イネの根におけるカルスの形成と生長のセルロース阻害剤2,6-dichlorobenzonitrileによる促進

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Enhancement of Callus Initiation and Growth with 2,6-Dichlorobenzonitrile, a Cellulose Inhibitor, in Rice Roots

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It was previously reported that root segments of rice cultured on modified LINSMAIER and SKOOG medium in the absence or presence of 2,4-dichlorophenoxyacetic acid (2,4-D : 5×10^{-5}M) formed lateral roots or callus tissue, respectively, from the pericycle and endodermis[3]. The structure and content of cell walls were different in cells of lateral root primordia and callus initials. In the present investigation certain metabolic inhibitors, which affect cell wall composition, were used to study the functions of cell wall on the morphogenesis of the pericycle cells of root segments. We found that 2,6-dichlorobenzonitrile (2,6-DB), an inhibitor of cellulose synthesis[1], enhanced callus initiation and growth in root segments of rice.

Materials and Methods

Decapitated root segments (2 cm length) of seminal roots of rice (*Oryza sativa*. L. cv. Aichi-asahi) were aseptically cultured at 30 ℃ for 30 days on modified LINSMAIER and SKOOG medium as described in a previous paper[3]. This basal medium was supplemented with 2,4-D and 2,6-DB as indicated. Development of lateral roots and callus tissue was observed during the culture period. The cultured roots taken out at 30 h after inoculation, were estimated and stained with Delafield's hematoxyline. The distal 1 cm of the root segment was smeared and the number of lateral root primordia or callus initials were counted under the light microscope.

Results and Discussion

The number of primordia (lateral roots or callus initials) increased with increasing 2,4-D concentration (Fig. 1), and roots formed more primordia with 2,4-D (10^{-6}M) and 2,6-DB (2×10^{-6}M) than with 2,4-D (10^{-6}M) treatment alone.

Fig. 2 shows the development of lateral roots or callus tissue on (1) basal medium alone ; (2) basal medium with low concentration of 2,4-D (10^{-6}M) ; (3) basal medium with low concentration of 2,4-D (10^{-6}M) and 2,6-DB (2×10^{-6}M) ; and (4) basal medium

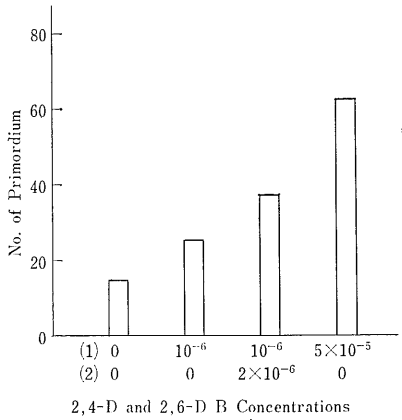

Fig. 1. The number of lateral root primordia or callus initials that arose from root segment 30 h after culture on the media with various concentrations of 2,4-D and 2,6-DB. (1) and (2) represent the concentrations of 2,4-D and 2,6-DB, respectively.

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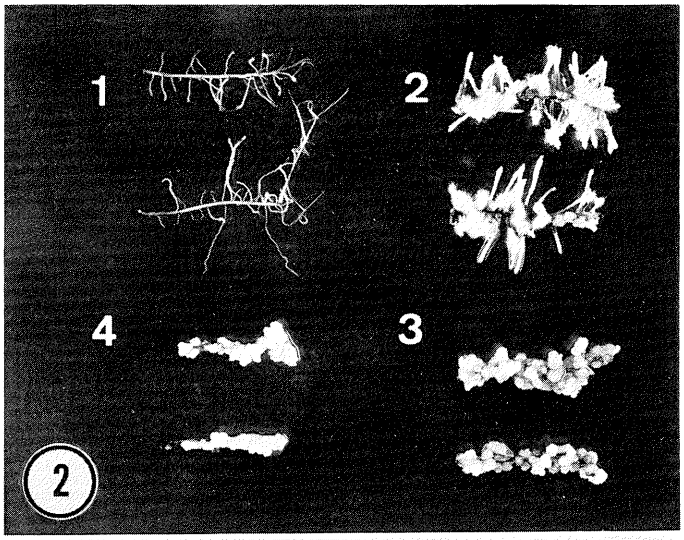

Fig. 2. Development of lateral roots and callus tissue from root segments 14 days after culture on the following media: (1) basal medium; (2) basal medium with low concentration of 2,4-D (10^{-6}M); (3) basal medium with low concentration of 2,4-D (10^{-6}M) and 2,6-DB (2×10^{-6}M) and (4) basal medium with high concentration of 2,4-D (5×10^{-5}M).

Fig. 3. Growth of callus tissue from root segments 30 days after culture on the basal medium with 2,4-D (10^{-6}M) and 2,6-DB (2×10^{-6}M).

