CHAPTER II
LITERATURE REVIEWS

Rice (*Oryza sativa* L.)

Rice is one of the important cereal crops since it is staple food feeding more than half of population in the world (Salekdeh et al., 2002). Furthermore, it is used as a model of monocot plants for molecular biological study as it has a small genome size and its genome is completely sequenced (Goff et al., 2002). In Thailand, rice is one of the top ten agricultural export products. Its value is 158,434 million bahts in 2012 (Centre for Agricultural Information, 2013).

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

'Leung Pratew123' ('LPT123') rice is a Thai indica rice. It is a photo-sensitive variety. Leung Pratew123-TC171 (LPT123-TC171) rice is a salt tolerant mutant line derived from somaclonal variation of the LPT123 rice under salt stress condition (Vajrabhaya and Vajrabhaya, 1991). Physiological and molecular responses to salt and drought stresses in these two rice lines have been studied (Chamnanmanoontham, 2009; Chantarachot, 2012; Klomsakul, 2004; Maneeprasopsuk, 2004; Pongprayoon et al., 2012; Pongprayoon et al., 2013; Sa-nguanmoo, 2013; Saeng-ngam et al., 2012; Sripinyowanich, 2010; Sripinyowanich et al., 2013; Thikart et al., 2005; Udomchalotorn et al., 2009). Thikart et al. (2005) showed that the LPT123-TC171 rice was resistant to drought stress. It could maintain plant height, shoot fresh weight, shoot dry weight, root fresh weight and root dry weight at higher level than that of its original variety, LPT123, under drought stress condition (Thikart et al., 2005). Moreover, its survival rate could support these salt and drought resistant characteristics (Klomsakul, 2004).

Exogenous application of abscisic acid by foliar spraying could improve salt and drought tolerance in the LPT123 and LPT123-TC171 rice but appropriate concentration was different between these rice lines (Klomsakul, 2004). Physiological responses to chitosan application prior to drought stress were studied in these LPT123 and LPT123-TC171 rice. Four types of chitosan, polymeric chitosan with 90% and 80% degree of deacetylation and oligomeric chitosan with 90% and 80% degree of deacetylation at 20 and 40 mg/L were applied to rice three times prior to drought stress by seed soaking and foliar spraying. It was found that 80% deacetylated oligomeric chitosan solution (O80) at a concentration of 40 mg/L could enhance shoot growth in LPT123 rice, but it had no positive impact on LPT123-TC171 rice (Pongprayoon et al., 2012; Pongprayoon et al., 2013). This indicates genetic

dependent chitosan responses of plant. Chitosan application increased photostynthetic pigments in both rice lines. It could maintain the pigments in LPT123 rice under drought stress condition but not in LPT123-TC171 rice. Drought stress induced H_2O_2 accumulation in both lines. With chitosan treatment, the reduction of hydrogen peroxide content was observed in LPT123-TC171 rice under drought stress, but no drastic change found in LPT123 rice. Chitosan application together with H_2O_2 tended to increased plant growth in LPT123-TC171 rice under drought stress compared with chitosan treatment without H_2O_2 (Pongprayoon et al., 2013).

Plant responses to drought stress

Plant resistance to drought stress has been divided into 3 strategies (Chaves et al., 2003).

1. Drought escape: Plants have the capability to complete their life cycle before subjected to drought stress.
2. Drought avoidance: Plants reduce water loss while maximising water uptake to keep water potential as high as possible.
3. Drought tolerance: Plants can tolerate to low water potential by osmotic adjustment and/or antioxidant capacity.

Plants responses to drought stress involve in changes at the morphological, physiological, biochemical and molecular levels.

