STUDIES ON THE HYPERTROPHIC DISEASE CAUSED BY TAPHRINA SPECIES (V) DNA LEVEL OF NUCLEUS IN THE INFECTED TISSUE

STUDIES ON THE HYPERTROPHIC DISEASE CAUSED BY TAPHRINA SPECIES

(V) DNA LEVEL OF NUCLEUS IN THE INFECTED TISSUE

By

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Introduction

In the leaf curl caused by *Taphrina deformans*, diseased tissue cells divide vigorously and their nuclei become very stainable by haematoxylin. In addition, giant nuclei and nucleolus, or multinuclear cells were also observed (3). Therefore, it was assumed that changes occured in the nucleic acid metabolism of the diseased tissue cells. Especially, it was supposed that the swelling of the nucleus was due to the increment of nucleic acids and proteins which compose the nucleus.

Taphrina fungi produced IAA from tryptophane or other amino acids and from indole with serine (5). Accordingly, it was presumed that the IAA excreted by these fungi promoted the synthesis of DNA and RNA. For the purpose to ascertain this assumption, this experiment was carried out. There are few investigations on the changes of the nucleic acid level in the nucleus, which include measuring the diseased tissue by the micro-technique.

The outline of results already reported (4) and the details will be presented here.

Material and Method

The smaple used was leaf curled tissue caused by *Taphrina deformans* Tul. The infected leaf tissue was divided in to three groups according to the degree of disease — initial stage, middle stage and final stage. And the healthy tissue was sampled at a given day concurrent with the sampling of diseased tissue. Methods of measurement were Senoo and Utsumi's (9). Samples were fixed in 10% formalin for 24 hrs and after washing by water, dehydrated by n-butanol series. They

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were then embedded in paraffin, sectioned at 10 μ in thickness, and deparafinized. Sections were transfered into water through the alcohol series and hydrolysed in 1 N HCl at 60°C for 15 min. After washing by water, sectioned tissues were stained with Schiff's reagent for 4 hrs. Then excess staining reagent was removed from the section with the decolorant solution (Mixture of 9 volume of glycine buffer-pH 2.28 and 1 volume of 15% sodium metabisulfite). The decoloration was repeated twice for 30 min. After these treatments, sections were dehydrated with ethanol series and mounted in Canada balsam. The photometry of the DNA content in the individual cell was done by using a microspectrophotometer (Olympus A-IV type). The wavelength used was 560 mμ. The DNA content in the nucleus was measured on upper and lower epidermis, palisade- and spongy-cells by the Swift method (9). The DNA content was estimated for one hundred cells in each tissue respectively.

Results and Discussion

In the diseased tissue, cells divided remarkably with periclinal division and were abnormally enlarged. And increment of stainability, swelling of nucleus and nucleolus, and occurrence of multinuclear cell and multinucleolar nucleus were observed. Therefore, it was supposed that an abnormality of nucleic acid metabolism occured in the nucleus of the diseased tissue. Fig. 1 shows swelling of nucleus in the diseased tissue cell. In the diseased parenchyma tissue cell of the middle stage, the diameter of nuclei were 4.1–8.0 μ. On the other hand, nuclei of healthy palisade and spongy-tissue cells were 2.0–4.0 μ in diameter. Especially, the distinct difference between diseased and healthy tissue was observed in the palisade tissue in which the most vigorous cell division and swelling occured. Increasing of DNA content is shown in Fig. 2, 3, 4. DNA level rised in the diseased tissue cell in every stage from initial to final. The degree of increase was extremely high in the palisade and spongy tissue. And the most remarkable increase was observed in the palisade tissue at the middle stage.

As shown in Fig. 2, the nucleus of the palisade tissue cell swelled and DNA content increased even in the initial stage. The same tendency was observed in the spongy tissue cell, but the degree of increase was lower than that in the palisade tissue cell. The DNA content apparently increased in most outside cells of the infected tissue (Fig. 2; f). But its increment was not observed in adjacent cells which appeared to be morphologically healthy (Fig. 2; c).

