPT123 (Vajrabhaya and Vajrabhaya, 1991b). The LPT123-TC171 rice or SR line is a salt- and drought-resistant rice line (Pongprayoon et al., 2013; Sripinyowanich et al., 2013; Udomchalothorn et al., 2009; Vajrabhaya and Vajrabhaya, 1991b). Seeds of LPT123-TC171 were provided by the Center of Excellence in Environment and Plant Physiology, Department of Botany, Faculty of Science, Chulalongkorn University.

Two Arabidopsis lines used in this study are *Arabidopsis thaliana* ecotype Columbia-0 (Col-0) and *Arabidopsis thaliana* mutant (*gtl1-4*) (SALK_005972). The seeds of both wild type and *gtl1-4* were kindly provided from Associate Professor Michael V Mickelbart, Department of Horticulture and Landscape Architecture, Purdue University, West Lafayette, Indiana, USA

2. Equipment

2.1. General uses (planting, collecting sample and RNA/protein extraction)

- Balances (Mettler Toledo AG285, Mettler Toledo, Switzerland)
- -80 ºC deep freezer (Thermo-Scientific, USA)
- -20 ºC freezer (SANYO biomedical freezer, Japan)
- Autoclave (Taichung, Taiwan)
- Refrigerated centrifuge (Universal 32R, Hettich, Germany)
- Microwave oven (Toshiba, Thailand)
- Mortar and pestle
- Spatula
- Forceps
- Liquid nitrogen container
- Spectrophotometer (Agilent Technology, USA) and cuvettes
- Micropipette (Gilson, France) and micropipette tips
- Vortex mixer (Labnet, USA)
- Water bath (LabTech, USA)
- Dry bath incubator (MD-01N model, Major Science, Taiwan)
- Cylinder
- Plastic tray
- Aluminum foil
- Microcentrifuge tube
- Ice box
- Shaker (Biosan, USA)
- Scalpel

- Parafilm (Whatman®, GE healthcare, USA)

2.2. For proteomics study

- ESI ion Trap MS (HCT ultra PTM Discovery System, Bruker Daltonik, Germany)
- Ultimate 3000 LC system (Dionex, USA)
- Vertical gel electrophoresis unit (Bio-Rad, USA)
- Vial and insert tube
- 96 well microplate
- Multi-channel micropipette 200 µl

2.3. For study-gene expression at transcriptional level

- Horizontal gel electrophoresis system (MiniRun GE-100, Hangzhou BIOER Technology, China)
- Gel documentation system (Gel DOC^{TM}2000, Bio-Rad, USA)
- Microcentrifuge (Sorvall® Biofuge Pico, Germany)
- PCR tube (Axygen Inc., USA)
- NanoDrop™ 2000 Spectrophotometers (Thermo Fisher Scientific, USA)

2.3.1. Specific equipment for semi quantitative reverse transcription polymerase chain reaction (semi qRT-PCR)

- PCR thermal cycler (PTC-100TM, Peltier Thermal Cycler, MJ Research, USA)

2.3.2. Specific equipment for quantitative reverse polymerase chain reaction (qRT-PCR)

- CFX96^{TM} Real-Time PCR Detection System (Bio-Rad, USA)
- 8 tube strip and flat cap (Bio-Rad, USA)

2.4. For study stomatal density

- Superglue
- Glass slide

2.4.1. Stomatal density in rice

- 20X objective lens (UPlanApp, Olympus, Japan) couple with Multipurpose Microscope (Olympus BX-51)

2.4.2. Stomatal density in Arabidopsis

- Nikon-OptiPhot 2 microscope (Nikon)

2.5. For photosynthesis measurement

- LI-6400 Portable photosynthesis system (LI-COR, Lincoln, NE, USA) with the LI-6400-40 leaf chamber fluorometer (LI-COR)
- Pocket PEA chlorophyll fluorimeter (Hansatech Instrument, King's Lynn, United Kingdom)

3. Chemicals and reagents

3.1. For rice planting

3.1.1. In solution

- Modified WP nutrient solution (appendix A)
- 10% Polyethylene glycol (PEG) 6000

3.1.1. In soil

- Clay soil

3.2. For Arabidopsis planting: in soil

- Fafard 2X Mix soilless media

3.3. For sample collection

- Liquid nitrogen (Linde, Thailand)

3.4. For protein identification

3.4.1. Protein extraction and precipitation

- 0.1% sodium dodecyl sulfate (SDS)
- 0.15% deoxycholic acid (DOC)
- 72% trichloroacetic acid (TCA)

3.4.2. Protein concentration measurement by Lowry method

- Bovine serum albumin (BSA) (2µg/µl)
- Reagent A (alkaline copper reagent; appendix A)

- Reagent B (diluted Folin-Ciocalteu's phenol reagent; appendix A)

3.4.3. Protein separation by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE)

- 0.5 M Tris HCl pH 6.8
- 1.5 M Tris HCl pH 8.8
- 10% sodium dodecyl sulfate (SDS)
- 10% ammonium persulfate (APS)
- 40% (w/v) acrylamide/bis-acrylamide solution (29:1)
- Distilled water
- Tetramethylethylenediamine (TEMED)
- Protein ladder 10-250 kDa (New England Biolabs, USA)
- Protein loading dye (appendix A)
- Tris-glycine electrophoresis buffer (appendix A)
- Staining solution (appendix A)
- Destaining solution (appendix A)

3.4.3. Protein in-gel digestion and peptide analysis (LC-MS/MS)

- 0.1% trifluoroacetic acid (TFA)
- 10 mM ammonium bicarbonate
- 10 mM dithiothreitol (DTT)
- 10 ng/mL trypsin (Promega, USA)
- 100 mM iodoacetamide (IAM)

- 100% acetronitrile (ACN)
- Bovine serum albumin (BSA)
- Steriled milli Q water

3.5. For analysis of transcription expression

3.5.1. For study of transcription expression in rice

- Purelink® Plant RNA Reagent (Ambion, Life Technologies, USA)
- DNase I, RNase-free (Thermo Fisher Scientific, USA)
- iScript^{TM} Reverse Transcription Supermix for RT-qPCR (Bio-Rad, USA)
- 5 M sodium chloride (NaCl)
- Chloroform (Merck, Germany)
- Isopropanol (Merck, Germany)
- Absolute ethanol (Merck, Germany)
- Phenol:chloroform:isoamyl alcohol (25:24:1) (v/v)
- 10 M lithium chloride ($LiCl_2$)
- 5x TBE buffer (appendix A)
- DEPC-treated RNA loading dye (appendix A)
- Ethidium bromide (Gibco BRL, USA)
- Agarose (USB Corporation, Ohio, USA)
- Forward primer
- Reverse primer
- Ultrapure water

3.5.1.1. Quantitative polymerase chain reaction (qPCR)

- SsoFast™ EvaGreen® Supermix (Bio-Rad, USA)

3.5.1.2. Semi quantitative reverse transcription polymerase chain reaction (semi qRT-PCR)

- Taq DNA Polymerase, recombinant (5 U/µL) (Thermo Fisher Scientific, USA)

3.5.2. For study of transcription expression in Arabidopsis

- RNeasy® Plant Mini Kit (Qiagen, USA)
- TURBO DNA-free™ kit (Life technologies, USA)
- High capacity cDNA Reverse Transcription kit (Life Technologies, USA)
- GoTaq ® Hot Start Polymerase (500 u) (Promega, USA)
- 10mM dNTP Mix (Life Technologies, USA)
- 5x TBA buffer (appendix A)

II. Methods

1. Proteomics study

1.1. Investigation of protein profiles after drought stress by using proteomic approach

1.1.1. Rice grown condition for protein identification

The experiment was performed with a completely randomized design (CRD) with three biological replicates. Three rice seedlings were pools for each biological replication. Rice germination and growing condition were used with the similar procedure as previous study (Chamnanmanoontham et al., 2015). Rice seeds were soaked in distilled water for 24 hours and then transferred to germinate on sterile sand fully soaked with distilled water. After 2 weeks of germination, modified WP solution No.2 (Vajrabhaya and Vajrabhaya, 1991b) was added. The seedlings were grown in the greenhouse under natural light. During growing period, the nutrient solution was refreshed every 7 days. After 4 weeks, seedlings of each line/cultivar were separated into 2 groups, one group continued to grow in the WP solution, while the other was transferred to the WP solution supplemented with 10 % (w/v) polyethylene glycol 6000 (PEG6000) for drought stress treatment (Pongprayoon et al., 2013). SS and SR leaves were collected at 0, 2, 6 and 24 hours after treatment. The leaf sample at each time point were frozen immediately in liquid nitrogen and stored at -80^{o}C for further analysis.

1.1.2. Protein extraction and separation by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE)

Total proteins for proteomics analysis were extracted from SS and SR leaves. Three hundred milligrams of leaf tissues were ground in liquid nitrogen to fine powder then, 900 µl of 0.1% SDS was added immediately to the ground tissues and incubated at 37 ºC for 3 hours. The mixture was centrifuged at 13,000 rpm for 15 minutes at 4 ºC to collect the total proteins in the supernatant.

The total protein extract was purified according to deoxycholate - trichloroacetic acid precipitation method (Peterson, 1983) with some modifications. The supernatant (50 µl) was mixed with 950 µl of 0.15% deoxycholic acid (DOC) and then incubated at room temperature for 10 minutes. Then, 100 µl of 72% trichloroacetic acid (TCA) was added and subsequently incubated once at 4 ºC overnight. The mixture was centrifuged at 13,000 rpm for 15 minutes at 4 ºC. The protein pellet was collected and dried at room temperature approximately 5-10 minutes. The dried protein pellet was re-suspended in 50 µl of 0.15% DOC.

The protein concentration was determined according to Lowry's method (Lowry et al., 1951). The bovine serum albumin (BSA) was used as a protein standard. The purified proteins (5 µl) were mixed with 200 µl of reagent A (alkaline copper reagent; appendix A) and kept at room temperature for 30 minutes. Then 50 µl of reagent B (diluted Folin-Ciocalteu's phenol reagent; appendix A) was added and followed by incubation for 30 minutes at room temperature. The absorbance was recorded at 750 nm using spectrophotometer and the protein concentration was calculated as indicated below.

Protein concentration (µg/µl)

= (average OD750 of sample/m) X dilution factor/testing volume

m is slope of standard curve.

Fifteen micrograms of extracted proteins was dissolved in 10 µl of 0.5% SDS and 20 µl of protein loading dye was added. Then, the well-mixed mixture was boiled for 5 minutes before loaded into SDS-PAGE. The total protein was separated on 12.5% SDS-PAGE (Laemmli, 1970). The gel was stained with Coomassie Brilliant Blue R-250 ((Meyer and Lamberts, 1965); see in Appendix A) until the protein bands appeared. After that the staining solution was removed and then the destaining solution was added to remove background color. The destaining solution was changed around 3-4 times and the gel was de-stained overnight until the background was clear. The protein gels were stored in 0.1% acetic acid for further study.

1.1.3. In-gel digestion

The protein gel from each sample was segmented into 6 ranges according to protein molecular weight (see in Appendix C; Fig C.2). Each of which was cut into small cube about 1 mm^3. The protein cubes were subjected to in-gel digestion as previously described method (Jaresitthikunchai et al., 2009). The gel plugs were located into 96-well microplate and washed twice with sterile mili Q water (200 µl). Next, the gel was dehydrated with 200 µl of 100% ACN for 5 minutes and then dried for another 5 minutes. Carbamidomethyl reaction was conducted by incubating the dried gel plugs with 50 µl of 10 mM dithiothreitol/10 mM ammonium bicarbonate for an hour before incubating the gel plugs with 50 µl of 100 mM iodoacetamide/10 mM ammonium

bicarbonate in the dark for an hour. After that the gel pieces were dehydrated three times. All of these processes, the former solution in the plate was always taken away before new solution was added. Proteins were digested with 40 µl of trypsin solution (10 ng trypsin in 50% acetronitrile/10 mM ammonium bicarbonate) at room temperature for 20 minutes, subsequently immersed in 30 µl of 30% acetronitrile and incubated overnight. The digested peptide solution was carefully transferred to a new plate (avoiding any of gel pieces) and the residues in the gel pieces were extracted twice by adding 30 µl of 50% acetronitrile/0.1% trifluoroacetic acid and agitating for 10 minutes. All of the procedures were carried out at room temperature. The extracted peptide solution was dried at 40 ºC overnight and stored at -80 ºC for further analysis.

1.1.4. Protein quantification and identification

The digested protein will be injected to Ultimate 3000 LC system (Dionex) coupled with ESI-Ion Trap MS (HCT ultra PTM Discovery System, Bruker Daltonik) with electrospray at a flow rate of 20 µL/min to µ-precolumn (Monolithic Trap Column, 200 µm i.d. x 5 cm). The raw data from LC-MS/MS analysis were converted into mzXML format with CompassXport 1.3.10 program (Bruker Daltonik GmbH). Proteins were quantified with DeCyder MS Differential Analysis software (DeCyderMS, GE Healthcare) (Johansson et al., 2006; Thorsell et al., 2007) and identified with MASCOT software (Matrix Science, London, UK) (Perkins et al., 1999) by searching against non-redundant database of National Center for Biotechnology Information (NCBInr) 20170221 with the following parameters, taxonomy: *Oryza sativa* (rice), enzyme: trypsin, allow up to: 1 missed cleavage, fixed modifications: carbamidomethyl (C), variable modifications: oxidation (M), peptide tolerance: ± 1.2

Da, MS/MS tolerance: ± 0.6 Da, peptide charge: 1+, 2+ and 3+ (monoisotopic) and instrument: ESI-TRAP.

1.1.5. Gene ontology

Protein loci and functions in biological process were assigned by using blastp and gene ontology (GO) browsers in rice genome annotation project (http://rice.plantbiology.msu.edu) (Kawahara et al., 2013), respectively. For the proteins assigned to the same locus, a protein having the highest Mascot score was selected. In the case of equal Mascot score, ANOVA p-value derived from analysis with DeCyder MS Differential Analysis software would be considered. The protein with the lowest p-value was chosen.

1.1.6. Identification of drought responsive patterns

The identified proteins were searched against the Rice Genome Annotation Project database (http://rice.plantbiology.msu.edu) (Kawahara et al., 2013) using BLASTP to annotate proteins and assign functions based on gene ontology described as above. The identified proteins in each set of treatments that matched the above criteria were visualized and analyzed with the MultiExperiment Viewer (MeV) program to identify the osmotic-stress responsive proteins with t-test ($P<0.05$) (Saeed et al., 2003). The hierarchical clustering was conducted using the Pearson correlation.

1.2. Comparison of SS and SR proteomics data

After the significantly differential expression profiles due to drought stress of SS and SR lines obtained, the overlapping significantly different expressed proteins between SS and SR lines were determined and presented in Venn diagram.

2. Identification and characterization of the drought responsive genes from the gene/protein expression patterns.

2.1. Selection and expression analysis of the drought responsive genes in 'LPT123' and 'LPT123-TC171' rice lines

2.1.1. Co-expression analysis

The co-expression network analysis of proteins that were significantly affected by osmotic stress in the SR line was generated using a 'guide gene approach' by RiceFREND with hierarchy of 2 and mutual rank (MR) of 5 (Sato et al., 2013).

2.1.2. Planting and stress condition for gene expression analysis

Rice seeds were soaked in distilled water for 24 hours in dark and then transferred to germinate in sterilized water under natural light for 7 days. Leaves of 7-day-old rice seedlings of both lines were cut and air-dried for 2 hours to create drought stress condition. The transcription level of control and stressed-plants were conducted using three biological replicates.

2.1.3. Total RNA extraction and cDNA synthesis

Plant total RNA was extracted by using PureLink® Plant RNA Reagent (Invitrogen, USA) as described in manufacture's protocol with some modifications. Briefly, the plant sample approximately 0.1 mg was ground in liquid nitrogen to fine

powder. The powder was homogenized with the 500 µl of chilled (4ºC) Plant RNA reagent and the tube was incubated in horizontal at room temperature for 5 minutes. The mixture was centrifuged at 12,000 rpm for 5 minutes and the supernatant was transferred into clean RNase-free tube. Then, 100 µl of 5M NaCl was added and followed by 300 µl of chloroform. The aqueous phase was harvested by centrifugation at 12,000 rpm for 10 minutes at 4 ºC and transferred into new tube. RNA was precipitated by incubation with equal volume of isopropanol at room temperature for 10 minutes. After that the RNA pellet was harvested by centrifugation at 12,000 rpm for 10 minutes at 4ºC. The pellet was washed with ice-cold 80% ethanol and air dried at room temperature. The RNA pellet was re-suspended in 20 µl of DEPC-treated water. The total RNA concentration and quality were measured by NanoDrop™ 2000 Spectrophotometers

Then, the total RNA was treated with RNase free DNaseI, (Thermo Fisher Scientific, USA) to cleave contaminated genomic DNA according to manufacturer' s protocol and purified with phenol-chloroform extraction. The reaction was incubated at 37ºC for 30 minutes and followed by 65ºC for 10 minutes in PCR thermo cycler. The RNA was purified by adding 150 µl of phenol:chloroform:isoamyl alcohol (25:24:1, v/v) to the mixture and then centrifuged at 12,000 rpm for 5 minutes at 4ºC to collect supernatant. To precipitate RNA, 0.1 volume of 3M sodium acetate and 0.6 volume of isopropanol were added into supernatant. The mixture was kept at -20ºC for 30 minutes. After that the mixture was centrifuged at 12,000 rpm for 10 minutes at 4ºC to collect the DNA-free RNA pellet. The pellet was washed with chilled 80% ethanol and air dried at room temperature. The pellet was dissolved in 10 µl of DEPC-treated water. The 0.8% agarose gel electrophoresis in 0.5x TBE buffer (see in Appendix A) was

performed to clarify that genomic DNA was removed. The DNA-free RNA concentration and quality were measured using NanoDrop™ 2000 Spectrophotometers.

One microgram of purified RNA was reverse-transcribed to first strand cDNA using iScript Reverse Transcription Supermix for RT-qPCR (Bio-Rad, USA) according to the supplier's protocol. The RNA template (1 µg) was mixed with 4 µl of iScript supermix and the nuclease-free water (supplied in the box) was added to the total volume of 20 µl. The reaction was incubated at 25 ºC for 5 minutes for priming, followed by reverse transcription at 46ºC for 5 minutes and inactivation at 95ºC for 1 minute.

2.1.4. Determination of *trihelix transcription factor* (*GTL1*) expression by quantitative RT-PCR (qRT-PCR)

The qRT-PCR was performed with three biological replicates and three technical replicates for each sample. The qRT-PCR was done in 10 µl reaction using SsoFast™ EvaGreen® Supermix (Bio-Rad, USA) with CFX96™ Real-Time PCR Detection System (Bio-Rad, USA). The reaction contained 2 µl of cDNA , 5 µl of 2x SsoFast™ EvaGreen® Supermix, 0.5 µl of 5 µM forward primer, 0.5 µl of 5 µM reverse primer and 3 µl of sterile water. The thermal cycle was performed at 95 °C for 30 second for enzyme activation, then 40 cycles of denaturation at 95 °C for 5 second, annealing/extension at 57 °C for 5 second and finally, melting curve analysis at 70-95 ºC for 5 seconds. The *OsGTL1* primers were designed from CDS of LOC_Os03g02240 which was retrieved from Rice Genome Annotation Project (Kawahara et al., 2013). The primers for detection of *OsEF-1α* were the same primers as previously indicated (Saeng-ngam et al., 2012). Moreover, the expression of *OsDREB2A* was used as a

control for drought inducible gene and the primer were designed according to the cDNA sequence obtained from NCBI GenBank (AK067313.1). The lists of primer are shown in Appendix B. For qRT-PCR analysis, the expression of *OsGTL1* and *OsDREB2A* were normalized by a housekeeping gene,*OsEF-1α*, in each sample and the level of expression was determined according to Pfaffl method (Pfaffl, 2001), as shown below.

R = Relative expression ratio of target gene

$= [(E_{target})^{\Delta CP\ target\ (control-sample)}] / [(E_{ref})^{\Delta CP\ ref\ (control-sample)}]$

E_{target} = $10^{-1/slope}$ of the target gene

E_{ref} = $10^{-1/slope}$ of the reference gene

$\Delta CP_{target(control-sample)} = CP_{0\ hour} - CP_{\text{any time point of the target gene}}$

$\Delta CP_{ref(control-sample)} = CP_{0\ hour} - CP_{\text{any time point of the reference gene}}$

The CP is defined as the point at which the fluorescence signal rises appreciably above the background fluorescence.

The relative expression level of transcription level was tested by analysis of variance (ANOVA) at $p < 0.05$ with SPSS Statistics 20.0 software (IBM SPSS Modeler) and the means were compared by Duncan's multiple range test (DMRT). The significant difference was accepted at $p \leq 0.05$. The data were shown as mean ± S.E.

2.1.5. Determination of *glyceraldehyde-3-phosphate dehydrogenase* (*GAPDH*) by semi-quantitative RT-PCR

The CDS of *LOC_Os04g38600* retrieved from Rice Genome Annotation Project was used to design the forward and reverse primers (see in Appendix B) to detect *GAPDH* gene expression. A semi-quantitative RT-PCR was conducted in 50 µl of samples using *Taq* DNA Polymerase (Thermo Fisher Scientific, USA). The 50 µl

reaction contained 5 µl of 25 mM $MgCl_2$, 1 µl of 10mM dNTP, 2.5 µl of 2 µM forward primer, 2.5 µl of 2 µM reverse primer, 1 µl of cDNA template, 0.25 µl of *Taq* Polymerase, 5 µl of 10x Taq Buffer with KCl and 32.75 µl of water. The thermal cycler was started at 95°C for 3 minutes, then 35 cycles of 95°C for 30 second, 57°C for 30 second and 72°C for 30 second, followed by a final extension at 72 °C for 5 minutes. *OsEF-1α* was used as the internal control and amplified with the same primer set as indicated above. The *OsDREB2A* primers were used according to (Dubouzet et al., 2003) to serve as drought-responsive gene expression control. In order to check transcription level, the PCR product was run on 0.8% agarose gel electrophoresis (Appendix A).

2.2 Phenotyping of SS and SR lines under drought stress condition

2.2.1 Determination of relative water content (RWC) and stomatal density (SD) in 'LPT123' and 'LPT123-TC171' rice lines

2.2.1.1 Plant growing condition

SS and SR rice seedlings were germinated as mentioned above. After two weeks, each seedling was transferred to grow in clay soil in three-inch diameter plastic pot, which placed in greenhouse under natural light. Plants were grown at 100 % field capacity (FC) for another two weeks before leaf water content and stomatal density (SD) were collected at the first time point. Two treatments of soil water content, 100 % FC (control) and 55-60 % FC (drought treatment), were performed with two rice lines, SS and SR. In order to reach 55-60 % FC, water withholding was performed and FC target was reached in five days after water withholding. Field capacity was maintained by calibrating the water level 3 times a day throughout the experiment.

2.2.1.2 Experimental design and data collection

The experiment to compare the phenotypes, RWC and SD, of SS and SR in normal and drought stress conditions was performed in completely randomized design (CRD) with six biological replicates. Plant tissues were harvested for data collection after 0, 10 and 20 days of treatment. Fresh weight and dry weight of rice shoot from each plant were recorded. RWC was collected from the first fully expanded leaves. After cutting the leaf, it was immediately weighed to get fresh weight. Then, the leaf was placed in 1.5 ml microcentrifuge tube filled with sterilized water for 24 hours. The excess water was removed from the leaf surface before weighting to get turgid weight. Finally, the leaf tissues were dried at 60ºC for three days to get dry weight. RWC was calculated as [(fresh weight − dry weight) / (turgid weight − dry weight)] × 100.

Abaxial stomatal density (SD: number of stomata per area) was also determined in the middle part of first fully expanded leaves that were used to collect the RWC. The abaxial epidermis of rice leaves were attached briefly to a slide by using super glue and then the leaves were pulled-out. Hence, the abaxial epidermis imprint remained on the slide. The stomatal imprinted images were captured with high resolution under 20X objective lens (UPlanApp, Olympus, Japan) coupled with Multipurpose Microscope (Olympus BX-51). The SD was obtained from a leaf area of 0.586 mm^2. Three positions from each imprinted image were used for stomata counting.

2.2.2 Determination of leaf gas exchange parameters in SS and SR lines

2.2.2.1 Plant growing condition

The rice seeds were germinated in growth chamber with distilled water for 3 days and then, transferred to half strength of Yoshida solution (Yoshida et al., 1976). Seven days after that all rice plants were grown in Yoshida solution under natural light condition in a netted-house at the Tropical Vegetable Research Development Center at Kasetsart University, Kamphangsaen Campus, Nakhon Pathom, Thailand. At 30-day-old, rice plants were separated into two groups; control and drought stress. The control plants were maintained in Yoshida solution and the drought-treated plants were cultured in Yoshida solution supplemented with PEG6000. Drought stress condition was applied in two steps. First, rice plants were grown in the nutrient solution containing 12.5 % PEG6000 for a week and then the solution was changed to contain 22.5 % PEG6000 for another week. Each level of the stress was applied to the plants for 7 days.

2.2.2.2 Experimental design and data collection

The experiment was designed in CRD with six biological replicates of SS and SR lines. At the beginning of the experiment, the youngest fully expanded leaf of 30 day-old plant was tagged and it was used for the measurement every 3 days until the end of the experiment. This leaf was called “old leaf” as it was used for the measurement repeatedly. During the experimental period (9 days), a new leaf emerged and became the new youngest fully expanded leaf at the later time point. The new youngest fully expended leaf occurred in any time point was used for the measurement, so it was called “young leaf”.

Before sunrise, the maximum quantum efficiency of PSII (Fv/Fm) and performance index (Pi) were recorded using the Pocket PEA portable chlorophyll fluorimeter (Hansatech Instrument, King's Lynn, United Kingdom). After sunrise, the leaf gas exchange parameters were recorded using LI-6400 Portable Photosynthesis System (LI-COR, Licor Inc., Lincoln, NE, USA) with the LI-6400-40 Leaf Chamber Fluorometer (LI-COR, Licor Inc.). Net photosynthetic rate (*A*) was determined under specific conditions, as follows: saturating light at 1500 µmol PPFD m^{-2} s^{-1} (with 10 % blue light), air CO_2 concentration (C_a) of 400 µmol mol^{-1}, chamber block temperature of 28°C, and relative humidity 70–75 %, resulting in an air vapor pressure deficit of 1.0–1.5 kPa.

2.3 Phenotyping of wild type and *gtl1-4* under drought stress condition

2.3.1 Planting and stress condition

Arabidopsis thaliana (wild type and *gtl1-4* mutant) was used in all experiments conducted in Arabidopsis. The seed of both wild type and *gtl1-4* were embedded in distilled water and kept in dark condition at 4°C for 5-7 days. Then, the seeds were germinated in 115 ml tubes containing soilless media (Fafard 2X Mix soilless media) in a mist house for 10 days. After that all seedlings were transferred to a growth room under short-day conditions (eight hour / day of light period). At the 6-leaf stage which is around four-week-old after germination, plants were separated into two treatments; well-watered (WW) and water-stressed (WS). Well-watered plant was watered as needed and water-stressed plant was stopped watering. All experiments were conducted in CRD with least three biological replicates.

2.3.2 Determination of gene expression by semi quantitative RT-PCR

Five-week-old Arabidopsis was used to study the transcription level with three biological replicates. The leaf tissue was ground in liquid nitrogen until the fine powder was obtained. The RNA was extracted by RNeasy® Plant Mini Kit (Qiagen, USA) according to kit's protocol and followed by elimination of genomic DNA with TURBO DNA-freeTM kit (Life technologies, USA). The DNaseI-treated RNA concentration was measured by NanoDrop™ 2000 Spectrophotometers. After that 500 ng of DNA-free RNA was reverse-transcribed to first strand cDNA using High capacity cDNA Reverse Transcription kit (Life Technologies, USA) according to manufacturer's protocol.

The *GTL1, SDD1, ACT2* primer were retrieved from previous study (Yoo et al., 2010) and *DREB2A* primers were used according to (Liu et al., 1998) (Appendix B). The *ACT2* was used as an internal control. A semi-quantitative RT-PCR experiment was conducted in 20 µl of samples. The semi-quantitative RT-PCR was performed by GoTaq ® Hot Start Polymerase (Promega, USA) according to kit's protocol. The reaction contained 0.2 µl of GoTaq ® Hot Start Polymerase, 4 µl of 5X Green GoTaq® Flexi Buffer, 1.6 µl of 25 mM $MgCl_2$, 0.4 µl of 10 mM dNTP, 2 µl of 2 µM forward primer, 2 µl of 2 µM reverse primer, 2 µl of cDNA template and 7.8 µl of water. The thermal cycle was started at 95°C for 2 minutes, then 30 cycles of 95°C for 30 seconds, 61°C (for *GTL1* and *DREB2A)* or 63°C (for *ACT2*) or 53°C (for *SDD1*) for 45 seconds and 72°C for 45 seconds, followed by a final extension at 72°C for 5 minutes. In order to check transcription level, the PCR product was run on 0.8% agarose gel electrophoresis.

2.3.3 Determination of media water content and relative water content during water deficit

Media water content (MWC) and leaf relative water content were used to monitor the stress level of each treatments. During the experiment, every tube was watered until it saturated. The media saturated weight was recorded at the beginning of the experiment and then, the tubes were weighted throughout the experiment to get fresh weight of media. At the end, the media were dried at 80^{o}C to get a media dry weight. The relative MWC was calculated as [(fresh weight – dry weight) / (saturated weight – dry weight)]. The RWC at each time point was calculated as mentioned in 2.2.1.2. However, to get turgid weight, Arabidopsis leaf was kept in 5 ml vial containing distilled water.

2.3.4 Determination of survival rate in wild type and *gtl1-4*

Wild type and *gtl1-4* were treated with water withholding for 16 days. Ten biological replicates in each time point were performed. The first time point of RWC and survival rate evaluation was 8 days after withholding water. The plants that can/cannot survive were counted and their RWC was determined.

2.3.5 Determination of stomatal density (SD), stomatal index (SI) and leaf development during water deficit

To study stomatal density, stomatal index and leaf development under water deficit, wild type and *gtl1-4* were used. Photograph was taken every day to monitor the leaf development in all plants. The pictures were also used to identify timeline of leaf

development. The MWC was collected as described previously to ensure that targeted leaf was developed before or after the stress.

The stomatal density of abaxial and adaxial epidermis was determined as mentioned above. The whole leaf of Arabidopsis was attached briefly to a slide with super glue. The imprint of abaxial and adaxial epidermis was photographed under Nikon-OptiPhot 2 microscope. Each treatment had at least 13 biological replicates and each replicate was photographed in three different positions. Stomatal index (SI) was calculated from the ratio of stomata to total epidermal cells (including stomata, stomatal precursor cells, and pavement cells). The SD and SI were obtained from a leaf area of 0.1141 mm^2. Stomatal precursor cells were identified as cells at the meristemoid or guard mother cell stage.

CHAPTER IV
RESULTS

1. Proteomics study

1.1. Investigation of protein profiles after drought stress by using proteomic approach

1.1.1 Protein profiles of SS and SR lines

The stress-susceptible rice (SS) and the stress-resistant rice (SR) were grown in WP solution until four-week-old and then each of them were separated into 2 groups; control (WP) and stress-treated (10%PEG). The samples were collect at 0, 2, 6, and 24 hours after stress. Total proteins were extracted and separated by one-dimensional polyacrylamide gel electrophoresis (SDS-PAGE). The tryptic peptides from each gel plug were subjected to LC-MS/MS.

The proteomics analysis was done two times in year 2013 and 2017. The proteomics data was firstly analyzed in 2013. From the GeLC-MS/MS, approximately 1,400 proteins were obtained and there were 352 proteins remained after cut out the false positive. The 352 proteins were statistically analyzed and the gene locus number and their function were identified base on the Rice Genome Annotation Project database (http://rice.plantbiology.msu.edu) (Kawahara et al., 2013). After statistical analysis by MeV, there were 54 and 43 significant different expression in SS and SR, respectively. The significant protein were classified into 10 functional groups in SS and 11 functional groups in SR (Fig. D1 see in Appendix D).

In 2017, the GeLC-MS/MS reveals 4,310 proteins detected in SS and SR. After that the false positive data were cleaned out before actual data analysis were processed.

Then, 1,246 proteins were performed Blastp in the NCBI database (Coordinators 2016) by using their peptide sequences. Only 357 proteins (Table D1. see in Appendix D) were showed highest similarity with rice proteins. Then, 357 proteins were identified a locus ID from MSU and Rice Annotation Project Database (RAP-DB) (Kawahara et al., 2013; Sakai et al., 2013). Most of the proteins (69%) found in both MSU and RAP-DB. Around 22% of the proteins found in MSU and only five percentage found in RAP-DB. Finally, 357 proteins were used for further analysis.

1.1.2 Significant different protein profiles between drought-treated LPT123 (SS) and LPT123-TC171 (SR)

Since SS and SR have the same genetic background, gene/protein expression should behave similarly in the control condition. Therefore, a comparison of proteins found in drought-treated SS and SR were performed. From the analysis, 67 proteins significantly expressed differently in SS and SR ($P \leq 0.05$) (Fig. 1). Their functional groups were categorized into eight groups which are unknown (28%), metabolic process (22%), transcription (16%), defense (12%), retrotransposon (11%), development (5%), signalling (3%), and post-transcription (3%) (Fig. 2A). The number of up-/down-regulated proteins was different in each of the categories. The proteins which were up-/down-regulated in SS when compared to SR are shown in Figure 2B.

Disregarding the unknown function, the largest group of up-regulated protein was involved in transcription (nine proteins e.g. Myb-like DNA-binding domain containing protein, PWWP domain containing protein and Osfbx334 - F-box domain containing protein). The second largest group was metabolic process (seven proteins e.g hydrolase, guanylate kinase and Ulp1 protease family). The other were proteins

involving in defense (four proteins e.g stripe rust resistance protein Yr10, NB-ARC domain containing protein, and peroxiredoxin), retrotransposon (four proteins), development (two proteins; FG-GAP repeat-containing protein and SCAR-like protein 2), post-transcription (two proteins; RNA recognition motif containing protein and PPR repeat domain containing protein), and signaling (one protein; leucine-rich repeat family protein) (Fig. 2B and Table D2 in Appendix D).

The metabolic process related proteins were the largest group of down-regulated proteins. These included glycosyl hydrolases, aspartic proteinase nepenthesin precursor and ribulose bisphosphate carboxylase large chain precursor. Down-regulated proteins categorized into the defense mechanism were AMP-binding domain containing protein, BTBA2 - Bric-a-Brac,Tramtrack, Broad Complex BTB domain with Ankyrin repeat region, CAF1 family ribonuclease containing protein and NBS-LRR disease resistance protein. The other proteins down-regulated in SS belonged to retrotransposon (three proteins), transcription (two protein e.g. DDT domain-containing protein), development (one protein; SWP), and signaling (one protein; receptor-like protein kinase 5 precursor) (Fig. 2B and Table D2 in Appendix D).


0
10
20
SS + PEG6000
SR + PEG6000
Hour(s)
0 2 6 24 0 2 6 24
Replicate
1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3
Description
Retrotransposon protein
Retrotransposon protein, putative, Ty3-gypsy subclass
Expressed protein
ZOS4-02 - C2H2 zinc finger protein
SCAR-like protein 2
RNA recognition motif containing protein
Helix-loop-helix DNA-binding domain containing protein
Ulp1 protease family, C-terminal catalytic domain containing protein
Expressed protein
Hypothetical protein
Expressed protein
Protein of unknown function DUF803 family protein
Guanylate kinase
ISA3, ISOAMYLASE_3
SET domain containing protein
BTBA2
Hypothetical protein
FG-GAP repeat-containing protein
Expressed protein
Stripe rust resistance protein Yr10
Oxidoreductase, 2OG-Fe oxygenase family protein
Expressed protein
Unknown
RING finger and CHY zinc finger domain-containing protein 1
Retrotransposon protein
Hydrolase, NUDIX family, domain containing protein
Expressed protein
WD domain, G-beta repeat domain containing protein
Leucine-rich repeat family protein
Expressed protein
Osfbx320 - F-box domain containing protein
Retrotransposon protein
Hypothetical protein LOC_Os12g23849
Enolase
Expressed protein
PPR repeat domain containing protein
NB-ARC domain containing protein,
Peroxiredoxin
PWWP domain containing protein
Osfbx334 - F-box domain containing protein
GDSL-like lipase/acylhydrolase
Hypothetical protein OsJ_34111
Hypothetical protein osi_35919
Myb-like DNA-binding domain containing protein
Unknown
DNA-directed RNA polymerase III subunit RPC1
NBS-LRR disease resistance protein
SWP
Retrotransposon protein
Retrotransposon protein
ATROPGEF7/ROPGEF7
Glycosyl hydrolases
Ribulose bisphosphate carboxylase large chain precursor
AMP-binding domain containing protein
S1 RNA binding domain containing protein
Lipase
Hypothetical protein osj_21571
Retrotransposon protein
Receptor-like protein kinase 5 precursor
BTBA2
DDT domain-containing protein
Expressed protein
Mur ligase family protein
Aspartic proteinase nepenthesin precursor
Expressed protein
Glycosyl hydrolase family 5 protein
CAF1 family ribonuclease containing protein
-1.172215
-0.586107
-0.0

Figure 1. Expression profile of the significantly different proteins found in the comparison of the proteins expressed in SS and SR under drought stress. MultiExperiment Viewer (MeV) software was used to create the heat map. The heat map shows significant up- or down- regulated leaf proteins under 10% PEG 6000 for 0, 2, 6 and 24 hr. Each column represents treated-time and each row represents an individual protein. Light green to dark red bars indicate low to high protein abundance. Sixty seven proteins were found to be significantly different ($P < 0.05$). The identified proteins are listed in Table D2 in Appendix D.

