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Review

Biological roles of crop NADP-malic enzymes and molecular mechanisms involved in abiotic stress

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The abiotic stress tolerance of plants is very important for plant growth, development, survival and functional performance. NADP-ME is one of the most important enzymes in plants. Studying the role that NADP-malic enzyme plays in many metabolisms may help researchers improve the plant abiotic tolerance. The studies on NADP-ME in plants focus on its activity under different stresses. The regulation of NADP-ME gene expression in transgenic plants and the mechanism about abiotic stress resistance are less. In this paper, we reviewed the characteristics of the activity and genes expression of NADP-ME under drought, salt and temperature stresses. We also focused on the role of NADP-ME when it resists these varying stresses and the mechanism on how it performs.

Key words: Plant NADP-malic enzyme, abiotic stress, gene expression, molecular mechanism.

INTRODUCTION

The growth of plant is limited usually by many adverse factors. These factors include biotic factors and abiotic factors. With the change of the environment, abiotic stresses would bring more extensive influence compared with biotic factors for plant growth. So, the influence caused by abiotic stress is of great importance for plant growth, especially for cultivating crops. Abiotic factors mainly include drought, salt and temperature. Abiotic stress can induce the expression of anti-stresses genes in plant. The protein expressed from these genes can improve the plant tolerance. So focusing on these proteins and the anti-stress gene expression, regulation is very significance for plant cultivation.

NADP-ME is widely distributed in plant, which mainly appear in mitochondria, chloroplast and cytoplasm (Edwards and Andreo, 1992; Detarsio et al., 2003). It can catalyze the oxidative decarboxylation of malate to produce pyruvate, CO_2 and NADPH under metallic ion (Mg^{2+}, Mn^{2+} etc.) (Edwards and Andreo, 1992). Therefore, it is one of the critical enzymes in malate metabolism, which play an important role in plant development. It can keep osmotic potential of cell, stabilize pH of cytoplasm and keep balance the ion absorption (Detarsio et al., 2003; Martinoia and Rentsch, 1994; Drincovich et al., 2001). NADP-ME has different isoforms from varied plants. The nucleotide sequences of the gene may be different in C_3, CAM and C_4 plants (Martinoia and Rentsch, 1994; Drincovich et al., 2001). It could be classified to photosynthetic NADP-malic enzyme, non-photosynthetic NADP-malic enzyme and root NADP-ME according to it different functions in plant (Table 1) (Drincovich et al., 2001; Maurino et al., 1997) The photosynthetic NADP-ME is found in chloroplast, which mainly takes part in L-malate oxidative decarboxylation and provides CO_2 to Rubisco for C fixing (Edwards and Huber, 1981). This process can be

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Table 1. Different NADP-malic enzymes in different type plants.

Name*	Plant found	Main subcellular localization	Classification	The characteristic and property of enzyme
$C_{4(1)}$-NADP-ME	Maize, sugar cane, sorghum, C_4 *Flaveria* species, *H. persicum*	Bundle sheath chloroplasts	Photosynthetic	Its gene expression is regulated by light. It can repair the damage caused by UV-induced.
$C_{4(2)}$ -NADP-ME	Maize, *F. bidentis*	Plastids	Non-photosynthetic	Related to plant defense responses.
$C_{4(3)}$ -NADP-ME	Maize	Cytosol	Non-photosynthetic	Produce multiple transcripts in leaves that are differentially expressed in response to leaf development, injury and illumination.
$CAM_{(1)}$-NADP-ME	Ice plant, *A. arborescens*	Cytosol	Photosynthetic	Function like $C_{4(1)}$NADP-ME, but they have different subcellular localization.
$CAM_{(2)}$-NADP-ME	*M. crystallinum, A. arborescens*	Cytosol	Non-photosynthetic	This enzyme is possibly involved in non-photosynthetic functions.
$C3_{(1)}$ -NADP-ME	Bean, poplar, grape berries, tomato, *A. graveolens*	Cytosol	Non-photosynthetic	This cytosolic enzyme is involved in plant defense responses, possibly by providing NADPH for the biosynthesis of lignin and favonoids.
$C3_{(2)}$ -NADP-ME	Rice, *F. pringlei*, tomato, grape berries, *A. thaliana*, *R. communis*	Plastids	Non-photosynthetic	this enzyme could provide NADPH to support lignin biosynthesis in defense mechanisms