As shown in Fig. 3, at the middle stage DNA content of the palisade tissue and spongy tissue increased considerably. This stage was the most vigorous stage of cell division and enlargement during the overgrowth of diseased tissue. Accordingly, the increase of nuclear DNA content was of course remarkable. As stated previously, the nuclear volume increased extremely in this middle stage. From

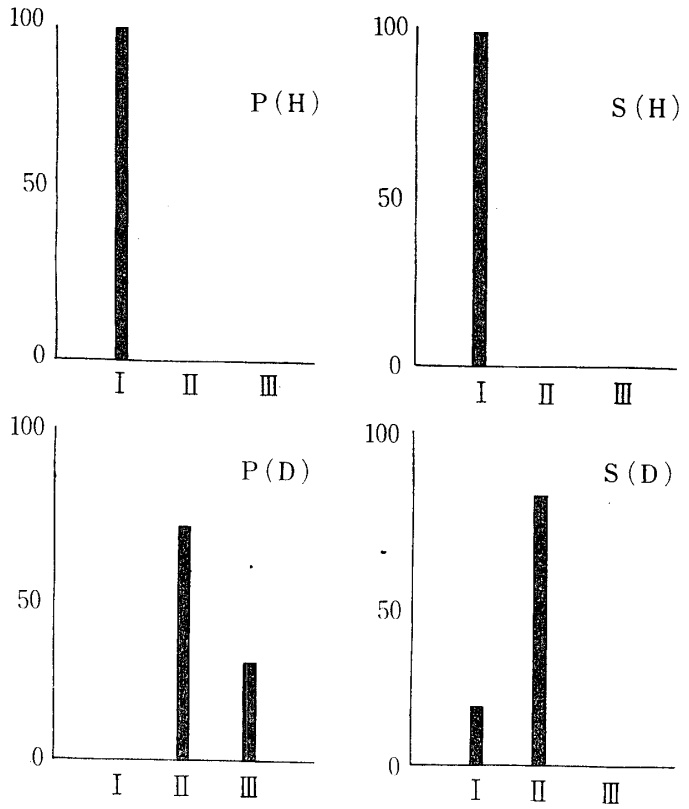

Fig. 1 The nucleus diameter of diseased tissue cells.
Ordinate: Number of nucleus
Abcissa; Grouping of nuclei by the diameter
I; 2.1–4.0 μ, II; 4.1–6.0 μ, III; 6.1–8.0 μ
Abbreviation: P; Palisade tissue, S; Spongy tissue, (H); Healthy, (D); Diseased

the above result, it is assumed that the enlargement of nucleus may be due to partly the increase of DNA.

Cells having two nuclei were observed at the middle stage (3). In this case, DNA content per one nucleus was much more than that of the single nucleus of the healthy cell, but about half of that of the diseased cells having a single nucleus (Fig. 3; c,f). In upper and lower epidermal cells, nuclear DNA also increased in the diseased tissue. But the nuclear DNA content was quite variable. This tendency was mostly distinct in diseased lower epidermal cells. This state was also investigated in healthy epidermal cells.

At the final stage, nuclear DNA content in the palisade and spongy tissue was much more than that in the healthy tissue (Fig. 4). However the difference between them was not so remarkable as in the middle stage.

There are many reports concerning the increase of DNA content caused by the infection of parasite. For example, Klein (2) reported for the crown gall caused by *Agrobacterium tumefaciens* that the nuclear DNA level in tomato stem tissue cell

