黄化葉を光にあてたとき、第2葉に stripe 病徴が現われてくる様子を観察した。

前と同じように、1カップ当り15粒ずつ播種したオオムギを、25°C、暗黒下で育てる。播種後6日、第1葉が十分展開したとき、これにBSMVを摩擦接種した。明条件下でオオムギ第1葉にBSMVを接種すると、接種葉には病徴が現われないか、または灰白色の大型えそ斑を生じ、典型的な stripe 病徴は、第2葉に現われるのが普通である。しかも第2葉にみられるこの病徴は、第2葉が十分展開する前に感染しないと現われない。したがって本実験で、第2葉の病徴を観察するに当って、光をあてたのちに第2葉がよく伸びるものでなければならない。この点で劔吉3号が横綱にまさっていたので劔吉3号を用いた。

接種直後から光にあてた区、接種後2, 4, 7日のそれぞれの期間さらに暗所においてから光にあてた区のおのおのについて、第2葉に stripe 病徴が現われる様子を観察してまとめたのが第2表である。播種直

後から暗黒で育てたオオムギ葉はクリーム色をおびた乳白色を呈している(第1図)。これに光をあてると、第2葉では葉基部からうす緑色に着色しはじめ、2-3日で全体に黄緑色を呈する。このとき、すでに stripe 病徴を認めることができる。さらに2-3日たつと、ますます緑色味を増し、病徴も激しくなる。最終的な stripe 病徴は、明所で感染したオオムギに見られるものと差がない(第2図)。光にあててから、はじめて stripe 病徴が認められるまでの日数は、接種後光にあてるまでの日数に関係なく、どの区でもおよそ3日であった。すなわち、黄化葉が光にあたって緑化しはじめるのと同時に stripe 病徴を認めることができた。この観察事実は、黄化オオムギ葉中でBSMVが増殖する過程で、proplastidに何らかの異常が起こっており、光にあたって chloroplast に転化する過程で部分的に緑化できず、その結果 stripe 病徴を呈するにいたることを示すものと思われる。

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Incorporation of Tritiated Phenylalanine into Lignified Cell Walls of Japanese Radish Root Infected by *Peronospora parasitica**

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浅田泰次**・久郷 毅**：べと病菌感染ダイコン根木化細胞壁へのフェニールアラニン-³Hのとりこみ*

Brown and Neish¹⁾ showed that lignin was synthesized from phenylalanine and related aromatic monomers. Radiotracers have been used to study lignin biosynthesis in higher plants²⁾. We reported that lignin was formed only in the parenchyma cell walls of downy mildew-infected Japanese radish root as a result of the host-parasite interaction³⁾. By microspectrophotometry, we found that greater accumulation of lignin occurred in the middle lamella of the parenchyma cell walls of *Peronospora*-infected Japanese radish root than that of the noninfected ones⁴⁾.

* This paper constitutes Part 5 of a series of papers on the lignin formation in fungus-infected plants. Contribution No. 9.

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Hepler *et al.*⁵⁾ reported on lignification during secondary wall formation in *Coleus blumei* as observed by electron microscopy. They mentioned that lignin was first detected as conspicuous electron-opaque granules in the primary wall. It was also found in the region where secondary wall thickening arose as fine granular striations extended into very young secondary wall. In order to determine precisely the exact site of lignin deposition in the cell wall, we examined the incorporation of L-phenylalanine-G-³H into the infected cell walls by electron microscopic autoradiography.