When we compared the protein profiles of SS and SR in normal grown condition, a number of significant proteins was found (Table D2 see in Appendix D). This revealed that we could not assume the similar protein expression profile of SS and SR in normal grown condition. In order to identify the drought-responsive proteins in SS and SR, the comparison among the significant protein profiles changed by drought stress in each line was performed. Then, the list of significant different expressed proteins was compared between lines.

After analysis with the MeV program, 68 and 55 proteins from the SS and SR lines, respectively, were significantly changed in stressed plants relative to their levels in untreated control plants ($P \leq 0.05$) (Fig. 3 and 4). The list of all significant proteins is shown in Table D2 (Appendix D). Surprisingly, only six drought responsive proteins were found in both rice lines (Fig. 5). Three proteins including helicase domain-containing protein, cytochrome P450, and stripe rust resistance protein Yr10 were significantly up-regulated in SS and SR according to drought stress. The other three proteins, BTBA2-Bric-a-Brac, Tramtrack, Broad Complex BTB domain with Ankyrin repeat region, DDT domain-containing protein and NBS-LRR disease resistance protein are expressed contrarily. All of them were up-regulated in the SR line, but down-regulated in the SS line.

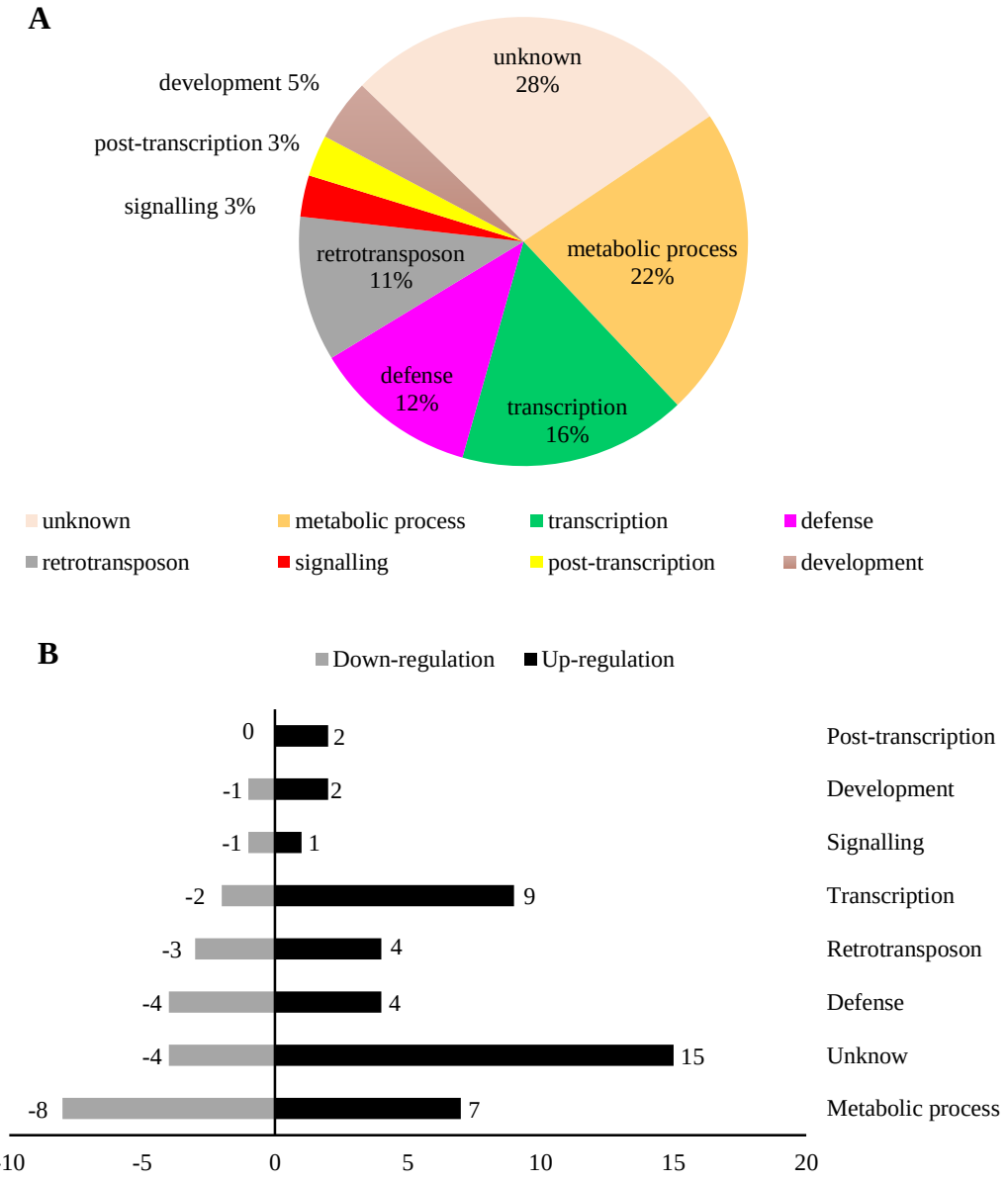

Figure 2. Functional classification of proteins from a comparison between SS and SR line under drought stress (A) and the number of down-and up-regulated proteins in each functional group (B). The negative sign and grey bar indicates down-regulated proteins and the black bar indicates up-regulated proteins. Number of proteins are presented at the end of bar. The functional groups were categorized according to Gene Ontology annotations from the Rice Genome Annotation Project (http://rice.plantbiology.msu.edu).

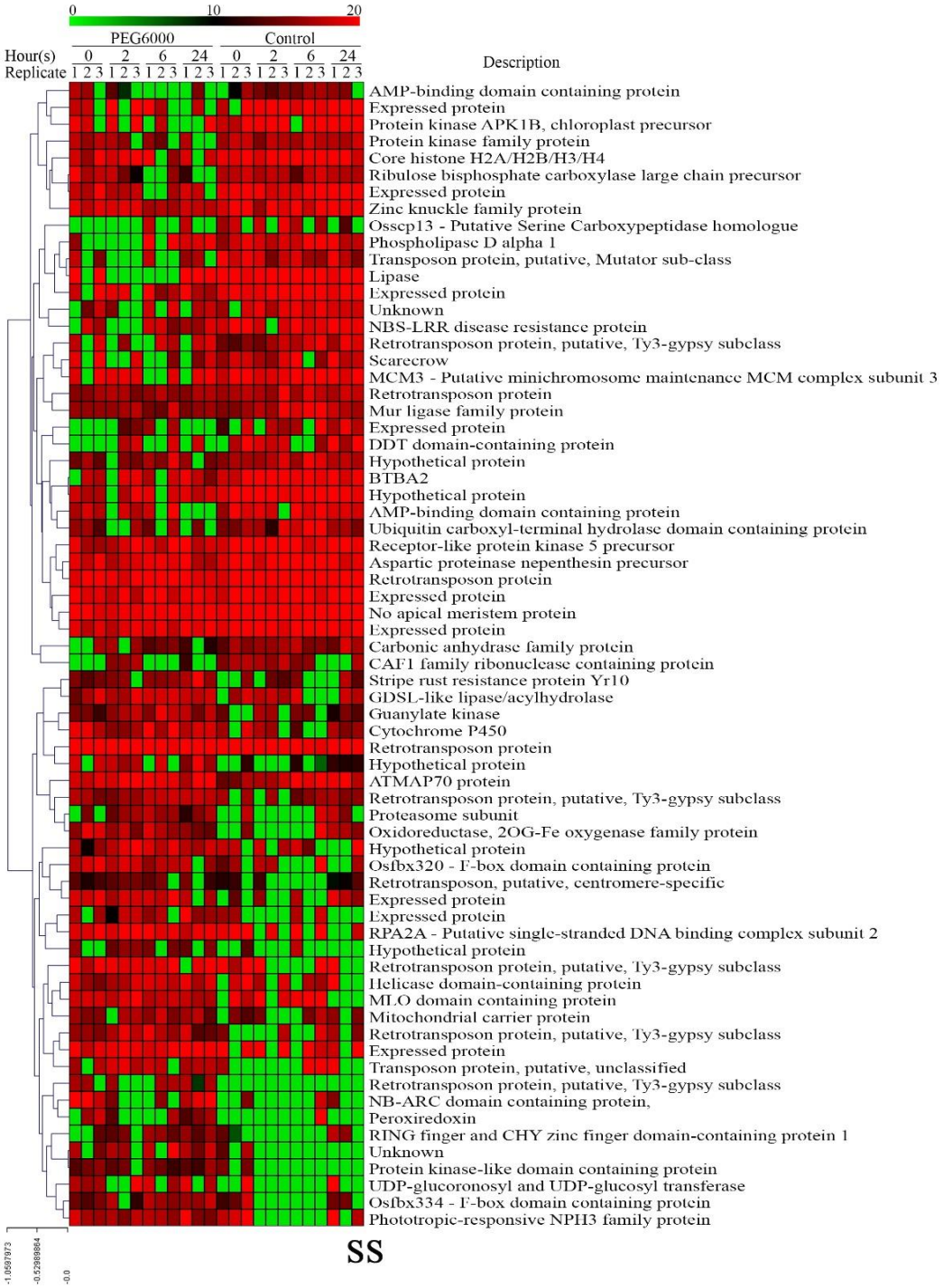

Figure 3. Expression profile of the significantly different expressed proteins found in the comparison of the proteins expressed in plants grown under normal and drought stress condition in SS. MultiExperiment Viewer (MeV) software was used to create the heat map. The heat map shows significant up- or down- regulated leaf proteins under 10% PEG 6000 for 0, 2, 6 and 24 hr. Each column represents treated-time and each row represents an individual protein. The light green to dark red bars indicate low to high

protein abundance. The significant different expressed proteins are listed in Table D2 in Appendix D. The significant difference was cut at $P< 0.05$.

The significant different expressed proteins were categorized by their functions from the Gene Ontology annotations (Rice Genome Annotation Project). The significant different expressed proteins in SS were categorized into ten functional groups which are unknown (24%), metabolic process (16%), retrotransposon (13%), defense (13%), transcription (12%), signalling (10%), cellular process (6%), transposon (3%), post-translation (1%) and transport (1%) (Fig. 6A). The categories of cell wall and post-translation were the two groups found only in SS line.

Disregarding the unknown group, the biggest group of up-regulated protein under drought stress were involved in retrotransposon (six proteins) and followed by metabolic process (five proteins e.g. UDP-glucoronosyl and UDP-glucosyl transferase, guanylate kinase and cytochrome P450) and transcription (four proteins e.g. Osfbx320- and Osfbx334- F-box domain containing protein). Defense had four proteins in this group. For example, stripe rust resistance protein Yr10, MLO domain containing protein and peroxiredoxin were categorized into the defense mechanism. The other proteins were involved in signaling (two proteins), cellular process (two proteins), post-translation (one protein), transport (one protein) and transposon (one protein) (Fig. 7A and Table D2 in Appendix D).

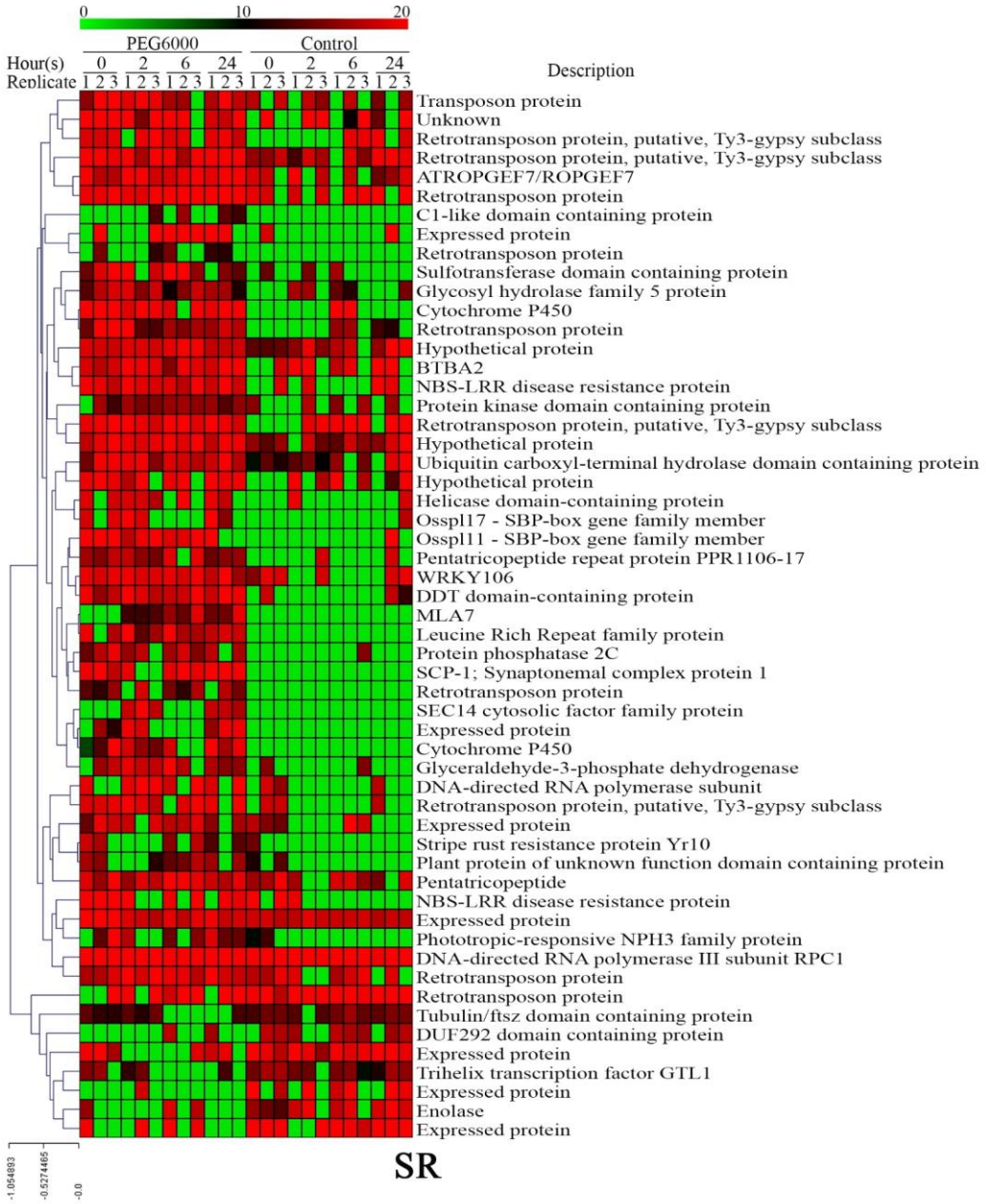

Figure 4. Expression profile of the significantly different expressed proteins found in the comparison of the proteins expressed in plants grown under normal and drought stress condition in SR. MultiExperiment Viewer (MeV) software was used to create the heat map. The heat map shows significant up- or down- regulated leaf proteins under 10% PEG 6000 for 0, 2, 6 and 24 hr. Each column represents treated-time and each row represents an individual protein. The light green to dark red bars indicate low to high protein abundance. The significant different expressed proteins are listed in Table D2 in Appendix D. The significant difference was cut at $P< 0.05$.

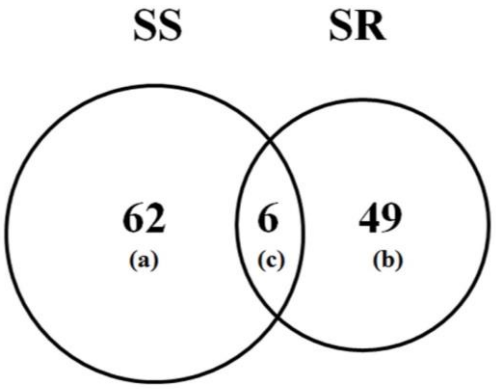

Figure 5. Venn diagram of drought-responsive proteins in SS and SR. There were 62 (group a) and 49 (group b) identified proteins present only in the SS or SR lines, respectively. Six (group c) identified proteins were detected in both lines.

For the down-regulated proteins in SS, the largest group of proteins was involved in the metabolic process such as aspartic proteinase nepenthesin precursor, lipase and carbonic anhydrase family protein. The others were proteins involved in defense (five proteins e.g. AMP-binding domain containing protein, NBS-LRR disease resistance protein and BTBA2 - Bric-a-Brac,Tramtrack, Broad Complex BTB domain with Ankyrin repeat region), signalling (five proteins e.g. protein kinase family protein, receptor-like protein kinase 5 precursor and Osscp13 - putative serine carboxypeptidase homologue), transcription (four proteins e.g. zinc knuckle family protein and DDT domain-containing protein), retrotransposon (three proteins), cellular process (two proteins) and transposon (one protein) (Fig. 7A and Table D2 in Appendix D).

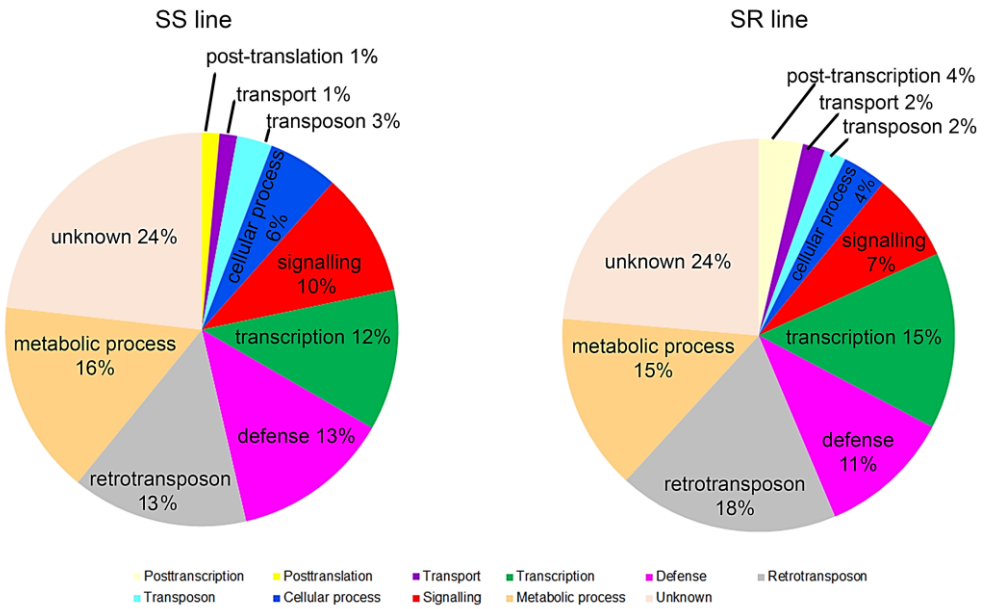

Figure 6. Functional classification of drought-responsive proteins detected in SS and SR rice leaves. The functions were categorized according to Gene Ontology annotations from the Rice Genome Annotation Project (http://rice.plantbiology.msu.edu).

The proteins in rice resistant line (SR) were categorized into ten groups. The number of proteins associated with unknown (24%), retrotransposon (18%), metabolic process (15%), transcription (15%), defense (11%), signalling (7%), cellular process (4%), post-transcription (4%), transport (2%), and transposon (2%) were discovered (Fig. 6B). Disregarding the unknown protein group, retrotransposon function was the main group of proteins affected by osmotic stress in SR plants. The percentage of the genes in categories of transport, transcription and retrotransposon was higher in SR line, suggesting the importance of changes in these functions for drought tolerance (Fig. 6). In addition, the post-transcription group was the category found only in SR plants.

Among proteins that were up-regulated in SR, those involving in transcription (seven proteins e.g. trihelix transcription factor GTL1, Osspl11 - SBP-box gene family member and WRKY106) and retrotransposon (nine proteins) were two main groups.

Seven proteins were found in metabolic process e.g. Sulfotransferase domain containing protein, Cytochrome P450 and Ubiquitin carboxyl-terminal hydrolase domain containing protein. The other proteins were related to defense (six proteins), signalling (four proteins), post-transcription (two proteins), cellular process (one protein), transport (one protein) and transposon (one protein) (Fig. 7B and Table D2 in Appendix D).

Down-regulated proteins in SR were only found in eight proteins from 55 significant proteins. The proteins belonging to unknown protein were the largest group (four proteins) and the others functions were only one protein in each function; cellular process, metabolic process, transcription and retrotransposon (Fig. 7B and Table D2 in Appendix D).

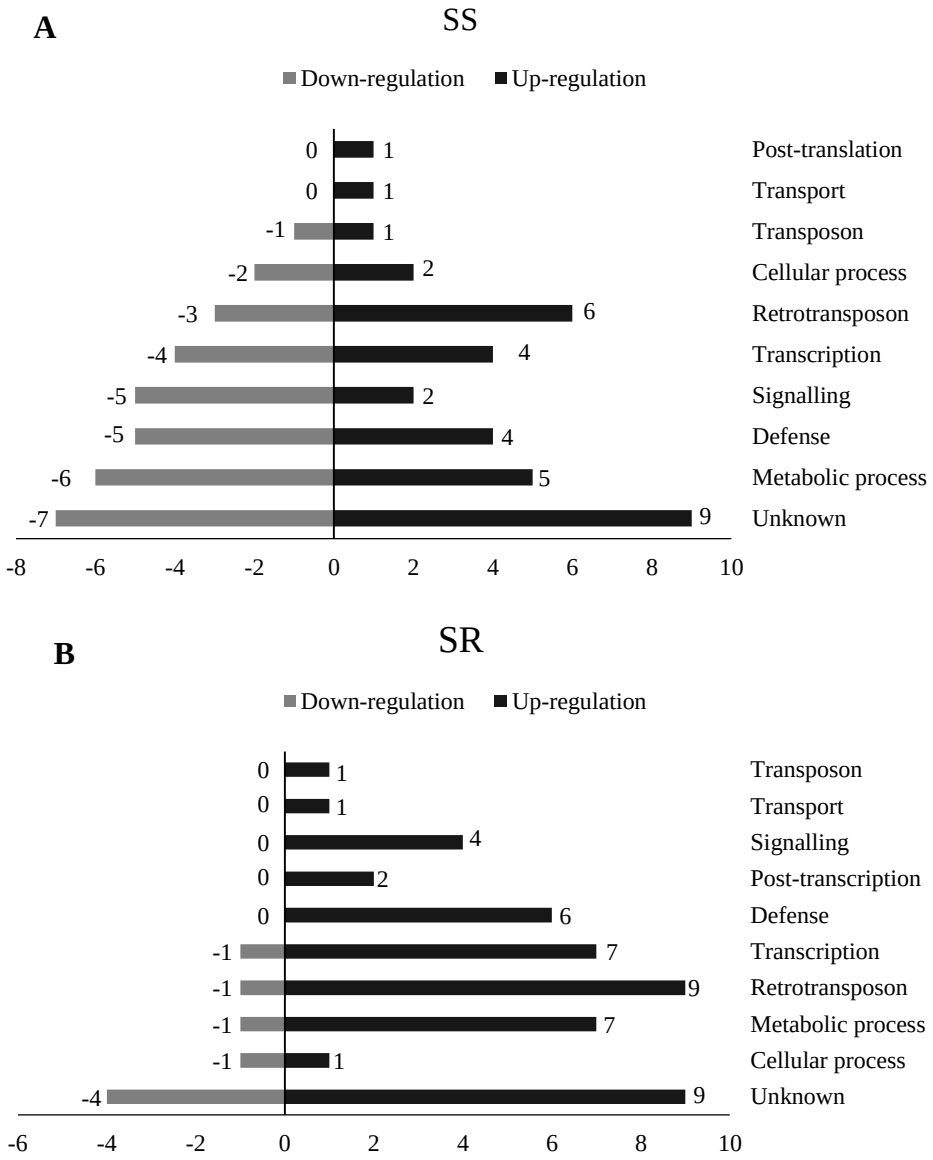

Figure 7. Number of down-and up-regulated protein in each category found in SS (A) and SR (B). The negative sign and grey bar indicates down-regulated proteins and the black bar indicates up-regulated proteins. Number of proteins are presented at the end of bar.

1.2 Comparison of SS and SR proteomics data

Due to the different assumption of the expression in normal condition, two different approaches for protein profile comparison were performed. The assumption of the similarity of SS and SR lines' expression in normal condition revealed 67 proteins different between the two lines (Group 1).

The comparison based on the assumption of different protein profiles in SS and SR lines in normal condition revealed 111 different proteins. Comparison for drought responsive proteins in SS, 68 proteins were found significantly difference and this group was called as Group 2. In SR, there were 55 proteins significantly changed because of drought stress and was called as Group 3. And 6 proteins were found in both SS and SR lines as described above.

The Venn's diagram was done to show the overlapping data of all three groups. Only four proteins were found commonly in all analyzes. Interestingly, group 1 and 2 shared 25 proteins together. The majority of this group was metabolic process. Group one and three shared only eight proteins. Interestingly, group three shared fewer proteins with the other groups (Fig. 8). Based the Venn's diagram, 78 proteins could not be detected by the comparison of the drought treated profiles only and 30 proteins could not be detected if we the significant drought responsive protein in each line before the comparison between lines. Taken together, combining both comparison methods may contribute to the overall significant proteins that should be considered as the contributors for drought tolerance.

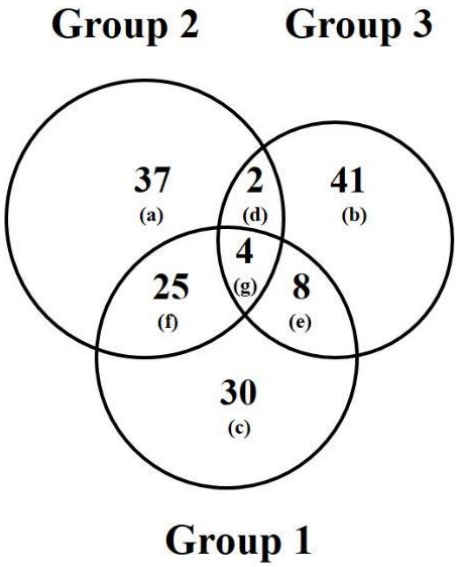

Figure 8. Venn diagram of significant different expressed proteins in all comparison methods. There were 37 (group a), 41 (group b) and 30 (group c) identified proteins present in each calculation, respectively. The comparison of control and drought treatment in SS and SR found two common proteins (group d). There were eight proteins (group e) found commonly between group one and group three only and 25 proteins (group f) found commonly in group one and group two only. Four (group g) identified proteins were detected in all.

2. Identification and characterization of the drought responsive gene(s)

2.1. Selection and expression analysis of the drought responsive genes in 'LPT123' and 'LPT123-TC171' rice lines

2.1.1. Co-expression analysis

Based on the comparison of drought responsive genes in SR lines, 55 proteins were detected. These loci were analyzed with co-expression network analysis using the RiceFrend (Sato et al., 2013). Seven proteins node presented in the co-expression network are shown in Fig. 9. The seven proteins with the co-expression network were transcription factor GTL1 (A), cytochrome P450 (B), GAPDH (C), LOC_Os08g17020 (expressed protein, D), tubulin/ftsz domain containing protein (E), cytochrome P450 (LOC_Os10g05020) (F), and stripe rust resistance protein Yr10 (G). Node F and G were also expressed in SS line, while node A-E were the proteins significantly changed

in SR line only. All of these proteins have been reported that they are involved in stress response and have interesting function. The list of genes that are presented in each main node were presented in Table D3-D9 see in the Appendix D.

Overall, two identified networks are very interesting network. GTL1 and GAPDH are two proteins that have high complexity network and KEGG function. GTL1 is an only network that show a connection with transcription factor. It has been proposed to regulate stomata development which is a first stage for preventing water loss. GAPDH showed the connection to metabolic pathways, especially the genes in photosynthesis. This suggests the importance of the GAPDH function in response to osmotic stress.

OsGTL1 (LOC_Os03g02240) is a transcription factor (indicated as red box) and interact with two other transcription factors (LOC_Os10g37240 and LOC_Os02g43300) which are their orthologs, surprisingly. Yoo et al. (2010) reported that *AtGTL1* is involved in stomatal development regulation which enhances drought tolerance ability in Arabidopsis. However, there have been no reports for rice and OsGTL1 is closely related to AtGTL1 (Weng et al., 2012). The OsGTL1 level in SR was significantly reduced after treated with 10% PEG. In contrast, OsGTL1 in SS trended to increase compared to control group (Fig. 10A). A comparison of protein expression pattern with the microarray database (GSE6901) found that a lower expression of *OsGTL1* under drought stress. However, *OsGTL1* did not change their expression because of salt stress while increased the expression in cold stress (Fig. D2.B see in Appendix D). This result was consistent with the proteomic data that SR has lower expression after osmotic stress (Fig. 10A).

Glyceraldehyde-3-phosphate dehydrogenase (GAPDH; LOC_Os04g38600) is well known to play an important role in the photosynthetic processes and associated with drought tolerance in many plant species such as wheat(Cheng et al., 2015), *Thellungiella halophila* (Chang et al., 2015) and potato (Kappachery et al., 2014). In addition, among the 55 proteins tested for co-expression network, GAPDH is the only gene showing the connection to metabolic pathways, especially the genes in photosynthesis. Protein expression level of GAPDH was significantly higher after 2 hours of stress in SR while SS showed a similar trend of GAPDH expression compared to the control (Fig. 10B). Base on microarray database, *GAPDH* had extremely high expression under control condition but it reduces in the stress treatment (Jain et al., 2007) (Fig. D2.D see in Appendix D). However, the proteomic data of SR showed an opposite direction. GAPDH of SR line was up-regulated under osmotic stress (Fig. 10B).

Since OsGTL1 and GAPDH have a potential for being a candidate to crops improvement against drought stress, these two genes were selected for further analysis.

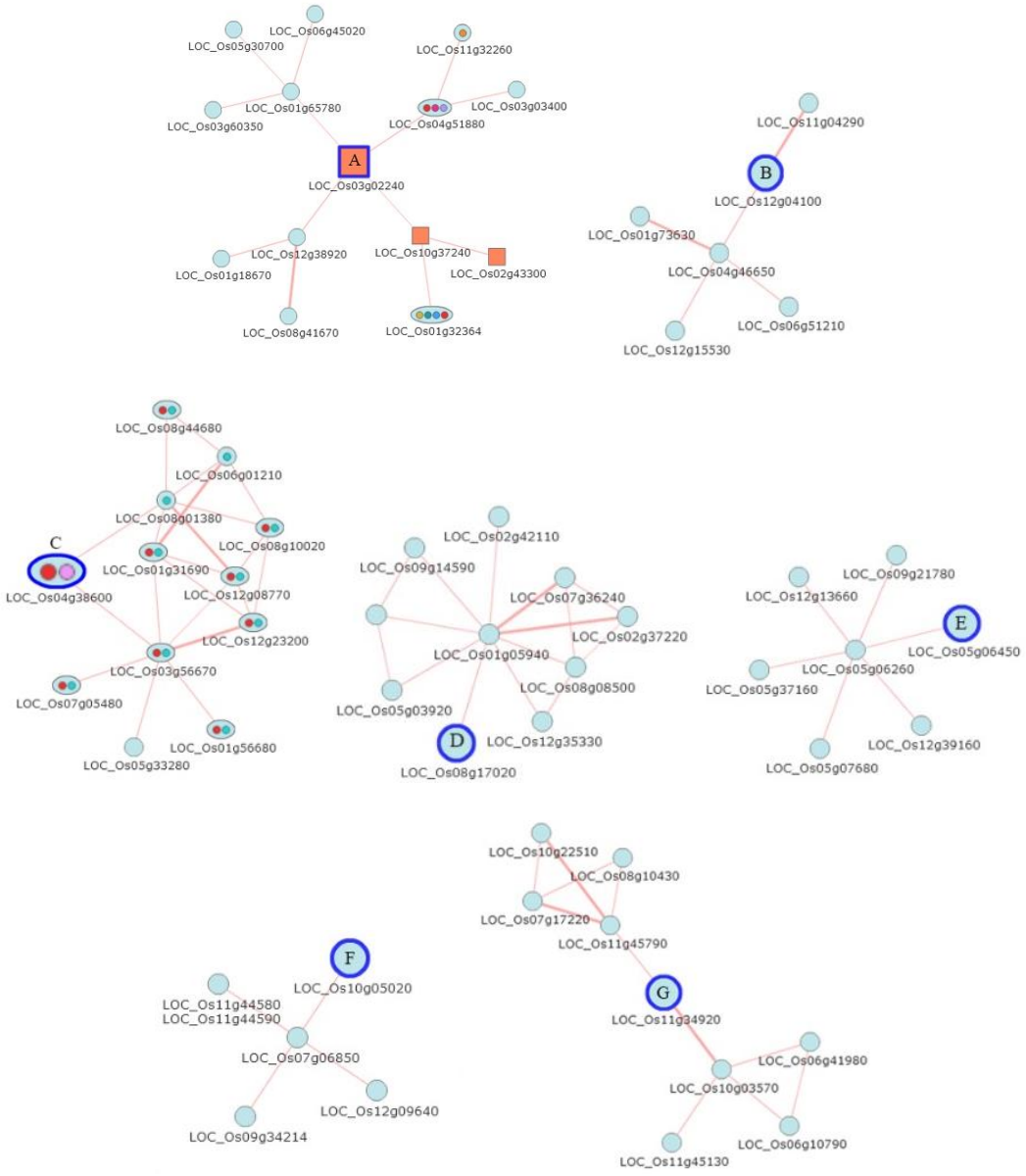

Figure 9. Co-expression networks of the significant changed proteins from SR line. A-G indicate the genes are significantly expressed in SR lines. Squares represent the transcription factors. Blue circles indicate nodes in the network, while the green, red and pink circles in the ellipses represent the metabolic pathways in which the node genes (ellipses) are involved.

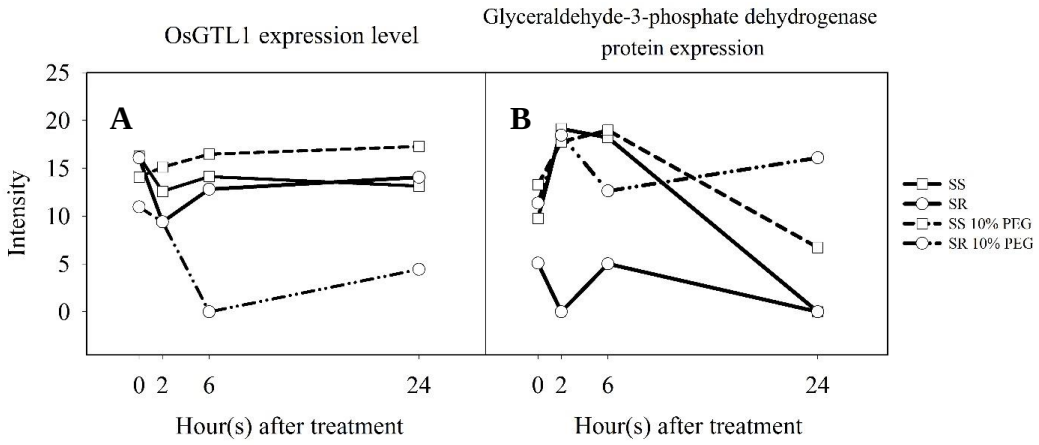

Figure 10. Protein expression patterns of OsGTL1 and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) after 10% PEG6000 treatment for 0, 2, 6, and 24 hours. Expression level of four-week-old SS (open square) and SR (open circle) leaves in normal (solid line) and drought stress (dash line), based on proteomic analysis.