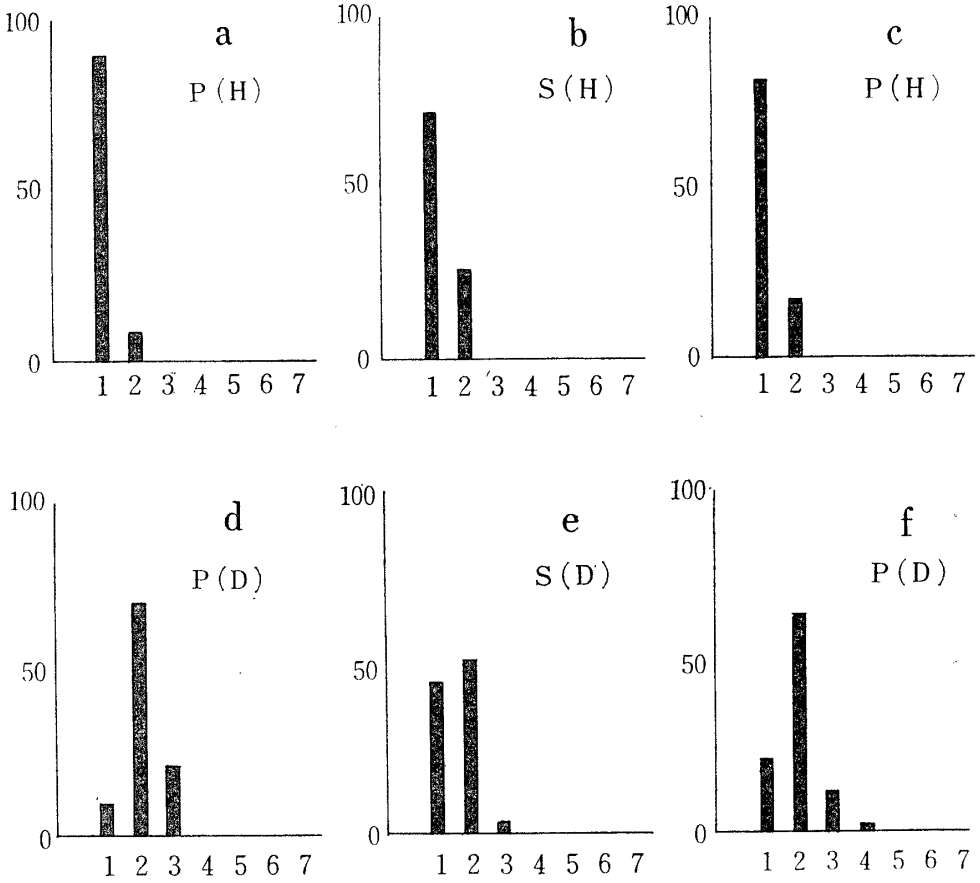

Fig. 2 The DNA level of diseased tissue cells in the initial stage.
Ordinate: Number of nucleus
Abcissa: The arbitrary unit. Number 1 shows the lowest DNA level and number 7 the highest level.
Abbreviation: P; Palisade tissue, S; Spongy tissue (H); Healthy, (D); Diseased
(c): Cells adjacent to the diseased portion, which appear to be morphologically healthy.
(f): The perimeter cells of the diseased portion.

which was inoculated with a virulent strain, doubled in 24 hrs. And the same phenomenon was observed in gall tissue caused by *Meloidogyne* (6). On the other hand, Whiteney et al (10) observed the swelling of nucleus and nucleolus in rust infected tissue, and reported the increase of RNA content in the nucleolus and no increase of nuclear DNA.

Thus, it is presumed that the abnormal increase of DNA content and abnormality of nucleus are a special property in the tissue of the hypertrophic disease in which vigorous cell division occurs.

Recently, it was clarified that the auxin was related quantitatively to the metabolism of nucleic acids (1) (7) (8). Also, it has been supposed that auxins and phytokinins are indispensable for the cell divisions. Therefore, auxins and

