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Transcriptional Regulation of Two Chloroplast Superoxide Dismutases, Fe-SOD and CuZn-SOD, by Copper in a Moss

Barbula unguiculata

Tadahiko SHIONO, Tomonori MIYATA, Masaru NAKATA, Takanori SUZUKI, Isamu YAMAMOTO, Toshio SATOH; Dept. Biol. Sci., Grad. Sch. Sci., Hiroshima Univ. Hiroshima

Bryophytes are considered to have been the most primitive land plant group and to occupy a critical position for studying the evolution of oxidative stress adaptation. Then, we have studied on their isozymes of superoxide dismutases (SODs), which take a role in reactive oxygen scavenging systems. The kinds of SOD isozymes of a moss, *Barbula unguiculata* were similar to those of higher plant.

B. unguiculata had two chloroplast SOD isozymes, Fe-SOD and CuZn-SOD, the expression of which was regulated by copper ion concentration in the culture medium. Fe-SOD activity was expressed in the initial copper concentration less than $0.1 \mu\text{M}$ and repressed more than $1 \mu\text{M}$. On the other hand, CuZn-SOD activity was expressed more than $1 \mu\text{M}$. Northern-blot analyses showed the similar results as to the effect of copper on the expression of both SOD genes. These results suggested that chloroplast SODs were regulated by copper ion concentration at the transcriptional level. In the liverwort *Marchantia paleacea* var. *diptera*, however, only Fe-SOD was present in the chloroplast and no repression by copper was observed.

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ROLE OF TREHALOSE IN TURGOR AND CELL GROWTH IN SENESCING TULIP TEPAL TISSUES

Man IWAYA-INOUE, Mutsumi TAKATA, Satoko TODA¹, Hiroshi WADA¹, Toshio FUKUYAMA¹, Takashi IKEDA², Hiroshi NONAMI¹ (Kyushu Univ., ¹Ehime Univ., ²Kyoto Pref. Univ.)

Treatment with 50mM trehalose was markedly effective in prolonging vase life without abscission in tulip tepals¹⁾. Role of trehalose on water status was determined by ¹H-NMR and isopiestic psychrometers²⁾. Long *T₁* of the intracellular water in tulip tepal tissues showed that trehalose functioned to protect vacuolar water. Trehalose did not promote elongation of epidermal parenchyma cells in tepal tissues, but maintained radial enlargement of the cells. Additionally, the hydraulic conductance of soybean embryos grown on trehalose-containing media became smaller than that in sucrose-containing media, suggesting that trehalose might reduce hydraulic conductance in soybean embryos, resulting in growth retardation of soybean embryos³⁾. From these results, trehalose did not act as an energy source or directly participate in osmoregulation and these characteristics distinguish it from other sugars such as sucrose. In cut tulip flowers trehalose may play an important role to prevent loss of water with protecting membrane integrity during senescence stages.

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410(S424)

DOES SALINITY ACCELERATES DEVELOPMENT OF ENDODERMAL CASPARIAN BAND IN MAIZE ROOTS?

Ichirou KARAHARA, Takanori KONDO, Atsuo IKEDA; Dept. Biol., Fac. Sci., Toyama Univ., Toyama 9308555

The Casparian band plays an essential role for retaining solutes in stele as an apoplastic barrier and, thus, suggested to be regulated by environmental factors. The distance between the lowermost position of the band and the root tip had been reported to decrease under salt stress in cotton roots. We tested whether the band development was accelerated actually in individual endodermal cells.

Maize roots were grown on vermiculite for 8d in the presence or the absence of 0.2 M NaCl and the band development was examined. The distance between the lowermost position of the band and the root tip decreased under salt stress. Then segments were cut from the region between the lowermost position of the band and the root tip, embedded in resin, and number of endodermal cells per cell file in this region was counted. The number of endodermal cells in roots grown under salt stress was the half of that of control. But the apparent production rate of endodermal cells under salt stress, determined in 4d old roots, was also the half of that of control. Therefore, the time necessary for individual endodermal cells to develop the band under salt stress, which was calculated from these results, was about the same as that in control.

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EFFECT OF H₂O₂ ON OXIDATIVE STRESS RELATING METABOLISM IN HAIRY ROOT CULTURES OF *HYOSCYAMUS NIGER*

Sachiko TAKAHASHI, Shinsaku TAKAYAMA; Dept. Biol. Sci. Technol., Tokai Univ., Numazu 410-0395.

Active oxygen species are generated by various metabolic processes or exogenously supplied agents, and become the cause of the damage to the cell. In order to avoid these damage, antioxidants and antioxidative enzymes are localized in the cell. In this study, effect of H₂O₂ on oxidative stress relating metabolism were analyzed in hairy root cultures of *Hyoscyamus niger*.

Hairy root of *Hyoscyamus niger* transformed by *Agrobacterium rhizogenes* ATCC15834 was cultured in B5 liquid medium and subjected to the experiment. The medium used was 100 ml B5 supplemented with 30 g/l sucrose, inoculated with 0.5 g hairy root, incubated at 25°C under continuous irradiation, and shaken at 100 rpm. After 14 days of culture, 0 to 100mM H₂O₂ was added to culture medium and after 3 days, cell growth, oxygen uptake and oxidative stress relating metabolism were analyzed.

Addition of H₂O₂ inhibited the growth, but oxygen uptake was preserved almost the constant level until 25 mM of H₂O₂, but was inhibited higher than 50 mM, especially 75 to 100mM. Activities of Catalase(CAT), SOD, ascorbate peroxidase (AsAPOX) were inhibited by H₂O₂, while the activities of dehydroascorbate reductase (DHAR), Monodehydroascorbate reductase(MDAR) or glutathione reductase(GR) were stimulated especially at 10mM of H₂O₂. Total ascorbate content was inhibited, but MDA level was increased by H₂O₂ especially at 10mM. GSH level was decreased but GSSG level was increased by addition of 10 to 50mM H₂O₂.

From these results, addition of H₂O₂ stimulated the oxidative stress, but antioxidative pathway such as ascorbate-glutathione pathway was induced by H₂O₂ especially 10mM, and will protect the cell.