2.1.2. Determination of *trihelix transcription factor, GTL1* by qRT-PCR

The effect of drought on an expression of *GTL1* was monitored with qRT-PCR in the first step. Rice seedling (one-week-old) were used in this experiment. According to microarray database (GSE6893) in Rice eFP Browser, *LOC_Os03g02240* is expressed very low in mature and young leaves (Fig D2.A see in Appendix D). The seedling shoots were cut and dried on the bench lab for 2 hours to create a dehydration stress condition. It was found that relative expression of *OsGTL1* transcript in SR leaves was significantly decreased after dehydration while the relative expression of *OsGTL1* in SS leaf tissues was increased (Fig. 11A). *DREB2A* was used as osmotic stress-inducible reference gene. In the dehydration stress, the relative expression of *DREB2A* induced in both SS and SR. The data were normalized by *EF1-alpha* expression. This data suggested that drought stress induced *OsGTL1* gene expression differently in SS and SR led to the difference in drought tolerant ability.

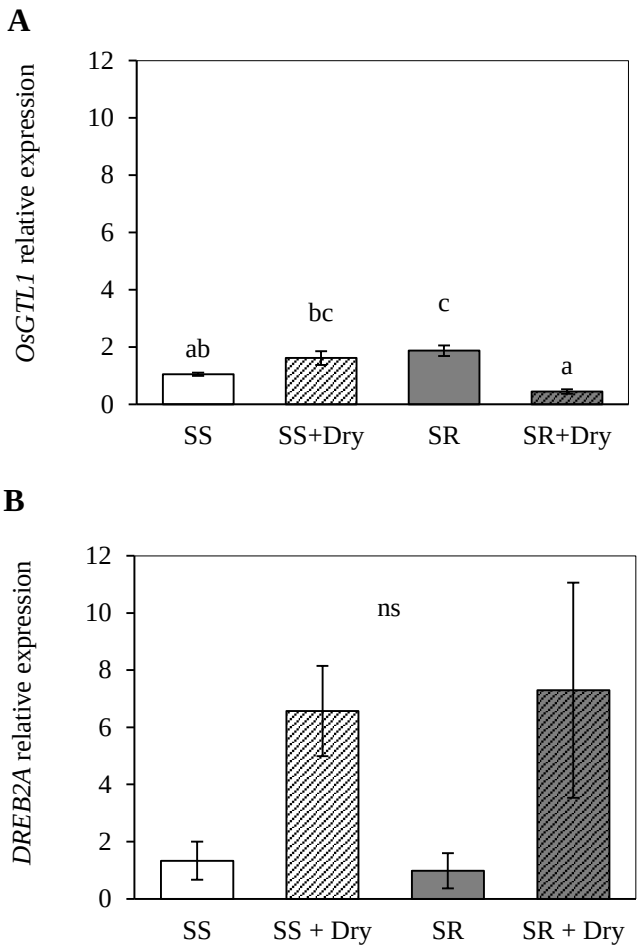

Figure 11. Relative expression of *OsGTL*1 and *DREB2A* transcripts. SS (white bar) and SR (grey bar) leaves of seven-day-old seedlings were used to perform qRT-PCR in normal (solid color) and dehydrated (dry) conditions (upward diagonal fill). The different letters above the bars represent the significant difference of the mean at $p<0.05$, analyzed with DMRT. Error bars present SE of each experiment.

2.1.3. Determination of *glyceraldehyde-3-phosphate dehydrogenase* (*GAPDH*) by semi qRT-PCR

The semi-quantitative RT-PCR was performed to validate the expression of *GAPDH* genes. *EF1-α* were also used as an internal control, while *DREB2A* served as a stress-responsive gene control. In the control plants, there were no expression of *GAPDH* in SS but low intensity of expression in SR. The *GAPDH* expression was up-regulated in both SS and SR leaves treated with air-dry for 2 hours, however; the increase of gene expression was greater in the SR leaves (Fig. 12). This was consistent with the increase in GAPDH protein abundance during our proteome-level analysis (Fig. 10B). These observations indicate that these genes are regulated at the transcriptional level in response to dehydration.

A gene which encode Ferredoxin-NADP reductase (FNR) was also investigated as it functions in photosystem I which regulates plant NADP(H) levels. Both GAPDH and FNR play an important role in the photosynthetic process which protects from photosystem damage by balancing the NADP(H) level. However, the *FNR* expression was similar in all treatments except in the drought condition of SR which slightly decreased (Fig. 12).

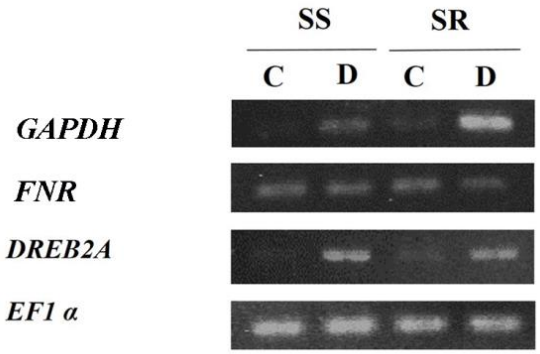

Figure 12. Semi-quantitative expression of *GAPDH, FNR, DREB2A* and *EF1-α* in SS and SR leaf. Seven-day-old rice were used. Control plants are indicated as C and cut and air-dried are indicated as D. The DREB2A was used as a stress indicator and EF1alpha was used as a housekeeping gene.

2.2. Function analysis for drought resistant ability

2.2.1. Determination of relative water content (RWC) and stomatal density (SD) in SS and SR rice lines

The phenotype the phenotype analysis of SS and SR was conducted to clarify drought tolerance ability of these two rice lines. In this study, all the rice plants were cultivated in soil pots. Fresh weight (FW), dry weight (DW), stomatal density (SD) and leaf relative water content (RWC) of SS and SR were monitored. Rice plants at four-week-old were used. In drought treatment, the experimental plants were not watered. Water withholding was stopped when the soil reached 55-60 % field capacity (FC) (approximately 5 days), and this FC was maintained to the end of experiment (20 days) by addition of water daily. The limited water led to significant growth reduction in both rice lines, compared to the plant grown in well-watered conditions (Fig. 13A and B). After 10 and 20 days of stress, shoot and root fresh weight of SS and SR were significantly reduced by the drought stress. However, SR showed significant higher

shoot FW (Fig. 13A) and DW (Fig. 13C) than SS after 20 days of drought stress. Shoot (Fig. 13C) and root (Fig. 13D) dry weight also showed the similar responses to fresh weight due to drought treatment. Therefore, this data confirmed that these two rice lines displayed contrasting growth responses to drought stress.

Leaf relative water content and stomatal density of SS and SR rice were investigated following a previous study of Yoo et al. (2010). The researchers showed that *GTL1* is a positive regulator of SD which also affects RWC under drought stress. The youngest fully expanded leaf was used to collect RWC and SD at each timing. After 10 days of drought stress, only RWC of drought-treated SS leaf was significantly lower compared to other treatments while drought treated SR still had similar RWC with the normal condition (Fig. 13E). RWC of SS and SR leaves were significantly reduced after 20 days of drought stress. However, the maintenance of RWC was higher in SR than SS lines (Fig. 13E).

For the stomatal density study, the youngest fully expanded leaves of drought treated SR showed a significant lower SD, when compared to SS and untreated-SR leaves (Fig. 13F). After 20 days of drought stress, SD of SS became lower but was not significantly different from normal conditions, while SD of SR was shown to be significantly lower than normal grown plants (Fig. 13F). In addition, the stomata imprint of all treatments were obvious that drought treated SS had lower SD than other treatments (Fig. 14). These data showed that *OsGTL1* might play a crucial role in regulating stomatal density during drought stress.

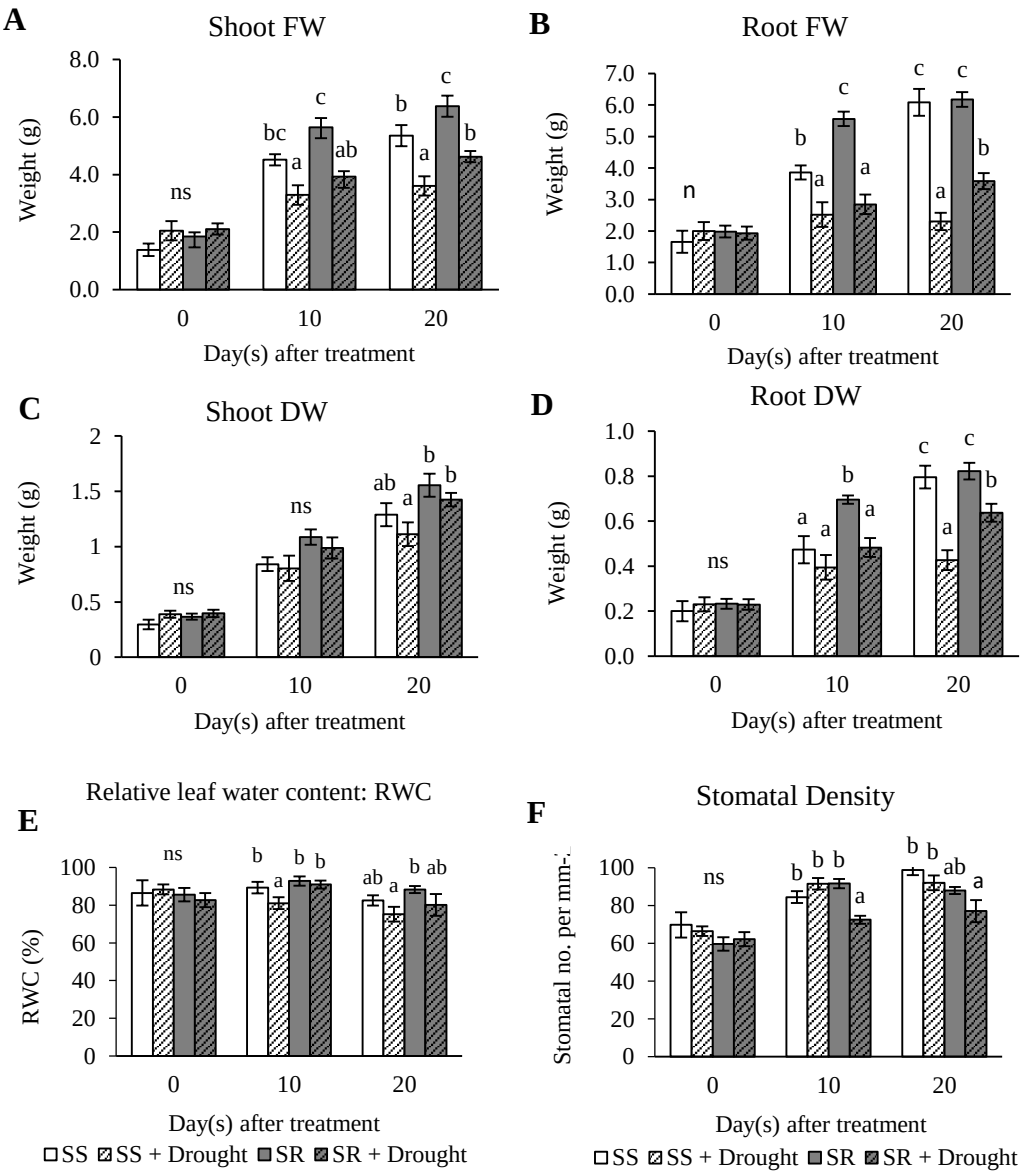

Figure 13. Growth and physiological responses to drought stress of SS and SR. An average fresh weight of shoots (A) and roots (B), dry weight of shoots (C) and roots (D), leaf relative water content (E) and stomatal density (F). Four-week old SS (white color) and SR (grey color) were planted in soil. Control plants were well-watered (plain color) and drought-treated plants were maintained at 55-60 % field capacity (FC) (upward diagonal fill). Data were collected on day 0, 10 and 20 after treatment. The different letters above the bars represent the significant difference of the mean at $p<0.05$, analyzed with DMRT. Error bars present SE.

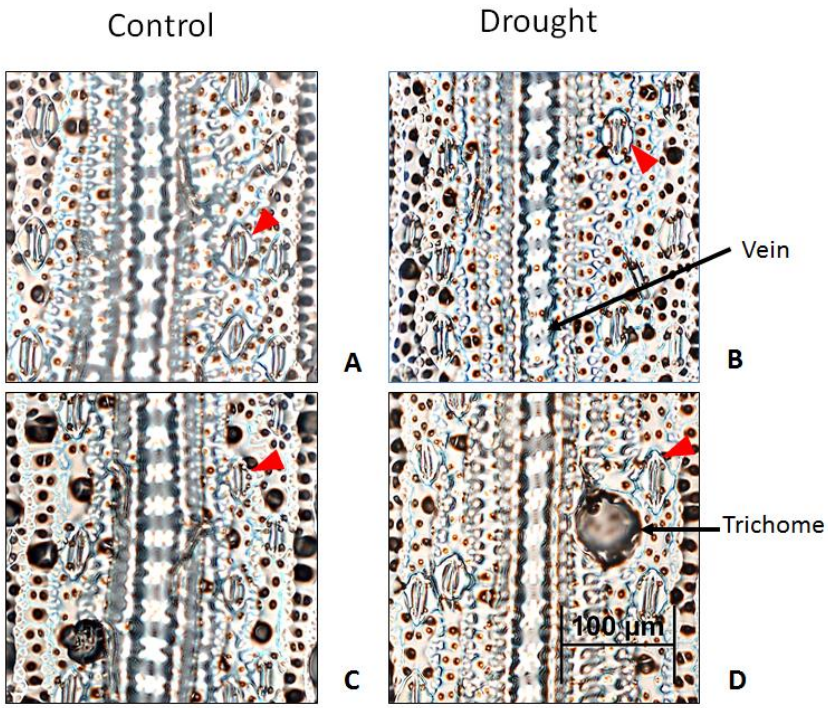

Figure 14. Images of abaxial surface imprint of SS (A and B) and SR (C and D) grown in normal (A and C) and drought (B and D) conditions. Plants were grown as described in Figure 11. The imprint was obtained from the middle part of the youngest fully expanded leaves after 20 days of the treatments. Stomata are shown at the red arrow tips. Vein and trichome images were also captured in the imprint as shown.

2.2.2. Determination of leaf gas exchange parameters in SS and SR rice lines

The photosynthesis process is one factor that can be altered by drought stress. Therefore, the photosynthesis parameters were measured. As mentioned in material and method section, the leaf gas exchange parameters were measured in two type of leaf; old leaf and young leaf. The old leaf was the youngest fully expanded leaf at day 0 and was measured repeatedly throughout the experiment, while, the young leaf was the newly youngest fully expended leaf at any time point of the measurement.

2.2.1.1. Effect of drought stress in the old leaf

In the old leaf, net photosynthesis rate (*A*) in SS and SR was significantly reduced after drought stress. *A* was extremely reduced after 3 day of drought stress from 31.76 to 7.09 µmol CO_2 m^{-2} s^{-1} in SS line and from 29.39 to 13.90 µmolCO_2 m^{-2} s^{-1} in SR line. SS and SR lines had similar net photosynthesis rates in each timing except for 3 days of the stress which SR line had higher net photosynthesis rate than SS line. The stress-treated rice almost died after 9 days of drought stress, so net photosynthesis rate was very low in both rice lines (1.99 µmolCO_2 m^{-2} s^{-1} in SS and 1.96 µmolCO_2 m^{-2} s^{-1} in SR) (Fig. 15A).

Another parameter is stomatal conductance or g_s which can refer to open/close stomata. Water enters the plant via stomata thus g_s and transpiration (*E*) values are frequently related. In this study, g_s and *E* also showed similar patterns (Fig. 15B and C). At the beginning of the experiment g_s of all leaves were similar, but after 3 days of drought treatment, g_s and *E* of both lines were significantly decreased (Fig. 15B and C). However, after 6 days of the treatment, g_s and *E* were increased back to the similar level of the normal grown plants, and then after 9 days, the significant lower of g_s and *E* were detected. The reverse of g_s and *E* were consistent with the tendency of A (Fig. 15A).

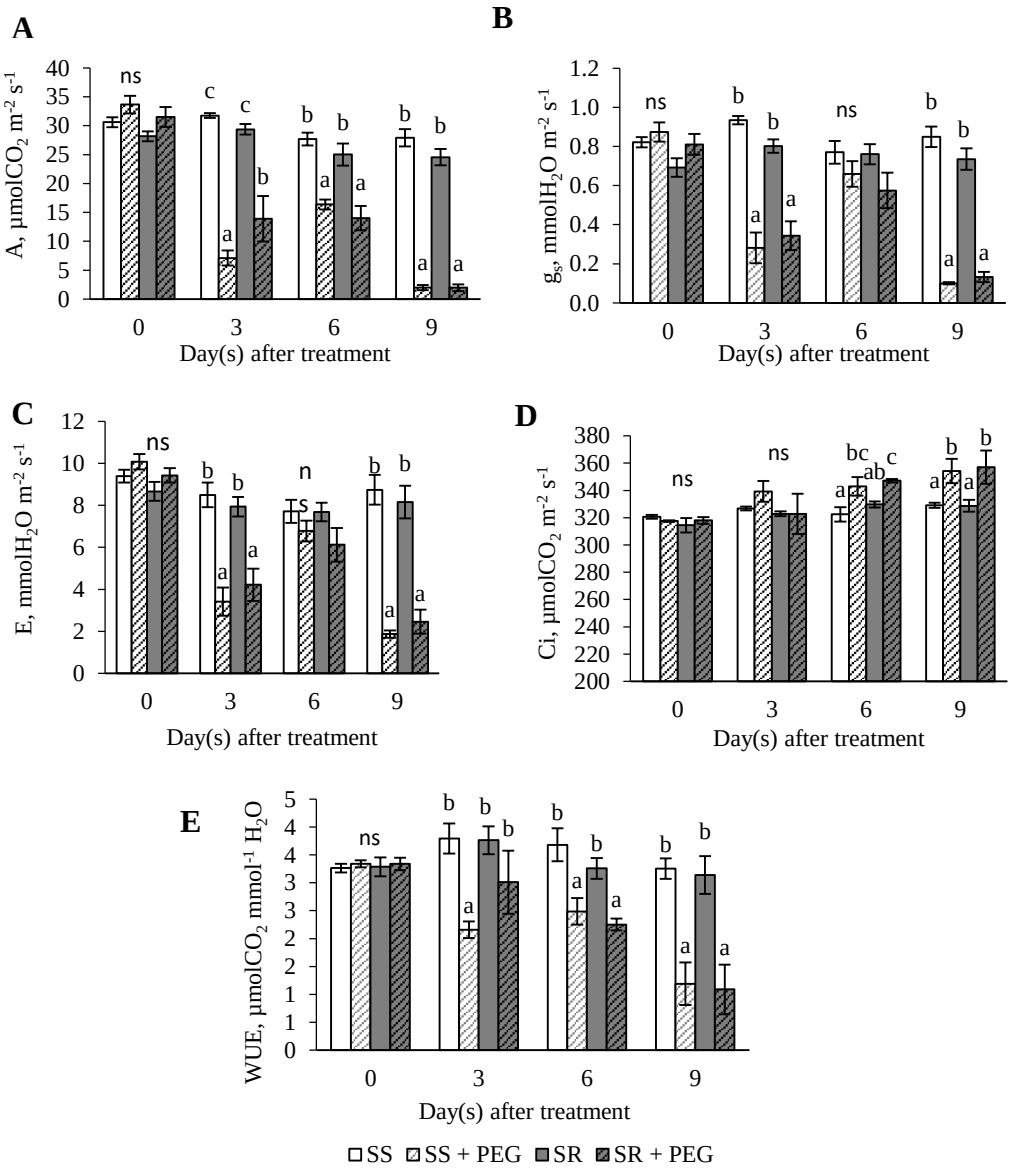

Figure 15. Photosynthesis parameters were measured in the old leaf. A net photosynthetic rate (*A*) (A), stomatal conductance (g_s) (B), transpiration rate (E) (C), intercellular CO_2 concentration (Ci) (D) and water use efficiency (WUE) (E)) of 4-week-old SS and SR. SS and SR are presented in white and grey color, respectively. Plain color represents control condition which grown in half strength Yoshida. Upward diagonal fill represents the drought stress condition as PEG600 was added to the solution. The different letters above the bars represent the significant difference of the mean at $p<0.05$, analyzed with DMRT. Error bars present SE.

At day 0 and 3, intercellular CO_2 concentration (Ci) did not change in the drought stress treatment of SS and SR. It was maintained at around 310- 340 $\mu molCO_2$ m^{-2} s^{-1} in all conditions whereas Ci significantly increased after 6 and 9 days after the drought stress. This increased from 322.51 $\mu molCO_2$ m^{-2} s^{-1} to 342.93 $\mu molCO_2$ m^{-2} s^{-1} in SS and from 329.71 $\mu molCO_2$ m^{-2} s^{-1} to 347.14 $\mu molCO_2$ m^{-2} s^{-1} in SR at day 6. At the last day of stress, Ci in the stress-treated plant grow bigger than day 6, at 354.19 $\mu molCO_2$ m^{-2} s^{-1} in SS and 357.05 $\mu molCO_2$ m^{-2} s^{-1} in SR (Fig. 15D).

Under normal condition, all the plants could maintain water use efficiency (WUE) to the level of approximately 3 $\mu molCO_2$ $mmol^{-1}$ H_2O in both rice lines. After 3 days of the treatment, only PEG-treated SS had significantly lower WUE (2.16 $\mu molCO_2$ $mmol^{-1}$ H_2O). However, PEG-treated SR seemed to have the reduction of WUE, but it was not significantly different to the control. WUE of SR continued to decline after 6 and 9 days of stress. On day 9, all the PEG-treated rice had very low WUE, which was about 1.1 $\mu molCO_2$ $mmol^{-1}$ H_2O in both lines (Fig. 15E).

This data suggested that the ability to retain net photosynthesis rate and WUE during the drought in the old leaf of SR is due to other factors. The lower SD was not a physiological character that helped maintain *A* and WUE in the fully developed leaf.

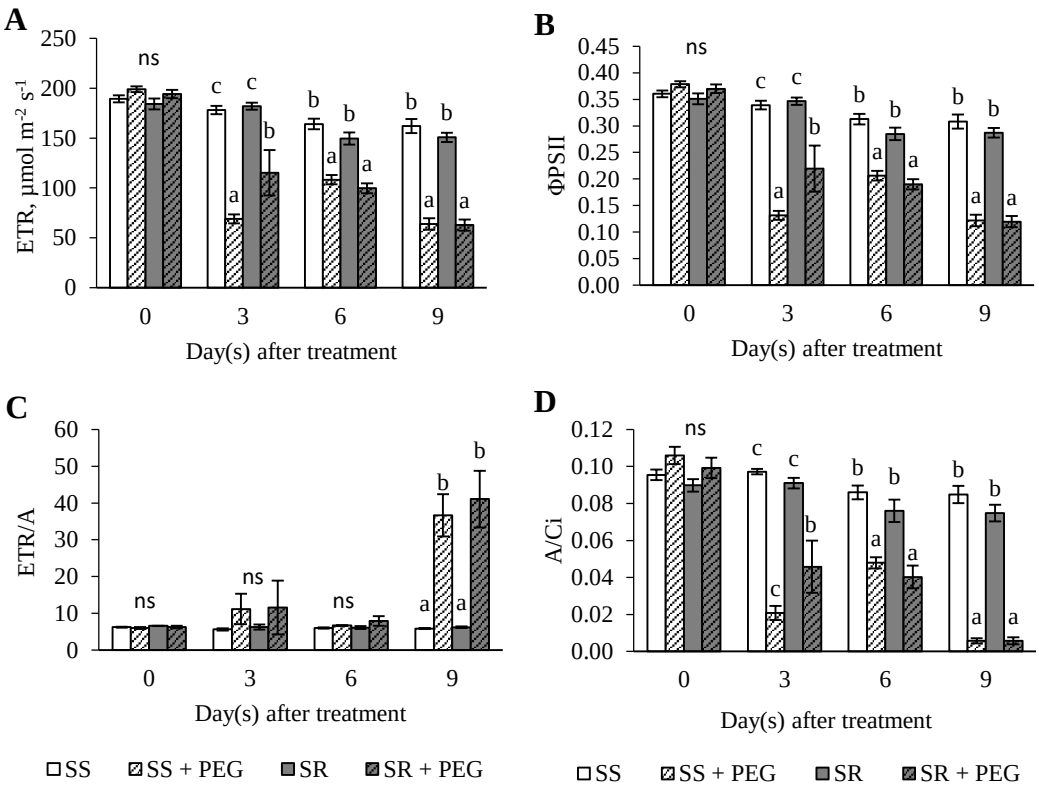

Figure 16. The measurement of electron transport rate (ETR) (A), effective quantum yield of photosystem II photochemistry (ΦPSII) (B), ETR/A ratio (C), and A/Ci ratio (D) in the old leaves. SS and SR are presented in white and grey color, respectively. Solid color represents normal grown treatment in half strength Yoshida solution. Upward diagonal fill represents drought stress condition treated by addition of PEG6000 to the solution. The different letters above the bars represent the significant difference of the mean at $p<0.05$, analyzed with DMRT. Error bars present SE of the experiment.

Electron transport rate (ETR) and ΦPSII are the two values representing electron transfer in the photosynthetic process. The ETR and ΦPSII results were correlated. The old leaf showed no significant difference in ETR and ΦPSII at the beginning of the experiment. After PEG-treatment, both parameters of SS and SR leaves significantly decreased (Fig. 16 and B). In the normal condition, ETR value is approximately 150-200 µmol m^{-2} s^{-1}. ETR gradually reduced to around 60 µmol m^{-2} s^{-1} in SS and SR after drought treatment. Interestingly, PEG-treated SS had significantly

lower ETR after 3 day of stress and then 3 days later, it was slightly increased. ΦPSII showed the similar response. The ETR and ΦPSII were reduced at day 9 after osmotic stress in both lines (Fig. 16A and B).

A/Ci was another parameter that had a similar response to ETR and ΦPSII. Osmotic stress significantly reduced *A*/Ci in both SS and SR after 3, 6 and 9 days of stress. After 3 days of stress, PEG-treated SR had significantly higher *A*/Ci ratio than SS. The values were 0.48 and 0.21 in SR and SS, respectively (Fig. 16D).

No significant changes in the ETR/*A* ratio were found in any treatments at day 0, 3 and 6. However, significant increase in ETR/*A* ratio was found only in the PEG-treated group after 9 days of stress. This raised up to approximately 5 times untreated group (Fig. 16C).

Fv/Fm was measured in order to investigate the efficiency of photosystem II under osmotic stress in the old leaves of both lines. At the beginning of the experiment, Fv/Fm was about 0.8 in both lines, and it was maintained until 3 days of osmotic stress. After 6 days of stress, the significant decrease of Fv/Fm was found in SS leaves, but SR leaves showed the ability to maintain Fv/Fm. However, after 9 days of the treatment, Fv/Fm of both lines was declined, but due to the large variation, no significant difference was found (Fig. 17A).

Pi or the performance photosynthetic index is the associated value with Fv/Fm. The Pi response was similar to Fv/Fm. There was no significant difference in Pi value at the beginning of the experiment and after 3 days of the stress. This values were maintained at 6-8. SS after treated with 12.5% PEG for 6 days showed significantly lower Pi value, while SR still had a slightly higher values. On day 9, SS and SR under stress condition had significantly lower Pi compared to the normal condition (Fig. 17B).

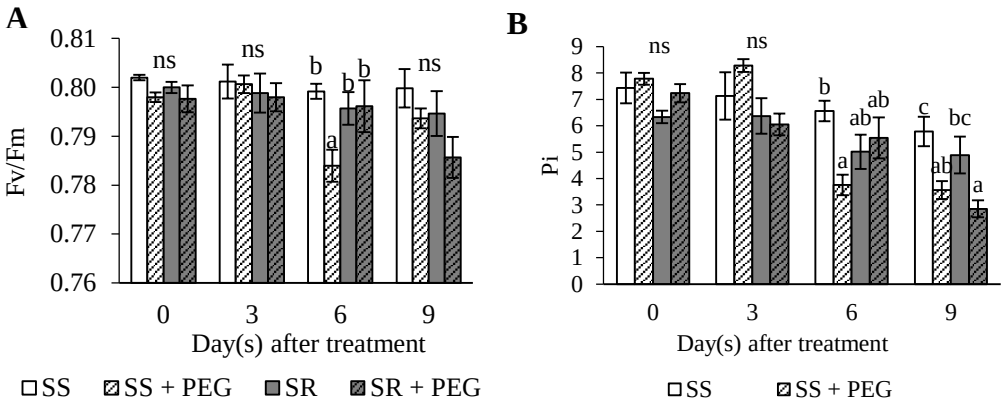

Figure 17. The measurement of maximum quantum efficiency of PSII (Fv/Fm) (A) and photosynthesis performance index (Pi) (B) in the old leaf. SS and SR are presented in white and grey color, respectively. Solid color represents control condition which grown in half strength Yoshida's solution. Upward diagonal fill represents drought stress condition as PEG6000 was added to the solution. The different letters above the bars represent the significant difference of the mean at $p<0.05$, analyzed with DMRT. Error bars present SE.

2.2.2.2. Effect of drought stress in the young leaf

The measurement of the photosynthetic rate in the young leaves started after 3 days of the experiment, when the new fully expanded leaves were completely developed. Both SS and SR plants had the significant lower net photosynthesis rate (*A*) after 3 days of osmotic stress. Interestingly, *A* of the young SR leaves was lower than *A* of young SS leaves. However, *A* of both lines was similar after the extended period of osmotic stress (Fig. 18A).

For the stomatal conductance of the new leaves, it was also declined in both lines after 3 days of drought stress, and the stronger reduction of g_s was found in SR line after 3 and 6 days of the treatment. However, g_s of the both treated plants' young leaves were similar after 9 days of the treatment (Fig 18B).

The transpiration of the young leaf also had a similar trend as g_s. After 3 days of drought stress, the transpiration value significantly reduced in SR. This might be due to lower SD in SR, leading to less water loss. However, treated SS and SR were not significant difference from the untreated plant after 6 days of stress. At 9 days after stress, the E was reduced under osmotic stress in both line (Fig. 18C).

Although the reduction of A and g_s was found in the young leaves of both lines after 3 days of osmotic stress, they could maintain the internal concentration of CO_2 (C_i). After 6 days of stress, the Ci of the stress treated plants was higher than the normal grown ones. The decline of Ci was found after 9 days of stress. However, they were not significantly different from the normal grown plants of both lines (Fig. 18D).

Similarly, water use efficiency of all treatments were not significantly different at day 3. However, WUE dropped after 6 days of osmotic stress in both SS and SR. After 9 days of stress, the WUE increased back to similarly level of untreated plants (Fig. 18E)

These data suggested that the regulation of stomatal development via *OsGTL1* caused the better preservation of water in the young leaves of SR line.

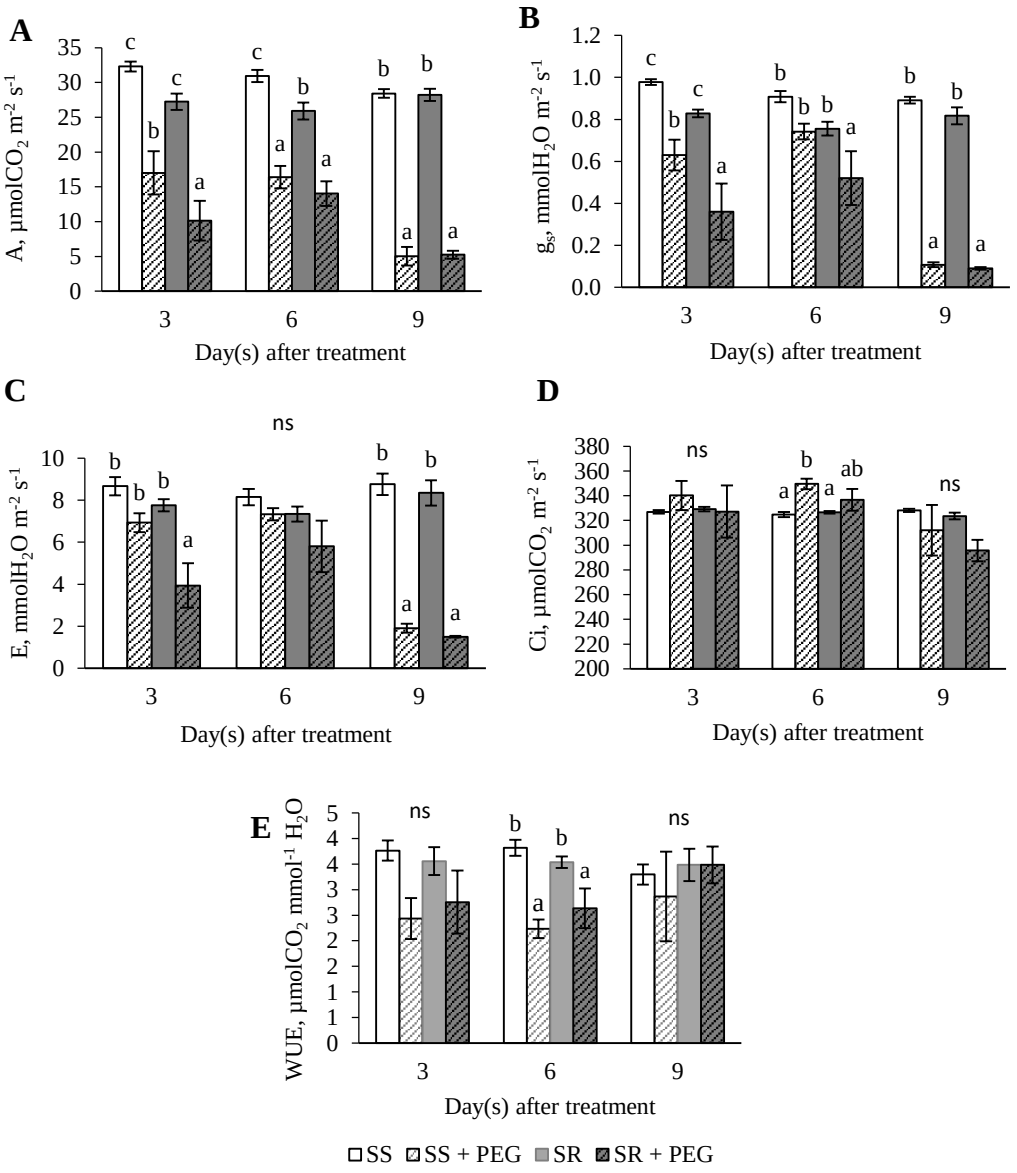

Figure 18. The measurement of net photosynthetic rate (*A*) (A), stomatal conductance (g_s) (B), transpiration rate (E) (C), intercellular CO_2 concentration (Ci) (D) and water use efficiency (WUE) (E)) in the new leaf. SS and SR are presented in white and grey color, respectively. Plain color represents control condition which grown in half strength Yoshida. Upward diagonal fill represents drought stress condition as PEG600 was added to the solution. The different letters above the bars represent the significant difference of the mean at $p<0.05$, analyzed with DMRT. Error bars present SE.

ETR, ΦPSII and *A*/Ci results had a similar trend in all timings (Fig. 19A, B and D). All three values in the PEG-treated plant were reduced by around 50% from the untreated plants. After 3 days of osmotic stress, PEG-treated SR seemed to be lower in all three values than PEG-treated SS.

The ratio of ETR/*A* was significant difference in all timings. The ETR/*A* ratio of PEG-treated SR was higher than other treatments around 60% after 3 days of the stress. However, the similarly ETR/*A* ratio in all treatments were showed in day 6 and it extremely increased in treated plant at day 9 (Fig. 19C)

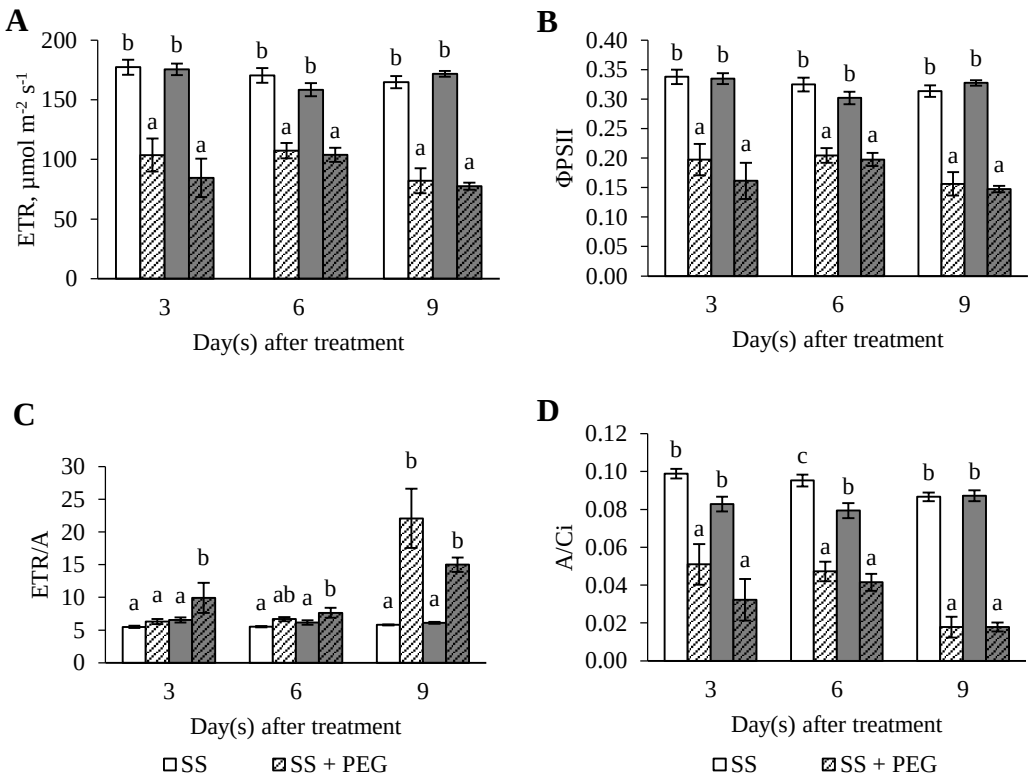

Figure 19. The measurement of electron transport rate (ETR) (A), effective quantum yield of photosystem II photochemistry (ΦPSII) (B), ETR/A ratio (C), and A/Ci ratio (D) in the new leaf. SS and SR are presented in white and grey color, respectively. Plain color represents control condition grown in half strength Yoshida. Upward diagonal fill represents drought stress condition as PEG600 was added to the solution. The different letters above the bars represent the significant difference of the mean at $p<0.05$, analyzed with DMRT. Error bars present SE.