* The name of the different isoforms of main NADP-ME.

regulated by light. So it has closed relationship with photosynthesis. The function of non-photosynthetic NADP-ME is not clearly understood presently. But it has higher *Km* value, lower specificity, narrower suitable pH and bigger molecular weight compared with photosynthetic NADP-ME (Pupillo and Bossi, 1979; Chi et al., 2004). The expression of non-photosynthetic NADP-ME genes was very particular, which is unlike those in different development stages and changing environments (Martinoia and Rentsch, 1994; Drincovich et al., 2001). The root NADP-ME is located in root cells, whose main function is joining plant defenses.

The gene coding NADP-ME is not unique but rather a gene family. There are four genes in rice. They have different expression specificity in different plant tissues. So it was deemed to have close relation to environment stresses (Zhang, 2003). Meanwhile, Fu et al. (2009) clone NADP-ME gene (TaNADP-ME_1 and TaNADP-ME_2) from wheat at first in the world and found TaNADP-ME_2 to belong to the group of photochemical reaction. It may be the first cytoplasmic NADP-ME gene found in C_3 plant. It was proved that TaNADP-ME_1 and TaNADP-ME_2 may play important role in stress tolerance (Fu et al., 2009; 2010). Through bioinformatical analysis, photosynthetic NADP-ME protein from maize had two function domains. One was 161 to 350 peptide segment in amino acid sequences, which is the N-terminal. The other was 352 to 605 peptide segment, which is a binding domain of NAD (P). Leu is the most abundant amino in NADP-ME protein sequence. Meanwhile, the protein did not include Asx, Glx and Xaa. It is a hydrophilicity protein (Li et al., 2009).

DROUGHT AND NADP-MALIC ENZYMES

NADP-malic enzyme and its gene expression under drought stress

The deficiency of water in natural environment causes dryness of soil and atmosphere, which can limit the plant growth. It would cause osmotic stress of plant cells. Drought stress could cause the accumulation of free radical and azotic acid and the membrane lipid peroxidation too. The plant metabolism would be disorder. It will suppress the extention of leaf, cause the close of stoma, reduce the absorption of CO_2, raise the resistance of mesophyll cell and decrease the activity of some enzymes. They will affect the CO_2 fixed, destroy the structure of chloroplast and reduce the content of chlorophyll. The last result is suppressing the photosynthesis and lowing the photosynthetic rate in wheat under drought stress (Fu et al., 2009; Sun et al., 2004)

Because of the function of NADP-ME in malate metabolism and photosynthesis, the NADP-ME plays an important role in anti-drought. The activity of NADP-ME in wheatear is higher than that in flag leaf. In light drought, the photosynthetic rate of all organ decline. But the activity of NADP-ME is increasing obviously. It means that, the expression

of NADP-ME gene is induced by drought to resist drought (Wu et al., 2008). The leaf stoma is closed because of drought stress, which would decrease the content of malate in cell and raise the NADP-ME activity. It was proved that some malate enter the mitochondrion (Wei et al., 2003). ^{14}C mark experiment in wheat proved that some NADP-MEs were synthesized in mitochondrion, which may join the malate metabolism (Wei et al., 2003) While, Outlaw et al.(1981) reported that, NADP-ME in wheat plays an important role in controlling stoma opening and closing through regulate the degradation of malic in day.

