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Genetic Engineering and Strategies to Cope with Plants chilling

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Abstract. One of the main environmental challenges in plants life is stress caused by abiotic environmental factors such as chilling and freezing which lead to cellular water deficit. Annually damage caused by spring frost leads to billions of dollars of economic losses. Cold stress with plants growth at low temperatures or cold climates has been studied. Plants have different adaptive mechanisms to cope with adverse effects of various abiotic stresses such as cold. chilling denotes physical and physiological changes which occur as a result of plants collision with cold. Cold stress is associated with a set of adaptive paths in plants such as activating various adaptive responses regulators and physiological and metabolic changes caused by stress. The studies have proven the relationship of biological materials such as osmolytes and compatible solutes, membrane lipids, heat shock proteins and antifreeze proteins, factors to remove active oxygen and their role in plants cold stress tolerance, controlling ice nucleating bacteria etc. In the present study, genetic engineering strategies are discussed to modify and achieve physiological and molecular adoption by changing the amount and composition of mentioned materials in living creatures and manipulating the number of ice nucleating active bacteria and creating mutants lacking ice nucleation and also future perspectives in the field of plants molecular modification for cold stress tolerance.

Keywords: Plants, chilling, Freezing, genetic, engineering, osmolytes, Ice nucleation bacteria

1. INTRODUCTION

Resistance or sensitivity to stress depends on the species, genotype and plants growth stage (1). Below optimal temperature can cause huge damage to plants; however, different plant species show different levels of tolerance at low temperature stress tolerance (2). Freezing stress is a combination stress which occurs by low temperature and also mechanical stress which causes forming ice in tissues. Freezing stress leads to damaging the membranes (3) and also like other stresses, causes the cellular water deficit (4). Damage caused by frost is a complex problem whose solving requires group efforts of concerned specialists; a research team consisting of the specialists of horticultural science, plant breeding, plant physiologist, plant biochemist, molecularbiologists and biotechnologist and plant genetic engineering is required to assess the aspects related to the phenomenon of coping with damages caused by freezing. Today many studies are being conducted to identify genes whose expression relates to freezing tolerance and resistance against winter, factors which affect ice nucleation and ice crystals growth in plants and vegetation and to identify factors affecting plants physiological and molecular adaption to cold and genetic engineering strategies for plants tolerance to cold (5). Factors which have the greatest impact on ice formation and expansion in plant tissues include: ambient temperature, duration of exposure to below zero temperature, population of ice nucleation active bacteria in the tissues and internal ice nucleation sites, tissue anatomy, plant water status, antifreeze proteins and heat transferring characteristics of plant tissues.

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A factor leading to ice nucleation in plant tissues is the presence of ice nucleation active (INA) bacteria. Therefore, genetic engineering application to decrease the population of INA bacteria, leads to the decrease in frost damage in plant tissues (6). Studies have proven relationship of biological materials such as osmolytes and compatible solutes, membrane lipids, reactive oxygen and their roles in plants cold stress tolerance. In the following parts, meanwhile overviewing these compounds' roles in plants cold tolerance, genetic engineering strategies will be investigated to modify plants compatibility to cold stresses with changes in the amount of these compounds in living creature, also to manipulate genetic sequences of ice nucleation active bacteria and create mutants lacking ice nucleation.Moreover future perspectives in the field of molecular modification and genetic engineering for cold stress tolerance will be discussed.

2. FREEZING TOLERANCE REGULATORS ENGINEERING

One of the ways to improve freezing tolerance in plants is to increase the target genes expression and metabolic engineering of osmotic protection biosynthesis. Another strategy to increase the stress tolerance in plants is the change in signals transduction pathways which leads to activating the stress response genes. It seems that expression of large clusters of genes which are controlled by such similar regulator pathways, has significantly greater effects in improving freezing tolerance than expression of particular genes. Recently, such acts have been performed, for example, increase in expression of CBF/DREB which is a transcription activatorleads to activating several cold responsive genes and more importantly, the increase in freezing tolerance in plants. Many plants have genetic potential to resist against low temperature and dehydration; recently it has been observed that transgenic plants containing AB13 gene, have increased freezing tolerance (7).

