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Review

Metabolic engineering of cold tolerance in plants

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ABSTRACT

Low temperature stress is one of the major abiotic stress challenging the growth and productivity of economically important crops. Both chilling and freezing temperatures have severe effects on growth of plants and have resulted in temperate plants, such as perennial rye grass and wheat to evolve mechanisms to avoid or, at the very least, minimize this damage. Accumulating osmoprotectants including glycine betaine, sugars (trehalose and fructans), polyamines, changes in lipid membrane profile, photosynthetic acclimation along with extensive reprogramming at molecular level help temperate plants acquire tolerance to low temperatures. In this review, we have focused mainly on metabolic engineering of plants by introduction of biosynthetic genes involved in various metabolic pathways. Availability of genomic, transcriptomic sequences combined with post-transcriptional data is beginning to link the gene function, regulatory networks and epigenetic states to different phenotypes. Generation of this knowledge together with our ability to manipulate genes involved in mediating tolerance to various stressors including low temperature will lead to the development of cold-resistant genotypes.

Contents

1. Introduction

Plants being sessile in nature are exposed to various abiotic stresses, which cause physiological, cellular, molecular and biochemical changes (Fig. 1). Cold stress is a major environmental factor that affects plant growth and productivity causing significant crop losses (Zhu et al., 2007). Low temperature, including chilling (0–15 °C) and freezing (< 0 °C) impose stress on a plant in two ways: the effect of low temperature alone and, dehydration of cells and tissues when cellular water freezes (Beck et al., 2007).

Several temperate plant species have the ability to increase their tolerance to freezing temperatures, following exposure to low non freezing temperatures, a process known as cold acclimation (Fig. 1) (Theocharis et al., 2012). Reduced leaf expansion, wilting, chlorosis and necrosis are some of the many phenotypic indicators of chilling stress (Mahajan and Tuteja, 2005). At the

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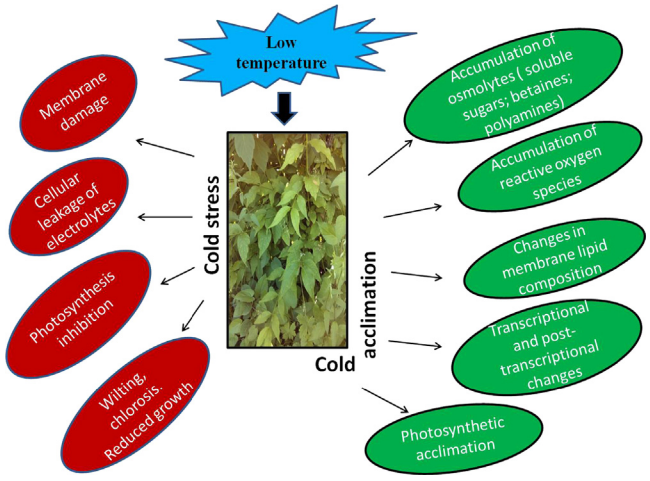

Fig. 1. Cellular, biochemical and molecular changes involved in response to cold stress and acclimation.

physiological level, reduced growth causes a feedback inhibition of photosynthesis (Ruelland and Zachowski, 2010), along with disruption in protein assembly, general metabolic reactions and production of free radicals (Mahajan and Tuteja, 2005).To cope up with the low temperature stress, temperate plants have evolved various physiological and molecular mechanisms. Changes in lipid composition of the membranes, protein and carbohydrate composition and activation of ion channels are some of these physiological alterations in response to low temperatures (Catala et al., 2003; Fernandez et al., 2012). The changes brought about by cold acclimation has also been shown to protect the photosynthetic machinery mediated through the enhanced expression of CBFs (Savitch et al., 2005) possibly through reduced sensitivity to feedback-limited photosynthesis (Dahal et al., 2012). Plants, like other organisms, accumulate low molecular weight water soluble compounds known as "compatible solutes" or "osmolytes" in response to osmotic stress caused by low temperature and other abiotic stresses (Giri, 2011). Glycine Betaine, a variety of sugars and sugar alcohols (trehalose, mannitol), polyamines and amino acid (proline) are some of the common osmolytes providing osmotic adjustment and reducing cellular dehydration during low temperature stress conditions (Giri, 2011).