Fv/Fm value had no significant difference in the young leaf from all timings. The PEG-treated and untreated plant showed Fv/Fm value around 0.8 in all treatments (Fig. 20A).

At day 3 of experiment, the photosynthesis performance index or Pi was no significant difference. PEG-treated SS had the highest Pi than other treatments after 6 days of osmotic stress, while PEG-treated SR had lowest Pi after 9 days of stress (Fig. 20B).

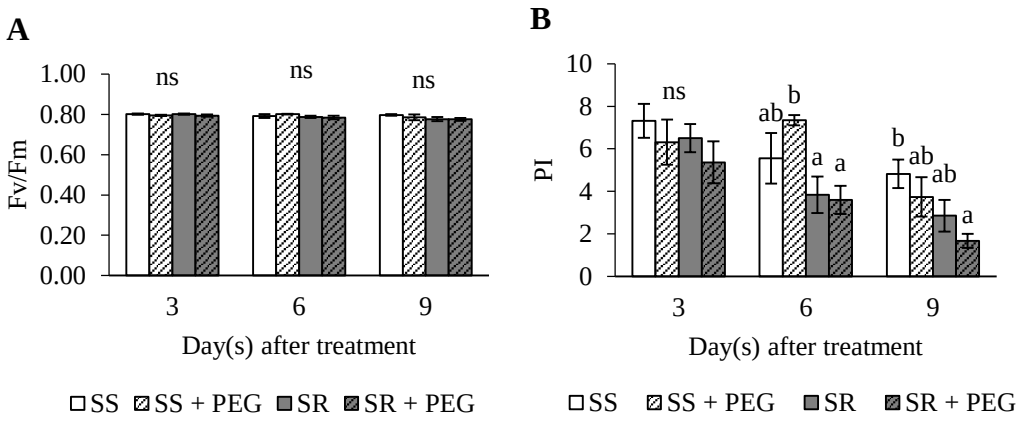

Figure 20. The measurement of maximal quantum efficiency of PSII (Fv/Fm) (A) and photosynthesis performance index (Pi) (B) in the new leaf. SS and SR are presented in white and grey color, respectively. Plain color represents control condition which grown in half strength Yoshida. Upward diagonal fill represents drought stress condition as PEG600 was added to the solution. The different letters above the bars represent the significant difference of the mean at $p<0.05$, analyzed with DMRT. Error bars present SE.

2.2.3. Investigation of drought stress effect in wild type and *gtl1-4*

2.2.3.1. Determination of gene expression by semi-quantitative RT-PCR

An investigation of Arabidopsis mutant, *gtl1-4* was selected according to a study of Yoo et al. (2010). It showed that *GTL1* negative regulates *SDD1* (*Stomatal Density and Distribution1*) expression which lead to lower SD, higher water use efficiency and higher survival rate. However, most of the data were conducted under normal condition in wild type and *gtl1-4*. Therefore, a few of the parameters need further investigation.

Firstly, *GTL1* expression in response to water stress was investigated by using semi-quantitative RT-PCR. In wild type, the expression of this transcription factor was gradually reduced under dehydration (shoot removal from roots and air dried on a bench lab). In contrast, *SDD1* transiently increased after air-drying for 30 minutes. *DREB2A* which is a drought-responsive gene was gradually increased overtime and *ACT2* (housekeeping gene) was constitutively expressed (Fig 21).

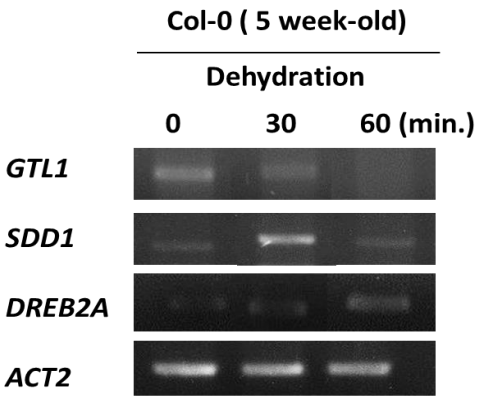

Figure 21. Transcription level of *GTL1*, *SDD1*, *DREB2A* and *ACT2* under dehydration in 5-week- old wild type (Col-0). The dehydration started after the shoot was cut and frozen immediately in liquid nitrogen (0 min). Other timings, the shoot was air-dried for 30 and 60 minutes before freezing in liquid nitrogen.

2.2.3.2. Determination of media water content and relative water content during water deficit

The next experiment was an observation of RWC overtime to confirm if *gtl1-4* had the ability of survival through the drought stress because of higher RWC. In the preliminary experiment, it was found that the fully expanded leaves wilted faster than expanding leaves during the water withholding period (Fig 22A). This suggested that the expanding leaves had the higher ability to maintain RWC than the fully expanded leaves under the drought stress. Hence, the RWC of both expanding leaves and fully expanded leaves were observed.

In this experiment, media water content (MWC) gradually reduced in drought treatment (Fig 22B). The analysis of RWC differences between expanding and fully expanded leaves was determined. The differences of RWC between well-watered and withholding water group could be detected on day 9 after treatment in both types of leaves and the RWC of stressed plants continually reduced until day 15 (Fig. 22C and

D). There was no significant difference in RWC between types of leaves. Interestingly, both types of leaves had the same decreasing slope of RWC. This suggested that they had a similar rate of water loss. In addition, the higher RWC was detected in *gtl1-4* expanding leaves (33%) and fully expanded leaves (24%) after fifteen days of withholding water when compared to wild-type plant (around 15% in both types) (Fig. 22C and D).

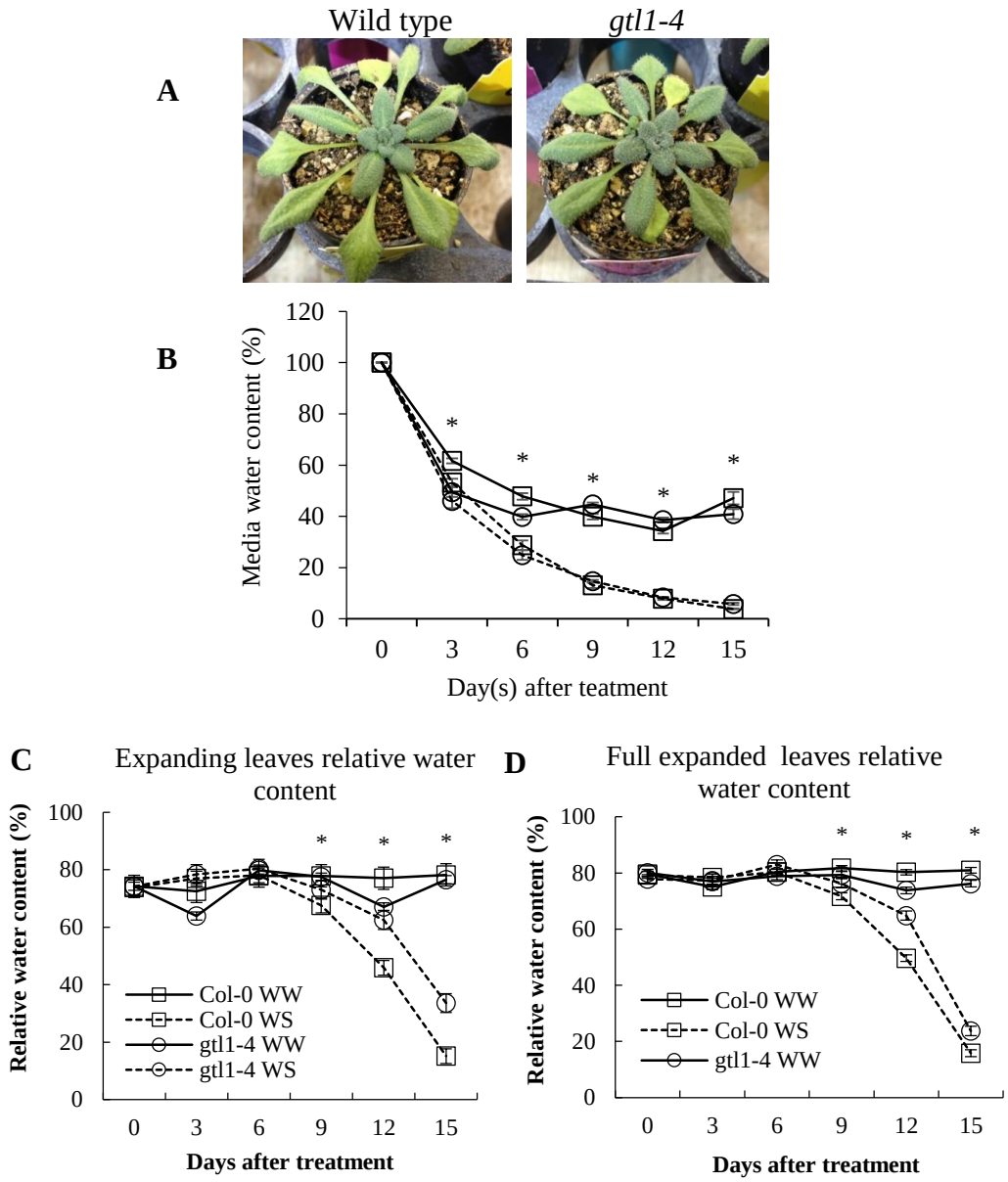

Figure 22. Photograph of wild type and *gtl1-4* under drought stress for 15 days (A). Media water content (B), relative water content of expanding (C) and fully expanded (D) leaves in well-watered (WW) (solid line) and water stress (WS) (dash line) were observed. Wild type (Col-0) is presented as squares and *gtl1-4* presented as circles. The asterisk above the lines represent the significant difference of the mean at $p<0.05$, analyzed with DMRT. Error bars present SE in the experiment.

2.2.3.3. Determination of survival rate in wild type and *gtl1-4*

When the stress-treated plants were re-watered, only the mutant line fully recovered, but the wild type could not survive. It was hypothesized that the recovery of *gtl1-4* was because the RWC was maintained better in the mutant than wild type during the stress period (Fig. 22C and D). Therefore, the next experiment was the observation of survival plants and RWC of death/survival plants overtime.

The Arabidopsis was watered normally until starting the experiment. It was found that *gtl1-4* had higher RWC than wild type (Col-0) throughout the experiment (Fig. 23A). The survival rate was also higher in *gtl1-4* than wild type (Fig. 23A). There is 100 % recovery of *gtl1-4* and wild type on day 8 through day 12. After withholding water for 13 days, *gtl1-4* still had 100% recovery but wild type showed 80% recovery. The recovery rate continued to reduce after that (Fig. 23A). The calculation of RWC from the plants that can/cannot recover were also determined. Interestingly, both genotypes could not recover after re-watering when RWC was lowers than 15% (Fig. 23B). This means that the survival rate depended on their relative water content.

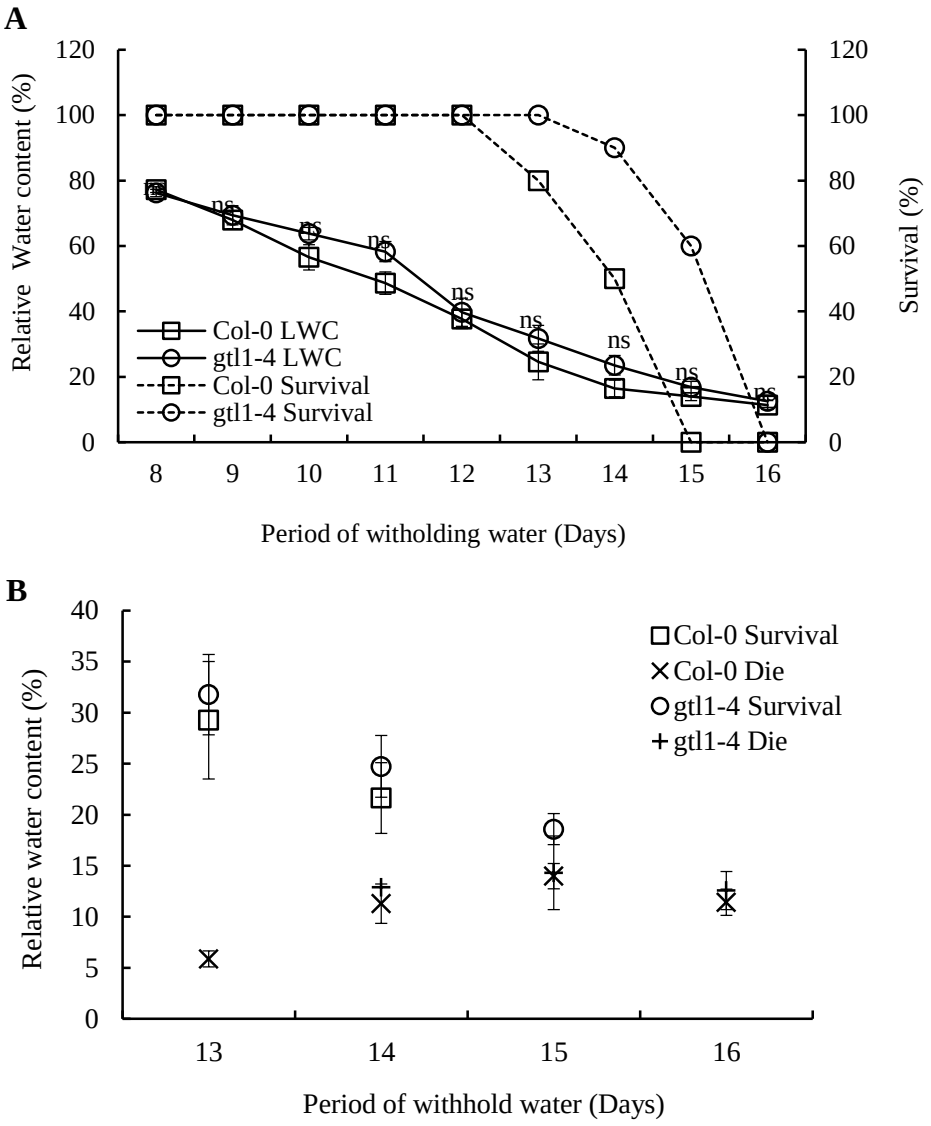

Figure 23. Percentage of relative water content during withholding water (primary y axil) and percentage of survival overtime after re-watering (secondary y axil) (A) and percentage of relative water content separately calculated of survival and die wild type (Col-0) and *gtl1-4* (B). The analysis was performed by DMRT with the significant difference of the mean at $p<0.05$. Error bars present SE.

2.2.3.4. Determination of stomatal density, stomatal index and leaf development during water deficit

Two leaves that developed before and during drought stress were used to collect the stomatal density to test if the knocked out of *GTL1* affected SD. The photograph was taken twice a day from 4-leaf-stage until finish the experiment and used to indicate position of leaf number 8 and 12. Leaf number 8 which fully developed before the experiment and leaf number 12 which emerged during drought stress were determined (Fig. 24).

SD on the abaxial leaf was not significantly different in any treatments (Fig. 25A). Leaf 8 and 12 had significant difference in SD on the adaxial leaves. In addition, the stress-treated mutant did not show the lower SD compared to the normal one (Fig. 25B).

Stomatal index (SI), the number of stomata per total epidermal cells, was also investigated. Significant difference of SI in the abaxial leaf was found in both leaf numbers, while the significant difference in SI in adaxial was found only in leaf 8 (Fig. 25C and D). From this results, the mutant did not showed phenotype as hypothesized.

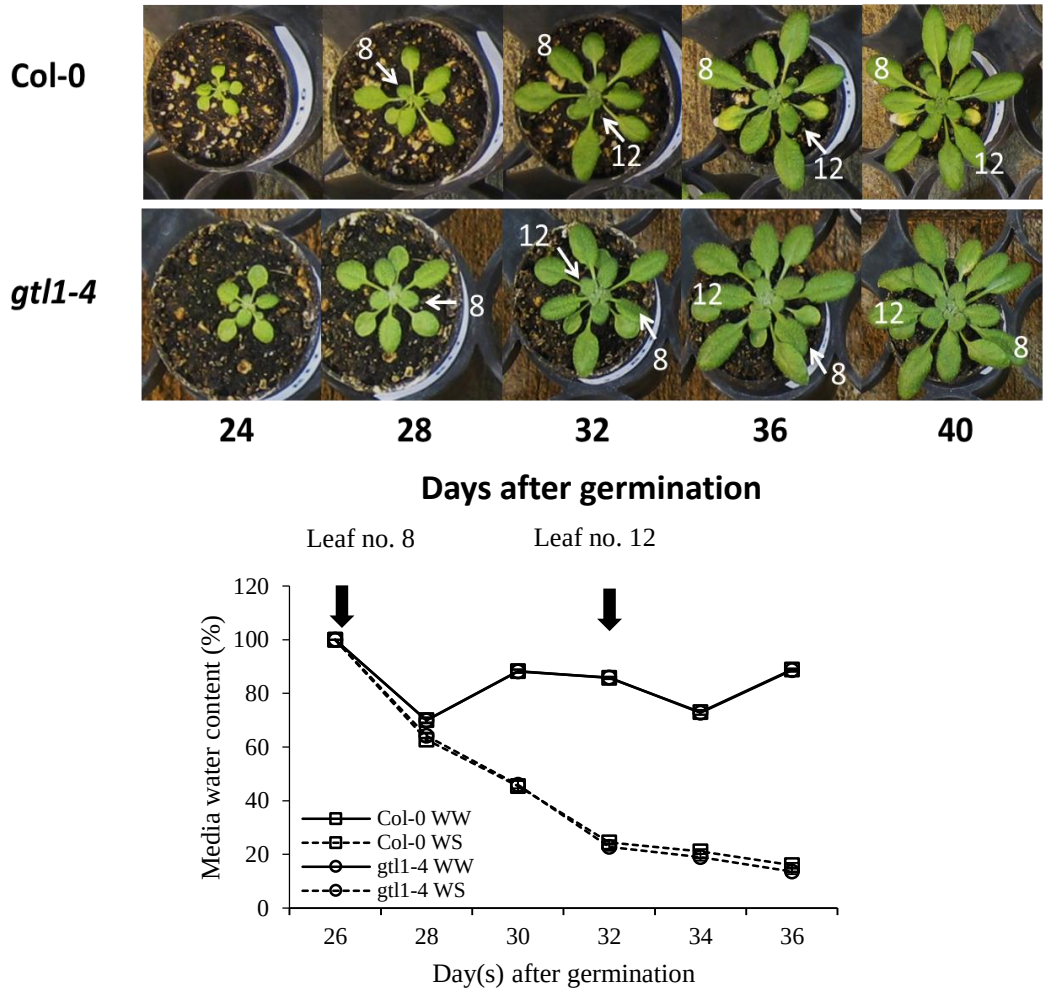

Figure 24. Photograph of leaf development in wild type (Col-0) and *gtl1-4*. The photographs were used to determined the number of leaves and number of fully expanded leaves. Leaf no. 8 and 12 were used for the stomatal density experiment. The media water content showed water status during the leaf was developed.

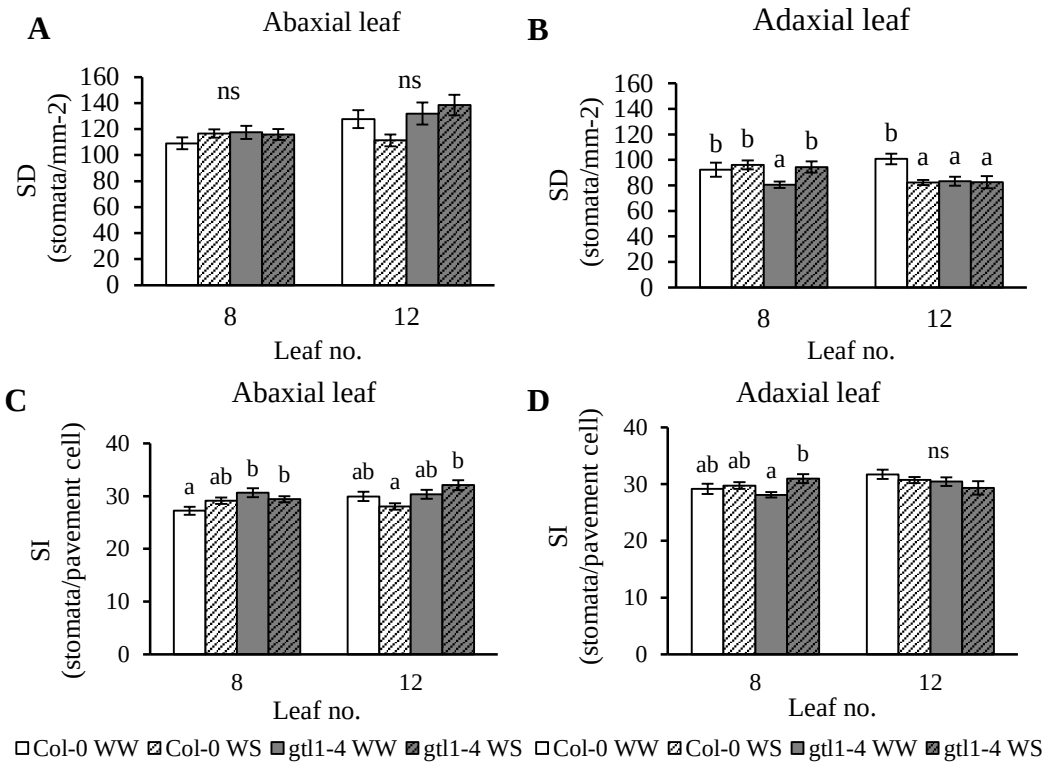

Figure 25. Stomatal density (SD) (A and B) and stomatal index (SI) (C and D) adaptation on abaxial leaf (A and C) and adaxial leaf (B and D) under drought stress. Leaf no. 8 and 12 of wild type (Col-0: white color) and *gtl1-4* (grey color) from the figure 18 were determined. Well-watered (WW) is presented in plain color and water stress (WS) is presented in upward diagonal fill. The different letters above the bars represent the significant difference of the mean at $p<0.05$, analyzed with DMRT. Error bars present SE.

To determine the leaf number, a photograph was taken. From the picture, it shows a few differences in leaf development. So total leaves number and total fully expanded leaves were investigated. The experiment started after 26 days of the germination and stress-treated groups were withholding water for 10 days. After germination for 26 days, wild type and *gtl1-4* had similar total leaf numbers (around 8 leaves) and it increased continuously overtime. The difference in the stress-treated plants and untreated plants were found at day 35. The significant difference in total leaf number was found on day 37, 39, 41 and 43 after germination. The wild type and *gtl1-*

4 under drought stress had lower total leaf number compared to normal condition but there was no difference between genotypes (Fig. 26A).

Wild type and *gtl1-4* under normal and stress conditions had a similar total fully expanded leaf number at the beginning of experiment. After 10 days of withholding water (36 days after germination), the differences of leaf development was found between the treatments. Wild type under withholding water had significantly lowest total fully expanded leaves number from day 34 afterward. While, *gtl1-4* under drought stress had a significantly different number of total fully expanded leaves compared to the normal condition only on day 37 and 39. After that, the fully expanded leaf number increased in *gtl1-4* and it seemed to catch up with the untreated-plant at day 46. However, wild type still had the lower number of fully expanded leaves (Fig. 26B).

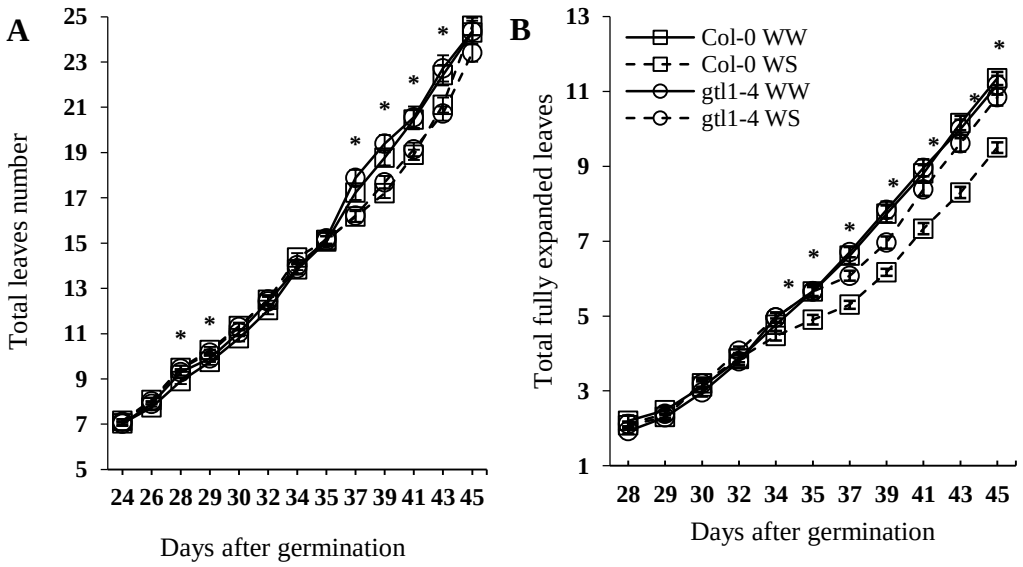

Figure 26. Leaf development of wild type and *gtl1-4*. Total leaf number (A) and total fully expanded leaves (B) were counted from the photograph. In control (WW) (solid line) and water stress (WS) (dash line) were observed. Wild type (Col-0) is presented as squares and *gtl1-4* is presented as circles. The asterisk above the lines represent the difference of the mean at $p<0.05$, analyzed with DMRT. Error bars present SE.

CHAPTER V
DISCUSSION

1. Proteomics study

1.1. Investigation of protein profiles after drought stress by using proteomic approach

1.1.1 Protein profiles of SS and SR lines

Since the first analysis was done in year 2013 and the NCBI database rapidly expanded. Therefore, the proteomics data was re-analyzed in year 2017 with the most recent NCBI database which is expected to deliver some new and interesting finding for the study. There were difference in total proteins number obtained from GeLC-MS/MS. It was clear that the raw data from the re-analysis revealed a bigger protein list. It was raised up around 3 time in 2017. For SS, approximately 20% of significantly different expressed proteins were both found in the 2013 and 2017 analysis. Similarly, 30% of common significantly different expressed proteins in SR were found in both analyses. The low amount of the same proteins found in both analyses might be due to the fact that some proteins were not included in the 2013 database. Moreover, the Blastp was added into the process to validate the existence of the rice protein in 2017 database, so some of the proteins found in 2013 were eliminated from the list.

The functional groups from the analysis in 2013 and 2017 were showed similarly (Table D11. see in Appendix D). Some of the functional groups were present in both analysis, while some of them were found in either the 2013 or 2017 analysis. The proteins which were classified into proteinase inhibitor and replication were found only in the 2013 analysis. Two functional groups, cellular process and post-translation

were revealed only in the 2017 analysis. In SR, the post-transcription was discovered in both analyses; however, the percentage was different.

1.1.2 Significant different protein profiles between drought-treated LPT123 (SS) and LPT123-TC171 (SR)

Two strategies of the comparison between drought responsive protein profiles of SS and SR were designed. The first one was the direct comparison of drought expressed proteins in SS and SR and identified the significantly differential expressed proteins between SS and SR. The second one was the comparison between the proteins expressed in normal and drought stressed conditions of each line to identified the significantly responsive proteins due to drought stress in each line, followed by another comparison to determine the differentially expressed proteins between SS and SR.

For the first method of comparison, the total of 67 drought responsive proteins from SS and SR were detected, while the second method revealed a total of 117 proteins. Although the second method could reveal more drought responsive proteins than the first strategy, 30 proteins were missing (Figure 8). This group represented the proteins that were not significantly changed their expression under drought stress in both lines, but the level of their expression was significantly different between SS an SR lines. These proteins should be considered to be possible proteins responsible for drought tolerance in SR line.

If we combine two methodologies of the comparison together, we will obtain the total of 147 proteins that have the potential to be responsible for drought tolerance.

In addition, exome study of Udomchalothorn et al. (2014) showed that there are 35,431 SNPs found in SR genome compared its background, SS. The point mutations

spread throughout their genome. Approximately 10,000 genes are affected by the mutation. Among the genes, 212 genes are abiotic stress-associated genes and the researcher found that 23 genes in SR seemed to be lacking in function. The resistant rice still has normal growth while they had a huge difference in exome compared with SS. In addition, the phenotype of SS and SR are similar. This evidence suggested that SS and SR are different in their genome and the genes did not express similarly in normal growth. Therefore, it is needed to include control condition in the analysis of each rice line. Moreover, to get all the important protein, two comparing methods should be performed.

From all analysis methods, DDT domain-containing protein, stripe rust resistance protein Yr10, NBS-LRR disease resistance protein and BTBA2-Bric-a-Brac, tramtrack, broad complex BTB domain with ankyrin repeat region were found commonly in all analysis method (group g) (Fig. 8).

DNA binding homeobox and different transcription factors (DDT) domain has been characterized as a domain in bromodomain PHD finger transcription factors (BPTFs) (Doerks et al., 2001). It was shown to have the DNA-binding function. A study of maize PHD finger family showed that DDT domain was found only in *ZmPHD27*. However, the function of *ZmPHD27* was not stated in the research (Wang et al., 2015). In addition, the function of DDT domain-containing protein encoded from LOC_Os04g35864 has not been reported.

The largest group of R protein contained a nucleotide binding site (NBS) and leucine-rich repeats (LRRs) (Dangl and Jones, 2001). Stripe rust resistance protein, is encoded from Yr10. It has evolutionary-conserved and unique CC–NBS–LRR sequence (Liu et al., 2014). This protein was up-regulated in stress condition of all rice

lines when compared with control condition. However, SS had higher up-regulation than SR. This gene is conserved among plant species, including wheat, maize, sorghum and rice. Another NBS-LRR disease resistance protein, LOC_Os07g29820, was also found to be up-regulated in SR line, but down regulated in SS line when compared between control and drought condition. A study of Prasch and Sonnewald (2013) showed that a signaling network was affected by multiple stress treatments (heat, drought, and virus treated Arabidopsis). Different combination of stress showed significant different expression patterns of TIR-NBS-LRR genes. The stresses alter the disease defense in Arabidopsis which lead to the deactivation of other defense response.

BTB (Broad-complex, Tramtrack, and Bric a brac) proteins have been identified in poxviruses, Arabidopsis, rice and other eukaryotes which have diverse functions e.g. transcriptional regulation, chromatin remodeling to protein degradation and cytoskeletal regulation (Chaharbakhshi and Jemc, 2016). BTB domain is known to be present in conjunction with the MATH domain. The MATH-BTB proteins have a main function in ABA signaling (Kushwaha et al., 2016). The expression of *BTAB2* was higher in SR than SS under drought stress condition. This may result in rapidly response to the stress in SR. However, there is no report about function of *BTAB2*.

1.1.3. Two drought-responsive genes commonly found in group two and three

From Figure 7, six proteins were significantly affected by osmotic stress in SS and SR (group d and g). Two drought-responsive genes were found only in group two and three but not in group one that was a comparison of drought-treated plants. These

two proteins are a helicase domain-containing protein and cytochrome P450. Both proteins are up-regulated in SS and SR rice responding to osmotic stress.

A biggest group of RNA helicase genes is DEAD-box genes such as *STRS1*, *STRS2*, *TaRH1*, *SlDEAD31*, and *OsBAT1* (Barak et al., 2014; Chen et al., 2014; Kant et al., 2007; Tuteja et al., 2015; Zhang et al., 2014; Zhu et al., 2015). *STRS1* and *STRS2* was reduced by salt, drought, and heat stress in Arabidopsis, and in turn induced the expression of several stress responsive genes (Barak et al., 2014; Kant et al., 2007). In wheat, low temperature, dehydration and salt stress induced accumulation of *TaRH1* (*Triticum aestivum* RNA helicase) (Zhang et al., 2014). In tomato, *SlDEAD31* was induced by heat, cold, and dehydration and *SlDEAD31*-overexpressed resulted in enhanced salt and drought resistance (Zhu et al., 2015). A transgenic rice which *OsBAT1* constitutively expressed can germinate normally and tolerate to high concentration of salt (Tuteja et al., 2015). *OsSUV3*, encoding DNA/RNA helicase and belonging to the Ski2 family of DExH/D-box helicases was shown to function in salt tolerance in rice by maintaining photosynthesis and antioxidant machinery (Tuteja et al., 2014). Therefore, the helicase domain-containing protein detected in this study may play a role in drought stress response in rice.

Cytochrome P450s (CYPs) is one of the largest protein coding gene family and play an important role in plant hormone biosynthesis, catabolism and primary and secondary metabolites synthesis. However, the majority of CYPs was still unknown (Nelson and Werck-Reichhart, 2011; Tamiru et al., 2015). A cytochrome P450, CYP707A family member was identified as ABA 8'-hydroxylase, which degraded ABA under dehydration stress condition. The knock-out mutant of *CYP707A3* gene led to drought tolerant phenotype (Umezawa et al., 2006). However, the ectopic expression

of *PtCYP714A3* from *Populus trichocarpa* improved salt tolerance in transgenic rice (Wang et al., 2016). Moreover, the expression of LOC_Os08g01480, encoding CYP-like protein, in Arabidopsis caused the tolerance to heavy metal, salt and dehydration stress (Rai et al., 2015). The up-regulated cytochrome P450 (LOC_Os10g05020) suggests the involvement of this protein in osmotic stress response. The *dss1* rice mutant had higher drought tolerant ability compared with wild-type rice. The DSS1, belong to P450 families regulate growth and enhance drought tolerant by balancing gibberellin and ABA (Tamiru et al., 2015). The percent induction of LOC_Os10g05020 in SR was higher than SS. This suggested that cytochrome P450s is one of the proteins that regulate rice development under stress.

1.1.4 Significant different protein profiles found in group three

Expression of fifty seven proteins were significantly different when compared between control and drought stress in SR and the proteins were categorized into 10 functional groups. Some of the protein functions were described here.

Transposable elements

The genes encoding the proteins that accumulated only in the drought-tolerant line in response to osmotic stress may be useful as drought-tolerance genes. Protection from environmental stresses may be mediated by epigenetic events, such as the induction of the expression of adjacent genes by transposable elements. More than one fifth of 51 proteins detected only in the SR line were consisted of a combination of retrotransposons and transposons. Transposable elements (TEs) are classified as Class I (copy-and-paste mechanism via an RNA intermediate or retroelement) or Class II

(cut-and-paste mechanism via a DNA intermediate) transposons, and are major components of eukaryotic genomes (Anca et al., 2014; Chadha and Sharma, 2014). Additionally, the LTR retrotransposons, which may mediate somaclonal variation, are the major plant TEs (Grandbastien, 2015; Wessler, 1996). For example, copper and heat shock stresses induce TE activities, leading to instability in the *Magnaporthe oryzae* genome (Chadha and Sharma, 2014). The *Hordeum vulgare* DEMETER gene (HvDME) contains an LTR retrotransposon element. Its expression is induced in drought-tolerant barley exposed to drought conditions, resulting in differential DNA methylation in drought-sensitive (e.g., 'Caresse') and drought-tolerant (e.g., 'Demetra') cultivars (Kapazoglou et al., 2013). The activation of TEs is one of the mechanisms that enables self-protection and self-repair. It also stimulates the expression of other genes responsible for stress responses (Grandbastien, 2015).