Morphological response

Growth

Reduction of turgor pressure under drought stress condition affects cell growth. It impairs mitosis and inhibits cell elongation and enlargement, which lead to diminished growth (Jaleel et al., 2009). Leaf number and leaf area were reduced under water deficit condition (Ghanbari et al., 2013). Shoot extension rate decreased in drought treated creeping bentgrass (Xu and Huang, 2010). Decreased total root length due to drought stress was reported in CT9993 and IR62266 rice (Wang et al., 2009). Biomass accumulation and allocation were also affected under drought condition. Shoot and root dry weight reduced in drought-treated plants (Ji et al., 2012; Pongprayoon et al., 2013; Wang et al., 2009). Drought-stressed plants increase biomass allocation to root in order to increase water uptake. Reduction of shoot:root ratio was found in KDML105 rice treated with polyethylene glycol 6000 (Maksup et al., 2012).

Physiological responses

Photosynthesis

Photosynthetic rate, *A*, is correlated with relative water content and leaf water potential. It decreases under drought stress. This effect of drought stress involved in stomatal limitation and metabolic impairment. Plants respond to drought stress by closing stomata, which is ABA-dependent response, to reduce water lose. There is good correlation between leaf water potential and stomatal conductance (Reddy et al., 2004). The rate of photosynthesis depends on ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) and RuBP regeneration. The abundance of Rubisco is low under drought condition (Ji et al., 2012). There has been reported that drought stress cause shrinkage of chloroplast (Jia et al., 2008). This lead to conformation changes in Rubisco. Its activity is regulated by Rubisco activase which removes Rubisco inhibitor, and then allows the Rubisco to undergo rapid caboxylation. The abundance of Rubisco activase was affected by drought stress. Moreover, drought inhibits rate of RuBP regeneration. The reduction of CO_2 assimilation under drought stress may result in imbalance between light capture by photosynthetic pigments and utilization in Calvin cycle and consequently lead to excessive light and hence reactive oxygen species (ROS) generation which can damage cellular components (Miller et al., 2010). The plant processes can get rid of excessive light by preventing its absorption, losing chlorophyll and non-photochemical quenching. Plants cope with such oxidative stress by activation of antioxidant system to eliminate the ROS (Ji et al., 2012; Miller et al., 2010; Xu and Huang, 2010).

Chlorophyll content

Chlorophyll content decreases under drought stress (Anjum et al., 2011; Pongprayoon et al., 2013). This may be the result of chlorophyll degradation, oxidative stress and pigment photo-oxidation. Concentration of photosynthetic pigment can directly affect photosynthetic potential and then primary production (Anjum et al., 2011; Pongprayoon et al., 2013).

Osmotic adjustment

Under drought stress, the media water potential is low. Therefore, plants reduce cellular water potential in order to take up water by decreasing the osmotic potential via compatible solute accumulation (Ashraf, 2010; Chaves et al., 2003; Farouk and Abdul Qados, 2013). The osmotic compounds include proline, aspartic

acid, glutamic acid, glycine betaine, alanine betaine, manitol and sorbitol (Chaves et al., 2003).

Biochemical response

Antioxidant system

An early event in plant responding to biotic and abiotic stresses is the oxidative burst. Besides being toxic substance, reactive oxygen species have been considered to act as a signaling molecule to trigger downstream responses (Anjum et al., 2011; Pongprayoon et al., 2013). Reactive oxygen scavenging system protects plants from oxidative damage. It composes of enzymatic and non-enzymatic antioxidants. The enzymatic antioxidants include glutathione reductase (GR) superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (Apx), peroxide (POD), and monodehydroascorbate reductase (MDAR). Non-enzymatic components include ascorbic acid (AA), carotenoids, and anthocyanins and flavanones.

Molecular responses

Gene expression

Drought responsive genes have been identified in several species including rice (Rabbani et al., 2003). These genes can be classified into transcriptional regulation, post-transcriptional RNA or protein phosphorylation, and osmoprotectant metabolism or molecular chaperones (Yang et al., 2010).

Transcriptional regulation

Transcription factors act as molecular switches for gene expression in responses to environmental factors. A number of families of transcription factors have been characterized with their involvement in plant stress responses such as APETALA2 (AP2), bZIP, NAC, zinc-finger, MYB transcription factor families (Hadiarto and Tran, 2011).

