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Inhibitory Effects of *s*-Triazine and Carbamate Anticytokinins on Light-induced Adventitious Shoot Formation in Horseradish Hairy Roots

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In many species of dicotyledonous plants, hairy roots were formed by infection with *Agrobacterium rhizogenes* harboring Ri plasmid. It is well known that this a phenomenon is due to the transfer of a part of the Ri plasmid, called T-DNA, into the plant's genomic DNA. Several genes on the T-DNA are responsible for inducing changes in physiological conditions in the plant cells[1]. One of the physiological changes in hairy roots transformed with Ri plasmid is that they grow well on hormone-free medium and remain root organs whilst untransformed roots normally required the addition of plant growth regulators to grow. Regenerated plants were usually obtained by treatment of hairy roots with phytohormones[2]. In some plant species, however, adventitious shoots were formed from hairy roots when they were cultured in hormone-free medium under light and/or dark conditions[1,3,4].

In horseradish hairy roots, adventitious shoot were formed when they were cultured on hormone-free MS medium under light conditions, but not under dark conditions[5-7]. Whereas, shoot formation could be induced even when the hairy roots were cultured in complete darkness provided that cytokinins were supplied exogenously[8]. In our previous report[8], we demonstrated that treatment with pyrrolo [2, 3-*d*] pyrimidine anticytokinins, which showed strong anticytokinin activity in tobacco tissue culture[9], inhibited the adventitious shoot formation from horseradish hairy roots under light conditions. These results indicate that endogenous cytokinins might be involved in the process of the light-induced adventitious shoot formation in horseradish hairy roots.

In this study, we report the effects of 4 new types of anticytokinins (**Fig. 1**) on light-induced shoot formation in horseradish hairy roots.

One cm of distal ends of horseradish (*Armoracia rusticana*) hairy roots transformed with *A. rhizogenes* strain 15834 were cultured on semi-solidified (0.2% Gelrite) hormone-free Murashige & Skoog's (MS) medium in the dark for more than 12 weeks. One cm of proximal ends of hairy roots with 5 mm of lateral roots which were also pre-cultured under dark condition were excised and cultured on semi-solidified MS medium containing one of the 4 anticytokinins. These anticytokinins

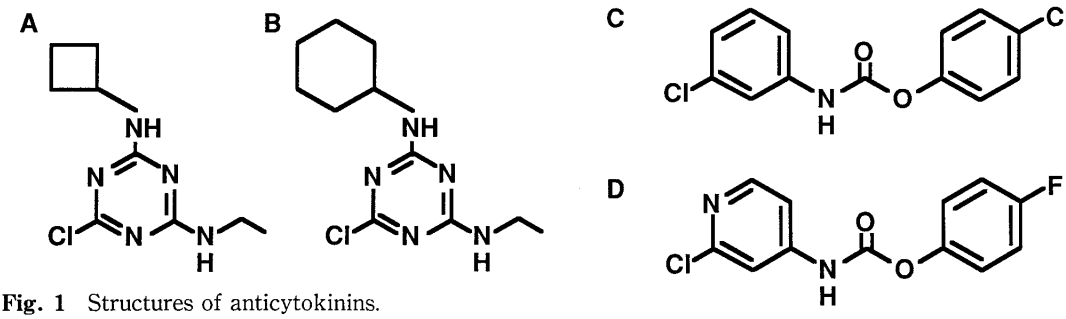

Fig. 1 Structures of anticytokinins.
A: N^2-cyclobutyl-2-amino-4-chloro-6-ethylamino-*s*-triazine (*s*-triazine No. 11), B: N^2-cyclohexyl-2-amino-4-chloro-6-ethylamino-*s*-triazine (*s*-triazine No. 12), C: *p*-chlorophenyl *N*-(*m*-chlorophenyl)-carbamate (carbamate No. 48), D: *p*-fluorophenyl *N*-(2-chloro-4-pyridyl)-carbamate (carbamate No. 55).

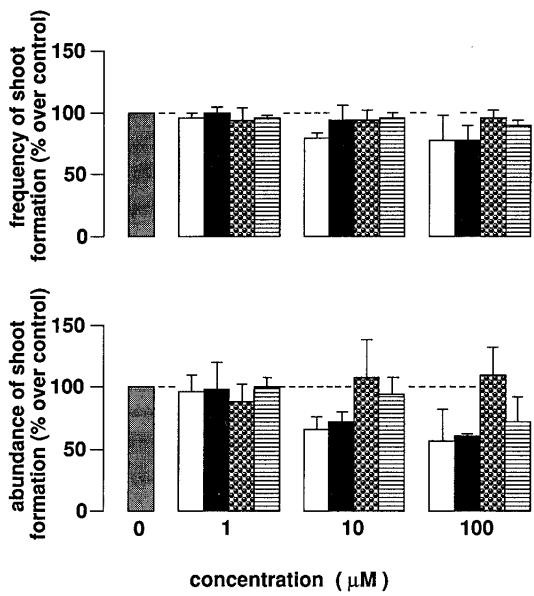

Fig. 2 Effects of anticytokinins on adventitious shoot formation under light conditions.
Proximal ends (1 cm) of horseradish hairy roots cultured for more than 12 weeks under complete darkness were excised and cultured on MS medium containing anticytokinins indicated under light conditions. After 4 weeks of culture, frequency of shoot formation [(no. of explants forming shoots/total no. of explants)×100] and abundance of shoot formation [(no. of shoots formed/total no. of explants)×100] were recorded. Both values (frequency and abundance of shoot formation) on control (hormone-free) MS medium (▒) were determined as 100 and values in each treatment were calculated by comparing to control value. Explants were treated with *s*-triazine No. 11 (□), *s*-triazine No. 12 (■), carbamate No. 48 (▩) and carbamate No. 55 (▤). Experiments were repeated 3 times with 25 replicates for each treatment. Vertical bars indicate S.E..