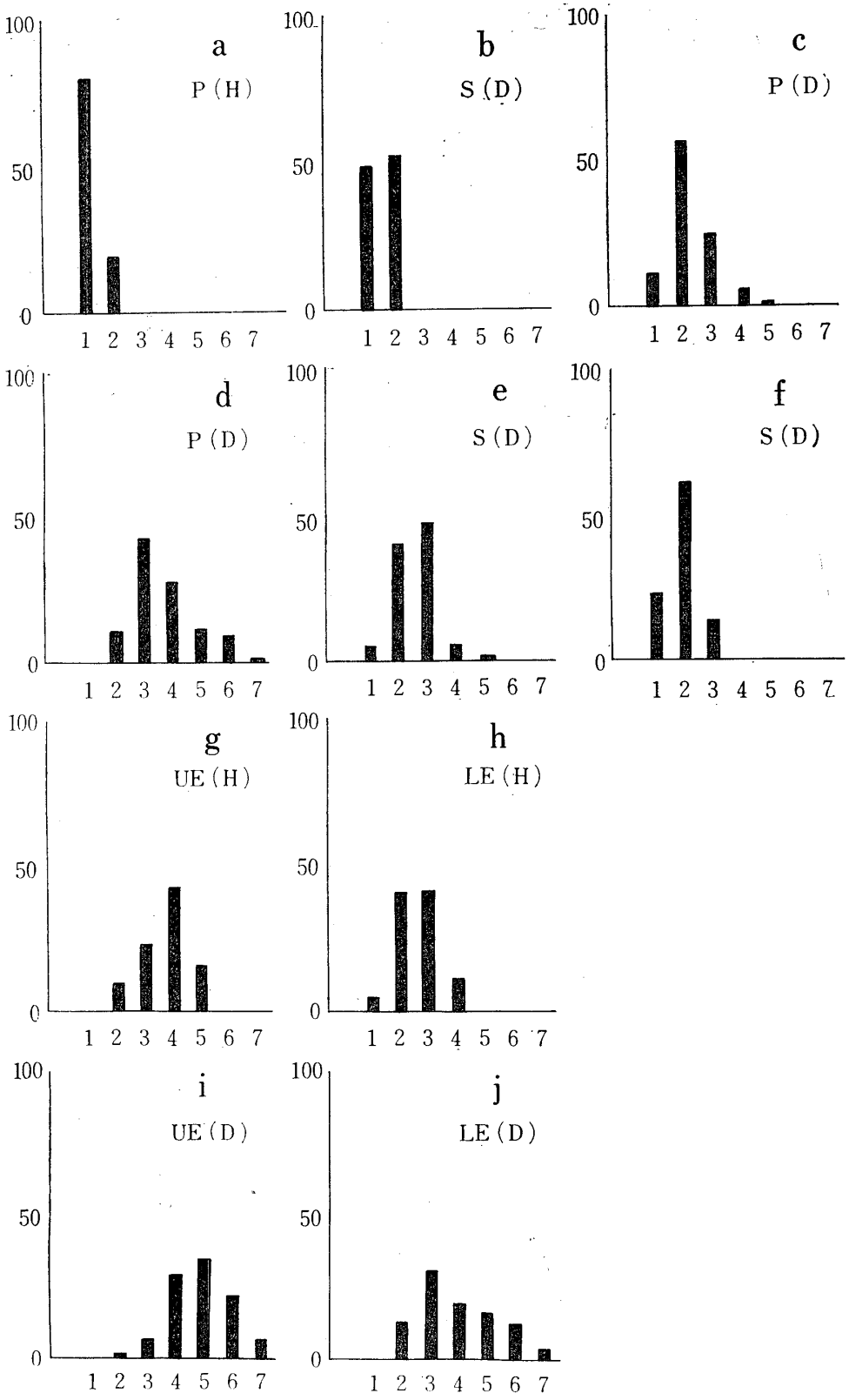

Fig. 3 The DNA level in the middle stage
Abbreviation: UE: Upper epidermis, LE: Lower epidermis,
(c), (f): The DNA level per one nucleus of multinuclear cell