Well-developed roots of about 3-month-old Japanese radish (*Raphanus sativus* L. var. *hortensis* Backer f. *minowase* Kitam.) were aseptically cut into slices about 0.7 cm thick. One ml of L-phenylalanine-G-³H (40-100 μ Ci/ml) was uniformly supplied to one surface of each slice. After incubation for 4 days at 20°C in moistened petri dishes under 110 lux illumination from white fluorescent lamps, the slices were inoculated with conidia suspension of *Peronospora parasitica* Pers. ex Fr. or treated with distilled water, and incubated again in the moistened petri dishes for 7 days under the same conditions. Root slices treated with distilled water served as control. Slices were cut into about 1 mm³ cubes in 0.1 M phosphate buffer (pH 7.2) containing 5.5% sucrose, and fixed with 2% KMnO₄ for 4 hr at 4°C. They were washed with the same buffer for 1 hr, dehydrated through graded acetones and embedded in a cross-linked epoxy resin mixture. Ultrathin sections (ca. 70 m μ) were picked up on 2% collodion-coated copper grids (100 mesh) and stained with 12% (w/v) uranyl acetate (dissolved in 50% ethanol) for 30 min. Finally they were coated by the touching method⁶⁾ with 7% Sakura NR-12 monolayer emulsion and exposed for 20 days at 4°C in the dark. The specimens were developed with EAA solution (0.045 g Elon, 0.3 g ascorbic acid, 0.5 g Na₂B₄O₇, 0.1 g KBr in 100 ml H₂O) after latentification of chloroauric acid solution (0.4 g AuCl₃HCl·4H₂O, 0.5 g KSCN, 0.6 g KBr in 1 l H₂O) and fixed with Kodak fixer. The gelatin was removed by Mizuhira-Kurotaki's solution (0.04 g NaOH, 0.016 g Rochelle salt, 0.001 g PbO in 100 ml H₂O). A carbon layer (ca. 6 m μ) was evaporated over the specimens.

As already reported,³⁾ noninoculated parenchyma cell walls were found to give a negative

Fig. 1. Electron microphotographs showing the site of tritiated phenylalanine incorporation in the parenchyma cell walls of Japanese radish root.

The scales represent 0.5 μ (A), 1 μ (B, C), 2 μ (D). I: Inner layer, M: Middle lamella, C: Cytoplasm.

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reaction for phloroglucinol-HCl reagent, whereas inoculated walls gave a positive reaction. Fig. 1 shows the site of tritiated phenylalanine incorporated in the parenchyma cell walls. Control slices did not produce silver grain (Fig. 1, A). In inoculated slices, noticeable silver grains were observed first in the inner layers of the cell wall and then in the middle lamella (Fig. 1, B, C, D). Since lignification was different from site to site in the infected parenchyma³, various sites of incorporation were observed in the same material. However, there is no evidence for the presence of lignin deposition in the middle lamella alone and the absence of the deposition in the inner layers, the lignification seems to take place first in the inner layers. Also, this result seems to show that the silver grains were not due to any chemical fogging as artifacts. It is generally assumed that inner layers of a cell wall may be composed mainly of cellulose and a middle lamella may be of pectic substance. Therefore, we supposed that the lignification of the parenchyma cell walls induced by the infection was initiated first in the cellulose microfibrils of the inner layers and then in the pectic layer of the middle lamella.

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Interrelationship of a Fungus, *Curvularia lunata* with Root-Knot Nematode, *Meloidogyne javanica* in Sugarcane Seedling Blight**

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S.M. Paul Khurana* and Shatrughna Singh** : サトウキビ苗立枯病における病菌,
Curvularia lunata と根こぶ線虫, *Meloidogyne javanica* との協合**

Disease complexes involving plant pathogenic nematodes and fungi have been well established in the past as reviewed by Goswami, *et al.*¹⁾ and Pitcher²⁾.

Recently a serious decline of Indo-American hybrid sugarcane seedlings (developed from crosses between Indian *Saccharum spontaneum* and Puerto Rican or Hawaiian sugarcane cultivars) due to blight was observed at this Institute**. Uprooting of these seedlings revealed that their root system was poor, discolored, rotting and had root-knots indicating infection with a fungus and nematode. In some cases collar rot was also observed. The fungus and nematode were isolated, tested for their pathogenicity and identified as *Curvularia lunata* (Walker) Boed. and *Meloidogyne javanica* (Treub.) Chitwood, respectively. Therefore, a study was undertaken to determine the relationship of the isolated fungus and nematode on infection to seedlings of sugarcane.

The experiment was conducted in a glasshouse. Healthy seedlings of hybrid sugarcane (PR.

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