In addition, there are C_4 enzyme systems in C_3 plants. The activity of C_4 enzyme will be to strengthen metabolism under drought stress, whose goal is to compensate the loss in C_3 pathway caused by drought. Some studies indicate that the C_3 pathway would be suppressed in some plants by drought. At the same time, the C_4 pathway was reinforced (Laporte et al., 2002). Through increasing the activity of NADP-ME to compensate other enzyme, activity decrease can prove that increasing the activity of NADP-ME is an ecological adaptation mechanism under drought stress (Wu et al., 2008; Laporte et al., 2002; Li, et al., 1999).

There is a gene family to code the NADP-ME. So it is meaningful to investigate the gene family expression under stress. Four genes coding NADP-ME were found in rice. Three of them could increase expression under 10% PEG; only one was inclining. It means that, not all NADP-ME genes have association with drought stress. The ways of stress signal introduction were mainly classed ABA dependence and ABA independence. It was proved that the concentration of NADP-ME increases with the content of ABA raise in guard cell under drought stress (Cushman, 1992). It means the NADP-ME gene expression may have close relation with ABA.

The mechanisms of NADP-malic enzyme resist drought stress

There are many pathways to resist drought. One of them is a defensive system, which is made up of some protein and enzyme induced by drought. Now, many investigations found that the stress signal stimulate one signal system which can regulate the defensive genes expression in plant to resist drought (Shao et al., 2008; Shao et al., 2008). There are two groups for drought-induced genes regulation. One includes genes encoding proteins whose catalytic activities are responsible for protecting the cells and organs against stress, while the other includes genes encoding proteins necessary for signal transduction and regulation of gene expression (Bray, 1997; Yamaguchi-Shinozaki and Shinozaki, 2005), because NADP-ME is mainly joined in some metabolisms directly. So, NADP-ME may belonged to the first group. It was reported that, drought can induce the NADP-ME gene expression abundant in ice grass in 1992, which could be used to strengthen malate metabolism (Ni et al., 2009).

The function of NADP-ME is to catalyze the delivery of CO_2 in photosynthesis II (Zhang, 2003). In order to keep the reaction of photosynthesis II, plant can increase the content of NADP-ME to reinforce the delivery and fixing of CO_2 when the absorption of CO_2 is decreased because of the shut of stoma under drought stress. The goal is to compensate the deficiency of CO_2 in this situation. This theory can explain the reinforcement of C_4 enzyme system in C_3 plant under drought stress (Wu et al., 2008). At the same time, the protein of NADP-ME is hydrophilicity, which can protect cells through increasing the osmotic pressure of cell and decreasing the water loss (Schroeder et al., 2001; Li et al., 2009).

SALT AND NADP-MALIC ENZYMES

NADP-malic enzyme and its gene expression under salt stress

Salt stress can cause harm to plant growth. The salinity in soil is 0.2 to 0.5% which can hamper the plant development (Sun et al., 2002). The harms are obvious, which mainly includes suppression of growth and differentiation of unhalophytic plant tissues and organ which makes plant enter the development stage beforehand (Jiang et al., 2001). In order to limit the harm caused by salt, plant produces some proteins and enzymes (POD and SOD, etc.). Many defense genes were induced by salt (Jiang et al., 2001). Salt signal can induce NADP-ME gene expression and increase its activity (Liao et al., 2007). It is generally accepted that, enzymes exhibit slightly increased activity under low concentrations of ions, whereas, they start to be inhibited in the presence of NaCl concentrations higher than 100 mM (Munns, 2002). But, Fu et al. (2009) found that the NADP-ME activity in leaf achieved its peak level after 6 h at 200 mM NaCl. It is two times higher than the in normal. But the photosynthetic and transpiration rate were decreased gradually. It means that salt stress can induce NADP-ME gene expression and NADP-ME may have close relation with salt stress (Sun et al., 2003). It was reported that, the mRNA and the activity of NADP-ME in *Aloe vera L.* and *Aloe saponarea Haw* increased gradually after 12 h under salt drought. It was continued until the 72nd hour. It indicated that, the expression of NADP-ME gene and the protein accumulation was induced by salt. Sun et al. (2003) reported that, the expression of NADP-ME gene induced by salt had close relation with plant salt tolerance. The content of protein in the leaf and root increased under Na_2CO_3, $NaHCO_3$ and NaCl stresses (Liao et al., 2007). CAM plant has a specific photosynthesis pathway. Its stomas open at night to absorb CO_2 which would be fixed by PEP and PEPC to produce four carbonates (most of them are malate) (Zhang, 2003; Sun et al., 2003). Some facultative CAM plants can change their C_3 metabolism pathway to C_4 metabolism pathway under salt stress. At the same time, the activity of NADP-ME would increase 4 to 10