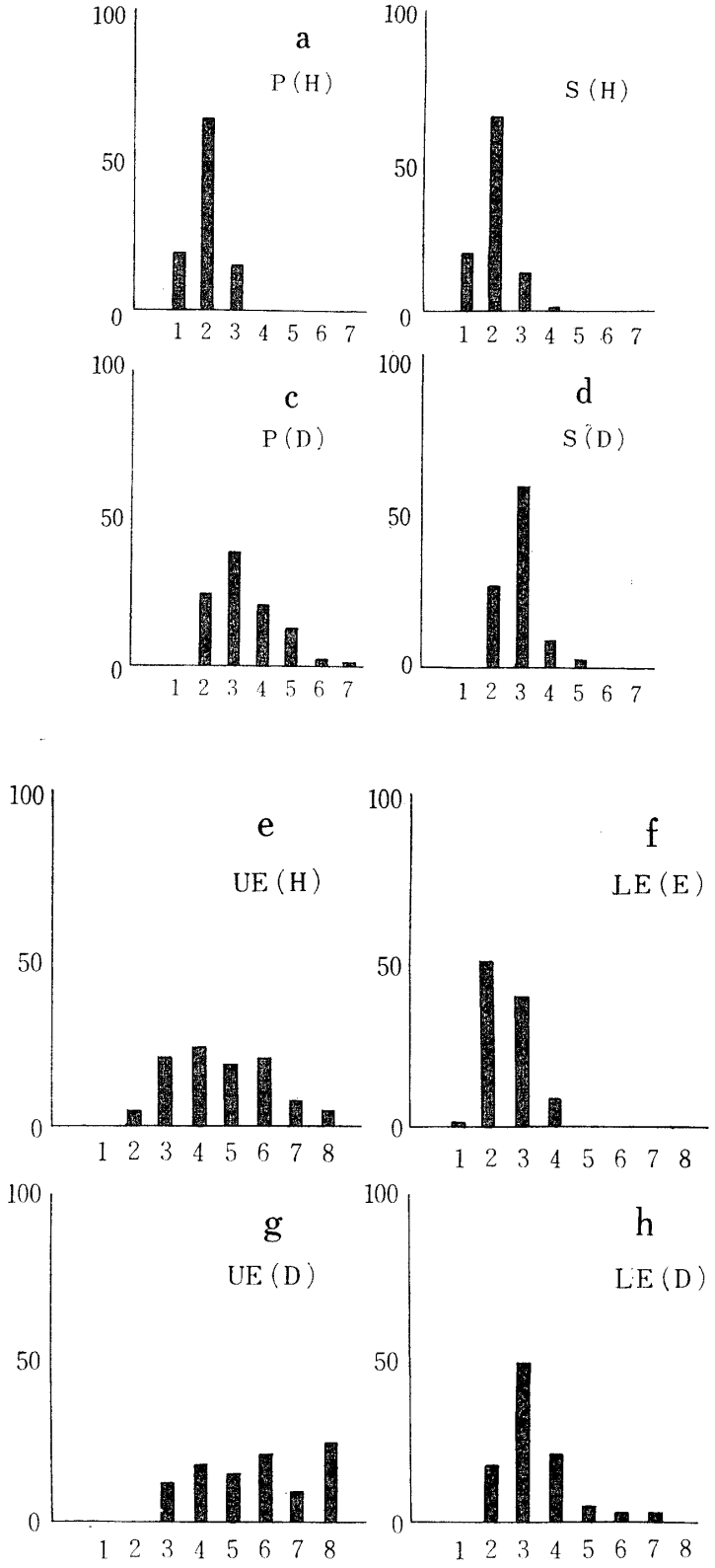

Fig. 4 The DNA level in the final stage

phytokinins excreted by *Taphrina* fungi may promote the biosynthesis of nucleic acids, especially of DNA, and nuclear division.

Summary

In the leaf curl disease caused by *T. deformans* Tul., the nucleus and nucleolus of diseased tissue swelled extremely. The DNA level in the nucleus rose soon after the fungal invasion. In the middle stage of the disease, the cell division was most vigorous and the increased of DNA content was most remarkable. The increment of DNA content occured in the palisade-, spongy-tissue and epidermis. But the greatest increase was observed in the palisade tissue where the cell division and cell swelling was most remarkable. In the diseased tissue, multinuclear cells were observed. Their DNA content per one nucleus was much more than that of heatlhy cells, but less than that of one nuclear cells in the diseased tissue.

References

1) Biswas, B.B., S.P. Sen (1959), Nature, **183**, 1824–1825.
2) Klein, R.M. (1953). Amer. J. Bot. **40**, 597.
3) Matsuyama, N. and T. Misawa (1961). Tohoku J. Agric. Res., **12** (4), 317–325.
4) Matsuyama, N. and T. Misawa (1965). Ann. Phytopath. Soc. Japan, **30**(2), 96.
5) Matsuyama, N. and T. Misawa (1966). Tohoku J. Agric. Res., **17** (1),
6) Nishizawa, T. (1957). Japanese J. Appl. Ent. Zool. Symposium I, **17**.
7) Patau, K., N.K. Das and F. Skoog (1957). Physiol Plant., **10**, 949–966.
8) Roychoudhury, R. and S.P. Sen (1964). Physiol Plant., **17**, 352–362.
9) Senoo, S. and K. Utsumi (1961). Protein Nucleic acid Enzyme, **6** (8), 482–486.
10) Whiteney, H.S., N. Shaw and J.M. Nayler (1962). Can. J. Bot., **40**, 1533–1544.