412(S426)

EFFECT OF H_2O_2 ON RELEASE AND UPTAKE OF TROPANE ALKALOIDS IN HAIRY ROOT CULTURES OF *HYOSCYAMUS NIGER*

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

Addition of H2O2 to culture medium stimulated the release of tropane alkaloids from hairy root cultures of *Hyoscyamus niger*. The release and uptake of tropane alkaloids were examined in relation to H2O2 treatment.

Hairy root of *Hyoscyamus niger* transformed by *Agrobacterium rhizogenes* ATCC 15834 was cultured in B5 liquid medium containing 30 g/l sucrose at 23C under continuous irradiance. At 14 days, 2 g cells were inoculated into 100 ml of deionized water and treated by 10 mM H2O2, and EC and tropane alkaloids were analyzed. Time course of release and uptake of tropane alkaloids after 10 mM H2O2 treatment was also analyzed.

Release of hyoscyamine, scopolamine and littorine were stimulated just after H2O2 treatment until 6 to 12 hours, but the release of 6β-hydroxy hyoscyamine was not stimulated. After 24 to 48 hours, the alkaloids released into culture medium absorbed by hairy roots and completely disappeared from culture medium. Time course of the content of the alkaloids in the cell and in the medium were negatively correlated. The amount of 6β-hydroxy hyoscyamine and hyoscyamine were decreased and littorine and scopolamine were increased during 48 to 240 hours, and after 240 hours, the composition of the alkaloids changed comparing to the composition before H2O2 treatment.

413(S427)

Absorption and Translocation of ^{52}Fe, ^{52}Mn and ^{62}Zn Visualized by PETIS in Barley and Rice

Hiromi NAKANISHI[1], Shoichiro Kiyomiya[1], Masashi Yoshimura[1], Satoshi Watanabe[2], Noriko S. Ishioka[2], Akihiko Osa[2], Hiroshi Uchida[3], Atsunori Tsuji[3], Shinpei Matsuhashi[2], Shoji Hashimoto[2], Toshiaki Sekine[2] and Satoshi Mori[1]

[1]Department of Applied Biological Chemistry, The University of Tokyo, [2]Japan Atomic Energy Research Institute, [3]Hamamatsu Photonics Co.

^{52}Fe, ^{52}Mn and ^{62}Zn were supplied to barley and rice plants via roots and, real-time movement was monitored using a PETIS (Positron Emitting Tracer Imaging System). ①^{52}Fe was chelated with mugineic acid and supplied to barley plants. Absorbed ^{52}Fe was first translocated to the basal part of the leaf, discrimination center (d.c.), within 15 min, and then to the youngest and 2nd youngest leaves. In Fe-deficient plants, translocation to d.c. was 1.5 times increased. ②^{52}Mn translocation to d.c. took 40 min and Mn-deficiency increased about 10 times. Dark treatment and 10^{-4} M abscisic acid treatment suppressed the translocation to d.c. about 1/3~1/5. Fe-deficiency treatment did not influence the ^{52}Mn translocation, while Zn-deficiency treatment depressed about half of the translocation. ③^{62}Zn was translocated to the d.c. within 40 min in rice. Zn-deficient d.c. accumulated ^{62}Zn more than 10 times higher. Dark treatment and abscisic acid treatment remarkably enhanced the translocation to d.c., but depressed to the leaves.

414(S428)

COPPER INHIBITIONS OF THE PHYTOCHELATIN SYNTHESIS AND CADMIUM TOLERANCE IN PLANTS

Masahiro INOUHE, Satoyo TERAOKA, Shoko ITO, Hiroshi TOHOYAMA and Masanori JOHO, Dept. Biol. Earth Sci., Ehime Univ., Matsuyama 790-8577.

Suspension-cultured cells of tomato (*Lycopersicon esculentum*) exhibit a substantial tolerance to Cd but not to Cu. This difference may depend on the cell ability to produce phytochelatins (PCs). The PCs were produced rapidly in the cells exposed to Cd but not apparently in those to Cu. Tomato cells that had been adapted to excess levels of Cd (100-400 μM) by an enhanced PC formation, however, exhibited no significant tolerance to Cu (>100 μM) even in the presence of Cd. In turn, tomato cells sub-cultured with Cu (50 μM) had no enhanced but a reduced tolerance to Cd. Determinations of the PC contents in the cells showed that Cu inhibited PC formation in the cells. Furthermore enzyme assays *in vitro* revealed that Cd stimulates the PC synthase activity but Cu strongly inhibits the activity in a counteracting manner to Cd.

From these results, we concluded that Cu is a potent inhibitor of PC synthase hence inhibiting the response of tomato cells to Cd.

415(S429)

INCREASED ANTIOXIDANT STATUS IN AN ALUMINUM TOLERANT TOBACCO CELL LINE ALT301: A POSSIBLE MECHANISM OF ALUMINUM TOLERANCE

S. RAMA DEVI[1,2], Yoko YAMAMOTO[1], Hideaki MATSUMOTO[1]; [1]Res. Inst. Bioresour., Okayama Univ., Kurashiki 710-0046, [2]BRAIN

An aluminum (Al) tolerant cell line ALT301 isolated in a simple calcium solution exhibited higher growth capability after Al treatment than parental cell line (SL). To elucidate the mechanism of Al tolerance of ALT301, we have examined the plasma membrane integrity (evans blue uptake), lipid peroxidation and antioxidant status of SL and ALT301. During post-Al treatment culture in MS medium, evans blue uptake and lipid peroxidation increased, but much less in ALT301 than SL. ALT301 contained higher levels of ascorbate and glutathione (GSH) compared to SL under normal growth conditions. Al treatment did not alter the ascorbate or GSH levels. However, during post-Al treatment growth in MS medium GSH level decreased by ten fold in SL cells and only two fold in ALT301. Thus it is suggested that Al tolerance of ALT301 might be due to increases in ascorbate and GSH contents which can protect the cells from lipid peroxidation and plasma membrane damage during post-Al treatment growth.