times (Lou et al., 2008; Holtum and Winter 1982). That means NADP-ME is a critical enzyme in CAM pathway. It may play an important role to resist salt stress in CAM plant and facultative CAM plants.

The mechanism of NADP-malic enzyme resistance to salt stress

In the long evolution history, plant form some special physiology and biochemistry paths itself to resist or limit the harm caused by salt stress. It mainly includes synthesizing and accumulating organic substances, increasing Na^+/K^+ pump and H^+-ATP activity, enhancing the aquaporins genes expression and changing the metabolism way (Winter et al., 1982; Dajic, 2006; Zhang et al., 2009). The study about NADP-ME gene expression regulation under salt stress is not much now. Different regulation mechanism may cooperate with each other under salt stress to give plant vital signs. The mechanism of Na^+ crossing cell membrane is not certain. There are two imaginable pathways for crossing: K^+ channel and non-selective channel. So to balance these input positive ions, many negative ions are accumulated. Malate and Cl^- are the main members (Li, et al., 1999; Cushman, 1992; Zhang et al., 2009). Malate is synthesized through the reaction catalyzed by phosphoenolpyruvate carboxylase, which was stored in the vacuole. The destiny of malate is not clear. But there are some studies that prove that NADP-ME was synthesized in this stage, which may be taking part in the malate metabolize (Wu et al., 2008; Shao et al., 2009).

TEMPERATURE AND NADP-MALIC ENZYMES

NADP-malic enzymes and gene expression under temperature stress

Because of different seasons in one year, plants have to experience temperature change. Specifically for the crops, the change of temperature would slow the growth and decrease the yield. High temperature may cause enzyme deactivation. Its result is that, enzyme system would be destroyed and the water in the body would be lost. The root vitality, the liquidity of protoplasm and the activity of NADP-ME are inclined when the temperature is very low. At the same time, the chlorophyll would be decomposed. The result of all the stated can cause metabolic disorder (Zhang et al., 2000; Xu and Yan 2003).

Plant can adapt to the environment within affordable temperature range. When the temperature was at 22 to 55°C, the activity of NADP-ME was increased as the temperature ascended in *Orostachys fimbriatus.* (CAM plant), *Sedum spectabile* (facultative CAM plant) and *Mesembryanthemum cordifolium*. The highest activity was in *O. fimbriatus*. (CAM plant) (Xu et al., 2007). This study proved that NADP-ME could resist high temperature and that the CAM plants had the highest ability to anti-high temperature. High temperature can regulate NADP-ME activity and the activity of CAM (Xu et al., 2007; Wang et al., 1994).

Low temperature can affect plant growth significantly. It could be classified as chilling and frozen injury. Frozen injury can destroy plant fatally, but chilling injury can slow the growth rate and change the leaf color, which cannot freeze the cellular fluid; the injury could be restorable under certain conditions (Xu and Yan, 2003). It is known that the activity of NADP-ME in wheat leaf was increased gradually fewer than 4°C in 24 h. But the index of photosynthesis was not the same trend. It means that the activity of NADP-ME was affected seriously by low temperature. The enzyme responded to the stress at the protein level. From RT-PCR study, the gene $TaNADP\text{-}ME_1$ and $TaNADP\text{-}ME_2$ had the same variation trend, which content was increased after 3 h under low temperature stress (Sun et al., 2003). These evidences indicate that cold stress can induce the expression of NADP-ME gene, which have close relationship with resisting cold in plant.

