CHAPTER II

LITERATURE REVIEW

2.1 Rice

Rice (*Oryza sativo* L.) is an annual grass belonging to the Poaceae (Gramineae) family. Rice is cultivated in different regions including Asia, Europe, America, Africa, and Australia. In Asia, *Japonica* and *Indica* are two major subspecies of *Oryza sativa*. *Japonica* is a sticky, short-grained variety. This upland rice variety is thoroughly cultivated in dry fields in temperate East Asia. A non-sticky, long-grained *Indica* variety is lowland rice that is usually cultivated in submerged areas throughout Southeast Asia (Lim 2012). Rice is a staple food and an economic cereal crop in Thailand. Therefore, various *Indica* rice cultivars have been distributed and are cultivated throughout the country. To date, the pericarp color of rice grains can be used to classify rice cultivars into colored and white rice. Colored rice grains possess colored pericarps ranging from red to dark purple, whereas the pericarp of white rice is pale (Deng et al. 2013).

Rice grains are commonly consumed as cooked rice. Therefore, the nutritional content, active compounds, and antioxidant and biological activities of rice grains have been widely investigated by examining their grains (Lin and Lai 2011), germinated grains (Moongngarm and Saetung 2010; Sutharut and Sudarat 2012), and bran (Laokuldilok et al. 2010; Nam et al. 2006) of colored and white rice. Those studies have been indicated that extracts from colored rice exhibit greater antioxidant activity than those of white rice. The presence of phenolic compounds and anthocyanins such as cyanidin-3-glucoside, peonidin-3-glucoside, and cyanidin

diglucoside influence the high antioxidant efficacy of colored rice (Chen et al. 2006; Sadabpod et al. 2010).

2.2 Promoters used in gene expression system

Plant morphology, secondary metabolite production, stress-tolerant traits, and other characteristics are products of gene functions (Pérez-Torres et al. 2009). The knowledge of gene functions is necessary for plant molecular biology. To understand the expression and regulation of genes involving different pathways, a number of gene expression systems have been developed and used for plant functional genomic analysis. The regulatory region or 'promoter' that is used in expression systems plays a key role in gene expression studies (Komarnytsky and Borisjuk 2003). Promoter locates in the 5' upstream region of the coding sequence of a gene. This regulatory region contains the binding sites for RNA polymerase II and transcription factors that are required for gene transcription (Porto et al. 2014). Several promoters that are commonly used to regulate genes of interest include constitutive, tissues-specific, and chemical-inducible promoters (Li et al. 2005).

2.2.1 Constitutive promoters

The constitutive promoters regulate a constitutive expression of target genes throughout tissues and developmental stages of plants. Constitutive promoters are originated from both viruses and plants such as the cauliflower mosaic virus 35S (CaMV 35S), ubiquitin, and actin promoters. These promoters have been used to control a wide range of genes in expression analysis in plants (Gurr and Rushton 2005). However, constitutive overexpression of the target genes can alter metabolic and developmental pathways leading to deleterious or lethal effects in target plants (Fu et al. 2001; Li et al. 2005). Therefore, constitutive promoters are unsuitable for

the regulation of lethal genes and genes that lead highly detrimental plant phenotypes (Corrado and Karali 2009).

2.2.2 Tissue-specific promoters

The tissue-specific promoters are suitable for expression studies of genes that strictly express in specific tissues or at specific developmental stage of plants (Li et al. 2005). Although, tissue-specific promoters are rare and limited to only a few cell types in some plants. These promoters cannot be activated by external inducers. Thus, it is impossible to quantitatively regulate the expression level of target genes in the tested organisms. Additionally, the background activity of these promoters appears to be increase during plant regeneration (Corrado and Karali 2009; Li et al. 2005).