Plant metabolism

Several proteins involved in metabolic processes increased or decreased abundance under osmotic stress (Table D1). When plant cells experience abiotic stress, glyceraldehyde 3-phosphate dehydrogenase (GAPDH) is one of the most prominent protein targets for oxidative modification (Hildebrandt et al., 2015). In a proteomic analysis of overexpression of *TRRF1* in sugarcane displayed an up-regulation of GAPDH after treated with PEG (Rahman et al., 2014). A similar result was found in a protein identification of two contrasting drought-tolerant wheat. The GAPDH level increased when treated with PEG6000 (Cheng et al., 2015). Another protein that involved in plant metabolism is enolase. Enolase is an enzyme in glycolytic pathway which categorized into metabolic process. The significant reduction was found in SR

line only. In a previous study, enolase protein abundance was significantly higher in drought-tolerant Chinese spring wheat than the drought-sensitive cultivar after treated with PEG6000 for 48 hours (Cheng et al., 2015). This pointed out that drought tolerance in different species might use different metabolic pathways for drought adaptation.

Both GAPDH and enolase changes suggested the adaptation in carbohydrate metabolism to drought stress in SR line. The regulation of photosynthetic efficiency under drought stress leads to the maintenance of grain yield in rice (Ambavaram et al., 2014). Sugar accumulation is also the mechanisms for tolerance to abiotic stresses, including drought (Pandey and Shukla, 2015), salt (Udomchalothorn et al., 2009) and chilling stresses (Morsy et al., 2007).

Plant signaling

Protein phosphatase 2 C (PP2C) is a big group of protein which interact with a wide range of targets such as receptor-like kinase (RLKs) and mitogen-activated protein kinase (MAPK). PP2C was found in only SR responding to drought. PP2C involves in signal transduction network activated by drought, salinity, and especially abscisic acid (ABA) (Himmelbach et al., 2002). ABA is an important hormone regulates many genes in stress-signaling pathway. *ABI1* is one of PP2Cs interacting with *ATHB6* as a negative regulator of ABA signaling pathway and the overexpression of this gene decreased ABA sensitivity and led to water loss more than the detached leaves of wild type (Himmelbach et al., 2002). Consistently, *ZmPP2C* overexpression decreased drought tolerance ability. The transgenic plant had more rapid water loss than wild type (Liu et al., 2009).

Defense mechanism

Stripe rust resistance protein Yr10, NBS-LRR disease resistance protein and BTBA2 were categorized into plant defense. As mentioned above, SR might respond to the stress quicker than SS by the regulation of BTBA2 protein and SS had a lesser chance to survive during the stress due to the activation of disease resistance proteins.

Osmotic stress caused the changes in proteins with signaling and defense functions differently between SS and SR lines. These suggested that these two lines had different signaling pathways and used different defense responses in order to cope with osmotic stress.

Transcription

TFs trigger other stress-responsive genes and have been reported to involve with drought stress. TFs that were found in this study are WRKY106, ZOS11-11 - C2H2 zinc finger protein, trihelix transcription factor GTL1, OsSPL11 and OsSPL17 - SBP-box gene family member. *WRKY* genes play an important role in developmental process of plant under normal condition. The overexpression of *OsWRKY45* and *OsWRKY72* in Arabidopsis (Song et al., 2010) and the constitutive expression of *GmWRKY54* from *Glycine max* (Zhou et al., 2008) enhanced drought and salt tolerance ability. However, the function of WRKY106 found in this study has not been reported. A C2H2 zinc finger protein from soybean (*GmZFP3*) was reported as a negative regulator of drought stress. In addition, the expression of *GmZFP3* increased after treated with PEG and ABA (Zhang et al., 2016). Another transcription factor, trihelix transcription factor GTL1, have been reported as a positive regulator of stomatal density which led to better

drought tolerant ability. The loss-mutant plant (*gtl1-4*) showed lower stomatal density and higher survival rate than wild type (Yoo et al., 2010). Expression of WRKY 106 and GTL1 was significantly different in SR but not in SS.

Posttranscriptional

Posttranscriptional regulation of gene expression is controlled by gene activities in mitochondria. In this experiment, pentatricopeptide repeat (PPR) protein was up-regulated after drought stress. Mitochondrial pentatricopeptide repeat (PPR) proteins are associated with many plant biological processes, including RNA sequence changes, translation, and seed and embryo development. Salt, ABA, and oxidative stresses inhibit plant growth in an *A. thaliana* mutant (*ppr40*), and results in the accumulation of reactive oxygen species. Because PPR proteins are very important to plant organelles, defects in these proteins lead to retarded growth, diverse defects in embryo morphology, and irregular photosynthesis (Cushing et al., 2005; Manna, 2015; Meierhoff et al., 2003; Pusnik et al., 2007).

Transport

One protein with transport functions is SEC 14 cytosolic factor family protein. A comparison of transcriptomes among several sorghum genotypes revealed that SEC14 cytosolic factor protein is more abundant in the nitrogen stress-tolerant sorghum genotypes than in the susceptible sorghum lines. Additionally, the production of this protein can lead to greater membrane stability and stress tolerance (Gelli et al., 2014).

1.2. Validation the proteomic data: comparison between our data and microarray database

Rice eFP Browser is a web tool which has representations of expression patterns of genes base on microarray databases. GSE6901 is a collection of gene profiles under three types of stress; drought, salt and cold. From the significantly different expressed proteins in Group 3 (Fig. 8), 41 proteins were found an expression in micro array database. Eighteen proteins were found with similar expression pattern with the database (Table D10 see in Appendix D).

The comparison between expression of protein from proteomics data and transcription level from Rice eFP browser showed that only half of the proteins had similar pattern of expression induced by drought stress. In general, it was assumed that there are strong correlation between mRNA abundance and generated protein expression. However, some studies show that the correlation between transcripts and protein expression is unpredictable due to different half-lives and post-transcription (Haider and Pal, 2013). In addition, mRNAs level can be translated, degraded, or temporarily stored during the stress condition which affect the protein expression level (Urano et al., 2010).

2. Identification and characterization of the drought responsive genes from the gene/protein expression patterns.

2.1. Selection and expression analysis of the drought responsive genes in ‘LPT123’ and ‘LPT123-TC171’ rice lines

2.1.1. Co-expression network

RiceFREND (Sato et al., 2013) was used for creating a co-expression network to see which protein has a potential of further studies. The co-expression network was created from transcriptome profiling of various tissues and rice organs at different developmental stages throughout their life cycle. A co-expression network represents a relationship between similar expressions profiling of genes across microarray database. The linkage of each node gene suggested that they have a potential interaction between them. Therefore, the co-expression network helps to illustrate a candidate gene from massive data.

A study in cotton (You et al., 2016), arbuscular mycorrhizal (Garcia et al., 2017), Arabidopsis (Li and Hu, 2015) and rice (Huang et al., 2016) used co-expression network as a tool for selecting candidate gene(s) or illustrating mechanisms of the interesting gene. In this study, the co-expression network revealed four proteins that involved with drought stress as previously mentioned.

2.1.2. Function of *trihelix transcription factor GTL1*

Trihelix DNA binding proteins involve with plant development programs. The transcription factors GT-1, GT-2, GT-3 and Nt SIP1-like proteins are a subfamily of the trihelix proteins. All the subfamilies have a conserved N-terminal trihelix I domain and C-terminal alpha-helical regions. However, the GT-2 subfamily is the only one that has a trihelix II domain at C-terminal and α-helical in the center (Gao et al., 2009). *GT-2 LIKE 1 (GTL1)* has conserved N-and C-terminal trihelix DNA binding domains (Breuer et al., 2009). A phylogenetic study of GTL1 family showed that the highly identical sequences was found between PtaGTL1 through 7 and AtGTL1 while 4 rice orthologs display phylogenetically different from other AtGTL and PtaGTL proteins

((Weng et al., 2012). However, a central region between N-and C- terminal trihelix domain are conserved in both Arabidopsis and rice (Kuhn et al., 1993). A Cam-binding site and PEST sequence are found in both PtaGTL1 and AtGTL1 but not found in rice (Weng et al., 2012).

Some of the GTL1 functions were studied in Arabiodopsis, wheat and *Populus trichocarpa*. *Arabidopsis thaliana GTL1* loss-of-function mutations (*gtl1-4*) had a higher integrated WUE, leading to higher survival rate after water deficit in the mutant. The *AtGTL1* repressed the expression of *SDD1* (*Stomtal Density and Distribution 1*) which regulates stomatal density (Yoo et al., 2010). The *sdd1* Arabidopsis mutant increased 2 to 4 fold of stomatal density and formed an arrested stomata (von Groll et al., 2002). In contrast, the 25% of stomatal density lower in *gtl1-4* compared to wild type was found. The expression of *SDD1* up-regulated in *gtl1-4*. The lower SD compensated water lost and improved drought tolerance in *gtl1-4* (Yoo et al., 2010). A complementation test by using *PtaGTL1* transcript regulated by *AtGTL1* promoter revealed a drought responsive mechanism in Poplar. The transgenic plant and wild type showed a similar stomatal density number and survival rate. Contrastingly, the *gtl1-4* had lower SD and higher survival rate when compared to wild type and the transgenic plant. The similar results also found in the study of overexpression of *TaGT2L1D* in Arabidopsis. The *TaGT2L1D*-overexpressed had similar stomatal density number as wild type but significant higher than *gtl1-3*. Both wild type and the transgenic plant significant reduced the survival rate compared to *gtl1-3*. The expression of *TaGT2L1D* was also found in floral organ development and overall plant growth (Zheng et al., 2016). GTL1 was proposed to be a regulator of trichome cell growth due to the *gtl1-3* plant exhibited larger trichome compared to wild type (Breuer et al., 2009). The

overexpression of *TaGT2L1D* restored the trichome phenotype of *gtl1-3* (Zheng et al., 2016). In addition, *AtGTL1pro:PtaGTL1* showed a similar size of trichome branch to wild type (Weng et al., 2012). In contrast, the *gtl1-4* mutant showed a large trichome branch (Breuer et al., 2009). It suggested that *TaGT2L1D, AtGTL1* and *PtaGTL1* role have a similar function in drought tolerance (Zheng et al., 2016).

The transcription factors, trihelix transcription factor GTL1 was shown a significant change in protein levels in SR line, but not in SS line, suggesting the role in the regulation of osmotic stress tolerance. Further validation is required for the further study. The trihelix transcription factor GTL1 was reported to be involved in regulation of stomatal development resulting in enhance drought tolerance ability in Arabidopsis. However, there have been no reports on the study of *GTL1* function in rice yet. Interestingly, LOC_Os03g02240 is closely related to AtGTL1 (Weng et al., 2012).

Actually, the fully expanded leaves used in the study of stomatal density were also collected for detection of *GTL1* (LOC_Os03g02240) transcripts. However, quantitative PCR could not detect *GTL1* expression in 4-week-old SS and SR leaves under control and drought condition. This result is consistent with Rice eFP Browser data (http://bar.utoronto.ca/efprice/cgi-bin/efpWeb.cgi) (Patel et al., 2012; Toufighi et al., 2005) which Trihelix transcription factor *GTL1* has almost no express in mature leaf (Fig. 11). These data suggested that *OsGTL1* detected in this proteomic experiment was a gene product that had been synthesized in very young tissues (developing stage). Moreover, the study in Arabidopsis with *STOMAGEN* expression suggested that stomata finish the development before reaching the mature state (Sugano et al., 2010). Therefore, it is possible that *OsGTL1* transcripts may not be detectable in the mature leaves.

Plant seedlings are usually used in many studies (Ali and Komatsu, 2006; Minh-Thu et al., 2013; Yang et al., 2012) because it is very fragile and sensitive to abiotic stresses. Microarray study (GSE6901) in Rice eFP Browser showed *OsGTL1* expression in 7-day-old seedling which was air-dried for 3 hr. and the transcript level decreased compare to the control (Jain et al., 2007). Therefore, the similar experiment were performed to investigate the *OsGTL1* expression at transcriptional level in SS and SR. It was found that *OsGTL1* transcript level from SR shoot was significantly decreased after dehydration for 2 hr., while the expression of *OsGTL1* in SS was increased (Fig. 11). These data suggested the different drought-stress induced *OsGTL1* gene expression in SS and SR, leading to the difference in drought tolerant ability.

2.1.3. Function of *glyceraldehyde-3-phosphate dehydrogenase* (*GAPDH*)

GAPDH exists in most organisms as a ubiquitous enzyme. There are three type of GAPDH, including GAPA/B encode by *gapA* and *gapB*, cytoplasmic GAPDH (GAPC) and plastids GAPDH (GAPCp) (Zaffagnini et al., 2013). GAPDH is a key enzyme for converting glycerate-3-phosphate (3-PGA) to glyceraldehyde-3-phosphate (G3P). 3-PGA is an electron acceptor that receives electrons from NADPH and protects photosystem II from ROS activity (Takahashi and Murata, 2006). Under oxidative stress, antioxidant cofactor NADPH are needed. NADPH is a reduced form of NADP which can be catalyzed by several enzymes including GAPDH (Ralser et al., 2007). A proteomics study of *Thellungiella halophila* chloroplasts under different saline conditions revealed several salt-responsive proteins, including glyceraldehyde-3-phosphate dehydrogenase beta subunit (GAPB) (Araus et al., 2002). Overexpression of

GAPB in transgenic Arabidopsis increased chlorophyll concentration, dry weight, water content, and survival rate.

In prior research, GAPDH was widely used as a housekeeping gene in protein and gene expression profile, especially of plant, animal and human studies (Chandna et al., 2012; Nicholls et al., 2012). However, our study showed that GAPDH could not be used as a housekeeping gene, because the difference levels and responses of GAPDH expression was found in SS and SR lines according to proteome and transcription level study (Fig 10 and 12).

GAPDH (LOC_ Os04g38600) increased in both SS and SR lines after osmotic stress for 2 hours. It suggested that photosystem II might be protected from the stress by the reducing an occurrence of ROS by GAPDH activity. Two wheat cultivars with contrast drought tolerant ability showed similar result with our study. An increasing of GAPDH after 48 h of PEG600 treatment in both wheat genotypes (different in levels of drought tolerance) were found (Cheng et al., 2015). In *Populus tremula*, an up-regulation of GAPDH was found due to water deficit treatment (Pelah et al., 1997). In rice, three *OsGAPC* respond to 20% PEG 6000, 200 mM NaCl, 50 uM abscisic acid and 50 uM methyl viologen treatments. The overexpression of *OsGAPC3* increased salt-tolerant ability through the regulation of the hydrogen peroxide during the salt stress (Zhang et al., 2011). Moreover, GAPDH have been proposed to be involved in root development (Muñoz-Bertomeu et al., 2010). However, the up-regulation of LOC_ Os04g38600 until 24 hours after stress was found only in SR line, but not in SS line (Fig 10B). It suggested the SR line has a better protection of photosystem II during osmotic stress. NADPH can be produced by the light reactions of photosynthesis to be utilized in the Calvin cycle. The expression of *GAPDH* genes up-regulated in both SS

and SR plants after dehydration for 2 hour however the induction of *GAPDH* was slightly larger in SR (Fig. 12). The photosystem I and II are a major site where ROS is generated. ROS can damage the photosystem which leads to be a stress susceptible. The limitation of CO_2 assimilation due to the stomatal closure can lead to over reduction of electron transport chain and cause the ROS generation (Asada, 2006; Miller et al., 2010). The up-regulation of GAPDH is needed for catalyzing the NADPH and preventing ROS induction.

Ferredoxin-NADP reductase (FNR) is another enzyme that important for balancing electron transport and redox homeostasis in chloroplasts. The activity of FNR is to catalyze the terminal stage of photosynthetic electron transport chain in photosystem I (PSI). FNR oxidizes ferredoxin which generates a reducing power (NADPH) to be used in CO_2 fixation in Calvin cycle (Gharechahi et al., 2015). Both GAPDH and FNR are involved in regulating plant NADP(H) levels (Hald et al. 2008). Therefore, the transcription level of *FNR* was investigated in both rice line to elucidate the importance of NADP(H) homeostasis in drought-tolerant plant. Only the stress-treated SR showed slightly decreased *FNR* expression but there was no change in SS. The reduction of FNR abundance because of drought stress has been reported transgenic tobacco (Gharechahi et al., 2015) , *P. cathayana* (Xiao et al., 2009), wheat (Budak et al., 2013), and rice (Nouri et al., 2015). In contrast, the induction of *FNR* level due to moderately high temperature (30 °C) was found in potato (Hancock et al., 2014). Salt (Zörb et al., 2009) and osmotic stress (Tai et al., 2011) induced FNR level in maize. However, a similar level of FNR in untreated and drought-treated wheat cultivars was found (Nikolaeva et al., 2010). These findings imply that different species

use different mechanisms to balance electron flow in photosynthetic processes under osmotic stress conditions.

Stomatal closure during the drought stress limits the carbon dioxide diffusion *via* stomata. The limiting carbon dioxide causes lower Calvin cycle activities, resulting in an induction of NADPH level in the stroma. As earlier mention, high level of NADPH can induce the ROS accumulation (Takahashi and Murata, 2005; Zavafer et al., 2015). To prevent photosystem damage from ROS, it needs to balance the NADP(H) level. The increasing of GAPDH activity will accelerate the NADP level. In addition, reduced FNR levels under drought conditions also contribute to NADPH homeostasis, delaying a NADPH production. Therefore, the NADPH/NADP ratios will be decreased which lead to protection of PSII. Consistent with our result, SR line show a greater reduction after the stress whereas *FNR* slightly down-regulated after dehydration (Fig. 12). In conclusion, SR rice showed that *GAPDH* were up-regulated while *FNR* reduced under the stress (Fig. 12), which imply that during the stress, plants try to use NADP(H) homeostasis mechanism to prevent photosystem damage by stress.

Interestingly, the co-expression network of GAPDH (LOC_Os04g38600) also showed a link to other genes which their function involving in photosynthetic process (Fig. 9 and Table.D5 see in Appendix D).

2.2. Function analysis for drought resistant ability

2.2.1. Determination of relative water content and stomatal density in SS and SR rice lines

Leaf RWC of SS was reduced after the drought stress (Fig. 13E) with the correlation of stomatal density. SR also had lower SD (Fig. 13F) under drought stress

compared to SS. Several researches showed that drought tolerance was increased by regulating stomata development, stomata closure or leaf expansion (Jung et al., 2008; Liu et al., 2011; Minh-Thu et al., 2013; Ouyang et al., 2010; Xie et al., 2012; Yoo et al., 2010). Stomata density reduction during drought might be the best way to impose the lower level of energy compared to normal condition because growth and development process need high level of energy to complete the process (Minh-Thu et al., 2013). The study of Arabidopsis mutants including positive SD regulators, *GTL1* and *STOMAGEN* showed the positively regulation of stomatal development. The mutation in these two genes resulted in SD reduction (Sugano et al., 2010; Yoo et al., 2010). In addition, decreased transpiration by *phyB* enhanced drought-tolerant in rice. The *phyB* mutant increased levels of *ERECTA* (*ER*) and *EXPANSIN* transcription, resulting in lower SD and a larger epidermal cells in the developed leaves (Liu et al., 2011). On the other hand, a knock-out mutant of *OsSIK1* caused 12.4–22.1% higher stomata density, compared to the control plants. In contrast, *OsSIK1*-overexpression reduced stomata density around 8.4-17.8%. In rice, OsSIK1 is a homolog of ER family proteins from Arabidopsis which control stomata pattern in *Arabidopsis thaliana.* Consequently, *OsSIK1* activated the anti-oxidative system and negatively regulated stomata development in rice leaf, leading to drought and salt tolerance (Ouyang et al., 2010). Not only in rice showed less SD in drought tolerance plant but also in *Medicago Truncatula*. An overexpression of *MtCAS31* significantly increased drought tolerance and caused SD reduction (Xie et al., 2012). The epidermal patterning factor (EPF) family of secreted signaling peptides regulate the frequency of stomatal development in dicot and basal land plant species. The overexpression of *HvEPF1* constrained the stomatal development pathway and reduced leaf gas exchange. The transgenic barley

plants also significantly reduced stomatal density with no grain yield penalty (Hughes et al., 2017). Recently, double mutant plants (*epf1epf2*) with induction of SD have been shown to have significantly lower water use efficiency. Conversely, the overexpression of *EPF2* resulted in lower stomatal density and led to minimize stomatal conductance and increased water use efficiency in transgenic plant (Franks et al., 2015). Therefore, the SR reduced SD after water stress might be a best mechanism to reduce water loss and keep growing normally.

2.2.2. Determination of leaf gas exchange parameters in 'LPT123' and 'LPT123-TC171' rice lines

According to GTL1 and GAPDH function, which can affect the photosynthesis, the photosynthetic parameters were measured. Photosynthesis on the abaxial leaf is independent of CO_2 concentration and largely relies on stomatal function (Driscoll et al., 2006). Increased stomatal density in constitutive expression of *STOMAGEN* plants rise about 30% of photosynthetic rate compared to the wild-type plants. The transgenic plants also increased the stomatal conductance under ambient CO_2 conditions and did not show alterations in the maximum carboxylation rate (Tanaka et al., 2013). The *HvEPF1*-overexpressed plant exhibit significantly enhanced water use efficiency. The quantum yield of photosystem II (ΦPSII) was measured. Under water withheld, the transgenic barley significantly maintained ΦPSII at higher level than that of wild-type plant approximately 4 days longer. In addition, the RWC of *HVEPF1* plants were significantly higher than that of the control under stress condition (Hughes et al., 2017).

In this study, the old leaf which was fully expanded at day 0 showed significant higher in net photosynthetic rate and water use efficiency in SR than SS after 3 days of

the stress. However, the significant difference of transpiration rate between genotypes was not found. There are also no significant difference in stomatal conductance and intercellular CO_2 concentration value. Since the WUE is the ratio of net photosynthesis and transpiration rate, it is suggested that the SR under drought stress enhanced net photosynthetic rate due to ΦPSII and ETR. It was not because of the lower SD in SR. The ΦPSII and ETR significantly up-regulated in SR after drought stress for 3 days. The ΦPSII and ERT present the efficiency of PSII and flow rate of electron in photosystem I. This data suggests that the light reaction might be a main factor caused higher net photosynthesis rate and water use efficiency in first fully expanded leaf under drought stress condition. Therefore, the old leaf of SR responds to the stress by regulating the light reaction to maintain *A* and WUE. In addition, the high level of *GAPDH* is required for protection of the photosystem from ROS accumulation.

The young new leaf might use different mechanism to protect itself from the stress. Stomatal conductance and transpiration rate were significantly lower in SR after drought stress for 3 days. The other parameters including the Fv/Fm, ETR and ΦPSII did not show the difference between genotypes. The maximum quantum efficiency of photosystem II photochemistry ((Fv/Fm) can also be used to indicate the efficiency of PSII. Since SR showed no significant difference in Fv/Fm, ETR and ΦPSII, it means SR had no or lower damage on photosynthesis. In transgenic sugarcane (overexpression of *P5SC)*, Fv/Fm was maintained under water deficit treatment because of the high level of proline production which helps to protect the photosynthetic apparatus (Molinari et al., 2007). Stomata is one of important factor that can control leaf gas and water exchange which can alter stomatal conductance and net photosynthetic rate (Wu et al.,

2014). This suggests that the new leaf might adjust themselves through the stomatal development via the *GTL1* function to reduce the water loss.

2.2.3. Investigation of drought stress effect in wild type and *gtl1-4*

Although the *gtl1-4* had lower SD when compared to wild type under normal condition (Yoo et al., 2010), the lower SD under control and drought stress was not found in this study. The investigation of stomatal density in Arabidopsis was no significant in abaxial leaf in all treatments. It is possible that environment influences on stomatal traits (Hetherington and Woodward, 2003). There are huge diversity of stomatal responses to the environmental changes. Carbon dioxide, humidity and light intensity have been reported to effect on stomatal development (Casson and Gray, 2008; Pillitteri and Torii, 2012). Arabidopsis from different altitude showed an increased SD and SI when grown at elevated CO_2 (Caldera et al., 2017). Stomatal index (SI) can represent relationship of cell enlargement and frequency of stomata. SI was sharply increased in maize that grown at high CO_2 concentration because it enhanced the epidermal cell size (Driscoll et al., 2006). In contrast, reduction of SD (14.3%) was found in many plant species at high CO_2 concentrations (Woodward and Kelly, 1995). Increased humidity resulted in a reduction of the stomatal index of *Scilla nutans* leaves (Salisbury, 1928). The similar result also found in *T. ciliate* which had significant lower SD when grew in high humidity (Carins Murphy et al., 2014). In addition, a study of (Hetherington and Woodward, 2003) showed a strong correlation between stomatal density and size. The study illustrated that high stomatal density tended to have small size of stomata. Therefore, the environmental is a factor that regulates stomatal density. Since our study had no significant difference of SD, it might be the effect of the severe

weather during the experiment was conducted. In addition, it is possible that there will be different on the size of epidermal cells due to the higher SI found in *gtl1-4* mutant.

In a study of *phyB* rice mutant, lower stomatal density was found when compared to wild-type rice. The *phyB* mutant increased the expression of *ER* and *EXPANSIN* which involved in cell expansion. This caused a large epidermal cell in fully expanded leaf of mutant (Liu et al., 2011). The Arabidopsis mutant (*ER*) increased SD and had smaller epidermal cells. While, the SI had no significant changes. Hence, it was hypothesized that *ER* regulates stomatal density via epidermal cell expansion (Masle et al., 2005). The *EXPANSIN* family genes also involved in cell expansion through the regulation of cell wall loosening (Choi et al., 2006; Lee et al., 2001). This suggested that the leaf expansion in *gtl1-4* might be one factor regulate stomatal density and total number of fully expanded leaf. The least water loss found in *gtl1-4* under drought stress also affected the cell expansion.

CHAPTER VI
CONCLUSIONS

1. **Investigation of leaf protein profiles of Leung Pratew 123 (*O. sativa* L. cv. Leung Pratew123) and its drought resistant mutated line responding to the drought stress**

According to GeLC-MS/MS analysis, leaf protein profiles were compared between control and drought stress treatment. For SS, there were 68 protein changes responding to 10% PEG while 55 proteins were found in SR induced by drought stress. The significant different protein expression from SS and SR were classified into ten functional groups. Disregarding the proteins with unknown functions, retrotransposons were the main group of proteins affected by osmotic stress in SR plants, while proteins related to metabolic processes were the most commonly affected proteins in SS plants. The categories of post-translation were the group found only in SS line, while post-trancription group was the category found only in SR plants. These differences suggest that SS and SR respond differently to osmotic stress.

2. **Determination the appropriate data analysis methods for the whole rice proteins after drought stress.**

The appropriate proteomics analysis to obtain the candidate proteins/genes responsible for drought tolerance in rice can be proposed by this research.

2.1. Replication of resources

At least three replications of the materials should be prepared in order to be valid for statistical analysis for the comparison.

2.2. Elimination of false positive prediction of LC-MS-MS data analysis

After the prediction of LC-MS-MS data, the potentially false positive data should be removed. The potentially false positive data are:

- The proteins identified by less than 5 amino acid residues
- The proteins present less than 2 replicates
- The same loci predicted by more than LC-MS-MS data, select only one with the highest significance of prediction.
- Check the existence by using blastp algorithm against NCBI database (Coordinators, 2016). If it does not exist, eliminate it from the list.

2.3. Statistical analysis for the significant responsive proteins and visualization

The identified proteins should be visualized and statistically analyzed with a t-test ($p<0.05$) using the MultiExperiment Viewer (MeV) program. The gene ontology (GO) can be obtained from rice genome annotation project (Kawahara et al., 2013).

2.4. Comparison of protein profiles to obtain the drought responsive proteins

Three sets of protein profiles, which are the significant drought responsive proteins from susceptible line, the significant drought responsive proteins from tolerant line and the significantly different proteins from susceptible and tolerant lines, should be obtained, and then create Venn's diagram to see the interception of three dataset. The union of all three datasets is the proteins of interest.

2.5. Identify the best candidate proteins/genes for further study and function validation

We use the co-expression network analysis based on the datasets available publicly to determine best candidate proteins/genes. The proteins with the highest network will be selected for further study.

3. Identification and characterization of drought responsive genes selected from the gene/protein expression patterns

The proteomics analysis revealed several candidate proteins with important roles in drought responses. A transcription factor, GT-2-LIKE1 (GTL1) protein showed the significantly differential expression only in the drought resistant line. Under drought stress condition, GTL1 protein of SR was decreased, but the *GTL1* transcripts could not be detected in leaves of 4-week-old plants in both rice lines. However, the dehydrated leaves of 7-day old SR seedlings showed the transcriptional expression reduction of the gene, while this gene transcripts were increased in 'SS' dehydrated leaves of 7-day-old seedlings, suggesting that *GTL1* was the dehydration responsive gene, which was transcriptionally expressed in young tissues of rice. This was also consistent with the pattern of GTL1 protein found with proteomic detection. The reduction of stomatal density was also found only in the SR line, but not in SS. These support the role of GTL1 regulation of stomatal density and lead to drought tolerant phenotype.

In addition, a major hub gene; *LOC_Os04g38600* (encoding a glyceraldehyde-3-phosphate dehydrogenase) was identified. *GAPDH* expression was up-regulated in

both SS and SR leaves treated with drought condition for 2 h. However, the increase was greater in the SR leaves. This was consistent with the increase in GAPDH protein abundance. The drought-resistant line, SR rice showed the higher fresh/dry weight, and relative leaf water content than ‘SS’ rice, under drought stress. An investigation of photosynthesis parameters using Li-6400XT in first fully expended leaf (old leaf) and youngest fully expended leaf (young leaf) in both SS and SR was conducted. In old leaf, net photosynthetic rate (*A*), water use efficiency (WUE), ΦPSII and electron transport rate (ERT) were higher in SR than SS after 3 days of drought stress (12.5% PEG). In young leaf, transpiration rate (E) is significant lower in SR. Overall, SR rice mediates drought stress by maintaining photosynthetic process. In addition, the studies in *gtl1* Arabidopsis mutants under drought stress condition, it was found that *gtl1-4* (knock-out mutant) has higher survival rate than wild type because of the higher maintenance of relative water content.

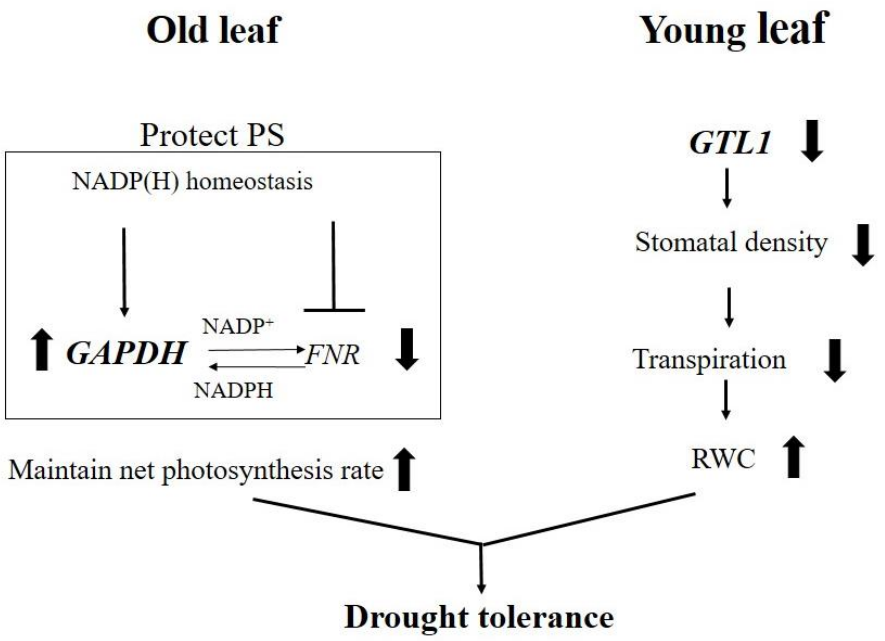

Figure 27. Summary of drought adaptation mechanism in old leaf (develop before drought stress period) and young leaf (develop during drought stress) of drought-resistant rice line (SR).

In summary, *GTL1* is another crucial gene which regulates stomatal density leading to less transpiration in young leaf, while *GAPDH* plays a role in protecting photosystem by NADP(H) homeostasis in old leaf contributes to drought tolerance in rice (Fig. 27). Therefore, *GTL1* and *GAPDH* are a potential candidate gene to improve drought stress tolerance crops in the future.

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APPENDIX

APPENDIX A
CHEMICALS AND REAGENTS

WP nutrient solution for the experiment that grow rice in the solution (Vajrabhaya and Vajrabhaya, 1991a)

The chemical listed below are for preparing 1 liter solution:

Chemicals	Content (mg)
Macroelements:	
Potassium nitrate (KNO_3)	580
Calcium sulfate ($CaSO_4$)	500
Magnesium sulfate ($MgSO_4.7H_2O$)	450
Triple superphosphate	250
Ammonium sulfate ($(NH_4)_2SO_4$)	100
Microelements:	
Di-sodium ethylene diamine tetraacetate (Na_2EDTA)[a]	160
Ferrous sulfate ($FeSO_4.7H_2O$)	120
Manganese sulfate ($MnSO_4.H_2O$)	15
Boric acid (H_3BO_3)	5
Zinc sulfate ($ZnSO_4.7H_2O$)	1.5
Potassium iodide (KI)	1.0
Sodium molybdate ($Na_2MoO_4.2H_2O$)	0.1
Copper sulfate ($CuSO_4.5H_2O$)	0.05
Cobalt chloride ($CoCl_2.6H_2O$)	0.05

2. Protein quantification and separation

2.1.Protein concentration measurement (Lowry's method)

- Reagent A (alkaline copper reagent)

CTC	5 ml
(0.2% $CuSO_4.7H_2O$ + 0.4% Tartaric acid)	
20% Na_2CO_3	5 ml
0.8 N NaOH	10 ml
5% SDS	20 ml

- Reagent B (diluted Folin-Ciocalteu's phenol reagent)

Folin-Ciocalteu phenol	1 ml
Distilled water	5 ml

2.2. Preparation of SDS-PAGE

- Separating gel (12.5 %)[a]

Reagents	**Content (µl)**
Distilled water	4,200 µl
40% (w/v) acrylamide/bis-acrylamide solution (29:1)	3,125 µl
1.5 M Tris. HCl pH 8.8	2,500 µl
10% SDS	125 µl
10% APS	50 µl
TEMED[b]	6 µl

- Stacking gel (4%)[a]

Reagents	**Content (µl)**
Distilled water	1,900 µl
40% (w/v) acrylamide/bis-acrylamide solution (29:1)	300 µl
0.5 M Tris. HCl pH 6.8	742 µl
10% SDS	30 µl
10% APS	23 µl
TEMED[b]	3.5 µl

[a] The components were mixed in the order shown.

[b] Polymerize will begin as soon as TEMED has been added.