At the molecular level, identification of the cold-responsive genes encoding a diverse array of proteins involved in various biosynthetic pathways including metabolism of carbohydrates, lipids, phenylpropanoids, antioxidants, antifreeze proteins, and others enzymes, may provide targets for improved stress tolerance via genetic modification (Sunkar et al., 2012). Cold stress tolerance mechanisms include cold signal perception, involvement of a complex and interactive network of transcription factors (TFs) through activation by signal transduction, resulting in expression of cold-responsive genes for mediating stress tolerance (Theocharis et al., 2012). Transcription factors such as dehydration responsive element (DRE), abscisic acid induced protein (*abi3*) has been found to be involved in response to low temperature stress conditions (Theocharis et al., 2012). Also, several cold regulated genes, known as *COR* (cold regulated), *KIN* (cold-induced), *LTI* (low temperature induced) and *RD* (responsive to dehydration) are triggered in response to cold treatment (Zhu et al., 2007). Furthermore, recent studies have also suggested the role of non-coding RNA molecules, microRNAs (miRNAs) in mediating cold response through repressive gene regulation and RNA silencing at the post-transcriptional level (Chinnusamy et al., 2010; Sunkar et al., 2012). miRNAs are small RNAs (20–22 nt) which impact almost all biological processes particularly growth, development and stress responses by modulating the expression of genes encoding transcription factors and regulatory proteins (Chen, 2012). This review will focus on the biosynthesis, degradation pathways and modulation of important metabolites and osmolytes including glycine betaine, sugars, and changes in membrane lipids and proteins in response to cold stress through genetic modification.

2. Compatible solutes

2.1. Glycine betaine

Glycine Betaine (GB), a quaternary ammonium salt (Chen and Murata, 2002) is found in a large variety of microorganisms, marine invertebrates, higher plants and mammals (Chen and Murata, 2002, 2008; Rhodes and Hanson, 1993, Takabe et al., 1998). Plants including spinach, maize, sugar beet, and barley are known as natural accumulators of GB, and upon exposure to salt, drought, and low temperature stresses show elevated endogenous levels of GB (Bohnert et al., 1995; Kishitani et al., 1994). The GB biosynthetic pathway utilizes choline as the precursor molecule and synthesizes GB in a two-step oxidation reaction involving betaine aldehyde as an intermediate (Rhodes and Hanson, 1993). The first oxidation reaction is catalyzed by choline monooxygenase (CMO) in plants, and by choline dehydrogenase (CDH) in animals and bacteria (encoded by the *betA* gene) (Chen and Murata, 2002; Takabe et al., 1998). The second oxidation reaction is catalyzed by the NAD^+- dependent betaine aldehyde dehydrogense (BADH) (Rathinasbapathi et al., 2001). With increasing knowledge of genes involved in GB biosynthetic pathway, transgenic approaches have been employed to enhance the abiotic stress tolerance in accumulators and major cereals and economically important crops, which are non-accumulators of GB.

Arthrobacter globiformis produces GB by single-step pathway where choline oxidase, encoded by *codA*, synthesizes GB from choline. Researchers took advantage of this single step biosynthesis to enhance cold-stress tolerance in *Arabidopsis* and rice (Alia et al., 1998; Sakamoto, 1998; Sakamoto et al., 2000). The *codA*-transgenic *Arabidopsis* exhibited enhanced tolerance to low temperature (0 °C) during imbibition of seeds and also showed accelerated rates of germination and growth at 10 and 15 °C (Alia et al., 1998) which could be attributed to the accumulation of GB preventing the disruption of membranes in the seeds, thereby, protecting the transformed seeds against chilling damage (Leopold, 1980). The targeted transformation of the chloroplasts with *codA* and subsequent accumulation of GB in chloroplasts resulted in enhanced protection of the photosynthetic machinery and freezing tolerance in rice (Sakamoto, 1998) and *Arabidopsis* (Sakamoto, 1998). Tolerance of tobacco to low temperature was enhanced by the introduction of genes *betA* and *betB* (encoding the enzyme choline dehydrogenase and betaine aldehyde dehydrogenase, respectively) from *Escherichia coli* resulting in GB accumulation and improved protection of the photosynthetic apparatus (Holmstrom et al., 2000). In another study, *cod*-transgenic tomato plants exhibited increased GB accumulation and were more tolerant to chilling stress at all developmental stages from seed germination to fruit production as compared to their wild type counterparts (Park et al., 2004).

Chilling is known to induce the production of reactive oxygen species (ROS) which cause damage to cellular components or hinder the repair of photosystem, PSII (Nishiyama et al., 2001). A moderate increase in the levels of H_2O_2 in *codA* transgenics could possibly activate the H_2O_2- inducible protective mechanism (Park et al., 2004). In a similar attempt, elite maize inbred DH4866 lines transformed with *betA* gene from *E. coli* accumulated higher levels of GB than wild type plants, and thus exhibited higher chilling