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RECENT RESEARCH ACTIVITIES

A molecular diagnosis for the resistant pine traits against pine wilt diseases

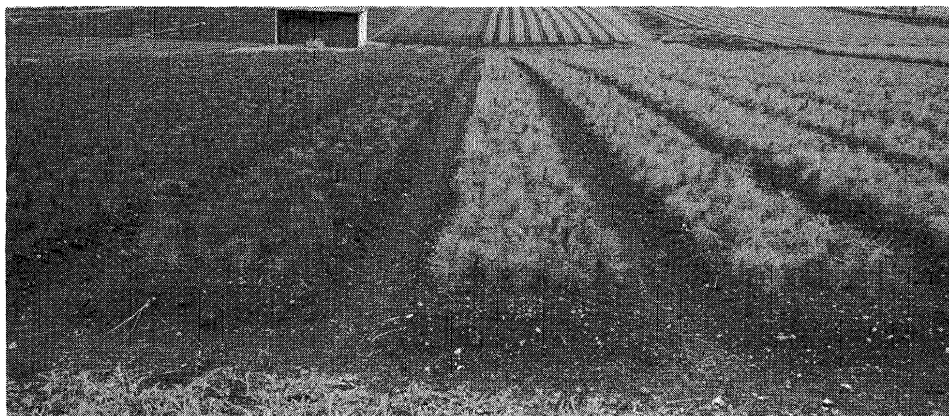
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Japanese pine forest has been severely suffered from pine wilt diseases and the dead trees are the biggest loss in biomass of Japan. Furthermore, the diseases have ignited in East Asia and Europe, causing political affairs. Pesticide application for controlling pine wilt diseases has gradually been diminished because of environmental concerns. New strategies are now required for controlling pine wilt disease, which is directly caused by pine wood nematodes. In the government-related facilities, survived pine trees have been collected from the natural pine forest stands, where they had been suffered from the pine wilt disease. Meantime, the pine EST libraries are available for analyzing gene expression under some stress in pine trees. The virtual full coding sequences, which may or may not represent substantial genes, are also easily obtained from the fragments. However, the expression data for pine wilt diseases in association with the resistant traits has little been accumulated. The author has focused attention on the resistant traits in Japanese red pine (*Pinus densiflora*).

In order to find a molecular clue, the expressed genes from a resistant tree have subtracted against the one from a susceptible tree after they had infected with the nematodes. In the course of the experiment, key processes were as follows: providing relevant plant materials (Fig. 1) for the resistant traits and the RNA preparations with reasonable quality and quantities. In the former case, the resistant traits against the nematodes are easily weakened by the pollination with susceptible pollen parents. The grown resistant family contains such weakened individuals since they are the seed descendants of a selected tree which carries resistant traits. The suspects had been under better control by counting nematode numbers after the infection. Thus the resistant tree was able to determine as the one which showed no nematode proliferation even after the nematode infection. In the latter case, resistant trees were more difficult to extract RNA, of which yield was always lower than the one from susceptible trees. Finally, the cDNA clones have successfully collected and over a thousand of sequenced clones have assigned for the public pine EST libraries.

The causes of the resistant traits may be compared to a gun. One component is corresponding to a kind of a trigger and the other is a kind of a bullet. The former trigger is a point mutation, which is usually expected to cause loss of function. The mutation may alter the metabolism, signal transduction and transport which are directly associated with the resistant traits. This direct resistance is the latter bullet case. The gain of function is possible in the latter case and it produces weapons which can defend against the worm and control them. In fact, obtained clones contain such families of "ecu and halberdier". The obtained sequences will provide molecular discrimination for selecting a resistant pine tree. The resistant tree selection is one of a key strategy for controlling pine wilt disease without environmental concerns. Further works for the diagnosis are now going on.



A view of pine nursery: the resistant families of Japanese red pine against the nematodes
The photo shows a nursery bed where the Japanese red pines with distinct resistance against the nematodes had been growing for two years after seeding.