2.3. SDS-PAGE running and staining		
- Protein loading dye	50 mM Tris.HCl pH 6.8	
	10% glycerol	
	2% SDS	
	1% β-mercaptoethanol	
	0.02% bromophenol blue	
	adjust volume with distilled water	
- Tris-glycine electrophoresis buffer (1 liter)		
	Tris	1.514 g
	Glycine	7.2 g
	0.1% SDS	0.5 g
	adjust volume to 1 L with distilled water	
- Gel staining (Coomassie Brilliant Blue)		
Staining solution	Coomassie Brilliant Blue R250	5 g
	Acetic acid	100 ml
	Methanol	500 ml
	Distilled water	400 ml
Destaining solution	Acetic acid	100 ml
	Methanol	200 ml
	Distilled water	

3. Transcription expression analysis in rice

3.1. Agarose gel electrophoresis		
- 6x RNA loading dye	30% (v/v) glycerol in water	
	0.25% (w/v) bromophenol blue	
	0.25% (w/v) xylene cyanol FF	
- 5x TBE buffer	Tris base	54 g
	Boric acid	27.5 g
	0.5 M EDTA pH 8.0	20 ml

adjust volume to 1,000 ml with distilled water

4. Transcription expression analysis in Arabidopsis

- 5x TAE buffer	**Tris base**	**48.4 g**
	Glacial acetic acid	10.9 g
	EDTA	2.92 g

adjust volume to 1,000 ml with distilled water

APPENDIX B

PRIMERS

List of gene primers for transcription analysis

Primer name	Primer sequences
LOC_Os03g02240(forward)	CTCTCTGGGAGGACATCTC
LOC_Os03g02240(reverse)	TAGTAGGGGCATGTCTTGGA
LOC_Os04g38600 (forward)	GGTGTCCAAGAAGACCC
LOC_Os04g38600 (reverse)	ATGACCTTCACCATGTCGTC
OsEF-1α (forward)	ATGGTTGTGGAGACCTTC
OsEF-1α (reverse)	TCACCTTGGCACCGGTTG
OsDREB2A (forward) (for qRT-PCR)	GGGAGCAATGGCTTGAAACG
OsDREB2A (reverse) (for qRT-PCR)	CCTATTGACCCGCAGCATGA
OsDREB2A (forward) (for semi qRT-PCR)	ATCGCGGCCGCATGGAGCGGGGGGAGGGGAG
OsDREB2A (reverse) (for semi qRT-PCR)	GGGGATCCTACTCTAATAGGAGAAAAGGCT
AtGTL1 (forward)	ATGGAGCAAGGAGGAGGTG
AtGTL1 (reverse)	AAAGGTGGTTCCGTATGG
SDD1 (forward)	GAAAGCGATAAAGGATGG
SDD1 (reverse)	GGTTACAGAGATTGGACTTC
ACT2 (forward)	AGAGATTCAGATGCCCAGAAGTCTTGTTCC
ACT2 (reverse)	TCCTGGACCTGCCTCATC
DREB2A (forward)	TCGAGCTGAAACGGAGGTAT
DREB2A (reverse)	GACCTAAATGGCGACGATGT

APPENDIX C

STANDARD CURVES AND PROTEIN LADDER

Identification of drought-responsive proteins in LPT123 and LPT123-TC171 rice during drought stress

Protein concentration measurement

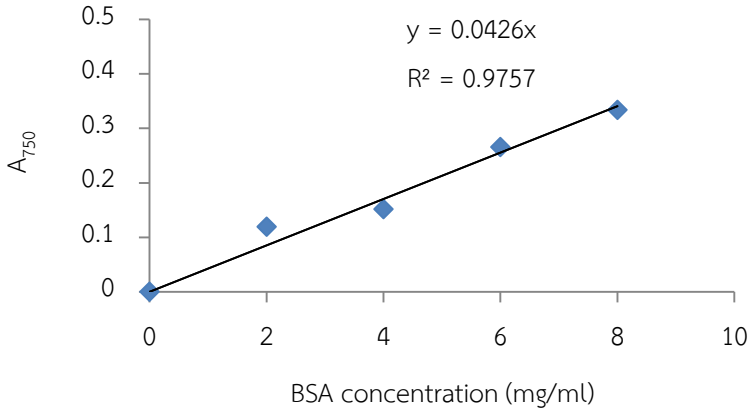


Figure C.1 Standard curve of standard protein (BSA)

Protein separation (SDS-polyacrylamide gel electrophoresis)

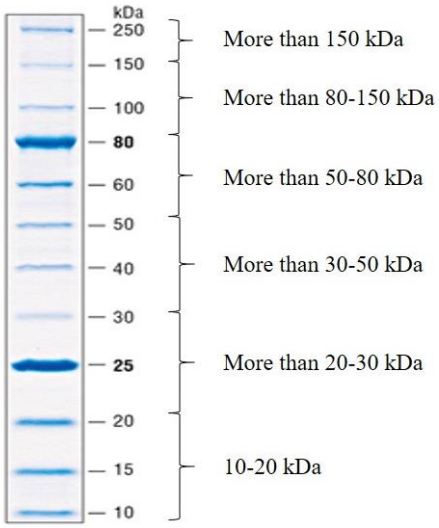
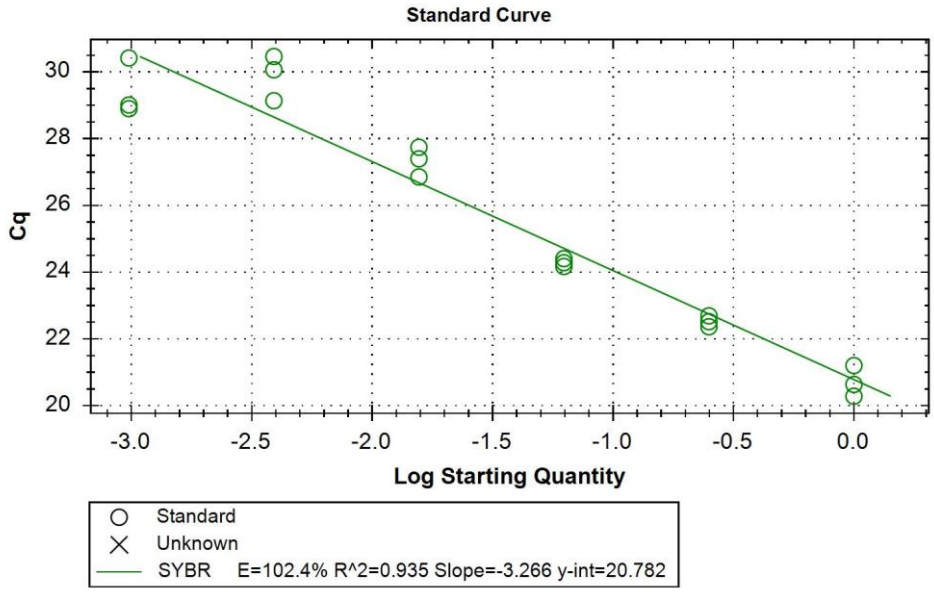


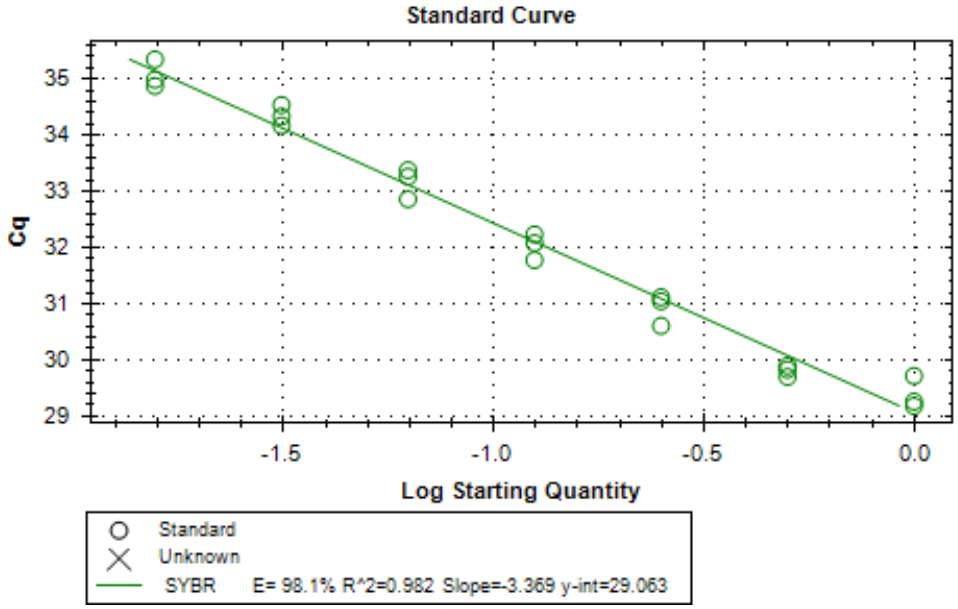
Figure C.2 Protein ladder 10-250 kDa (New England Biolabs, USA)

Expression analysis of a drought-responsive gene during drought stress, *EF1 alpha, OsGTL1 and DREB2A*

EF1 alpha



OsGTL1



DREB2A

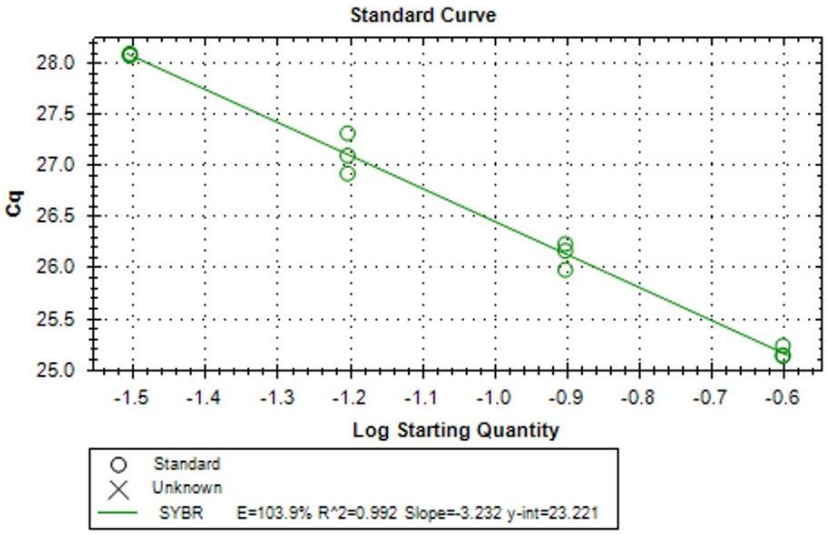


Figure C.3 Standard curve of (A) *EF-1α*, a reference gene, (B) *OsGTL1*, and *DREB2A*

APPENDIX D

DROUGHT-RESPONSIVE PROTEINS IN LPT123 AND LPT123-TC171 RICE

Table D1. List of 357 drought-responsive proteins in leaves of SS and SR seedling by proteomic analysis

Gene no.[a]	Locus [b]	RAP id [c]	Description [b]	Functional group	ID Score[a]	MH+ (Da) [a]	Peptide [a]
ABA97236.1	LOC_Os12g14320		Expressed protein	Unknown	10.14	894.77	GMTASSSVR
BAF03855.2	LOC_Os01g04230	Os01g0133900	Inactive receptor kinase At2g26730 precursor	Signalling	10.72	947.10	MRIASAAAR
BAS70681.1	LOC_Os01g08140	Os01g0176400	Phototropic-responsive NPH3 family protein	Signalling	25.90	1140.86	GGAAGAAAATPTPK
BAS70864.1	LOC_Os01g09990	Os01g0196300	Helix-loop-helix DNA-binding domain containing protein	Transcription	21.46	928.80	TSILIRAR
BAS70962.1	LOC_Os01g11040	Os01g0208600	SCAR-like protein 2	Development	7.76	1089.22	TENDTNGLPK
EEE54096.1	LOC_Os01g11220	Os01g0210400	Hydrolase, acting on carbon-nitrogen	Unknown	11.42	795.50	QGYGITR
BAB07986.1	LOC_Os01g15370		Retrotransposon protein, putative, Ty3-gypsy subclass	Retrotransposon	10.47	802.57	HMATGGGR
BAS71601.1	LOC_Os01g17420	Os01g0281301	Expressed protein	Unknown	6.25	882.55	DVAAAAPIR
BAD53958.1	LOC_Os01g26250		Expressed protein	Unknown	9.73	1124.16	AHEAAAGGGATGR
EEC70666.1	LOC_Os01g27340	Os01g0370900	Glutathione S-transferase	Metabolic process	11.03	1862.99	NPFGQDASFPGRGSASLR

[a] Gene no., ID Score, MH^+ (Da), predicted peptide from Mascot™
[b] Locus number and description retrieve from the Rice Genome Annotation Project
[c] RAP id from RAP-DB

Table D1. List of 357 drought-responsive proteins in leaves of SS and SR seedling by proteomic analysis *(cont.)*

Gene no.[a]	Locus [b]	RAP id [c]	Description [b]	Functional group	ID Score[a]	MH+ (Da) [a]	Peptide [a]
EEE54767	LOC_Os01g36550	Os01g0546000	Protein kinase family protein	Signalling	8.69	727.32	GVAGPSNK
BAS72886	LOC_Os01g40200	Os01g0584200	XIK	Metabolic process	9.84	1030.87	VDSLAAEVAR
XP_015646220	LOC_Os01g41750	Os01g0601625	Expressed protein	Unknown	17.77	701.19	HATMLK
BAS73047	LOC_Os01g41910	Os01g0603500	Receptor-like protein kinase 5 precursor	Signalling	19.03	965.44	AMIQGNSTK
BAS73183	LOC_Os01g43060	Os01g0617800	Expressed protein	Unknown	7.89	1122.20	KAGPPTDPLPK
BAS73439	LOC_Os01g46169	Os01g0650200	GDSL-like lipase/acylhydrolase	Metabolic process	8.33	968.63	MASSTSGRR
BAD72340	LOC_Os01g47440		Expressed protein	Unknown	8.19	978.76	GDGNGMVLIS
BAS73660	LOC_Os01g48500	Os01g0676200	Expressed protein	Unknown	5.87	1707.59	YGSGELLGTVAGVVTER
BAS73899	LOC_Os01g50616	Os01g0701900	Phosphatidylinositol transfer	Transport	6.61	1028.02	MLYDALMR
BAS73947	LOC_Os01g51050	Os01g0706800	Jacalin-like lectin domain containing protein	Defense	9.37	950.01	MVASSKAIK
BAS74069	LOC_Os01g52180	Os01g0719900	Lipase	Metabolic process	13.97	1653.69	MQISSLCCAEQPSK
BAS74447	LOC_Os01g55520	Os01g0760300	ATROPGEF7/ROPGEF7	Metabolic process	7.87	1016.78	AASGGGSLLER
BAS74525	LOC_Os01g56200	Os01g0767900	BTBA2 - Bric-a-Brac,Tramtrack, Broad Complex BTB domain with Ankyrin repeat region	Defense	4.58	984.27	MRFCELK

[a] Gene no., ID Score, MH^+ (Da), predicted peptide from Mascot™
[b] Locus number and description retrieve from the Rice Genome Annotation Project
[c] RAP id from RAP-DB

Table D1. List of 357 drought-responsive proteins in leaves of SS and SR seedling by proteomic analysis *(cont.)*

Gene no.[a]	Locus [b]	RAP id [c]	Description [b]	Functional group	ID Score[a]	MH+ (Da) [a]	Peptide [a]
EEE55443	LOC_Os01g56200	Os01g0767900	BTBA2 - Bric-a-Brac,Tramtrack, Broad Complex BTB domain with Ankyrin repeat region	Defense	7.29	919.86	EQGQESNK
BAF06391	LOC_Os01g57740	Os01g0787400	Expressed protein	Unknown	3.89	990.48	MAMDLVRR
EEE55604	LOC_Os01g60960	Os01g0825000	DUF260 domain containing protein	Unknow	10.45	1210.36	DLQAQVPAGRR
EEC71729	LOC_Os01g61110	Os01g0826651	Ulp1 protease family, C-terminal catalytic domain containing protein	Metabolic process	8.63	1272.78	DRMFVFLDSK
BAS75559	LOC_Os01g65840	Os01g0880900	Pentatricopeptide	Posttranscription	15.32	827.45	MALHGVGK
XP_015639963	LOC_Os01g65850	Os01g0881000	CHD3-type chromatin-remodeling factor PICKLE	Development	7.25	1934.97	HPGKSSDDYDENCSAPR
EEC71933	LOC_Os01g66650	Os01g0890300	Expressed protein	Unknow	5.16	866.58	GAVMSSCR
BAS75866	LOC_Os01g68610	Os01g0914600	Pentatricopeptide repeat protein PPR1106-17	Posttranscription	8.29	1027.26	KLMSAEGMK
BAS76046	LOC_Os01g70600	Os01g0931700	DUF567 domain containing protein	Unknown	8.93	1012.59	QEMAIAPPR
BAF07304	LOC_Os01g71960	Os01g0948100	Endonuclease	Defense	13.79	1107.28	SLKDLYQIK
BAS76556	LOC_Os02g01500	Os02g0105200	2-oxo acid dehydrogenases acyltransferase domain containing protein	Metabolic process	12.06	871.67	VSLGGLNGR

[a] Gene no., ID Score, MH^+ (Da), predicted peptide from Mascot™
[b] Locus number and description retrieve from the Rice Genome Annotation Project
[c] RAP id from RAP-DB

Table D1. List of 357 drought-responsive proteins in leaves of SS and SR seedling by proteomic analysis *(cont.)*

Gene no.[a]	Locus [b]	RAP id [c]	Description [b]	Functional group	ID Score[a]	MH+ (Da) [a]	Peptide [a]
EEE56150	LOC_Os02g01740	Os02g0106966	U5 small nuclear ribonucleoprotein 200 kda helicase	Transcription	3.48	1178.58	TWAAAPEAHAR
AAP55707	LOC_Os02g02670	Os02g0118875	NBS-LRR-like protein CR273	Defense	9.37	787.97	RIGTVNK
BAD10265	LOC_Os02g05000	Os02g0142800	Expressed protein	Unknown	5.87	1094.99	RLPSNPTPGR
BAS77209	LOC_Os02g07630	Os02g0172600	Copper-transporting atpase	Transport	9.07	1445.90	WILVSIVQFGVGK
BAS77588	LOC_Os02g11870	Os02g0209400	Expressed protein	Unknown	11.19	856.10	VLRLGGNK
BAS78380	LOC_Os02g21630	Os02g0321500	SEC14 cytosolic factor family protein	Transport	3.90	1106.01	KLSVDETVSK
BAS78390	LOC_Os02g21700	Os02g0322400	STE_MEKK_ste11_ MAP3K.8 - STE kinases include homologs to sterile 7, sterile 11 and sterile 20 from yeast	Signalling	19.86	1260.00	GTPMFLAPEAAR
BAS78414	LOC_Os02g22090	Os02g0326600	Pattern formation protein EMB30	Cellular process	11.02	813.54	NILLVLK
BAB86451	LOC_Os02g25760		Retrotransposon protein	Retrotransposon	24.73	760.15	GDLGGVGGK
BAS78873	LOC_Os02g30900	Os02g0513000	Protein kinase domain containing protein	Signalling	15.13	927.80	LPKMADPR
BAS78878	LOC_Os02g30974	Os02g0513800	Expressed protein	Unknown	8.85	756.51	EQPGGGGR
BAS79077	LOC_Os02g33450	Os02g0537700	Peroxiredoxin	Defense	19.90	1486.72	SFGVLIPDQGIALR
BAS79338	LOC_Os02g36140	Os02g0570400	Terpene synthase	Metabolic process	9.22	964.47	KSVLEVYK

[a] Gene no., ID Score, MH^+ (Da), predicted peptide Mascot™
[b] Locus number and description retrieve from the Rice Genome Annotation Project
[c] RAP id from RAP-DB

Table D1. List of 357 drought-responsive proteins in leaves of SS and SR seedling by proteomic analysis *(cont.)*

Gene no.[a]	Locus [b]	RAP id [c]	Description [b]	Functional group	ID Score[a]	MH+ (Da) [a]	Peptide [a]
EAY86554	LOC_Os02g38690	Os02g0599151	Protein phosphatase 2C containing protein	Signalling	13.48	978.55	RPSSKGTSC
EEE57326	LOC_Os02g39140	Os02g0603600	Helix-loop-helix DNA-binding domain containing protein	Transcription	11.79	1518.84	MASTSALEMAGMDR
BAS79656	LOC_Os02g39290	Os02g0605500	TRAF-type zinc finger family protein,	Transcription	6.99	902.60	EATAGTILK
XP_015626092	LOC_Os02g46650	Os02g0693400	Ubiquitin carboxyl-terminal hydrolase domain containing protein	Metabolic process	18.81	936.82	VESASKSTK
BAS80476	LOC_Os02g47360	Os02g0702000	PPR repeat domain containing protein	Posttranscription	8.50	940.13	FNFALATR
BAS80804	LOC_Os02g50320	Os02g0736100	ATMAP70 protein	Cellular process	24.35	958.65	ISPNGMLAR
BAS80810	LOC_Os02g50370	Os02g0736600	Helicase domain-containing protein	Transcription	10.27	1061.20	AFPGPSKDDK
BAD38452	LOC_Os02g50520		Transposon protein	Transposon	9.67	857.95	TPAEEGKK
BAF10117	LOC_Os02g52470	Os02g0762300	Cytoplasmic tRNA 2-thiolation protein 1	translation	14.22	964.10	MDSAVDGPR
BAF10141	LOC_Os02g52810	Os02g0767000	Remorin C-terminal domain containing protein	Unknown	8.17	927.46	HVTRGSDR
BAS81173	LOC_Os02g53680	Os02g0776800	RPA1A - Putative single-stranded DNA binding complex subunit 1	Cellular process	13.32	884.51	GNLRPAQK

[a] Gene no., ID Score, MH^+ (Da), predicted peptide Mascot™
[b] Locus number and description retrieve from the Rice Genome Annotation Project
[c] RAP id from RAP-DB

Table D1. List of 357 drought-responsive proteins in leaves of SS and SR seedling by proteomic analysis *(cont.)*

Gene no.[a]	Locus [b]	RAP id [c]	Description [b]	Functional group	ID Score[a]	MH+ (Da) [a]	Peptide [a]
EAY87757	LOC_Os02g54020	Os02g0780800	DEAD-box ATP-dependent RNA helicase	Transcription	10.32	849.64	AGLDAKFK
BAS81334	LOC_Os02g55030	Os02g0793300	Hydrolase, NUDIX family, domain containing protein	Metabolic process	12.44	936.68	NISEAKFK
BAS81593	LOC_Os02g57290	Os02g0817900	Cytochrome P450	Metabolic process	4.71	820.86	CAASGGNGK
BAB40535	LOC_Os02g58220	Os02g0829100	RPA2A - Putative single-stranded DNA binding complex subunit 2	Cellular process	8.86	826.64	LRLPEAK
EAZ25193	LOC_Os02g58410	Os02g0830801	Expressed protein	Unknown	6.00	723.26	MGAGGDAK
BAH91957	LOC_Os03g01670	Os03g0107100	Retrotransposon protein	Retrotransposon	9.07	1608.39	LRLHGPNPNVSLYK
BAS81932	LOC_Os03g02090	Os03g0111600	Expressed protein	Unknown	7.78	847.96	KHNMFR
EAY88249	LOC_Os03g02100	Os03g0111700	Valyl-trna synthetase	Translation	9.44	1738.40	VYLHPMIWDAHGRK
XP_015628078	LOC_Os03g02240	Os03g0113500	Trihelix transcription factor GTL1	Transcription	6.86	1044.26	RGGGGIGGGGGGK
EAY88587	LOC_Os03g05880	Os03g0153500	Monooxygenase	Metabolic process	13.74	881.08	AAAVPIPSR
ABF94046	LOC_Os03g05960		Retrotransposon protein, putative, Ty3-gypsy subclass	Retrotransposon	27.97	809.45	MASLMEK
CBC97088	LOC_Os03g06654	Os03g0162000	Flavin monooxygenase	Metabolic process	4.84	1927.75	NITGKSPVLDEGAWSLIK
ABF94151	LOC_Os03g06950	Os03g0165600	Ubiquitin carboxyl-terminal hydrolase domain containing protein	Metabolic process	9.41	1250.21	ALAALQRGNHAK

[a] Gene no., ID Score, MH^+ (Da), predicted peptide Mascot™
[b] Locus number and description retrieve from the Rice Genome Annotation Project
[c] RAP id from RAP-DB

Table D1. List of 357 drought-responsive proteins in leaves of SS and SR seedling by proteomic analysis *(cont.)*

Gene no.[a]	Locus [b]	RAP id [c]	Description [b]	Functional group	ID Score[a]	MH+ (Da) [a]	Peptide [a]
ABF94262	LOC_Os03g07940	Os03g0176300	AP2 domain containing protein	Transcription	3.65	1031.46	KPAQTFGQR
BAS83373	LOC_Os03g12200	Os03g0222300	Osfbx81 - F-box domain containing protein	Transcription	11.75	986.96	SIAAVGSEVR
ABF94733	LOC_Os03g12400		Transposon protein	Transposon	10.21	930.32	TAPGGDAGER
BAS85805	LOC_Os03g12750	Os03g0229000	Hypothetical protein	Unknown	23.50	1306.35	NGLYLMAFSFK
BAS83290	LOC_Os03g14690	Os03g0251500	Vacuolar ATP synthase 98 kda subuni	Transport	9.19	2153.90	ICDAFNANRYPFPEDVAR
BAS83540	LOC_Os03g17010	Os03g0278300	RNA recognition motif containing protein	Posttranscription	22.46	1477.64	AETLDMTLDDIIK
BAS83549	LOC_Os03g17060	Os03g0278800	RNA recognition motif containing protein	Posttranscription	19.97	1496.51	MALSSSLLHRLLR
BAF11659	LOC_Os03g17340	Os03g0281800	Expressed protein	Unknown	12.63	932.83	SSPADYHR
BAS83729	LOC_Os03g18550	Os03g0296800	Mitochondrial carrier protein	Transport	3.22	1805.04	LQLTSSPYTGVSHCVR
ABF95470	LOC_Os03g18770	Os03g0299600	Wound-induced protein 1	Defense	10.75	1588.83	MMRLLTGADHGESR
ABF95862	LOC_Os03g22190	Os03g0341200	Expressed protein	Unknown	9.00	843.14	GPGRGGEGR
ABF95876	LOC_Os03g22310	Os03g0343250	Expressed protein	Unknown	6.37	857.78	GEAAADVPK
BAS84244	LOC_Os03g24100	Os03g0356526	Expressed protein	Unknown	6.63	968.14	IVVAAVSPGR
BAS84292	LOC_Os03g24730	Os03g0362200	Expressed protein	Unknown	9.14	811.27	ATMTGSDK

[a] Gene no., ID Score, MH^+ (Da), predicted peptide Mascot™
[b] Locus number and description retrieve from the Rice Genome Annotation Project
[c] RAP id from RAP-DB

Table D1. List of 357 drought-responsive proteins in leaves of SS and SR seedling by proteomic analysis *(cont.)*

Gene no.[a]	Locus [b]	RAP id [c]	Description [b]	Functional group	ID Score[a]	MH+ (Da) [a]	Peptide [a]
BAH92250	LOC_Os03g39900	Os03g0596300	Retrotransposon protein	Retrotransposon	25.54	923.59	ILLIGIPGK
BAS85845	LOC_Os03g48490	Os03g0691500	SCP-1; Synaptonemal complex protein 1	Cellular process	8.03	829.43	LDVAGLIK
BAF13076	LOC_Os03g52070	Os03g0730500	Osscp20 - Putative Serine Carboxypeptidase homologue	Metabolic process	13.32	1446.20	LQGYIVGNPITGSK
ABF98946	LOC_Os03g54840		Hypothetical protein	Unknown	13.36	927.41	AVAPTSRAR
BAS86453	LOC_Os03g54890	Os03g0756000	Ribosomal protein L13	Translation	9.58	927.02	GMIPHKTK
EAY91980	LOC_Os03g55810	Os03g0767332	Uncharacterized PE-PGRS family protein PE_PGRS46	Unknown	12.02	1099.32	GAAGGGGDPGARR
BAS86628	LOC_Os03g56400	Os03g0775400	Pentatricopeptide	Posttranscription	22.05	819.85	SGSVENAR
ABF99478	LOC_Os03g59520		Retrotransposon protein, putative, Ty3-gypsy subclass	Retrotransposon	15.09	1480.82	GDAGSGDGFGRADSGR
BAF13607	LOC_Os03g60190	Os03g0816500	Oxidoreductase, 2OG-Fe oxygenase family protein	Metabolic process	6.47	923.18	SGLANASFR
BAS87151	LOC_Os03g61050	Os03g0825700	FG-GAP repeat-containing protein	Development	9.64	2070.99	LPTVPVRTTGTVLVEMVDK
BAH01076	LOC_Os03g61490	Os03g0830400	Expressed protein	Unknown	7.74	1000.14	GQGLDVVRR
XP_015616316	LOC_Os03g61690	Os03g0832400	Protein phosphatase 2C	Siganlling	8.07	1032.52	FNVGLSLQR
BAS87848	LOC_Os04g08034	Os04g0162100	ZOS4-02 - C2H2 zinc finger protein	Transcription	10.49	995.38	DNMRTEVN

[a] Gene no., ID Score, MH^+ (Da), predicted peptide Mascot™
[b] Locus number and description retrieve from the Rice Genome Annotation Project
[c] RAP id from RAP-DB

Table D1. List of 357 drought-responsive proteins in leaves of SS and SR seedling by proteomic analysis *(cont.)*

Gene no.[a]	Locus [b]	RAP id [c]	Description [b]	Functional group	ID Score[a]	MH+ (Da) [a]	Peptide [a]
CAI44652	LOC_Os04g09980		Transposon protein	Transposon	5.61	973.44	AAGLLPLYR
CAE01993	LOC_Os04g11560		Retrotransposon, putative, centromere-specific	Retrotransposon	8.04	1119.01	FLHDAFLEK
CAE03907	LOC_Os04g15510		Retrotransposon protein	Retrotransposon	14.17	1822.13	TPTIPTCSKMEAAEGGR
BAS88326	LOC_Os04g20260	Os04g0270900	UDP-glucoronosyl and UDP-glucosyl transferase	Metabolic process	9.65	1031.02	DGAMSHQLR
CAD40415.3	LOC_Os04g22020		Retrotransposon protein	Retrotransposon	10.11	899.46	DSSMANFK
CAE05607	LOC_Os04g25890		Retrotransposon protein	Retrotransposon	14.85	1035.94	RGCEACQR
CAH67848	LOC_Os04g27420	Os04g0341966	Retrotransposon protein	Retrotransposon	14.41	824.59	ISIGNGHK
BAS88759	LOC_Os04g28880	Os04g0358300	Retrotransposon protein, putative, Ty3-gypsy subclass	Retrotransposon	11.98	861.35	AIAVESDR
BAS89134	LOC_Os04g33720	Os04g0413200	Glycosyl hydrolases	Metabolic process	12.63	802.13	VSLDLTR
BAF14662	LOC_Os04g33740	Os04g0413500	Glycosyl hydrolases	Metabolic process	5.62	846.71	KPVMMNGA
CAI44652	LOC_Os04g09980		Transposon protein	Transposon	5.61	973.44	AAGLLPLYR
CAE01993	LOC_Os04g11560		Retrotransposon, putative, centromere-specific	Retrotransposon	8.04	1119.01	FLHDAFLEK
CAE03907	LOC_Os04g15510		Retrotransposon protein	Retrotransposon	14.17	1822.13	TPTIPTCSKMEAAEGGR
BAS89333	LOC_Os04g35864	Os04g0439300	DDT domain-containing protein	Transcription	5.16	1046.38	QSVQSNSLGK

[a] Gene no., ID Score, MH^+ (Da), predicted peptide Mascot™
[b] Locus number and description retrieve from the Rice Genome Annotation Project
[c] RAP id from RAP-DB

Table D1. List of 357 drought-responsive proteins in leaves of SS and SR seedling by proteomic analysis *(cont.)*

Gene no.[a]	Locus [b]	RAP id [c]	Description [b]	Functional group	ID Score[a]	MH+ (Da) [a]	Peptide [a]
BAF14799	LOC_Os04g36580	Os04g0442900	Retrotransposon protein	Retrotransposon	11.24	849.51	GKGSIIFK
BAS89533	LOC_Os04g38600	Os04g0459500	Glyceraldehyde-3-phosphate dehydrogenase	Metabolic process	87.70	1787.64	VIAWYDNEWGYSQR
BAS89615	LOC_Os04g39260	Os04g0467400	CAF1 family ribonuclease containing protein	Defense	6.15	1396.55	YQFDFNTCFR
BAS89740	LOC_Os04g40510	Os04g0481200	Glycosyl hydrolase family 5 protein	Metabolic process	12.04	1426.52	LSPRDSPLLCLR
BAS89792	LOC_Os04g40910	Os04g0485800	Osfbx146 - F-box domain containing protein	Transcription	9.94	1041.96	AVFGIKHLR
BAS89853	LOC_Os04g41490	Os04g0492300	DNA-directed RNA polymerase III subunit RPC1	Transcription	20.76	756.94	EIINAAK
BAS90195	LOC_Os04g44710	Os04g0529500	similar to OsCPL1, CPL1	Transcription	11.77	934.45	QAVNMSLR
BAS90244	LOC_Os04g45170	Os04g0534200	Protein kinase-like domain containing protein	Signalling	17.51	1722.73	MFACVDDDLLANVPK
CAE03883	LOC_Os04g51590		Retrotransposon protein	Retrotransposon	5.26	1070.20	MMLCTGKGR
BAS91002	LOC_Os04g52540	Os04g0615700	Retrotransposon protein	Retrotransposon	5.93	1808.94	RNLFTCAELPDGLFR
BAS91054	LOC_Os04g52900	Os04g0620000	ABC transporter family protein	Transport	32.05	814.55	LLILSAGK
CAH67816	LOC_Os04g54670	Os04g0639700	Retrotransposon protein	Retrotransposon	9.81	932.11	LSNSLLSAK

[a] Gene no., ID Score, MH^+ (Da), predicted peptide Mascot™
[b] Locus number and description retrieve from the Rice Genome Annotation Project
[c] RAP id from RAP-DB

Table D1. List of 357 drought-responsive proteins in leaves of SS and SR seedling by proteomic analysis *(cont.)*

Gene no.[a]	Locus [b]	RAP id [c]	Description [b]	Functional group	ID Score[a]	MH+ (Da) [a]	Peptide [a]
BAS91282	LOC_Os04g55060	Os04g0643300	3-oxoacyl-synthase III, chloroplast precursor	Metabolic process	11.36	779.24	GCGGHHR
BAF16092	LOC_Os04g56990	Os04g0665600	Myb-like DNA-binding domain containing protein	Transcription	19.17	985.43	YMPASSEGK
XP_015639814	LOC_Os05g05660	Os05g0149200	PWWP domain containing protein	Transcription	12.20	880.08	LPKSPIPK
BAS92333	LOC_Os05g06140	Os05g0153300	Lipase	Metabolic process	18.15	992.83	SMIAMIDGR
XP_015639857	LOC_Os05g06450	Os05g0156600	Tubulin/ftsz domain containing protein	Cellular process	11.08	1025.88	QAFLDNYR
EEE62671	LOC_Os05g08370	Os05g0176100	CESA1 - cellulose synthase	Cell wall formation	6.90	913.50	RPAVGVSVK
AAV31207	LOC_Os05g09716		Transposon protein, putative, CACTA, En/Spm sub-class	Transposon	13.64	1217.69	LMAQLRVELK
BAS93042	LOC_Os05g16460	Os05g0253700	Expressed protein	Unknown	5.11	929.79	QGPGWTAGR
BAS93081	LOC_Os05g18730	Os05g0269500	Generative cell specific-1	Development	9.18	861.92	DGHYSPSV
BAS93239	LOC_Os05g23720	Os05g0302700	Mitochondrial carrier protein	Transport	13.04	1210.31	QFNGLVDVYR
EEE63327	LOC_Os05g27740		Expressed protein	Unknown	4.27	892.55	ASNAKFQK
BAS93896	LOC_Os05g33030	Os05g0397900	Kinesin motor domain containing protein	Cellular process	6.54	1000.02	NNALSPQQK

[a] Gene no., ID Score, MH^+ (Da), predicted peptide Mascot™
[b] Locus number and description retrieve from the Rice Genome Annotation Project
[c] RAP id from RAP-DB

Table D1. List of 357 drought-responsive proteins in leaves of SS and SR seedling by proteomic analysis *(cont.)*

Gene no.[a]	Locus [b]	RAP id [c]	Description [b]	Functional group	ID Score[a]	MH+ (Da) [a]	Peptide [a]
BAF17386	LOC_Os05g33050	Os05g0398100	ABTB1 - Armadillo repeats with a Bric-a-Brac, Tramtrack, Broad Complex BTB domain	Defense	18.94	973.30	SGSSQLIQR
BAS92588	LOC_Os05g34550	Os05g0183566	MLO domain containing protein	Defense	5.69	889.61	AMVEALEK
BAS94532	LOC_Os05g39850	Os05g0476200	MCM3 - Putative minichromosome maintenance MCM complex subunit 3	Cellular process	6.50	808.96	GSVSGVFR
AAT01370	LOC_Os05g40110	Os05g0479250	Retrotransposon protein	Retrotransposon	5.66	838.15	RFQSGML
BAF17988	LOC_Os05g44570	Os05g0521300	Histidine-containing phosphotransfer protein	Signalling	6.27	805.25	GLPGFSVK
AAV44039	LOC_Os05g44880		Retrotransposon protein	Retrotransposon	6.88	866.44	GDRPHRK
XP_015638673	LOC_Os05g46180		Expressed protein	Unknown	15.63	817.68	NLLMVVK
BAS95121	LOC_Os05g46350	Os05g0541100	IQ calmodulin-binding motif domain containing protein	Signalling	15.79	987.33	LNSLSLKGR
BAS95587	LOC_Os05g50990	Os05g0587300	TTL3	Signalling	11.90	845.68	GGGGMTPPR
BAS95627	LOC_Os05g51400	Os05g0591800	Protein kinase APK1B, chloroplast precursor	Signalling	5.70	857.50	QEGNGAEPG
CAD40837.3	LOC_Os06g04030	Os06g0130900	Histone H3	Cellular process	18.83	993.46	RCSSTDLR

[a] Gene no., ID Score, MH^+ (Da), predicted peptide Mascot™
[b] Locus number and description retrieve from the Rice Genome Annotation Project
[c] RAP id from RAP-DB

Table D1. List of 357 drought-responsive proteins in leaves of SS and SR seedling by proteomic analysis *(cont.)*