DREBs/CBFs are well-known AP2 transcription factors. They regulate expression of downstream stress-responsive genes by specifically binding to DRE/CRT motif in promoter region in ABA-independent manner (Hadiarto and Tran, 2011). In rice, a number of OsDREB transcription factor encoding genes have been studied (Chen et al., 2008; Dubouzet et al., 2003; Gutha and Reddy, 2008; Ito et al., 2006; Matsukura et al., 2010; Tian et al., 2005; Wang et al., 2008). *OsDREB1A* was up-regulated under cold stress while *OsDREB2A* and *OsDREB2B* were up-regulated under dehydration and salt stresses (Dubouzet et al., 2003; Matsukura et al., 2010).

Overexpression of *OsDREB1* and *OsDREB2B* could improve drought tolerance in transgenic plants (Ito et al., 2006; Matsukura et al., 2010).

The 89 genes encoding bZIP transcription factors were identified and characterized in rice genome (Nijhawan et al., 2008). Among these genes, 24 genes were up-regulated whereas nine genes were down-regulated under drought treatment (Nijhawan et al., 2008).

Genome-wide analysis identified 151 non-redundant *NAC* genes in rice (Nuruzzaman et al., 2010). Microarray analysis showed 46 of which responded to at least one of the abiotic stresses (cold, drought, submergence, osmotic, salt and hormone) (Nuruzzaman et al., 2010). Overexpression of *OsNAC045* could enhance drought tolerance in rice (Zheng et al., 2009).

A number of genes encoding transcription factor in zinc finger family have been characterized. Overexpression of *ZPF252* in rice increased free proline content, expression of stress-related genes and tolerance to drought and salt stresses (Xu et al., 2008). Functional analysis of *ZFP245* showed that *ZFP245*-overexpressing transgenic plants had higher proline content, activities of reactive oxygen species-scavenging enzymes and relative shoot length than wild type plants under drought stress (Huang et al., 2009).

MYC and MYB transcription factors regulate target genes by interacting with MYC and MYB recognition sites in promoter region, respectively. Transgenic plants overexpressing the *AtMYC2* and *AtMYB2* were drought tolerance (Abe et al., 2003). In rice, *OsMYB2* has been characterized. Its expression was induced by salinity, low temperature and dehydration. Overproduction of *OsMYB2* in rice enhanced salt, cold and dehydration tolerance (Yang et al., 2012a).

Post-transcriptional RNA or protein phosphorylation

Protein kinases regulate their target genes through phosphorylation. A number of protein kinases play crucial role in signal transduction. In rice, 1,429 members of protein kinase family have been reported. These can be classified into 6 groups including AGC, GMGC, CAMK, CK1, TKL and STE (Dardick et al., 2007). Some of which have been characterized under abiotic stresses. Drought induced expressions of *OsMPK5, OsMPK7, OsMPK8* and *OsMPK12* while it repressed *OsMPK4* expression (Rohila and Yang, 2007). Xiang et al. (2007) studied drought-inducible *OsCIPK* genes by using RNA gel blot and qRT-PCR and found that 15 *OsCIPK* genes responded to drought stress including *OsCIPK01, 02, 05, 09, 11, 12, 15, 17, 20, 21, 22, 23, 24, 29 and 30.*

Osmoprotectant metabolism or molecular chaperones

Several genes that encode enzymes involving in osmoprotectant biosynthetic pathway have been studied. Proline is one of the compatible solutes that accumulate in plant following exposure to abiotic stress such as drought and salinity (Choudhary et al., 2005; Hien et al., 2003; Igarashi et al., 1997; Montesinos-Pereira et al., 2014; Sripinyowanich et al., 2013; Yooyongwech et al., 2012). *OsP5CS* encoding an enzyme involved in the biosynthesis of proline, was induced by dehydration treatment (Gao and Han, 2009; Hur et al., 2004; Igarashi et al., 1997; Yooyongwech et al., 2012). Northern blot analysis showed that *P5CS* expression was higher in drought tolerant cultivar, N-22, as compared with drought susceptible cultivar, Panidhan (Choudhary et al., 2005). This correlated with higher P5CS activity, proline content and relative water content in drought tolerant cultivar than that of susceptible one (Choudhary et al., 2005).