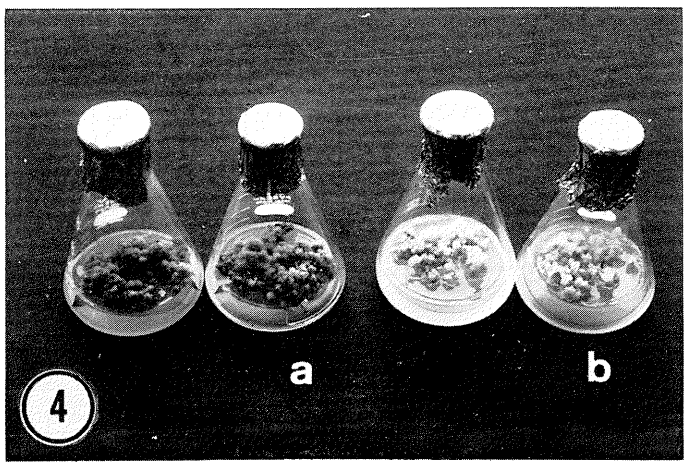

Fig. 4. Growth of callus tissue 45 days after culture on the basal medium with high concentration of 2,4-D (5×10^{-5}M) and 2,6-DB (2×10^{-6}M) (a); and basal medium with high concentration of 2,4-D alone (b). Note the growth of the callus tissue in (a) flasks is more vigorous than that in (b) flasks.

with high concentration of 2,4-D (5×10^{-5}M). Primordia developed as lateral roots on basal medium (1) or low 2,4-D(2), but lateral roots were stunted with many root hairs with low 2,4-D. However,at higher 2,4-D concentration (4), primordia grew as callus. Interestingly, a low concentration of 2,4-D (10^{-6}M) and 2,6-DB (2×10^{-6}M) (3) induced callus formation. The experiments were repeated three times and almost the same results were obtained.

Fig.3 shows the growth of callus tissue after 30 days of culture on medium (3). The growth of the callus tissue was good and the color of the callus was pale brown, but creamy white on the medium with high concentration of 2,4-D alone medium (4).

Enhancement of callus growth was also observed when high concentration of 2,4-D (5×10^{-5}M) was used in combination with 2,6-DB (2×10^{-6}M) as compared to 2,4-D (5×10^{-5}M) alone (Fig. 4).

In addition, we tested an effect of nojirimycin[2], a beta-glucosidase inhibitor in cell wall growth[4], on morphogenesis of root segments. No effect was observed in the initiation of lateral root primordia or callus initials except for an inhibition of lateral root growth.

In summary, the cellulose synthesis inhibitor, 2,6-DB, affected the morphogenesis of the lateral root by enhancing callus growth. This result suggests that the composition of cell wall may play an important role in the differentiation of plant tissues. Furthermore, it is speculated that cell surface, including cell wall, would play not only intercellular adherence but also intercellular communication in tissue development.

Acknowledgement

We wish to thank Dr. Taro NIIDA, Central Research Laboratory of Meiji Seika Kaisha, Ltd., for the kind gift of nojirimycin.

Summary

The effect of a cellulose synthesis inhibitor, 2,6-dichlorobenzonitrile, on callus initiation and callus growth was studied in rice (*Oryza sativa*) root cultures. The addition of 2,6-dichlorobenzonitrile to a growth medium containing 2,4-D enhanced callus initiation and growth. Microscopic examination confirmed the close relationship between the formation of callus initials and lateral root initials. These results indicate a possible function for the cell wall in callus initiation.

References

1. HOGETSU T., H. SHIBAOKA and M. SHIMOKORIYAMA 1974. Involvement of cellulose synthesis in actions of gibberellin and kinetin on cell expansion. 2,6-Dichlorobenzonitrile as a new cellulose-synthesis inhibitor. Plant & Cell Physiol. **15** : 389—393.
2. ISHIDA N., K. KUMAGAI, T. NIIDA, T. TSURUOKA and H. YUMOTO 1967. Nojirimycin, a new antibiotic. II. Isolation, characterization and biological activity. J. Antibioti. Ser. A **20**(2) : 66—71.
3. NISHIMURA S. and E. MAEDA 1982. Cytological studies on differentiation and dedifferentiation in pericycle cells of excised rice roots. Japan. Jour. Crop Sci. **51** : 553—560.
4. ROSENFIELD C.—L. and PH. MATILE 1979. Glycosidases in pear pollen tube development. Plant & Cell Physiol. **20** : 605—613.

〔和 文 摘 要〕

イネの根におけるカルスの形成と生長のセルロース阻害剤 2,6-dichlorobenzonitrile による促進

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イネの切断根からのカルス形成およびそのカルスの生長に対するセルローズ合成阻害剤の影響を研究した結果，2,4-dichlorobenzonitrile (2,6-DB) に促進作用のあることを認めた．

2,4-D 10^{-6}M と 2,6-DB 2×10^{-6}M を含む培地で無菌培養された切断根では，2,4-D 10^{-6}M のみを加えた培地のものより，多くのカルスが形成された．同様の結果は，5×10^{-5}M の 2,4-D と 2×10^{-6}M の 2,6-DB を基本培地に加えた場合でも得られた．これらの実験結果から，組織の分化(側根原基の形成)と組織の脱分化（カルスの形成）に対して，細胞壁の組成が一定の役割をもっていると言える．