Gene no.[a]	Locus [b]	RAP id [c]	Description [b]	Functional group	ID Score[a]	MH+ (Da) [a]	Peptide [a]
BAS96059	LOC_Os06g04690	Os06g0138700	Osfbx184 - F-box domain containing protei	Transcription	7.78	852.62	GMLDLMR
BAS96094	LOC_Os06g04980	Os06g0142100	Osfbx185 - F-box domain containing protein	Transcription	10.19	936.08	LGETLQMK
BAS96203	LOC_Os06g05920	Os06g0152500	pentatricopeptide repeat-containing protein	Posttrancription	12.92	917.65	AGELDGAER
AAX96737	LOC_Os06g06802		Retrotransposon protein	Retrotransposon	13.39	823.61	AGAIVKHK
EAZ36259	LOC_Os06g11060	Os06g0213900	Expressed protein	Unknown	19.66	994.12	GSVLMMSLR
EEE65432	LOC_Os06g13570	Os06g0244100	Expressed protein	Unknown	5.43	939.82	IVPTIELR
EEE65463	LOC_Os06g14270		Retrotransposon protein	Retrotransposon	10.78	1046.45	IVMAMAARGK
BAS97234	LOC_Os06g16450	Os06g0276300	Leucine Rich Repeat family protein	Unknown	11.69	888.18	MPEHTTR
BAS97292	LOC_Os06g17930	Os06g0287200	NBS-LRR disease resistance protein	Defense	5.09	890.11	DITVSISR
BAS97396	LOC_Os06g19980	Os06g0303700	MYB family transcription factor	Transcription	6.38	674.14	GDGEAVK
EAZ00688	LOC_Os06g20630	Os06g0311800	SAM dependent carboxyl methyltransferase	Signalling	9.85	972.85	APGDLKESR
EEC80502	LOC_Os06g21630		Hypothetical protein	Unknown	6.70	887.29	LLLPNYR
BAS98305	LOC_Os06g37300	Os06g0569500	Cytochrome P450	Metabolic process	9.34	1003.56	DAIMNALIK

[a] Gene no., ID Score, MH^+ (Da), predicted peptide Mascot™
[b] Locus number and description retrieve from the Rice Genome Annotation Project
[c] RAP id from RAP-DB

Table D1. List of 357 drought-responsive proteins in leaves of SS and SR seedling by proteomic analysis *(cont.)*

Gene no.[a]	Locus [b]	RAP id [c]	Description [b]	Functional group	ID Score[a]	MH+ (Da) [a]	Peptide [a]
BAD61967	LOC_Os06g37880		Expressed protein	Unknown	23.98	846.00	AGGEVAADR
BAS98557	LOC_Os06g40640	Os06g0608700	Fructose-bisphospate aldolase isozyme	Metabolic process	4.41	890.36	ANSEATLGK
BAS98800	LOC_Os06g43320	Os06g0640100	Cytochrome P450	Metabolic process	4.27	971.70	HGPVMMLR
BAS99010	LOC_Os06g45310	Os06g0663500	Osspl11 - SBP-box gene family member	Transcription	10.51	875.32	AGADSANIR
EEE66218	LOC_Os06g46475	Os06g0678650	WD domain, G-beta repeat domain containing protein	Transcription	14.31	1355.26	FTSVILVCIFR
BAS99296	LOC_Os06g48210	Os06g0697200	DEAD-box ATP-dependent RNA helicase	Transcription	11.17	842.93	DAFKSFK
BAS99483	LOC_Os06g50050	Os06g0714500	AAA-type atpase family protein	Cellular process	18.90	944.64	TMLAKAIAK
BAS99619	LOC_Os06g51220	Os06g0728000	HMG1/2	Transcription	4.11	855.43	DPNKPKR
BAS99674	LOC_Os07g01090	Os07g0100800	Transmembrane amino acid transporter protein	Transport	16.58	803.99	DGITTPAK
BAT00086	LOC_Os07g05670	Os07g0151500	Expressed protein	Unknown	10.03	979.43	KGGCSNGTAK
BAT00386	LOC_Os07g09000	Os07g0187700	WD domain, G-beta repeat domain containing protein	Transcription	16.67	819.68	MAGGGGGEGK
BAT00519	LOC_Os07g10350	Os07g0203300	S1 RNA binding domain containing protein	Metabolic process	11.66	820.37	SQGDEER
BAS83026	LOC_Os07g10560	Os07g0206300	Unknown	Unknown	10.11	767.44	ISGYAGAK

[a] Gene no., ID Score, MH^+ (Da), predicted peptide Mascot™
[b] Locus number and description retrieve from the Rice Genome Annotation Project
[c] RAP id from RAP-DB

Table D1. List of 357 drought-responsive proteins in leaves of SS and SR seedling by proteomic analysis *(cont.)*

Gene no.[a]	Locus [b]	RAP id [c]	Description [b]	Functional group	ID Score[a]	MH+ (Da) [a]	Peptide [a]
BAT00596	LOC_Os07g10960	Os07g0210900	Expressed protein	Unknown	8.14	786.72	ILGIQSR
BAF21115	LOC_Os07g12100	Os07g0222200	Expressed protein	Unknown	10.52	773.50	DPNPSDK
BAC83729	LOC_Os07g17730		Retrotransposon protein	Retrotransposon	4.99	922.90	RYQSAVAK
BAD82629	LOC_Os07g24930		Retrotransposon protein, putative, Ty3-gypsy subclass	Retrotransposon	9.78	1059.64	LTVAGDGNRR
BAC16105	LOC_Os07g26590		Expressed protein	Unknown	8.73	1133.55	KARPTTGFTR
EEC81953	LOC_Os07g29820	Os07g0481400	NBS-LRR disease resistance protein	Defense	5.52	948.28	SLRGLGAMK
EAZ04135	LOC_Os07g34420	Os07g0528200	Lipase class 3 family protein	Metabolic process	10.19	2237.03	NGFSSSSMVIAWDNEEHRR
BAD33476	LOC_Os07g34920	Os07g0533600	Aspartic proteinase nepenthesin precursor	Metabolic process	14.65	1124.14	ESSCSRMPR
BAT02000	LOC_Os07g36140	Os07g0545400	Core histone H2A/H2B/H3/H4	Cellular process	12.96	803.38	AIGSGAAKK
BAT02149	LOC_Os07g37560	Os07g0562800	Myosin-Vb	Cellular process	9.80	943.66	VSKAEAALR
BAT02329	LOC_Os07g39240	Os07g0580700	Expressed protein	Unknown	7.94	733.50	SGNGGGER
EEC82379	LOC_Os07g40360		Transposon protein, putative, CACTA, En/Spm sub-class	Transposon	13.14	1787.70	WMYPIERYLCTLK
EAZ40617	LOC_Os07g41850		C1-like domain containing protein	Signalling	8.93	1513.56	MSGIMMNVVVRED
EEC68954	LOC_Os07g43200	Os07g0625100	Retrotransposon protein, putative, LINE subclass	Retrotransposon	8.11	980.57	AHCTNHLK

[a] Gene no., ID Score, MH^+ (Da), predicted peptide Mascot™
[b] Locus number and description retrieve from the Rice Genome Annotation Project
[c] RAP id from RAP-DB

Table D1. List of 357 drought-responsive proteins in leaves of SS and SR seedling by proteomic analysis *(cont.)*

Gene no.[a]	Locus [b]	RAP id [c]	Description [b]	Functional group	ID Score[a]	MH+ (Da) [a]	Peptide [a]
XP_015647159	LOC_Os07g43380	Os07g0626900	Zinc finger, C3HC4 type domain containing protein	Transcription	4.32	794.86	YIPSTSK
BAT02801	LOC_Os07g44030	Os07g0634100	Myb/SANT domain protein	Transcription	25.16	767.60	QPNHSGK
BAF22350	LOC_Os07g44800	Os07g0642400	SWI/SNF-related matrix-associated actin-dependent regulator of chromatin subfamily A	Transcription	11.91	820.90	LISGAFGR
BAT03385	LOC_Os07g49520	Os07g0695800	2-oxoglutarate dehydrogenase E1 component, mitochondrial precursor	Metabolic process	8.18	711.73	LYASMK
BAT03394	LOC_Os08g01054	Os08g0100500	Retrotransposon protein	Retrotransposon	5.52	923.70	FQSQTGVR
BAT03406	LOC_Os08g01150	Os08g0101800	DTA2	Development	15.34	1030.86	MRGSSNNHK
BAT03494	LOC_Os08g01850	Os08g0110000	Esterase	Metabolic process	4.44	860.50	IGPGSTSSR
BAT03771	LOC_Os08g04460	Os08g0139200	NADPH-dependent FMN reductase domain containing protein	Metabolic process	14.30	591.52	MGAPSK
BAT04052	LOC_Os08g07774	Os08g0174800	Disease resistance protein RPM1	Defense	9.98	1197.30	CGSRIVMTTR
BAT04106	LOC_Os08g08210	Os08g0180100	SET domain containing protein	Transcription	17.90	827.77	LTHTLDK

[a] Gene no., ID Score, MH^+ (Da), predicted peptide Mascot™
[b] Locus number and description retrieve from the Rice Genome Annotation Project
[c] RAP id from RAP-DB

Table D1. List of 357 drought-responsive proteins in leaves of SS and SR seedling by proteomic analysis *(cont.)*

Gene no.[a]	Locus [b]	RAP id [c]	Description [b]	Functional group	ID Score[a]	MH+ (Da) [a]	Peptide [a]
BAT04140	LOC_Os08g08830	Os08g0187700	Expressed protein	Unknown	10.61	967.76	IPTNGPVDR
EEC83053	LOC_Os08g09810	Os08g0198100	WRKY106	Transcription	6.14	857.98	AAGDANAIR
BAD21881	LOC_Os08g10110		Hypothetical protein	Unknown	9.27	970.53	SVHLVQMR
BAT04314	LOC_Os08g10608	Os08g0207000	Ribosomal protein S1	Translation	7.72	941.84	VIPAGSTGGGK
CAH65865	LOC_Os08g12470		Retrotransposon protein, putative, Ty3-gypsy subclass	Retrotransposon	24.93	956.22	IIEEDIPK
AAS01958	LOC_Os08g13200		Retrotransposon protein	Retrotransposon	15.83	930.33	GGGVGRLTGR
EEE68316	LOC_Os08g14760	Os08g0245200	AMP-binding domain containing protein	Defense	7.74	808.89	APLGAPAGR
EEC83219	LOC_Os08g16030	Os08g0260400	Expressed protein	Unknown	7.93	1147.68	GSPAEAASAAGMK
BAT04655	LOC_Os08g17020	Os08g0271600	Expressed protein	Unknown	12.02	983.28	AAAMNDHVR
FAA01208	LOC_Os08g18820		Zinc knuckle family protein	Transcription	3.51	922.25	XAITNFGNK
EEC84860	LOC_Os08g18870	Os08g0285100	Expressed protein	Unknown	19.64	974.92	AFHVVCSR
XP_015649922	LOC_Os08g24420	Os08g0333000	SWP	Development	5.02	768.52	TSLTYGK
BAT05162	LOC_Os08g28214	Os08g0369600	Tesmin/TSO1-like CXC domain containing protein	Development	5.46	875.70	NPAAFMPK
BAH94292	LOC_Os08g30590	Os08g0396500	C1-like domain containing protein	Signalling	10.32	1415.55	QEEEGPDHCCR
BAT05342	LOC_Os08g31060	Os08g0401800	Phospholipase D alpha 1	Signalling	11.45	849.64	DGRMGAAR
EAZ42809	LOC_Os08g33170		Expressed protein	Unknown	3.91	1959.03	SNSLEVAQAGADPPMSTGVK

[a] Gene no., ID Score, MH^+ (Da), predicted peptide Mascot™
[b] Locus number and description retrieve from the Rice Genome Annotation Project
[c] RAP id from RAP-DB

Table D1. List of 357 drought-responsive proteins in leaves of SS and SR seedling by proteomic analysis *(cont.)*

Gene no.[a]	Locus [b]	RAP id [c]	Description [b]	Functional group	ID Score[a]	MH+ (Da) [a]	Peptide [a]
BAT05682	LOC_Os08g34790	Os08g0448000	AMP-binding domain containing protein	Defense	8.27	1077.82	MVISGAAPMGK
BAT06229	LOC_Os08g40330	Os08g0514600	Sulfotransferase domain containing protein	Metabolic process	15.43	1519.67	LLSTHMPPQLLPR
BAT06364	LOC_Os08g41630	Os08g0528100	Ubiquitin carboxyl-terminal hydrolase family protein	Metabolic process	8.03	931.41	AAALLSGGDR
BAD73095	LOC_Os08g42480	Os08g0537001	Expressed protein	Unknown	9.73	1015.18	AVGRRPFGR
BAD89365	LOC_Os09g01050		Retrotransposon protein, putative, Ty3-gypsy subclass	Retrotransposon	8.89	1331.74	AFGAADAWDPRR
EAY82517	LOC_Os09g14530		Hat dimerisation domain-containing protein	Transcription	5.87	980.21	NGAYEKGLK
BAT07589	LOC_Os09g17190	Os09g0342000	Osfbx320 - F-box domain containing protein	Transcription	15.55	927.45	GGLLLLSKK
BAT07878	LOC_Os09g21689	Os09g0385300	Expressed protein	Unknown	6.00	911.87	SKPSGLPAR
BAT07885	LOC_Os09g21760	Os09g0386400	Proteasome subunit	Posttranslation	8.67	1098.40	LQSAGSFLMK
XP_015610925	LOC_Os09g28130	Os09g0454400	Carbonic anhydrase family protein	Metabolic process	10.66	942.36	IVRAANMAP
EAZ09436	LOC_Os09g29350	Os09g0468600	Phototropic-responsive NPH3 family protein	Signalling	7.01	976.51	YSGDPPDAR
BAT08543	LOC_Os09g29404	Os09g0469400	ISA3, ISOAMYLASE_3	Metabolic process	19.36	937.10	TMVPVLVY

[a] Gene no., ID Score, MH^+ (Da), predicted peptide Mascot™
[b] Locus number and description retrieve from the Rice Genome Annotation Project
[c] RAP id from RAP-DB

Table D1. List of 357 drought-responsive proteins in leaves of SS and SR seedling by proteomic analysis *(cont.)*

Gene no.[a]	Locus [b]	RAP id [c]	Description [b]	Functional group	ID Score[a]	MH+ (Da) [a]	Peptide [a]
BAT08733	LOC_Os09g31438	Os09g0491532	Osspl17 - SBP-box gene family member	Transcription	4.61	1772.90	MATGGSGGGGGGGGGG DDVHGR
EEC78449	LOC_Os09g32600	Os09g0502800	Osfbx334 - F-box domain containing protein	Transcription	13.24	1207.44	MSRAQDEILK
EEE59343	LOC_Os09g32690	Os09g0504700	Zinc finger, C3HC4 type domain containing protein	Transcription	6.60	856.06	IMEGNHR
EAZ45490	LOC_Os09g36540	Os09g0536100	Retrotransposon protein	Retrotransposon	5.22	1602.47	DGDGSGGGVIGLGGGGG SAR
BAT09262	LOC_Os09g37510	Os09g0547200	DUF292 domain containing protein	Unknown	11.78	829.33	MMATAGSK
XP_015612482	LOC_Os09g38720	Os09g0560100	Mterf domain containing protein	Transcription	14.37	874.70	NELNCGAV
BAF25874	LOC_Os09g39380	Os09g0567300	Monodehydroascorba te reductase	Defense	12.19	1585.02	EVDDADKLVAAIQAK
AAN11188	LOC_Os10g04180	Os10g0131800	NB-ARC domain containing protein,	Defense	8.34	864.43	QRMSGGGR
BAT09838	LOC_Os10g05020	Os10g0139700	Cytochrome P450	Metabolic process	9.85	1345.19	MGSRLEVIVADR
EAY77685	LOC_Os10g05250		Protein kinase domain containing protein	Metabolic process	20.41	1646.86	IANESNMMVLVEIGK
BAF28384	LOC_Os10g08550	Os10g0167300	Enolase	Metabolic process	3.35	1029.90	LPEGMELPK
BAT10062	LOC_Os10g08850	Os10g0169900	Nodulin	Defense	8.18	934.23	MPAAALFAK
AAN16322	LOC_Os10g12630		Retrotransposon protein, putative, Ty3-gypsy subclass	Retrotransposon	18.00	928.29	ANVVADALR

[a] Gene no., ID Score, MH^+ (Da), predicted peptide Mascot™
[b] Locus number and description retrieve from the Rice Genome Annotation Project
[c] RAP id from RAP-DB

Table D1. List of 357 drought-responsive proteins in leaves of SS and SR seedling by proteomic analysis *(cont.)*

Gene no.[a]	Locus [b]	RAP id [c]	Description [b]	Functional group	ID Score[a]	MH+ (Da) [a]	Peptide [a]
BAF26245	LOC_Os10g16880		Retrotransposon protein	Retrotransposon	13.73	1591.77	QCAVMMEQGLIRR
BAD45680	LOC_Os10g17380		Retrotransposon protein	Retrotransposon	6.74	1027.06	LLPLPTASSK
YP_009305312	LOC_Os10g21268	Os10g0356000	Ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (chloroplast)	Metabolic process	64.44	1465.37	TFQGPPHGIQVER
YP_009305312	LOC_Os10g21268	Os10g0356000	Ribulose bisphosphate carboxylase large chain precursor	Metabolic process	49.45	1407.85	LTYYTPEYETK
YP_009305312	LOC_Os10g21268		Ribulose bisphosphate carboxylase large chain precursor	Metabolic process	25.59	1021.40	DTDILAAFR
ABB47454	LOC_Os10g24700		Retrotransposon protein, putative, Ty3-gypsy subclass	Retrotransposon	9.47	926.80	ADMYDAIK
BAD31471	LOC_Os10g24870		Hypothetical protein	Unknown	13.56	982.14	HSPTAGPCR
BAT10708	LOC_Os10g25660	Os10g0396100	Osfbx385 - F-box domain containing protein	Transcription	15.26	860.34	MEPGAALR
BAT10748	LOC_Os10g26280	Os10g0402200	ORC3 - Putative origin recognition complex subunit 3	Cellular process	8.42	1882.61	SGDNMVDGLSELMNIQK

[a] Gene no., ID Score, MH^+ (Da), predicted peptide Mascot™
[b] Locus number and description retrieve from the Rice Genome Annotation Project
[c] RAP id from RAP-DB

Table D1. List of 357 drought-responsive proteins in leaves of SS and SR seedling by proteomic analysis *(cont.)*

Gene no.[a]	Locus [b]	RAP id [c]	Description [b]	Functional group	ID Score[a]	MH+ (Da) [a]	Peptide [a]
BAT10984	LOC_Os10g29840	Os10g0435300	MBTB59 - Bric-a-Brac, Tramtrack, Broad Complex BTB domain with Meprin and TRAF Homology MATH domain	Defense	14.62	888.70	GETFAAHR
AAK98739	LOC_Os10g31850	Os10g0456800	RING finger and CHY zinc finger domain-containing protein 1	Transcription	9.80	1183.64	MASWPTSCTK
EAY78873	LOC_Os10g33080	Os10g0469000	Leucine-rich repeat receptor protein kinase EXS precursor	Signalling	6.05	1691.16	LSGWTRATPVCTWR
BAT11636	LOC_Os10g36880	Os10g0512800	Kinesin motor domain containing protein	Cellular process	5.99	863.67	ETDSLGDK
BAT11830	LOC_Os10g39200	Os10g0537300	Expressed protein	Unknown	7.22	1091.46	LKFLQSNLK
AAP54936	LOC_Os10g40390	Os10g0551200	Scarecrow	Transcription	12.03	894.29	EVGGYGGEK
EEE51439	LOC_Os10g41940	Os10g0569000	Expressed protein	Unknown	11.86	1018.24	YMSCLSEK
ABA91380	LOC_Os11g03830		Retrotransposon protein, putative, Ty1-copia subclass	Retrotransposon	8.36	1196.48	MNMTIISKAR
BAD38341	LOC_Os11g04090		Hypothetical protein	Unknown	3.91	1022.96	YGGAGLGAAMR
EAZ17371	LOC_Os11g04260	Os11g0138050	Expressed protein	Unknown	9.66	874.01	APSRVQSK
AAX92742	LOC_Os11g05130	Os11g0148700	PHD-finger family protein	Transcription	19.35	1003.12	ADLISPPYK

[a] Gene no., ID Score, MH^+ (Da), predicted peptide Mascot™
[b] Locus number and description retrieve from the Rice Genome Annotation Project
[c] RAP id from RAP-DB

Table D1. List of 357 drought-responsive proteins in leaves of SS and SR seedling by proteomic analysis *(cont.)*

Gene no.[a]	Locus [b]	RAP id [c]	Description [b]	Functional group	ID Score[a]	MH+ (Da) [a]	Peptide [a]
ABG22406	LOC_Os11g09919	Os11g0205400	Retrotransposon protein, putative, Ty1-copia subclass	Retrotransposon	12.60	992.98	LHKALNGLK
BAT13250	LOC_Os11g11400	Os11g0220900	Expressed protein	Unknown	14.98	877.03	AALMQPSC
ABA92214	LOC_Os11g11870		Retrotransposon protein	Retrotransposon	8.18	965.35	TMRVGDDR
ABA92480	LOC_Os11g16250		Expressed protein	Unknown	7.19	979.56	GAGSANSSSSR
BAT13728	LOC_Os11g20554	Os11g0310800	ATP-dependent RNA helicase	Transcription	3.35	1036.33	TRPGKCYR
AAX96706	LOC_Os11g21850		Retrotransposon protein, putative, Ty3-gypsy sub-class	Retrotransposon	3.65	974.37	MIMMMPR
AAX95813	LOC_Os11g22160		Retrotransposon protein	Retrotransposon	7.73	729.03	VAGVGNGR
AAX94813	LOC_Os11g23750		Retrotransposon protein, putative, Ty1-copia sub-class	Retrotransposon	8.83	996.40	FNMQDSKK
BAT13882	LOC_Os11g25860	Os11g0445300	Protein Kinase	Signalling	18.80	1590.28	KIVSIMDGNDEILK
XP_015616244	LOC_Os11g27440		MLA7	Defense	9.27	1030.72	DLGKLSELR
ABA93536	LOC_Os11g27750		Retrotransposon protein	Retrotransposon	7.85	773.94	ANPMAVR
ABA93607	LOC_Os11g28780		Retrotransposon protein	Retrotransposon	11.27	1201.14	RNEISSRPSR
EEC68161	LOC_Os11g29110	Os11g0481150	Leucine Rich Repeat family protein	Defense	3.28	520.89	TGGSAK
ABA93839	LOC_Os11g30650		Retrotransposon protein	Retrotransposon	12.27	907.58	LHGKIQGR
ABA93944	LOC_Os11g31760		Transposon protein, putative, unclassified	Transposon	4.43	798.71	VEAHSEK

[a] Gene no., ID Score, MH^+ (Da), predicted peptide Mascot™
[b] Locus number and description retrieve from the Rice Genome Annotation Project
[c] RAP id from RAP-DB

Table D1. List of 357 drought-responsive proteins in leaves of SS and SR seedling by proteomic analysis *(cont.)*

Gene no.[a]	Locus [b]	RAP id [c]	Description [b]	Functional group	ID Score[a]	MH+ (Da) [a]	Peptide [a]
BAT10042	LOC_Os11g32910	Os11g0533600	Expressed protein	Unknown	93.79	1573.71	VNQIGSVTESIEAVK
ABA94133	LOC_Os11g33440	Os11g0540800	Expressed protein	Unknown	10.27	909.75	MTAEAGSAR
EAY81215	LOC_Os11g33942	Os11g0541200	Plant protein of unknown function domain containing protein	Unknown	2.62	1848.71	QSGAMTRGSKPGHSIYR
EAY81230	LOC_Os11g34140	Os11g0544100	Expressed protein	Unknown	12.73	964.88	AAMGMEGKR
XP_015616874	LOC_Os11g34270	Os11g0545300	Ubiquitin carboxyl-terminal hydrolase domain containing protein	Metabolic process	4.96	1150.07	HQPVGLKNEK
BAT14423	LOC_Os11g34920	Os11g0550500	Stripe rust resistance protein Yr10	Defense	13.15	934.35	KTDDLVSR
BAT14701	LOC_Os11g38150	Os11g0593900	Expressed protein	Unknown	19.46	965.77	KRPNKPAR
BAF28629	LOC_Os11g39920	Os11g0613800	Expressed protein	Unknown	6.26	966.85	AMAGNTTSSK
XP_015615094	LOC_Os11g40070	Os11g0614900	Expressed protein	Unknown	9.64	1901.51	AAVTELLTKASMLYMSR
BAT14961	LOC_Os11g41590	Os11g0634100	Expressed protein	Unknown	11.77	1177.89	NNAAHAIDQPK
EEE65593	LOC_Os11g42590	Os11g0645886	Expressed protein	Unknown	16.02	702.09	QDQALK
BAT15094	LOC_Os11g43390	Os11g0654800	Expressed protein	Unknown	11.26	972.36	SHLKSICK
BAF28810	LOC_Os11g45130	Os11g0676500	Pollen signalling protein with adenylyl cyclase activity	Siganlling	9.87	2199.34	TTLAQQIYNDEKITGNFDK
BAT15245	LOC_Os11g45410	Os11g0680200	Expressed protein	Unknown	5.24	837.11	AIHGLAVR
ABA95421	LOC_Os11g46290		Transposon protein, putative, Mutator sub-class	Transposon	10.56	1053.68	EVMNMFKR

[a] Gene no., ID Score, MH^+ (Da), predicted peptide Mascot™
[b] Locus number and description retrieve from the Rice Genome Annotation Project
[c] RAP id from RAP-DB

Table D1. List of 357 drought-responsive proteins in leaves of SS and SR seedling by proteomic analysis *(cont.)*

Gene no.[a]	Locus [b]	RAP id [c]	Description [b]	Functional group	ID Score[a]	MH+ (Da) [a]	Peptide [a]
BAT15709	LOC_Os12g03400	Os12g0127500	Expressed protein	Unknown	5.01	898.42	RGIGNQVR
BAT15785	LOC_Os12g04100	Os12g0134900	Cytochrome P450	Metabolic process	4.51	881.90	KPTTHGLK
EAY82221	LOC_Os12g04630		Expressed protein	Unknown	14.44	1076.15	ETEASSAARR
ABA95817	LOC_Os12g05490		Retrotransposon protein, putative, Ty3-gypsy subclass	Retrotransposon	7.50	1009.88	TTDGDQGTSK
ABA95879	LOC_Os12g06760		DEFL17 - Defensin and Defensin-like DEFL family	Defense	4.24	1068.37	CCCTFHAN
ABA95991	LOC_Os12g08460		Retrotransposon protein	Retrotransposon	4.66	1945.19	ATVIPGRYPIMVELTIR
BAT16374	LOC_Os12g11940	Os12g0221000	Disease resistance family protein	Defense	4.44	1662.13	SMIMVTSDNDMLMK
BAD88127	LOC_Os12g12180		Retrotransposon protein, putative, Ty1-copia subclass	Retrotransposon	7.94	1048.16	GRLAPHTAAR
ABA96963	LOC_Os12g15330		Retrotransposon protein	Retrotransposon	6.57	1878.00	HSIEPTTILENGDIALR
ABA97339	LOC_Os12g16030		Retrotransposon protein	Retrotransposon	7.42	980.92	DAMGGDTAAR
AAB70544	LOC_Os12g17600	Os12g0274700	Ribulose 1,5-bisphosphate carboxylase small subunit	Metabolic process	31.59	1592.99	LPMFGCTDATQVLK
1WDD_W	LOC_Os12g19470	Os12g0292400	Ribulose bisphosphate carboxylase small chain, chloroplast precursor	Metabolic process	8.54	1230.59	XQVWPIEGIK

[a] Gene no., ID Score, MH^+ (Da), predicted peptide Mascot™
[b] Locus number and description retrieve from the Rice Genome Annotation Project
[c] RAP id from RAP-DB

Table D1. List of 357 drought-responsive proteins in leaves of SS and SR seedling by proteomic analysis *(cont.)*

Gene no.[a]	Locus [b]	RAP id [c]	Description [b]	Functional group	ID Score[a]	MH+ (Da) [a]	Peptide [a]
ABA97802	LOC_Os12g20010		Retrotransposon protein, putative, Ty3-gypsy subclass	Retrotransposon	11.91	1030.63	DKSSMGSFR
ABA97815	LOC_Os12g20140		Hypothetical protein	Unknown	2.71	899.29	HSVEGIEK
ABA97864	LOC_Os12g22300		Retrotransposon protein, putative, Ty3-gypsy subclass	Retrotransposon	14.58	1074.18	RMVVDPLTK
ABA97870	LOC_Os12g22360		Hypothetical protein	Unknown	5.52	939.60	AQPQTAAPR
ABA97607	LOC_Os12g23030		Retrotransposon protein	Retrotransposon	15.89	1294.88	VIVFGGDFRQR
EAY74022	LOC_Os12g24430		Retrotransposon protein, putative, Ty3-gypsy subclass	Retrotransposon	8.64	927.11	LVAAATLIR
ABA97724	LOC_Os12g25570		Retrotransposon protein, putative, Ty3-gypsy subclass	Retrotransposon	8.83	829.25	EPADVGNK
ABA98104	LOC_Os12g26720		Armadillo	Defense	5.42	1044.90	AVSERDAAAR
ABA98200	LOC_Os12g29060		Retrotransposon protein	Retrotransposon	7.90	943.37	MPILNPSR
EEC69385	LOC_Os12g31620	Os11g0138050	Disease resistance protein RPM1	Defense	15.61	1198.86	ARIQHLTTMK
BAT17372	LOC_Os12g33100	Os12g0515600	Guanylate kinase	Metabolic process	9.83	947.11	GTVVVAWSK
BAV53145	LOC_Os12g33958		NADH-ubiquinone oxidoreductase 49 kda subunit	Metabolic process	4.50	1033.76	GPGVCWDSR
ABA99409	LOC_Os12g42040	Os12g0614800	Oswak126 - oswak receptor-like protein kinase	Signalling	15.54	1002.84	QEALVLAMK

[a] Gene no., ID Score, MH^+ (Da), predicted peptide Mascot™
[b] Locus number and description retrieve from the Rice Genome Annotation Project
[c] RAP id from RAP-DB

Table D1. List of 357 drought-responsive proteins in leaves of SS and SR seedling by proteomic analysis *(cont.)*

Gene no.[a]	Locus [b]	RAP id [c]	Description [b]	Functional group	ID Score[a]	MH+ (Da) [a]	Peptide [a]
BAF30321	LOC_Os12g42660	Os12g0621500	AGC_AGC_other_gwld.1 - ACG kinases include homologs to PKA, PKG and PKC	Signalling	18.93	787.51	DILISAR
BAT18159	LOC_Os12g42870	Os12g0623800	Mur ligase family protein	Metabolic process	9.63	819.50	SMPASSLK
ABA99923	LOC_Os12g43480		Transposon protein, putative, CACTA, En/Spm sub-class	Transposon	9.10	718.20	GMSAHAK
ABA97950			Hypothetical protein LOC_Os12g23849	Unknown	8.83	1133.62	TTSLAILLFR
ABA99280			Hypothetical protein LOC_Os12g415800	Unknown	3.95	1677.11	RGLSGQRPGHDGGGGLR
BAC99856			Hypothetical protein	Unknown	5.16	1676.72	EETVDKFGELLIER
BAC99965			Hypothetical protein	Unknown	12.98	891.20	GNDGSTGQR
BAD10727			Hypothetical protein	Unknown	8.36	1165.46	TLALTMAATTR
BAD17457			Hypothetical protein	Unknown	4.43	1043.22	VLVGEARSGR
BAD20149			Hypothetical protein	Unknown	6.50	707.39	GASGGGMR
BAD29290			Hypothetical protein	Unknown	8.95	1059.17	SASAVTLALAR
BAD38326			Hypothetical protein	Unknown	12.93	767.21	GGGAVPPGR
BAD88381			Hypothetical protein	Unknown	9.85	750.07	EESAASR
BAD88390			Hypothetical protein	Unknown	10.41	907.40	MGATCRGR
BAH91908		Os02g0793150	Hypothetical protein	Unknown	3.63	1538.84	IIMPTNPPNHVFAA
BAK38947			Protein of unknown function DUF803 family protein	Unknown	18.86	936.87	YLNKALDT
BAS73449		Os01g0651350	Hypothetical protein	Unknown	9.18	858.37	VAEAEDAR
BAS74064		Os01g0719350	Hypothetical protein	Unknown	11.25	934.42	GEPSCKTR

[a] Gene no., ID Score, MH^+ (Da), predicted peptide Mascot™
[b] Locus number and description retrieve from the Rice Genome Annotation Project
[c] RAP id from RAP-DB

Table D1. List of 357 drought-responsive proteins in leaves of SS and SR seedling by proteomic analysis *(cont.)*

Gene no.[a]	Locus [b]	RAP id [c]	Description [b]	Functional group	ID Score[a]	MH+ (Da) [a]	Peptide [a]
BAS75207		Os01g0846700	Hypothetical protein	Unknown	8.83	1609.95	RPRGGGGGEGVGLVGVGK
BAS79735		Os02g0613150	Hypothetical protein	Unknown	8.54	2142.61	LPFPVRPDLEPPLHLHVAP
BAS85396		Os03g0637100	Unknown	Unknown	10.53	1136.47	ALLFTGVLESS
BAS91936		Os05g0112125	Hypothetical protein	Unknown	3.95	899.62	HGAGDGAASR
BAS98352		Os06g0575600	Unknown	Unknown	7.68	1427.95	RRPEQSGTAAQAR
BAT00645		Os07g0219600	Unknown	Unknown	10.71	1557.48	LRHVGVDDMPFVR
BAT05141		Os08g0366050	Unknown	Unknown	10.02	816.53	MPGSPLAK
BAT05579		Os08g0434150	Hypothetical protein	Unknown	6.28	1057.34	SAGSSSSASCR
BAT05739		Os08g0457501	Unknown	Unknown	11.24	823.45	ASSGKAMR
BAT06109		Os08g0500400	Hypothetical protein	Unknown	15.59	1217.36	QEPMAAAATRR
BAT06827		Os09g0110750	30S ribosomal protein S7	Translation	11.05	1041.99	MPHRGTAEK
BAT16944		Os12g0431200	Unknown	Unknown	15.74	886.98	EGVASPATR
EAZ08270			Hypothetical protein osi_30522	Unknown	12.61	1611.37	WFPFGGEQSKSWR
EAZ37233			Hypothetical protein osj_21571	Unknown	19.18	965.59	RSCGSGGER
EEC68066			Hypothetical protein osi_35919	Unknown	3.18	869.53	SVGLPELR
EEC83856			Hypothetical protein osi_29828	Unknown	14.56	914.46	TSSSDDFR
EEE57201			Hypothetical protein osj_07154	Unknown	5.19	854.93	AGSHDGRR

[a] Gene no., ID Score, MH^+ (Da), predicted peptide Mascot™
[b] Locus number and description retrieve from the Rice Genome Annotation Project
[c] RAP id from RAP-DB

Table D2. Significantly different expressed proteins

Gene no.	Locus no.	RAP ID	Description	ID Score	MH+ (Da)	Peptide	Group
Cellular process							
BAS94532	LOC_Os05g39850	Os05g0476200	MCM3 - Putative minichromosome maintenance MCM complex subunit 3	6.50	808.97	GSVSGVFR	A
BAT02000	LOC_Os07g36140	Os07g0545400	Core histone H2A/H2B/H3/H4	12.96	803.39	AIGSGAAKK	A
BAB40535	LOC_Os02g58220	Os02g0829100	RPA2A - Putative single-stranded DNA binding complex subunit 2	8.86	826.64	LRLPEAK	A
BAS80804	LOC_Os02g50320	Os02g0736100	ATMAP70 protein	24.35	958.65	ISPNGMLAR	A
Defense							
EEE68316	LOC_Os08g14760	Os08g0245200	AMP-binding domain containing protein	7.74	808.89	APLGAPAGR	A
BAS92588	LOC_Os05g34550	Os05g0183566	MLO domain containing protein	5.69	889.61	AMVEALEK	A
Metabolic process							
XP_015610925	LOC_Os09g28130	Os09g0454400	Carbonic anhydrase family protein	10.66	942.36	IVRAANMAP	A
XP_015626092	LOC_Os02g46650	Os02g0693400	Ubiquitin carboxyl-terminal hydrolase domain containing protein	18.81	936.82	VESASKSTK	A
BAS88326	LOC_Os04g20260	Os04g0270900	UDP-glucoronosyl and UDP-glucosyl transferase	9.65	1031.02	DGAMSHQLR	A
Retrotransposon							
AAS07079	LOC_Os03g30190		Retrotransposon protein	16.40	861.99	GKTSQVSR	A
ABA97802	LOC_Os12g20010		Retrotransposon protein, putative, Ty3-gypsy subclass	11.91	1030.63	DKSSMGSFR	A
AAN16322	LOC_Os10g12630		Retrotransposon protein, putative, Ty3-gypsy subclass	18.00	928.30	ANVVADALR	A
ABB47454	LOC_Os10g24700		Retrotransposon protein, putative, Ty3-gypsy subclass	9.47	926.80	ADMYDAIK	A
ABF96926	LOC_Os03g33328		Retrotransposon protein, putative, Ty3-gypsy subclass	9.52	849.80	EGLGFISK	A
CAE01993	LOC_Os04g11560		Retrotransposon, putative, centromere-specific	8.04	1119.01	FLHDAFLEK	A