The other compatible solute is trehalose, a non-reducing disaccharide. It serves as osmoprotectant under stress condition. Trehalose is synthesized in two-step process: trehalose-6-phosphate synthase catalyzes the reaction between UDP-glucose and glucose-6-phosphate to produce trehalose-6-phosphate which is then converted to trehalose by trehalose-6-phosphate phosphatase (Garg et al., 2002). Each of the rice and Arabidopsis genomes contain 11 *trehalose-6-phosphate synthase (TPS)* genes and Populus genome has 12 *TPS* genes. Some of them responded to abiotic stress including drought treatment (Yang et al., 2012b). Transgenic rice overexpressing *Escherichia coli otsA* and *otsB* (encoding trehalose-6-phosphate synthase and trehalose-6-phosphate phosphatase, respectively) showed fewer visual symptoms than that of non-transgenic plants after exposure to drought stress for 48 hours (Garg et al., 2002). These transgenic plants increased 3-to-6 fold higher trehalose content compared with wild type plants under drought stress. Furthermore, overexpression of these microbial trehalose biosynthetic genes in rice could improve photosynthesis under water deficit treatment (Garg et al., 2002). Overexpression of *OsTPS1* enhanced salt, drought, cold and high temperature tolerance in rice (Li et al., 2011).

Moreover, late embryogenesis abundant proteins (LEA) play important role in protection from desiccation. In rice, 34 *OsLEA* genes were identified (Wang et al., 2007). Among them, six genes (*OsLEA3,* 7, 21, 22, 27 and 28) were drought up-regulated (Wang et al., 2007). Overexpression of *OsLEA3-2* enhanced salt and drought tolerance in rice and Arabidopsis (Duan and Cai, 2012). Rice over-expressing *HVA1*, a

LEA gene from barley, had higher leaf relative water content and plant growth under drought stress than that of wild type plant (Chandra Babu et al., 2004).

Plant responses to chitosan

Chitosan, a derivative of chitin, is composed of N-acetylglucosamine (GlcNAc) and glucosamine (GlcN) residues. It is produced from deacetylation of chitin (Harish Prashanth and Tharanathan, 2007).

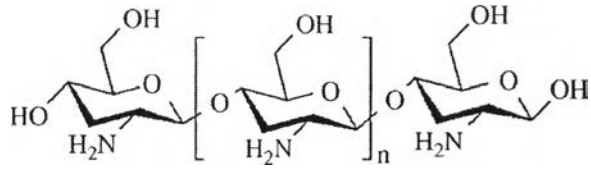

Figure 2.1 Structure of chitosan (Yin et al., 2010)

Early events in plant cells induced by chitosan were studied by Amborabe et al. (2008). It was found that chitosan stimulated rapid membrane transient depolarization. This effect was dose-dependent. Specific site of action was plasma membrane H^+-ATase. Povero et al. (2011) showed that chitosan was perceived through a CERK1-independent pathway. In addition, chitosan induced defense response genes (Povero et al., 2011) including phenylalanine ammonia lyase, chitinase, defense-related genes, β-1,3 glucanase and production of hydrogen peroxide (Lin et al., 2005; Lizama-Uc et al., 2007). Defense response was blocked when hydrogen peroxide production was inhibited (Lin et al., 2005). Chitosan activated MAPK-like protein shortly after calli imbibitions. Moreover, it induced genes that were similar to receptor-like kinases, *Verticillium*-like protein, and mitochondrial alternate oxidase (Lizama-Uc et al., 2007). Chitosan induced genes involved in primary metabolism, transcription and signal transduction. Some of which were induced by auxin and gibberellins. In addition, chitosan induced 2-oxophytodienoate-10,11-reductase, ATMPK4-homolog gene, ethylene receptor gene and ethylene responsive element binding protein (Yin et al., 2006). Chalcone synthase, chalcone-flavanone isomerase (CHI) were also induced in chitosan treatment (Ferri et al., 2009).