Plants stress responses are regulated by several signaling pathways which activate transcription of the gene and its downstream parts. Plant genome possesses a large number of these transcription factors (TF). For example, 5.9% of Arabidopsis genome codes about 1500 transcription factors most of which belong to several large multi-gene family such as AP_2/EREBP, MYB, bzIPand WRKY. The members of a multi-gene family often response differently to different stress triggers; on the other hand, some stress sensitive genes may have common transcription factors. Dehydration responsive element binding (DREB) proteins and C-repeat binding factors (CBF) are encrypted by multi-gene family of AP_2/EREBP and in response to cold and water stress, they affect in transcription of genes such as Kin, Cor, rdand etc. It has been indicated that the increase in Arabidopsis CBF_1expression induces expression of genes regulated by cold (Cor group) and increases Arabidopsis tolerance to freezing. Also, DRE BIA gene transmission to Arabidopsis leads toa significant increase in thisplant tolerance to different stresses such as cold. On the other hand, while additional expression of CBF_3 in the plant of Arabidopsis increases plant tolerance to freezing,, it leads to several biochemical changes which appear in response to cold stress. Among the changes, it can be referred to the increase in Proline, total soluble sugars such as Sucrose, Raffinose, Glucose and Fructose. Transferring Arabidopsis CBF genes to plants like tomato increases their tolerance to cold, while it also leads to tolerance to oxidative stress. Of course transmission of other transcription factors such as $ADH_{1,2}$ to corn, DESC to tobacco, Gal to petunia, GPAT to rice, NPK_1 to rye and etc. leads to an increase in plants tolerance to frost and freezing. These studies indicate the importance of transcription factors' role in tolerance to stresses which can ultimately be applied in environmental operations. Although producing transgenic plants with transcription factors which are sensitive to stress, causes additional expression of several genes placed in downstream, it may activate non-stress genes which may affect other plant traits; for example, one of the common negative effects among the plants which have been genetically modified by transcription factors, is their slow growth. Of course, these negative effects can be partially remedied by utilizing the inducible enhancements by stress which controls transcription factors expression (8).

3. CREATING BACTERIAL MUTANTS LACKING THE ICE CORE

Ice nucleation by heterogeneous method in which ice core is induced by an external agent such as bacteria, fungus or a special chemical composition, can reach to ice nucleation temperature to about 2°C in organic and inorganic materials (9).

Ice nucleation active (INA) bacteria are the agents which play an important role in losses caused by freezing in plants sensitive to cold. In 1998, Maki et al. diagnosed the INA^+ phenotype in gram negative bacteria Xanthomonas, Erwinia and Pseudomonas (10). All INA^+ bacteria are epiphytic and are nearly found on all plants. Frost damage in plants depends directly on logarithm of these bacteria population and logarithm of bacterial ice core number on plants at freezing time. In the presence of INA^+ bacteria, freezing may occur at temperature about -1.2°C (11). Burke and Lindow believed that each treatment which leads to the decrease in ice nucleation bacteria number or bacteria ice nucleation activity, decreases freezing damage in plants. One of the ways is to change the genetic structure of gene encoding INAZ protein so that this protein is not synthesized in bacterium or it is made defectively. As a result of the change, ice nucleation and therefore freezing are postponed and followed by the increase in growth season, decrease in costs required for freezing protection and preventing the decrease in performed and product quality (12).

With identification of INA^+ isolates in plants and creating INA^- mutants with genetic engineering technology, plants resistance to cold could be increased.

4. HEAT SHOCK PROTEINS, HSP, LEA AND ANTI-FREEZING PROTEINS

To cope with environmental stresses, plants activate many genes. *$AFPS_2$* causes accumulation of specific stress proteins. Heat shock proteins (HSP) and late embryogenesis (LEA) proteins are two groups of proteins which are accumulated in cells following the environment stresses. It has been specified that heat shock proteins with low molecular weight are accumulated in different environmental stresses such as low temperature. These proteins as molecular chaperone are responsible for the synthesis, targeting, investigation and destruction in cellular processes. It was observed that transmission of genes related to some of these proteins to plants, leads to plants resistance against oxidative stress (13). LEA proteins are accumulated in many plants in response to stresses, particularly to cold stress. These proteins as the water-binding proteins play a role in membrane and macromolecules stabilizing. Transmission of the genes related to LEA proteins to plants, has indicated their role in tolerance to stresses. For example, transmission of CoR15a (which protects proteins against freezing by prevention of hexagonal phase formation) to chloroplast, causes chloroplast protection against cold and transmission of WCS19 gene (an LEA- like protein) to Arabidopsis, leads to the increase in plant resistance against cold. Over wintering plants produce anti freezing proteins with the ability to be absorbed on the surface of ice crystals and affect in these crystals growth; recently several anti-freeze proteins have been isolated. The amino acid sequences obtained from these proteins indicated low homology in similar places. Transmission of the gene related to these proteins to the plant, indicated their role in tolerance to cold and freezing (14).