Table D2. Significantly different expressed proteins *(cont.)*

Gene no.	Locus no.	RAP ID	Description	ID Score	MH+ (Da)	Peptide	Group
Transposon							
ABA95421	LOC_Os11g46290		Transposon protein, putative, Mutator sub-class	10.56	1053.68	EVMNMFKR	A
ABA93944	LOC_Os11g31760		Transposon protein, putative, unclassified	4.43	798.71	VEAHSEK	A
Signalling							
ABF96316	LOC_Os03g26930	Os03g0386800	Osscp13 - Putative Serine Carboxypeptidase homologue	9.21	1131.80	MHTLPIKMK	A
BAS95627	LOC_Os05g51400	Os05g0591800	Protein kinase APK1B, chloroplast precursor	5.70	857.50	QEGNGAEPG	A
BAT05342	LOC_Os08g31060	Os08g0401800	Phospholipase D alpha 1	11.45	849.64	DGRMGAAR	A
EEE54767	LOC_Os01g36550	Os01g0546000	Protein kinase family protein	8.69	727.32	GVAGPSNK	A
EAZ09436	LOC_Os09g29350	Os09g0468600	Phototropic-responsive NPH3 family protein	7.01	976.51	YSGDPPDAR	A
Transport							
BAS83729	LOC_Os03g18550	Os03g0296800	Mitochondrial carrier protein	3.22	1805.04	LQLTSSPYTGVSHCVR	A
Transcription							
AAP54936	LOC_Os10g40390	Os10g0551200	Scarecrow	12.03	894.29	EVGGYGGEK	A
FAA01208	LOC_Os08g18820		Zinc knuckle family protein	3.51	922.25	XAITNFGNK	A
EEC75654	LOC_Os03g39100	Os03g0588000	No apical meristem protein	6.96	885.60	HQFGIKR	A
Post-translation							
BAT07885	LOC_Os09g21760	Os09g0386400	Proteasome subunit	8.67	1098.40	LQSAGSFLMK	A
Unknown							
ABA97870	LOC_Os12g22360		Hypothetical protein	5.52	939.60	AQPQTAAPR	A
BAS81932	LOC_Os03g02090	Os03g0111600	Expressed protein	7.78	847.96	KHNMFR	A
BAS93042	LOC_Os05g16460	Os05g0253700	Expressed protein	5.11	929.79	QGPGWTAGR	A
BAT02329	LOC_Os07g39240	Os07g0580700	Expressed protein	7.94	733.50	SGNGGGER	A
EEC80502	LOC_Os06g21630		Hypothetical protein	6.70	887.29	LLLPNYR	A
EEE51439	LOC_Os10g41940	Os10g0569000	Expressed protein	11.86	1018.24	YMSCLSEK	A
XP_015646220	LOC_Os01g41750	Os01g0601625	Expressed protein	17.77	701.19	HATMLK	A
BAD38341	LOC_Os11g04090		Hypothetical protein	3.91	1022.96	YGGAGLGAAMR	A
BAT05579		Os08g0434150	Hypothetical protein	6.28	1057.34	SAGSSSSASCR	A
BAT15094	LOC_Os11g43390	Os11g0654800	Expressed protein	11.26	972.36	SHLKSICK	A

Table D2. Significantly different expressed proteins *(cont.)*

Gene no.	Locus no.	RAP ID	Description	ID Score	MH+ (Da)	Peptide	Group
Cellular process							
XP_015639857	LOC_Os05g06450	Os05g0156600	Tubulin/ftsz domain containing protein	11.08	1025.88	QAFLDNYR	B
BAS85845	LOC_Os03g48490	Os03g0691500	SCP-1; Synaptonemal complex protein 1	8.03	829.43	LDVAGLIK	B
Defense							
BAS97292	LOC_Os06g17930	Os06g0287200	NBS-LRR disease resistance protein	5.09	890.11	DITVSISR	B
EEC68161	LOC_Os11g29110	Os11g0481150	Leucine Rich Repeat family protein	3.28	520.89	TGGSAK	B
XP_015616244	LOC_Os11g27440		MLA7	9.27	1030.72	DLGKLSELR	B
Metabolic process							
BAS89533	LOC_Os04g38600	Os04g0459500	Glyceraldehyde-3-phosphate dehydrogenase	87.70	1787.64	VIAWYDNEWGYSQR	B
BAT06229	LOC_Os08g40330	Os08g0514600	Sulfotransferase domain containing protein	15.43	1519.67	LLSTHMPPQLLPR	B
BAT15785	LOC_Os12g04100	Os12g0134900	Cytochrome P450	4.51	881.90	KPTTHGLK	B
XP_015616874	LOC_Os11g34270	Os11g0545300	Ubiquitin carboxyl-terminal hydrolase domain containing protein	4.96	1150.07	HQPVGLKNEK	B
Transport							
BAS78380	LOC_Os02g21630	Os02g0321500	SEC14 cytosolic factor family protein	3.90	1106.01	KLSVDETVSK	B
Transposon							
ABF94733	LOC_Os03g12400		Transposon protein	10.21	930.32	TAPGGDAGER	B
Transcription							
XP_015628078	LOC_Os03g02240	Os03g0113500	Trihelix transcription factor GTL1	6.86	1044.26	RGGGGIGGGGGGGK	B
BAS84618	LOC_Os03g28960	Os03g0403100	DNA-directed RNA polymerase subunit	3.96	779.11	DSPAVYK	B
BAS99010	LOC_Os06g45310	Os06g0663500	Osspl11 - SBP-box gene family member	10.51	875.32	AGADSANIR	B
BAT08733	LOC_Os09g31438	Os09g0491532	Osspl17 - SBP-box gene family member	4.61	1772.90	MATGGSGGGGGGGGGDDVHGR	B
EEC83053	LOC_Os08g09810	Os08g0198100	WRKY106	6.14	857.98	AAGDANAIR	B

Table D2. Significantly different expressed proteins *(cont.)*

Gene no.	Locus no.	RAP ID	Description	ID Score	MH+ (Da)	Peptide	Group
Signalling							
XP_015616316	LOC_Os03g61690	Os03g0832400	Protein phosphatase 2C	8.07	1032.52	FNVGLSLQR	B
BAH94292	LOC_Os08g30590	Os08g0396500	C1-like domain containing protein	10.32	1415.55	QEEEGPDHCCR	B
BAS70681	LOC_Os01g08140	Os01g0176400	Phototropic-responsive NPH3 family protein	25.90	1140.86	GGAAGAAAATPTPK	B
BAS78873	LOC_Os02g30900	Os02g0513000	Protein kinase domain containing protein	15.13	927.80	LPKMADPR	B
Post-transcription							
BAS75866	LOC_Os01g68610	Os01g0914600	Pentatricopeptide repeat protein PPR1106-17	8.29	1027.26	KLMSAEGMK	B
BAS86628	LOC_Os03g56400	Os03g0775400	Pentatricopeptide	22.05	819.85	SGSVENAR	B
Retrotransposon							
AAT01370	LOC_Os05g40110	Os05g0479250	Retrotransposon protein	5.66	838.15	RFQSGML	B
AAX95813	LOC_Os11g22160		Retrotransposon protein	7.73	729.03	VAGVGNGR	B
ABA95817	LOC_Os12g05490		Retrotransposon protein, putative, Ty3-gypsy subclass	7.50	1009.88	TTDGDQGTSK	B
BAB07986	LOC_Os01g15370		Retrotransposon protein, putative, Ty3-gypsy subclass	10.47	802.57	HMATGGGR	B
BAC83729	LOC_Os07g17730		Retrotransposon protein	4.99	922.90	RYQSAVAK	B
CAE03907	LOC_Os04g15510		Retrotransposon protein	14.17	1822.13	TPTIPTCSKMEAAEGGR	B
CAE05607	LOC_Os04g25890		Retrotransposon protein	14.85	1035.95	RGCEACQR	B
CAH65865	LOC_Os08g12470		Retrotransposon protein, putative, Ty3-gypsy subclass	24.93	956.22	IIEEDIPK	B
ABF94046	LOC_Os03g05960		Retrotransposon protein, putative, Ty3-gypsy subclass	27.97	809.45	MASLMEK	B

Table D2. Significantly different expressed proteins *(cont.)*

Gene no.	Locus no.	RAP ID	Description	ID Score	MH+ (Da)	Peptide	Group
Unknown							
BAT09262	LOC_Os09g37510	Os09g0547200	DUF292 domain containing protein	11.78	829.33	MMATAGSK	B
EEE65432	LOC_Os06g13570	Os06g0244100	Expressed protein	5.43	939.83	IVPTIELR	B
ABA97815	LOC_Os12g20140		Hypothetical protein	2.71	899.29	HSVEGIEK	B
BAC16105	LOC_Os07g26590		Expressed protein	8.73	1133.55	KARPTTGFTR	B
BAD21881	LOC_Os08g10110		Hypothetical protein	9.27	970.53	SVHLVQMR	B
BAD31471	LOC_Os10g24870		Hypothetical protein	13.56	982.14	HSPTAGPCR	B
BAS84292	LOC_Os03g24730	Os03g0362200	Expressed protein	9.14	811.27	ATMTGSDK	B
BAS98352		Os06g0575600	Unknown	7.68	1427.95	RRPEQSGTAAQAR	B
BAT04655	LOC_Os08g17020	Os08g0271600	Expressed protein	12.02	983.28	AAAMNDHVR	B
EAY81215	LOC_Os11g33942	Os11g0541200	Plant protein of unknown function domain containing protein	2.62	1848.71	QSGAMTRGSKPG HSIYR	B
Defense							
BAS74525	LOC_Os01g56200	Os01g0767900	BTBA2 - Bric-a-Brac,Tramtrack, Broad Complex BTB domain with Ankyrin repeat region	4.58	984.27	MRFCELK	C
Development							
XP_015649922	LOC_Os08g24420	Os08g0333000	SWP	5.02	768.52	TSLTYGK	C
BAS87151	LOC_Os03g61050	Os03g0825700	FG-GAP repeat-containing protein	9.64	2070.99	LPTVPVRTTGTVL VEMVDK	C
Metabolic process							
BAS89134	LOC_Os04g33720	Os04g0413200	Glycosyl hydrolases	12.63	802.13	VSLDLTR	C
BAT00519	LOC_Os07g10350	Os07g0203300	S1 RNA binding domain containing protein	11.66	820.37	SQGDEER	C
BAF13607	LOC_Os03g60190	Os03g0816500	Oxidoreductase, 2OG-Fe oxygenase family protein	6.47	923.18	SGLANASFR	C
BAF28384	LOC_Os10g08550	Os10g0167300	Enolase	3.35	1029.90	LPEGMELPK	C
BAS81334	LOC_Os02g55030	Os02g0793300	Hydrolase, NUDIX family, domain containing protein	12.44	936.68	NISEAKFK	C
BAT08543	LOC_Os09g29404	Os09g0469400	ISA3, ISOAMYLASE_3	19.36	937.10	TMVPVLVY	C
EEC71729	LOC_Os01g61110	Os01g0826651	Ulp1 protease family, C-terminal catalytic domain containing protein	8.63	1272.78	DRMFVFLDSK	C

Table D2. Significantly different expressed proteins *(cont.)*

Gene no.	Locus no.	RAP ID	Description	ID Score	MH+ (Da)	Peptide	Group
Post-transcription							
BAS80476	LOC_Os02g47360	Os02g0702000	PPR repeat domain containing protein	8.50	940.13	FNFALATR	C
BAS83549	LOC_Os03g17060	Os03g0278800	RNA recognition motif containing protein	19.97	1496.51	MALSSSLLHRLLR	C
Retrotransposon							
ABA98200	LOC_Os12g29060		Retrotransposon protein	7.90	943.37	MPILNPSR	C
CAH67848	LOC_Os04g27420	Os04g0341966	Retrotransposon protein	14.41	824.59	ISIGNGHK	C
ABA97724	LOC_Os12g25570		Retrotransposon protein, putative, Ty3-gypsy subclass	8.83	829.25	EPADVGNK	C
EAZ45490	LOC_Os09g36540	Os09g0536100	Retrotransposon protein	5.22	1602.47	DGDGSGGGVIGLGGGGGSAR	C
Transcription							
BAT04106	LOC_Os08g08210	Os08g0180100	SET domain containing protein	17.90	827.78	LTHTLDK	C
EEE66218	LOC_Os06g46475	Os06g0678650	WD domain, G-beta repeat domain containing protein	14.31	1355.26	FTSVILVCIFR	C
BAS87848	LOC_Os04g08034	Os04g0162100	ZOS4-02 - C2H2 zinc finger protein	10.49	995.383	DNMRTEVN	C
EEE57326	LOC_Os02g39140	Os02g0603600	Helix-loop-helix DNA-binding domain containing protein	11.79	1518.84	MASTSALEMAGMDR	C
XP_015639814	LOC_Os05g05660	Os05g0149200	PWWP domain containing protein	12.20	880.08	LPKSPIPK	C
Unknown							
EAZ37233			Hypothetical protein osj_21571	19.18	965.60	RSCGSGGER	C
EEC71933	LOC_Os01g66650	Os01g0890300	Expressed protein	5.16	866.58	GAVMSSCR	C
BAS73449		Os01g0651350	Hypothetical protein	9.18	858.37	VAEAEDAR	C
BAT04140	LOC_Os08g08830	Os08g0187700	Expressed protein	10.61	967.76	IPTNGPVDR	C
EEC68066			Hypothetical protein osi_35919	3.18	869.53	SVGLPELR	C
BAK38947			Protein of unknown function DUF803 family protein	18.86	936.87	YLNKALDT	C
BAT07878	LOC_Os09g21689	Os09g0385300	Expressed protein	6.00	911.87	SKPSGLPAR	C
XP_015615094	LOC_Os11g40070	Os11g0614900	Expressed protein	9.64	1901.51	AAVTELLTKASMLYMSR	C
EEE52213			Hypothetical protein OsJ_34111	9.07	656.75	RQLMSY	C

Table D2. Significantly different expressed proteins *(cont.)*

Gene no.	Locus no.	RAP id	Description	ID Score	MH+ (Da)	Peptide	Group
Metabolic process							
BAT09838	LOC_Os10g05020	Os10g0139700	Cytochrome P450	9.85	1345.188	MGSRLEVIVADR	D
Transcription							
BAS80810	LOC_Os02g50370	Os02g0736600	Helicase domain-containing protein	10.27	1061.203	AFPGPSKDDK	D
Defense							
AAN11188	LOC_Os10g04180	Os10g0131800	NB-ARC domain containing protein	8.34	864.43	QRMSGGGR	E
Metabolic process							
BAD33476	LOC_Os07g34920	Os07g0533600	Aspartic proteinase nepenthesin precursor	14.65	1124.14	ESSCSRMPR	E
BAS74447	LOC_Os01g55520	Os01g0760300	ATROPGEF7/ROPGEF7	7.87	1016.78	AASGGGSLLER	E
BAS89740	LOC_Os04g40510	Os04g0481200	Glycosyl hydrolase family 5 protein	12.04	1426.52	LSPRDSPLLCLR	E
Transcription							
BAS89853	LOC_Os04g41490	Os04g0492300	DNA-directed RNA polymerase III subunit RPC1	20.76	756.94	EIINAAK	E
BAF16092	LOC_Os04g56990	Os04g0665600	Myb-like DNA-binding domain containing protein	19.17	985.43	YMPASSEGK	E
Unknown							
BAS73183	LOC_Os01g43060	Os01g0617800	Expressed protein	7.89	1122.20	KAGPPTDPLPK	E
BAT10042	LOC_Os11g32910	Os11g0533600	Expressed protein	93.79	1573.71	VNQIGSVTESIEAVK	E
Defense							
BAS89615	LOC_Os04g39260	Os04g0467400	CAF1 family ribonuclease containing protein	6.15	1396.547	YQFDFNTCFR	F
BAT05682	LOC_Os08g34790	Os08g0448000	AMP-binding domain containing protein	8.27	1077.820	MVISGAAPMGK	F
BAS79077	LOC_Os02g33450	Os02g0537700	Peroxiredoxin	19.90	1486.717	SFGVLIPDQGIALR	F
Development							
BAS70962	LOC_Os01g11040	Os01g0208600	SCAR-like protein 2	7.76	1089.22	TENDTNGLPK	F
Metabolic process							
BAS74069	LOC_Os01g52180	Os01g0719900	Lipase	13.97	1653.694	MQISSLCCAEQPSK	F
BAT18159	LOC_Os12g42870	Os12g0623800	Mur ligase family protein	9.63	819.502	SMPASSLK	F
YP_009305312	LOC_Os10g21268	Os10g0356000	Ribulose bisphosphate carboxylase large chain precursor	25.59	1021.397	DTDILAAFR	F
BAS73439	LOC_Os01g46169	Os01g0650200	GDSL-like lipase/acylhydrolase	8.33	968.630	MASSTSGRR	F
BAT17372	LOC_Os12g33100	Os12g0515600	Guanylate kinase	9.83	947.105	GTVVVAWSK	F

Table D2. Significantly different expressed proteins *(cont.)*

Gene no.	Locus no.	RAP ID	Description	ID Score	MH+ (Da)	Peptide	Group
Retrotransposon							
ABA97607	LOC_Os12g23030		Retrotransposon protein	15.89	1294.88	VIVFGGDFRQR	F
BAB86451	LOC_Os02g25760		Retrotransposon protein	24.73	760.15	GDLGGVGGK	F
CAD40415	LOC_Os04g22020		Retrotransposon protein	10.11	899.463	DSSMANFK	F
Signalling							
BAS73047	LOC_Os01g41910	Os01g0603500	Receptor-like protein kinase 5 precursor	19.03	965.437	AMIQGNSTK	F
BAS90244	LOC_Os04g45170	Os04g0534200	Protein kinase-like domain containing protein	17.51	1722.726	MFACVDDDLLANVPK	F
Transcription							
BAT07589	LOC_Os09g17190	Os09g0342000	Osfbx320 - F-box domain containing protein	15.55	927.449	GGLLLLSKK	F
EEC78449	LOC_Os09g32600	Os09g0502800	Osfbx334 - F-box domain containing protein	13.24	1207.440	MSRAQDEILK	F
AAK98739	LOC_Os10g31850	Os10g0456800	RING finger and CHY zinc finger domain-containing protein 1	9.80	1183.637	MASWPTSCTK	F
Unknown							
ABA97950			Hypothetical protein LOC_Os12g23849	8.84	1133.62	TTSLAILLFR	F
BAF11659	LOC_Os03g17340	Os03g0281800	Expressed protein	12.63	932.82	SSPADYHR	F
BAT05739		Os08g0457501	Unknown	11.24	823.445	ASSGKAMR	F
EAY81230	LOC_Os11g34140	Os11g0544100	Expressed protein	12.73	964.880	AAMGMEGKR	F
BAT06109		Os08g0500400	Hypothetical protein	15.59	1217.357	QEPMAAAATRR	F
BAT00645		Os07g0219600	Unknown	10.71	1557.478	LRHVGVDDMPFVR	F
BAT11830	LOC_Os10g39200	Os10g0537300	Expressed protein	7.22	1091.461	LKFLQSNLK	F
EAZ42809	LOC_Os08g33170		Expressed protein	3.91	1959.027	SNSLEVAQAGADPPMSTGVK	F
Defense							
BAT14423	LOC_Os11g34920	Os11g0550500	Stripe rust resistance protein Yr10	13.15	934.351	KTDDLVSR	G
EEC81953	LOC_Os07g29820	Os07g0481400	NBS-LRR disease resistance protein	5.52	948.282	SLRGLGAMK	G
EEE55443	LOC_Os01g56200	Os01g0767900	BTBA2 - Bric-a-Brac,Tramtrack, Broad Complex BTB domain with Ankyrin repeat region	7.29	919.860	EQGQESNK	G
Transcription							
BAS89333	LOC_Os04g35864	Os04g0439300	DDT domain-containing protein	5.16	1046.382	QSVQSNSLGK	G

Table D3. List of gene co-expressed with LOC_Os03g02240 (Node A)

Locus ID	RAP ID	Description
LOC_Os01g18670.1	Os01g0290700	Similar to CjMDR1.
LOC_Os01g32364.1	Os01g0508000	Similar to Beta-glucosidase.
LOC_Os01g65780.1	Os01g0880200	Glycosyl transferase, family 8 protein.
LOC_Os02g43300.1	Os02g0648300	Homeodomain-like containing protein.
LOC_Os03g03400.1	Os03g0125400	Conserved hypothetical protein 147 family protein.
LOC_Os03g60350.1	Os03g0817900	Protein of unknown function DUF231, plant domain containing protein.
LOC_Os04g51880.1	Os04g0608100	Galactokinase family protein.
LOC_Os05g30700.1	Os05g0369900	Conserved hypothetical protein.
LOC_Os06g45020.1	Os06g0660800	Protein kinase domain containing protein.
LOC_Os08g41670.1	Os08g0528500	Protein of unknown function UPF0016 family protein.
LOC_Os10g37240.2	Os10g0516500	Conserved hypothetical protein.
LOC_Os11g32260.1	Os11g0525600	Similar to Alpha-mannosidase.
LOC_Os12g38920.1	Os12g0578400	Glycoside hydrolase family 79, N-terminal protein.

Table D4. List of gene co-expressed with LOC_Os12g04100 (Node B)

Locus ID	RAP ID	Description
LOC_Os04g46650.1	Os04g0552200	Beta-expansin 5.
LOC_Os11g04290.1	Os11g0138300	Cytochrome P450 family protein.
LOC_Os01g73630.1	Os01g0967200	Similar to Rac GTPase activating protein 1.
LOC_Os06g51210.1	Os06g0727900	Protein of unknown function DUF23 family protein.
LOC_Os12g15530.1	Os12g0257800	Similar to Laccase (EC 1.10.3.2) (Fragment).

Table D5. List of gene co-expressed with LOC_Os04g38660 (Node C)

Locus ID	RAP ID	Description
LOC_Os03g56670.1	Os03g0778100	Similar to Photosystem-1 F subunit.
LOC_Os08g01380.1	Os08g0104600	Ferredoxin I, chloroplast precursor (Anti-disease protein 1).
LOC_Os01g31690.1	Os01g0501800	Similar to Photosystem II oxygen-evolving complex protein 1 (Fragment).
LOC_Os01g56680.1	Os01g0773700	Similar to Photosystem II reaction center W protein (PSII 6.1 kDa protein) (Fragment).
LOC_Os05g33280.1	Os05g0401100	Protein of unknown function DUF477 family protein.
LOC_Os06g01210.1	Os06g0101600	Plastocyanin, chloroplast precursor.
LOC_Os07g05480.1	Os07g0148900	Photosystem I protein-like protein.
LOC_Os08g10020.1	Os08g0200300	Similar to Photosystem II 10 kDa polypeptide (Fragment).
LOC_Os08g44680.1	Os08g0560900	Similar to Photosystem I reaction center subunit II, chloroplast precursor (Photosystem I 20 kDa subunit) (PSI-D).
LOC_Os12g08770.1	Os12g0189400	Similar to Photosystem I reaction centre subunit N, chloroplast precursor (PSI- N).
LOC_Os12g23200.1	Os12g0420400	Similar to Photosystem I reaction center subunit XI, chloroplast precursor (PSI- L) (PSI subunit V).

Table D6. List of gene co-expressed with LOC_Os08g17020 (Node D)

Locus ID	RAP ID	Description
LOC_Os01g05940.1	Os01g0152600	Serine/threonine protein kinase domain containing protein.
LOC_Os02g37220.1	Os02g0583300	En/Spm-like transposon proteins family protein.
LOC_Os02g42110.1	Os02g0632100	Similar to Wall-associated kinase-like protein.
LOC_Os05g03920.1	Os05g0130100	Protein kinase domain containing protein.
	Os06g0527400	Non-protein coding transcript, unclassifiable transcript.
LOC_Os07g36240.1	Os07g0546500	Conserved hypothetical protein.
LOC_Os08g08500.1	Os08g0183900	NAD-dependent epimerase/dehydratase family protein.
LOC_Os09g14590.1	Os09g0314900	Proteasome maturation factor UMP1 family protein.
LOC_Os12g35330.1	Os12g0538600	Glutaredoxin-like, plant II family protein.

Table D7. List of gene co-expressed with LOC_Os05g06450 (Node E)

Locus ID	RAP ID	Description
LOC_Os05g06260.1	Os05g0154500	Spc97/Spc98 family protein.
LOC_Os05g07680.2	Os05g0168800	Prefoldin domain containing protein.
LOC_Os05g37160.1	Os05g0443800	Similar to Plastid division protein ftsZ1 precursor.
LOC_Os09g21780.2	Os09g0386600	Conserved hypothetical protein.
LOC_Os12g13660.1	Os12g0239000	Conserved hypothetical protein.
LOC_Os12g39160.1	Os12g0581300	Protein of unknown function DUF620 family protein.

Table D8. List of gene co-expressed with LOC_Os10g05020 (Node F)

Locus ID	RAP ID	Description
LOC_Os07g06850.1	Os07g0162600	Esterase/lipase/thioesterase domain containing protein.
LOC_Os09g34214.1	Os09g0517900	UDP-glucuronosyl/UDP-glucosyltransferase family protein.
LOC_Os11g44580.1	Os11g0668000	Disease resistance protein family protein.
LOC_Os11g44590.1		
LOC_Os12g09640.1	Os12g0198200	Protein phosphatase 2C family protein.

Table D9. List of gene co-expressed with LOC_Os11g34920 (Node G)

Locus ID	RAP ID	Description
LOC_Os10g03570.1	Os10g0124300	Similar to RGH1A.
LOC_Os11g45790.1	Os11g0684700	Disease resistance protein family protein.
LOC_Os06g10790.1	Os06g0210400	Legume lectin, beta domain containing protein.
LOC_Os06g41980.1	Os06g0625300	Peptidoglycan-binding LysM domain containing protein.
LOC_Os07g17220.1	Os07g0273600	Hypothetical protein.
LOC_Os08g10430.1	Os08g0205100	Disease resistance protein family protein.
LOC_Os10g22510.1	Os10g0370400	Disease resistance protein family protein.
LOC_Os11g45130.1	Os11g0676500	Similar to NBS-LRR type resistance protein (Fragment).

Table D10. Comparison of expression pattern between proteomics data and microarray database. Microarray data was retrieved from the Rice eFP Browser (GSE6893). The expression was showed in term of up-/down-regulated from the control condition and this is a comparison between drought and control treatment.

Locus	Protein expression level	Expression from Rice eFP
LOC_Os12g20140	Up-regulated	Up-regulated
LOC_Os07g26590	Up-regulated	Down-regulated
LOC_Os08g10110	Up-regulated	Down-regulated
LOC_Os10g24870	Up-regulated	Down-regulated
LOC_Os08g30590	Up-regulated	Down-regulated
LOC_Os01g08140	Up-regulated	Up-regulated
LOC_Os01g68610	Up-regulated	Unchanged
LOC_Os02g21630	Up-regulated	Down-regulated
LOC_Os02g30900	Up-regulated	Up-regulated
LOC_Os03g24730	Up-regulated	Up-regulated
LOC_Os03g28960	Up-regulated	Down-regulated
LOC_Os03g48490	Up-regulated	Down-regulated
LOC_Os03g56400	Up-regulated	Unchanged
LOC_Os04g38600	Up-regulated	Down-regulated
LOC_Os06g17930	Up-regulated	Up-regulated
LOC_Os06g45310	Up-regulated	Down-regulated
LOC_Os08g17020	Up-regulated	Up-regulated
LOC_Os08g40330	Up-regulated	Down-regulated
LOC_Os09g31438	Up-regulated	Unchanged
LOC_Os12g04100	Up-regulated	Down-regulated
LOC_Os04g15510	Up-regulated	Up-regulated
LOC_Os11g29110	Up-regulated	Up-regulated
LOC_Os11g34920	Up-regulated	Down-regulated
LOC_Os11g27440	Up-regulated	Down-regulated
LOC_Os03g61690	Up-regulated	Up-regulated

Table D10. (cont.). Comparison of expression pattern between proteomics data and microarray database. Microarray data was retrieved from the Rice eFP Browser (GSE6893). The expression was showed in term of up-/down-regulated from the control condition and this is a comparison between drought and control treatment.

Locus	Protein expression level	Expression from Rice eFP
LOC_Os11g34270	Up-regulated	Up-regulated
LOC_Os02g50370	Up-regulated	Down-regulated
LOC_Os10g05020	Up-regulated	Down-regulated
LOC_Os12g23030	Up-regulated	Up-regulated
LOC_Os01g43060	Up-regulated	Down-regulated
LOC_Os01g55520	Up-regulated	Unchanged
LOC_Os04g40510	Up-regulated	Down-regulated
LOC_Os07g29820	Up-regulated	Up-regulated
LOC_Os04g41490	Up-regulated	Down-regulated
LOC_Os03g02240	Down-regulated	Down-regulated
LOC_Os05g06450	Down-regulated	Down-regulated
LOC_Os03g17340	Down-regulated	Down-regulated
LOC_Os10g08550	Down-regulated	Down-regulated
LOC_Os06g13570	Down-regulated	Down-regulated
LOC_Os11g32910	Down-regulated	Unchanged
LOC_Os09g37510	Down-regulated	Down-regulated

Table D11. Functional group of significant protein in SS and SR from the analysis in year 2013 and 2017

Function group	SS		SR	
	2013	2017	2013	2017
Unknown	22%	24%	30%	24%
Metabolic process	22%	16%	12%	15%
Signalling	19%	10%	12%	7%
Transcription	13%	12%	7%	15%
Transport	9%	1%	2%	2%
Retrotransposon	7%	13%	19%	18%
Defense	4%	13%	9%	11%
Transposon	2%	3%	5%	2%
Proteinase inhibitor	2%	-	-	-
Cellular process	-	6%	-	4%
Replication	-	-	2%	-
Post-transcription	-	-	2%	4%
Post-translation	-	1%	-	-

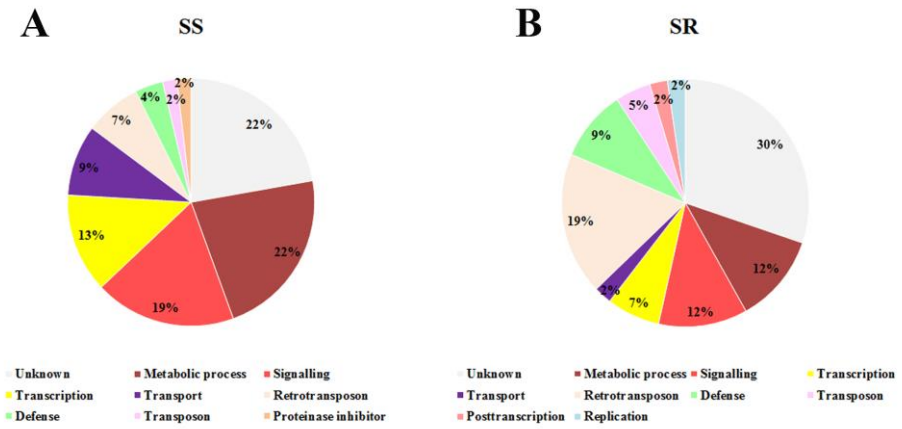


Figure D1. Functional classification of drought-responsive proteins found in LPT123 (SS) and LPT123-TC171 (SR) rice leaves. The analysis was done in year 2013. The functions were categorized according to Gene Ontology (GO) from Rice Genome Annotation Project (http://rice.plantbiology.msu.edu).

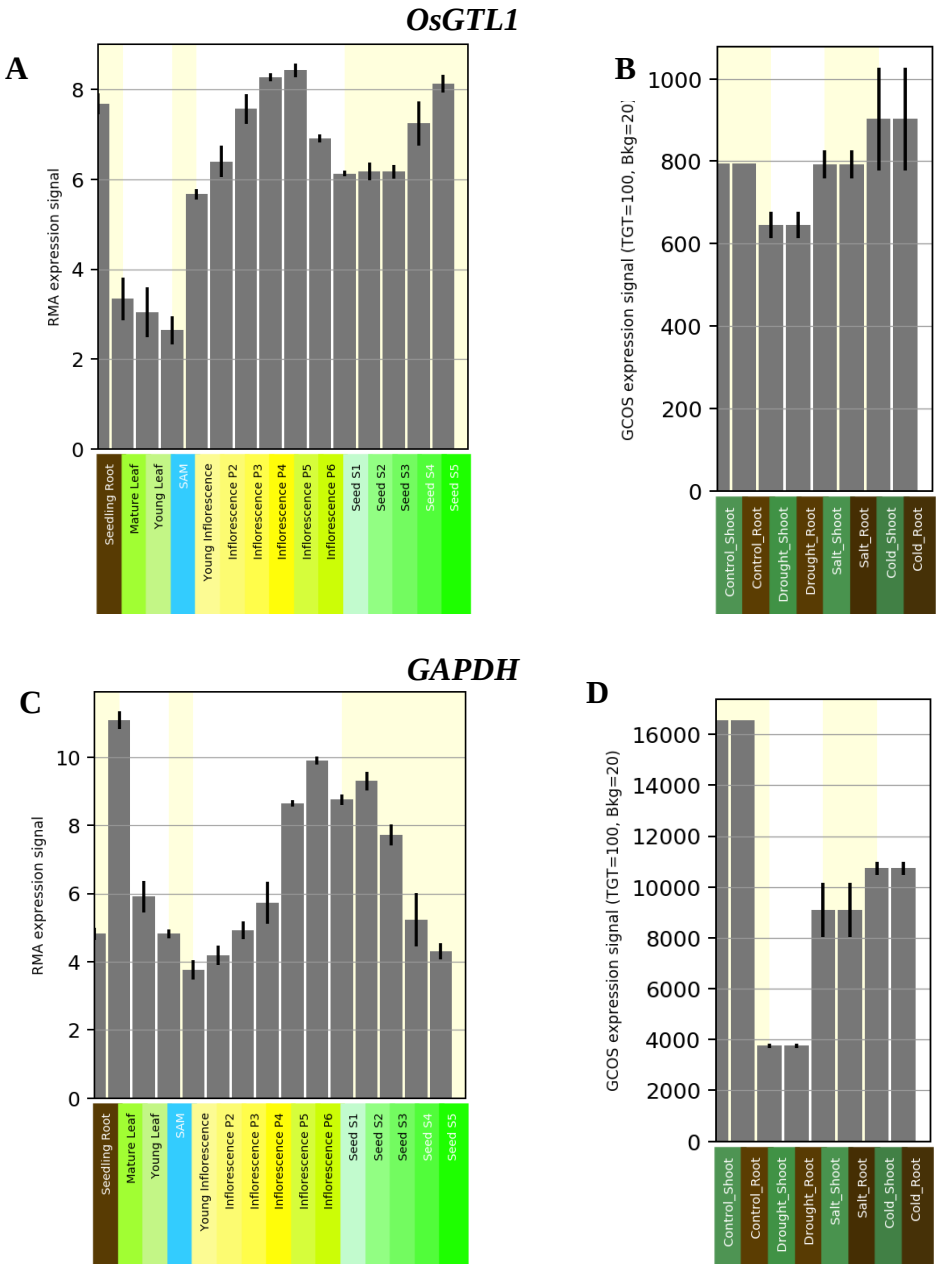


Figure D2. Gene expression profile of *OsGTL1* and *GAPDH*. Expression in different rice parts (A and C) and expression change due to stresses (B and D) retrieved from the Rice eFP Browser base on two microarray database GSE6893 and GSE6901.

VITA

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Journal paper:

Sanguanmoo, N., Pongprayoon, W., Chintakovid, N., Royrakul, S. and Chadchawan, S. 2012. Comparative proteomics of rice (Oryza sativa L.) root proteins under drought stress condition." Thai Journal of Botany, 4:125-134.

Proceeding:

Maipoka, M., Pongprayoon, W., Chintakovid, N., Roytrakul, S., Pichayangkura, R., and Chadchawan, S. 2012. "Comparison of chitosan-induced protein patterns in 'Leung Pratew123' rice (Oryza Sativa L. 'Leung Pratew123') and its drought resistant mutant line, Leung Pratew123-TC171, during drought stress." 13th FAOBMB International Congress of Biochemistry and Molecular Biology, November 25 – 29, 2012, BITEC, Bangkok, Thailand.