were disolved in 100% dimethyl sulfoxide (DMSO) and added to MS medium after autoclaving. The final concentration of DMSO in MS medium was 0. 1% (v/v). The cultures were then incubated under light conditions (16h light/8h dark, at 78 μmol/m^{2}s of light intensity). After 4 weeks of the culture, the frequency of shoot formation [(number of explants forming shoots/total number of explants)×100] and the abundance of shoot formation [(number of shoots formed/total number of explants)×100] were recorded.

It was found that adventitious shoot formation was partially inhibited by the application of

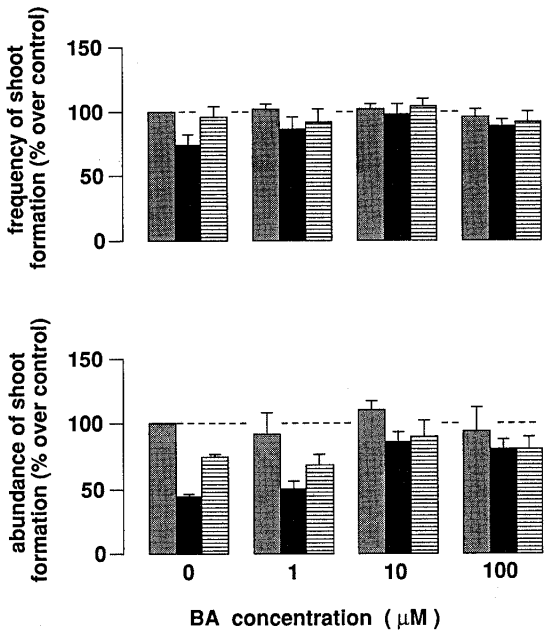

Fig. 3 Effects of simultaneously applied BA and anticytokinins on adventitious shoot formation under light conditions. Experimeutal procedure was same as that in **Fig. 1**, except that proximal ends (1 cm) of the hairy roots were cultured on MS medium containing both BA and each of the 4 anticytokinins indicated under light conditions. The basal ends were treated with BA alone (▒) and BA with 100 μM *s*-triazine No. 12 (■), or 100 μM carbamate No. 55 (▥). Experiments were repeated 3 times with 30 replicates per treatment. Vertical bars indicate S. E..

anticytokinins (**Fig. 2**). *s*-Triazine anticytokinins were found to be more effective than carbamate anticytokinins. One of the carbamate anticytokinins (No. 48) showed no inhibition on the adventitious shoot formation. In another experiment, BA was incorporated into MS medium together with either *s*-triazine No. 12 or carbamate No. 55. This was to clarify whether it is possible to nullify the inhibitory effect of anticytokinins on adventitious shoot formation by simultaneous application of cytokinin such as BA, in the presence of anticytokinin. **Fig. 3** shows that the inhibitory effects of anticytokinin were nullified by the addition of BA. The percentages of shoot formation from hairy root segments on media containing 10 or 100 μM BA and 100 μM anticytokinins were comparable to those without BA or anticytokinins.

In a bioassay system using tobacco tissue culture, the order of strength of anticytokinin activity was as follows: *s*-triazine No. 11 > *s*-triazine No. 12 = carbamate No. 55 > carbamate No. 48[10,11]. In the adventitious shoot formation from horseradish hairy roots, the degree of inhibition on shoot formation by anticytokinins was almost the same as that in tobacco tissue culture. However, the effects of carbamate anticytokinins in horseradish hairy roots were much lower than that in the tobacco tissue culture system. These differences might be caused by the difference in plant species, cytokinins and assay systems used. In spite of the different effects which depend on the types of anticytokinins tested, the adventitious shoot formation in horseradish hairy roots induced by light was inhibited by all types of anticytokinins tested. These results indicate the possible involvement of endogenous cytokinins in the process of light-induced shoot formation in horseradish hairy roots.

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《和文要約》

セイヨウワサビ毛状根からの光誘導不定芽分化における
s-トリアジン型およびカーバメイト型抗サイトカイニンの阻害効果

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セイヨウワサビ毛状根からの光誘導不定芽分化において，*s*-トリアジン型およびカーバメイト型抗サイトカイニンの効果を検討した．これら抗サイトカイニンを培地中に添加したところ，不定芽分化は阻害された．抗サイトカイニンと同時にベンジルアデニンを添加すると，抗サイトカイニンによる阻害効果は回復した．