5. OSMOLYTES

When plants are exposed to cold stress, they accumulate a group of compounds with low molecular weight. These compounds which are known as compatible solutes do not enter to cellular metabolic activities, even at high concentrations, and play the neutral role. Since many of these biological stresses cause dry cell, it is thought that these compounds play a role in water absorption by cells and in preventing water loss through the increase in intercellular osmotic pressure. Of course, other roles such as osmoprotectant, purging oxygen radicals and also chaperones with low molecular weight (maintaining membrane and protein structure) have been attributed to them. The most important of these compounds can be named Mannitol and other alcohols sugar, amino acids such as Proline and amino acid derivatives such as Glycine Betain. Some of these compounds like Prolineis practically accumulated in all species during the stress, while the other such as Glycine Betaine is only accumulated in plants which have high tolerance to cold or salinity (15). To increase the resistance against cold, biosynthetic genes of these compounds such as Glysin- Betaine, have been entered into different plants. For example, with transmission of Choline Oxidase gene to the plants like rice, Arabidopsis, tobacco and tomato, these plants' resistance against cold has increased. Transmission of Mannitol synthesis gene (mtld) to petunia has increased the resistance against cold. Transferring other genes such as Trehalose biosynthesis (TPSP) to rice, Spermidine synthesis (SPE) to Arabidopsis, Proline biosynthesis (P_5C_S)to tobacco and Dehydrine biosynthesis ($WCOR_{410}$) to strawberry has also increased the resistance to cold. What is certain is that accurate targeting of producing these compounds in cellular compounds can increase these genes transmission efficiency (16).

6. REACTIVE OXYGEN SPECIES (ROS)

When plants are exposed to cold stress, due to the disorder in plants metabolisms, production of oxygen radicals such as Superoxide ($-o_2$), Hydrogen Peroxide (H_2O_2) and Hydroxyle (-oh) increases. These very active radicals through oxidation of a variety of bio-molecules such as lipids, proteins and nucleic acids, besides adverse impact on plants, create a kind of secondary stress in plants which is known as oxidative stress. To cope with the reactive oxygen, plants are equipped with antioxidant systems which are divided into two groups of enzymatic and non-enzymatic. Enzymatic antioxidant systems include the enzymes of superoxide dismutase (SOD) which catalyzes superoxide dis-mutation to hydrogen peroxide; Catalase (CAT) and Ascorbate Peroxidase (APX) which plays a role in decomposition of hydrogen peroxide to water and oxygen; also subsidiary enzymes such as Mono DehydroAscorbateReductase (MDAR), DehydroAscorbateReductase (DHAR) and GlutathionReductase (GR) which involve in oxidized Ascorbic acid recycling by APX. Because in most cases, these enzymes' activity is induced in stress conditions, it is thought that it can increase tolerance to these stresses by inserting the genes into the plants. For example, transgenic plants of tobacco and rye which have received one of the SOD genes called*CU/zn-SoD* are more resistant to intense sunlight and low temperature (17). Transferring Mn- SOD gene to alfalfa and rye increases their resistance against cold. Also, by transmission of Catalase gene to plants such as rice, production of *H2O2* decreases. Transgenic plants such as cotton and tomato which have received one of Ascorbate Peroxidase genes are more resistance against frost. As mentioned, ROS detoxification systems are composed of several enzymes that operate in different cellular compounds depending on stress conditions. Therefore, it is possible that inserting each of these genes into the plants lonely has little protection activity. According to findings, apparently, increase in resistance against stress through inserting the genes involved in detoxification of ROS is limited. So, to create the plants resistance against cold and freezing, new strategies are required to activate the whole ROS detoxification system coordinately (18).

7. DISCUSSION AND CONCLUSION

While adaption to stress in natural conditions, has ecological benefits metabolic changes may cover the benefits and even be harmful for manufacturer; therefore, improving tolerance to plants abiotic stresses is partially achieved by combining conventional breeding methods and new molecular and genetic engineering methods. Therefore, an appropriate breeding strategy for tolerance to abiotic stresses should be based on the following stages and strategies: 1- Selecting germplasm particularly in related wild species. 2- Understanding control molecular mechanisms in resistant and susceptible genotypes. 3- Applying biotechnological and genetic engineering methods in selection and modification of a transgenic plant should be in a high setting power against stress, so that in the absence of tension, its characteristics are not affected. Although many transgenic products have been manufactured, a limited number of them have been tested in the field level.

With the rapid development of genomics and protemics sciences in recent years, accurate recognition of resistance mechanisms is possible and it is hoped to increase plants' tolerance to various abiotic stresses such as cold by applying modern genetic engineering methods.

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