2.2.3 Chemical-inducible expression systems

The suitable system for gene expression studies should be simple, easy to control, and provide a reversibly dose-dependent expression (Li et al. 2005). To meet this task, many chemical-inducible systems have been developed for gene expression analysis. The chemical-inducible system drives a spatiotemporal-regulated expression of desired genes in a dose-dependent manner. Chemical-inducible systems can be activated by adding or removing specific inducers. Generally, chemical-inducible systems comprise two transcription units. The first unit encodes a transcription factor that responds to specific chemicals. The expression of transcription factor-encoding gene may be regulated using a constitutive promoter or, for additional regulation, a tissue-specific promoter. In the presence of specific chemicals, an activated transcription factor only binds to its response element that locates in a chemical-inducible promoter in the second transcription unit and

activates gene transcription (Padidam 2003). Chemicals that are used to activate gene transcription include dexamethasone (Aoyama and Chua 1997), estrogen (Zuo et al. 2000), tetracycline (Weinmann et al. 1994), ecdysone (Martinez et al. 1999), copper (Mett et al. 1993), and ethanol (Caddick et al. 1998). Among them, ethanol is an ideal inducer since it is biodegradable, cheap, and environmentally safe. Moreover, only small amount of ethanol is required to induce the ethanol-inducible system in plants in which visible phytotoxic symptoms are not observed (Ait-ali et al. 2003; Caddick et al. 1998).

2.2.3.1 Ethanol-inducible expression system

An ethanol-inducible system or '*alc* switch' is derived from fungus, *Aspergillus nidulans* (Felenbok et al. 1988). This system consists of a constitutively expressed *alcR* gene that encodes an alcohol-regulated transcription factor (ALCR) and a chimeric ethanol-inducible promoter (*palcA:35S*). A *palcA:35S* consists of two ALCR binding sites of the *alcA* promoter isolated from the *alcA* gene that encodes alcohol dehydrogenase I and a 35S minimal promoter (**Figure 2-1**). ALCR is in an inactive form in the absence of ethanol. Under the ethanol treatment, ALCR is activated. The activated-ALCR binds to a response element in *alcA* promoter and activates gene transcription (Caddick et al. 1998; Li et al. 2005).

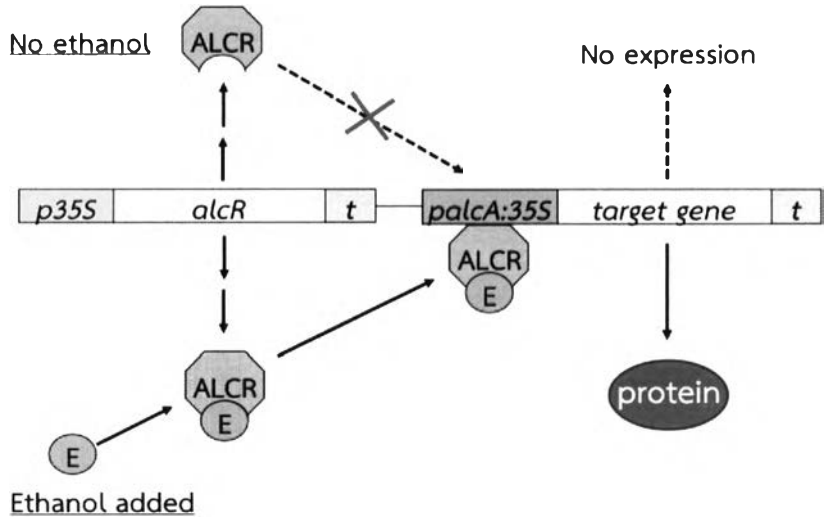

Figure 2-1 Schematic presentation of the ethanol-inducible gene expression system. The construct comprises two expression cassettes for a transcription factor (ALCR) and target gene. The CaMV 35S (*p35S*) promoter drives a constitutively expression of *alcR* gene. The target gene is fused to the *palcA:35S* promoter which consists of two ALCR binding sites of the *alcA* promoter from *Aspergillus nidulans* and a 35S minimal promoter. Adapted from Li et al. (2005).