Chitosan has been reported to promote plant growth in several species. It could increase seed germination percentage and germination rate in *Dendrobium* orchid (Kananont et al., 2010) and 'Pathum Thanee 1' rice (Suvanasara and

Boonlertnirun, 2013) and increase seedling survival rate in chili (Photchanacha et al., 2006). Dzung et al. (2011) found that foliar spraying of oligomeric chitosan could increase photosynthetic pigments, mineral uptake, growth and decrease fruit fallen rate in coffee seedling. Acceleration effect of chitosan on photosynthetic pigments was also found in Leung Pratew123 and Leung Pratew123-TC171 rice (Pongprayoon et al., 2013). Chitosan not only increased photosynthetic pigment but also chloroplast size in Dendrobium 'Eiskul' (Limpanavech et al., 2008) and photosynthetic rate in maize and soybean (Khan et al., 2002). Furthermore, chitosan could increase plant production. Chitosan application by seed soaking and soil application could promote rice yield (Boonlertnirun et al., 2008). Application of 80% deacetylated oligomeric chitosan in Dendrobium 'Eiskul' increased number of inflorescence and induced early flowering (Limpanavech et al., 2008).

Chitosan could improve drought tolerance. It was found that chitosan could reduce transpiration by inducing ABA-dependent stomatal closure via hydrogen peroxide mediated process (Bittelli et al., 2001; Iriti et al., 2009; Lee et al., 1999). Srivastava et al. (2009) revealed that nitric oxide acted downstream of the ROS production. MKK9 and MKK12 were shown to involve in chitosan-induced stomatal closure by function downstream of ROS production, cytosolic alkalization and $[Ca^{2+}]_{cyt}$ oscillation (Salam et al., 2012). Furthermore, foliar application of chitosan could improve water use efficiency in pepper plant (Bittelli et al., 2001). Iriti et al. (2009) sprayed 0.15% (w/v) chitosan to common bean leaf and found that chitosan could trigger stomatal closure. Non-photochemical quenching and ABA content were increased in chitosan treatment whereas stomatal conductance and transpiration rate were decreased. Chitosan did not affect maximal photochemical efficiency of PSII, photochemical quenching and water use efficiency (WUE) in common bean.

Boonlertnirun et al. (2007) showed that 'Suphanburi 1' rice treated with chitosan prior to drought stress had highest yield and yield component, showed good recovery and reduced percentage of damaged leaves. Moreover, application of chitosan prior to drought stress could increase superoxide dismutase and catalase activities and membrane stability in apple seedlings (Yang et al., 2009). Chitosan enhancing antioxidant enzyme activity under drought stress was also found in 'Favourite' potato and wheat. Foliar application of chitosan before drought stress increased superoxide dismutase and peroxidase activities, proline and soluble protein accumulation and membrane stability in 'Favourite' potato (Jiao et al., 2012). In wheat, germination rate, fresh weight, root length, activities of antioxidant enzymes such as superoxide dismutase, peroxidase and catalase, capability to maintain

membrane stability and chlorophyll content under drought stress were improved by chitosan seed coating (Zeng, 2012). Chitosan increased accumulation of osmolyte such as proline, glycine betaine and soluble carbohydrate, osmotic adjustment and sustained osmotic potential during water stress. This improvements led to increase leaf area and yield component in cowpea under drought stress (Farouk and Abdul Qados, 2013). Pongprayoon et al. (2013) found that application of 80% deacetylated oligomeric chitosan at a concentration of 40 mg/L three times by seed soaking and foliar spraying could improve drought tolerance in LPT123 rice whereas it had no positive impact on LPT123-TC171 rice. It could reduce hydrogen peroxide content in LPT123-TC171 rice and decreased lipid peroxidation in LPT123 rice (Pongprayoon et al., 2013).