The mechanism of NADP-malic enzymes resists temperature stress

The content of NADP-ME increases when the absorbility of CO_2 is decreased under cold stress. It proved that NADP-ME may resist cold stress besides catalyzing the reaction. The membrane system in cell is the most sensitive part under cold stress. Its liquidity and stability is the basis of the cell and the plant. When the phase transition temperature is lower, the ability of cold tolerance is better (Lin et al., 1995; Gareth, 1998; Yamaguchi-Shinozaki and Shinozaki, 2004). In order to decrease the phase transition temperature and strengthen the cold tolerance, the cold signal would induce some stress responsive genes express, which can produce some protein to keep the liquidity of the membrane (Lin et al., 1995; Yamaguchi-Shinozaki and Shinozaki, 2004; Chinnusamy et al., 2006; Floris et al., 2009; Wang et al., 1997). The aim is to stop the membrane phase change. NADP-ME is one of these proteins, which is mainly located in the membrane (cytomembrane, mitochondrial membrane, chloroplast membrane and thylakoids) (Jiang et al., 2001; Floris et al., 2009; Wang et al., 1997). Cold signal can induce NADP-ME gene expression, which produce the NADP-ME protein to keep the liquidity of the membrane and keep the metabolism as usual. Moreover, NADP-ME is a kind of hydrophilicity protein (Jiang et al., 2001) that can absorb more free water and change them to bound water. That course could decrease ice in cell under cold stress to raise the cold tolerance. This may be one of the paths to resist cold in plants.

CONCLUSION

The mechanisms to resist various stresses are cooperation with each other. The changing environment