The *alc* switch regulates expression of desired genes in spatial and temporal pattern. This advantage could diminish undesirable effects from overexpression of desired genes in the tested plants. The ethanol-inducible system has been widely applied to regulate several genes in different purposes including plant morphology control and hybrid seed production. This system could precisely control plant architecture of transgenic Arabidopsis. The transient ethanol-induced expression of a semi-dwarfing gene (*Arabidopsis gai*) could dwarf the stem of transgenic Arabidopsis. After the ethanol induction, the growth of tested plants was recovered without reducing seed production (Ait-ali et al. 2003). The expression of chloramphenicol

acetyltransferase (CAT)-encoding gene in different tested tissues of transgenic tomato (Garoosi et al. 2005) and in transgenic hairy root of *Catharanthus roseus* (Peebles et al. 2007) indicated that the *alc* switch could be used to regulate expression of genes in a wide range of plant species and tissues. Furthermore, the male fertility in the male sterile transgenic eggplant was restored by the ethanol-induced expression of *TBP-associated factor* (*TAF*). This application of the *alc* switch is a useful tool for the production of eggplant F1 hybrid seeds (Toppino et al. 2011).

2.3 Ethanol responses of non-transformed plant

It has been reported that non-transformed plants also respond to exogenously added ethanol. Vreugdenhil et al. (2006) reported that ethanol broke a dormancy period of non-transformed potato tubers and stimulated sprouting. Their study on gene expression analysis revealed that ethanol down-regulated the expression of genes involved in cell division and storing reserves in ethanol-treated potatoes (Vreugdenhil et al. 2006). Sugarcane is another example of a non-transformed plant that responds to ethanol. The transcription profiles of genes in sugarcane leaves were shown to be altered in the presence of ethanol, and the ethanol-responsive genes were identified (Camargo et al. 2007).

2.4 Cereal grasses

Cereal grasses are young plants, also called seedlings, of cereal crops that are members of the Poaceae family including rice and wheat. At the jointing stage prior to the emergence of the second leaf, cereal grasses are a rich source of antioxidants and phytonutrients (Gruenwald 2009; Kulkarni et al. 2006). A period of the jointing stage of cereals is variable, depending on the variety and growth conditions, for example, 6-10 days after sowing for wheat (Kulkarni et al. 2006) and 15-20 days for

rice (Moldenhauer et al. 2000). Juices squeezed from cereal grasses harvested at the jointing stage have been consumed as a health-promoting food and food supplements. Therefore, a number of studies have investigated on the active constituents and bioactivity of cereal grass juices and extracts.

2.4.1 Wheatgrass

Wheatgrass juice is a well-known example of a health-promoting food from cereal grasses. Wheatgrass juice has been popular in the functional food market since the 1980s (Falcioni et al. 2002; Wigmore 1985). The antioxidant and biological activities of wheatgrass juice and extracts have been investigated in numerous studies. Aqueous and ethanolic extracts from 6- to 15-day-old wheatgrass were found to exhibit high antioxidant activity. The highest antioxidant activity was found for aqueous extracts from 7-day-old wheatgrass (Kulkarni et al. 2006). Wheatgrass juice demonstrated potent immunomodulatory activity in normal and predsinolone-treated Swiss albino mice (Hemalatha et al. 2012). Furthermore, wheatgrass juice was found to be an effective treatment in clinical trials of active distal ulcerative colitis (Ben-Arye et al. 2002) and prevented myelotoxictiy from chemotherapy in breast cancer patients (Bar-Sela et al. 2007). Additionally, extracts from wheat sprouts grown over a period of 3-5 days inhibited the mutagenic activity of benzo[a]pyrene in rats. Apigenin and its derivatives that are present in wheat sprout extracts were identified as the active constituents (Peryt et al. 1992). A wheat sprout extract containing antioxidant glycosides also exhibited DNA protective effects (Falcioni et al. 2002). Moreover, wheat sprout extracts demonstrated higher antioxidant activity than extracts from seeds after sprout detachment and non-sprouted seeds. Non-sprouted

wheat seed extracts exhibited the lowest antioxidant activity, which was nearly undetectable (Calzuola et al. 2004).

2.4.2 Rice grass

In Thailand, rice grass juice has been developed and its antioxidant activity has determined. A colored rice cultivar and four white rice cultivars were selected and their juices were squeezed from 15-day-old seedlings. All tested rice grass juices exhibited a higher total antioxidant capacity than wheatgrass juice (Benjawan et al. 2010). Moreover, an extract from 7-day-old white rice seedlings exhibited cytotoxic effects on four tumor cell lines including P388, HSOS-1, X63-Ag8, and Jurkat, but not on normal cell lines (Okai et al. 1993).