Proteomics

Proteome is a complete set of proteins produced by cell or organism at a definite situation. The study of proteome is called proteomics. The scope of proteomics includes sequence and structural proteomics, expression proteomics, interaction proteomics and functional proteomics (Twyman, 2004).

Cells regulate abundance and activity of proteins to cope with environmental stimuli. The changes of proteome can provide snapshot of cells in the action of these responses (Twyman, 2004). Therefore, proteomics is a powerful approach and widely used to study plant responses to abiotic stress. Salekdeh et al. (2002) found 16 drought-responsive proteins in rice leaf and four of them were novel proteins including S-like RNase homologue, actin depolymerizing factor, Rubisco activase and isoflavone reductase-like protein. Ke et al. (2009) studied drought-responsive proteins in 'Nipponbare' rice. Up-regulation of LEA-like protein and chloroplast Cu-Zn superoxide dismutase and down-regulation of Rieske Fe-S precursor protein under drought stress were found. Ten phosphoproteins including NAD-malate dehydrogenase, abscisic acid- and stress-inducible protein, ribosomal protein, drought-induced S-like ribonuclease, ethylene-inducible protein, guanine nucleotide-binding protein beta subunit-like protein, OSJNBb0039L24.13 protein, r40c1 protein, OSJNBa0084K20.14 protein and germin-like protein 1, responded to drought stress.

Comparative proteomic between plants differing in drought stress tolerant ability will unravel drought-responsive mechanism that can be applied to improve drought tolerance. Ali and Komatsu (2006) studied drought-responsive proteins in rice leaf sheath of 'Nipponbare' and 'Zhonghua 8' rice. They found that abundance of

actin depolymerizing factor, light harvesting complex chain II, superoxidase dismutase and salt-induced protein were changed under drought stress. Moreover, the level of actin depolymerizing factor, light harvesting complex chain II, PSII oxygen evolving complex protein and oxygen evolving enhancer protein 2 were higher in 'Zhanghua 8' rice, a drought tolerant cultivar, than that of 'Nipponbare' rice. Drought-responsive proteins in two rice genotypes with contrast drought tolerance were investigated. Down-regulation of ATP synthase, orthophosphate dikinase, glycine dehydrogenase, glycine hydroxymethyltransferase and ribulose bisphosphate carboxylase were found in Zhenshan97B, a drought susceptible rice cultivar, under drought stress. In IRAT109, a drought tolerant rice cultivar, transketolase and Rubisco were down-regulated whereas Rubisco activase, peptidyl-prolyl cis-trans isomerase, chloroplastic superoxide dismutase and dehydroascorbate reductase were up-regulated under drought condition (Ji et al., 2012). Maksup et al. (2012) studied drought- responsive proteins in Thai jasmine rice (KDML105), drought tolerant cultivar (NSG19) and drought sensitive cultivar (IR20). It was found that proteins up-regulated in KDML105 under drought stress were putative H-protein promoter binding factor-2a, MtN3 and saliva-related transmembrane protein family protein, putative DEAD/DEAH box RNA helicase protein and protein that is similar to hypersensitive reaction associated Ca^{2+}-binding protein. Proteins up-regulated in IR20, a drought sensitive cultivar, under drought stress were WD-40 repeat protein-like, putative potassium transporter and curculin-like (mannose-binding) lectin domain containing protein. Tetratricopeptide repeat protein-like and protein that is similar to coronatine-insensitive 1, zinc finger, Ring-type domain containing protein and O-diphenol-O-methyl transferase-like were up-regulated under drought stress in NSG19, a drought tolerant cultivar.

Moreover, drought-responsive proteins between plants differing in stress tolerance were studied in creeping bentgrass (Xu and Huang, 2010). It was found that drought stress induced abundance of antioxidant enzyme proteins including ascorbate peroxidase, catalase and glutathione-S-transferase. The abundance of ascorbase peroxidase, glucan exohydrolase, UDP-sulfoquinovose synthase and actin were greater in 'Penn-A4' than in 'Penncross' which is more susceptible to drought than 'Penn-A4' (Xu and Huang, 2010).