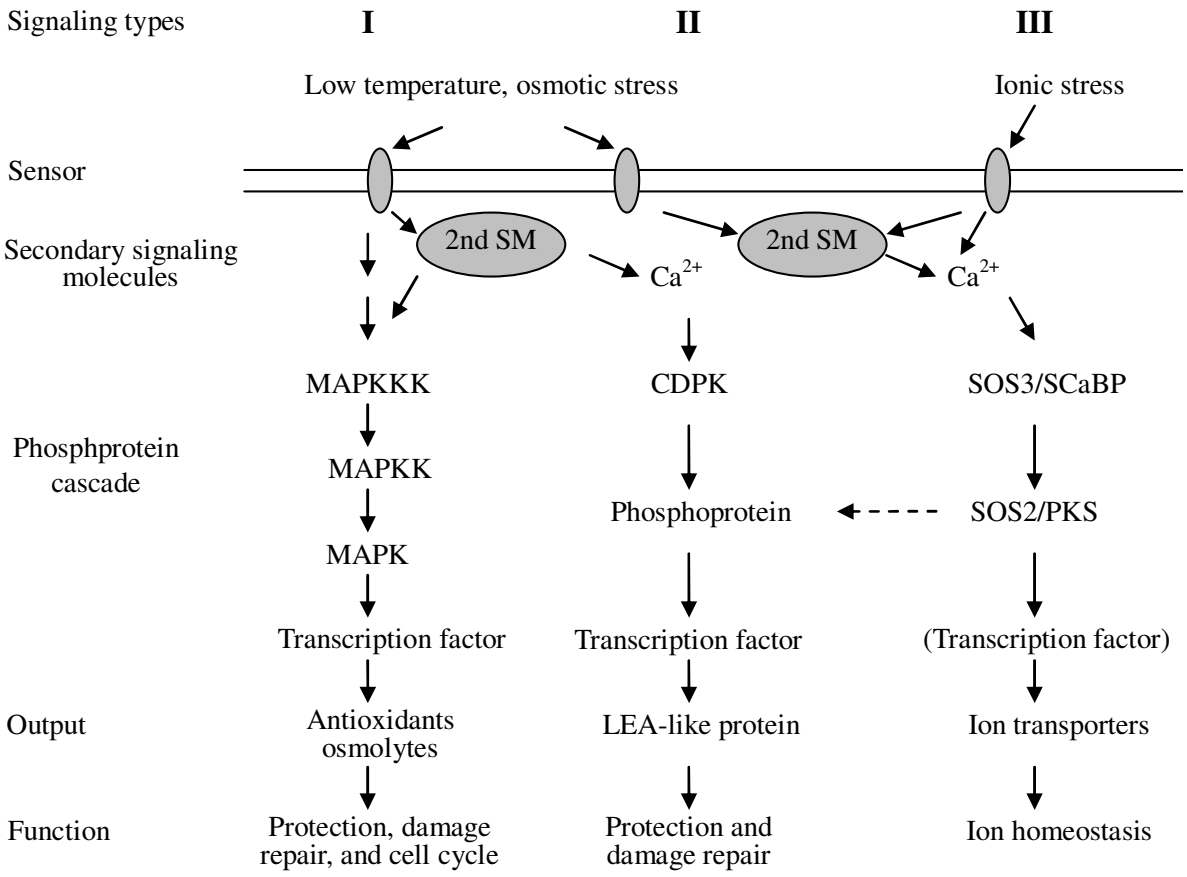

Figure 1. Major types of signaling for plants during drought, cold, and salt stress (Xiong et al., 2002).

factors make plant form its defensive quality. The main stress signal transduction is complex. Osmotic stress, oxidant stress and ion stress may happen simultaneously under different stresses. The stress signaling pathway may respond mutually to each other (Figure 1) (Xiong et al., 2002). They may regulate the NADP-ME gene expression at the same time. There are many factors which affect plant growth beside drought, salt and temperature (Ding et al., 2003; Tsuchida et al., 2001). NADP-ME is a critical enzyme in the photosynthetic pathway. There are many substances related to it. Its change can lead to other substance change, which would arouse anti-stress response (Shao et al., 2008). The activity of NADP-ME changes under drought, salt and temperature stresses. It means that the NADP-ME gene is a non-specific induced gene, which may have close relation with the stresses.

With the development of biomolecular technology, more and more resistance genes were found. It can help people to improve the plant stress resistance. But now, the study about the regulation of NADP-ME gene expression under different stresses is rare, particularly in transgenic research. The relation between NADP-ME gene expression and the metabolism system of transgenic plant has not be sufficiently studied, which limited it application in breeding. For example, one of directions of crop breeding is raising the photosynthetic rate of C_3 through transgenic C_4 genes technology. In C_4 plant, the expression quantity of NADP-ME gene is more than others. So it has high photosynthetic rate (Sun et al., 2003; Dajic, 2006). It was proved that NADP-ME in transfer NADP-ME gene rice was seven times than that in the ordinary rice. But the exchange of CO_2 and photosynthetic rate were not altered. Meanwhile, it can lead to leaf bleaching, slow growth and intense photo-inhibition (Sun et al., 2003; Ding et al., 2003; Casati et al., 1999). There are some researches to prove that the expression of NADP-ME gene from C_4 plants in C_3 plant may bring harm to chloroplast (Ding et al., 2003).It can be concluded that the transfer of NADP-ME gene from C_4 plant was not helpful for improving C_3 plant photosynthetic rate (Lou et al., 2008; Ding et al., 2003; Casati et al., 1999; Chi et al., 2004). The reason for the stated phenomenon is that, the study about the regulation of NADP-ME gene expression in transgenic plant under different stresses is rare. This is an important point for future research.

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