



The involvement of metallothioneins and stress markers in response to osmotic stress in *Avena sativa* L.

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Summary:

One of the major abiotic stresses that limits plant growth and development is osmotic stress. Osmotic stress occurs when the soil water potential is lower than the root water potential, reducing the uptake of water by plants. It can be caused by drought, salinity, and cold. Like many other stresses, osmotic stress causes the overproduction of reactive oxygen species (ROS), which, in turn, can lead to DNA, lipid, and protein damage. Since plants are sessile organisms, they have evolved complex mechanisms to adapt to various environmental stresses.

Metallothioneins are low-molecular-weight, cysteine-rich proteins. Based on the number and arrangement of cysteine residues, they are divided into four types. Metallothioneins are best known for their ability to bind to a variety of metal ions (such as copper, zinc, and cadmium), allowing them to maintain the homeostasis of metals in cells. However, the thiol groups of cysteine residues can also react with ROS, protecting cells from oxidative stress. Many reports show that the expression of metallothioneins is induced by various external factors, including drought/osmotic stress.

In our study, we characterized three novel metallothionein genes of oat and expressed them in *Escherichia coli* bacteria to evaluate their potential for reducing osmotic stress. Oat seedlings were exposed to osmotic stress induced by polyethylene glycol (PEG) and mannitol. The levels of phenols, soluble sugars, and abscisic acid were measured, as were the activities of antioxidant enzymes such as peroxidase, catalase, and superoxide dismutase. In addition, we analyzed metallothionein gene expression in the roots and shoots of stressed plants.

We observed an increase in levels of abscisic acid – a key plant hormone that regulates plant response to drought/osmotic stress. Stressed plants accumulated sugars that function as osmoprotectants. In stressed plants, we observed an increase in the activity of antioxidant enzymes and an increase in levels of phenolic compounds, indicating that osmotic stress led to the accumulation of ROS. Our results show that the expression of oat metallothionein genes is upregulated by osmotic stress and correlates with changes in levels of abscisic acid, phenolic compounds, soluble sugars, and the activity of antioxidant enzymes. Moreover, functional analysis in *E. coli* showed that the expression of oat metallothioneins increased the tolerance of bacteria to osmotic stress.



Overall, our results indicate that oat metallothioneins are involved in plant response to osmotic stress; however, further work is needed to elucidate exactly how their expression is regulated. In the future, metallothioneins may be used as molecular markers for the selection of stress-resistant crop cultivars or even for the generation of drought-resistant crops *via* conventional or transgenic breeding.