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Studies on the Auxin Metabolism of Some Cereal Crops in Relation to Growth and Development
III. Some Experiments in Vitro on the Indoleacetic Acid-destroying System in Wheat Seedlings*

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Summary

The mechanism of the IAA-destroying system in the wheat seedling is discussed in this paper.

The IAA-oxidase activity in the crude homogenate of dark-grown wheat seedlings is masked by the existence of IAA-oxidase inhibitors in the homogenate, but the addition of H_2O_2 to the homogenate eliminates this masking and much IAA-oxidase activity becomes visible.

The ferulic acid (one of the IAA-oxidase inhibitors), H_2O_2 and a dialysed enzyme solution from wheat coleoptile were mixed and incubated for the detection of changes in the UV-absorption spectrum of the mixture. As a result, it was observed that the ferulic acid in the mixture was oxidized by the H_2O_2-peroxidase system.

The masking of the IAA-oxidase activity in the crude homogenate can also be eliminated by the addition of FR (FMN) and light but the enhancement of the IAA-destroying action by FR (FMN) and light is observable without any enzyme preparation in the mixture. This result suggests that the destruction of IAA is non-enzymatic.

It was also observed that this non-enzymatic destruction of IAA by FR (FMN) and light is inhibited by the IAA-oxidase inhibitors, i.e. the phenolics contained in the wheat seedlings.

From the above-mentioned results, the authors have theorized about IAA-destroying system in wheat seedlings as shown in Fig. 5.

The authors have reported in the previous paper (1, 2) that the IAA-oxidase of wheat seedlings is a peroxidase and that its activity is masked by the IAA-

The following abbreviations will be used: IAA, 3-indoleacetic acid; FR, riboflavin; FMN, flavin mononucleotide.

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oxidase inhibitors, that is, the ferulic acid and other phenolcarboxylic acids contained in plant tissue. Yet, through what mechanism is IAA destroyed? And with this query, the following hypothesis has been presented by Galston et al (3): that IAA-oxidase is a complex enzyme of peroxidase and flavoprotein, the latter of which when activated by light produces H_2O_2 and is used for IAA-oxidation by the peroxidase as a hydrogen acceptor. However, after that, it was found that IAA is oxidized by peroxidase without exogenous H_2O_2 as a hydrogen acceptor, i.e. "oxidase action" of peroxidase, e.g. the cases of di-hydroxyfumaric acid, reduced co-enzyme I ($NADH_2$) and II ($NADPH_2$) (4). This finding was supported by the experimental results of the authors (1).

It has also been reported that the action spectrum of the tropism of coleoptile and the inhibition of stem growth, which seem to be closely related to the auxin level in tissues, is exactly the same as that in the absorption spectrum of flavin (5, 6). From this point of view, flavin seems to fulfill some roles in the IAA-destruction, as mentioned by Galston et al.

Thus, many workers have discussed the IAA-destroying system in plant tissue and have not yet reached an adequate conclusion.

The authors have undertaken some experiments on the IAA-destroying system in wheat seedlings and the results obtained are reported in this paper.

Material and Methods

Material

Wheat seedlings, variety AOBAKOMUGI, grown using the same method as that in the previous paper (1), were used.

Preparation of Enzyme

Crude and dialysed enzyme solutions were prepared using the same method as that in the previous paper (1).

Measurement of the Inhibiting Power of IAA-oxidation

Almost the same method as that in the previous paper (1) was used except with the use of Michaelis' (1/10 N Na_2HPO_4–1/10 N KH_2PO_4) buffer solution (pH 5.2) instead of McIlvaine's buffer, and the addition of 10^{-4}M $MnCl_2$ to the reaction mixture.

Spectrophotometric Observation of the Destroying Process of Ferulic Acid

Five ml of the reaction mixture containing Michaelis' buffer solution (3 ml) as mentioned above, final concentrations of 10^{-4}M of ferulic acid and H_2O_2, and 1 ml of dialysed enzyme solution, were incubated at 30°C in the dark and shaken 100 times per minute. After incubation for different periods of 0, 10, 20 and 30 minutes, 1 ml of 5% trichloroacetic acid was added to the mixture and it was centrifuged at

2000 × G for 30 minutes. The supernatant was diluted with the same amount of deionized water and this was used for the spectrophotometric observation using a HITACHI recording spectrophotometer.

Results and Discussion

Effect of H_2O_2 on the IAA-oxidizing Power of Crude Enzyme Solution

The IAA-oxidizing power of the crude enzyme solution was masked by the IAA-oxidase inhibitors mingling in the solution (1, 2). It showed, however, that a strong IAA-oxidizing power appeared when H_2O_2 was added to the solution (Fig. 1). On the other hand, it showed little effect when added to the dialysed enzyme solution (1). From the fact that the crude solution contains IAA-oxidase inhibitors, we contend that this enhancement of the IAA-oxidizing power of the crude enzyme by H_2O_2 may depend on the destruction of the inhibitors through the H_2O_2-peroxidase system.

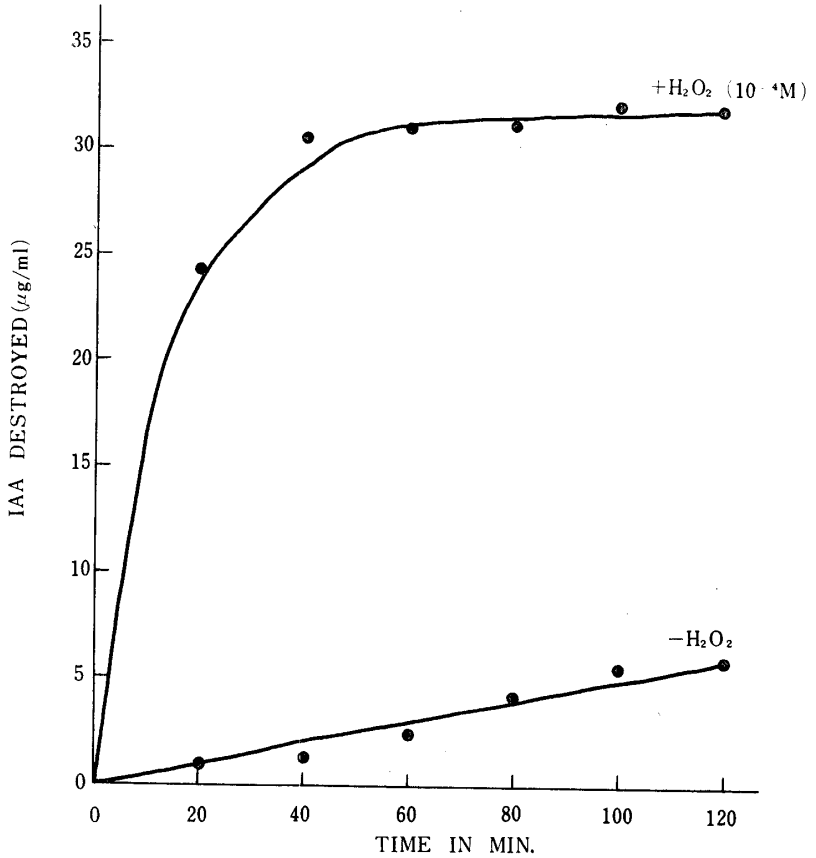

FIG. 1. Effect of H_2O_2 on IAA-oxidation by the crude homogenate of 5-day-old wheat coleoptiles.

Destruction of IAA-oxidase Inhibitor by H_2O_2-peroxidase System

By using ferulic acid, a sort of IAA-oxidase inhibitors in wheat seedlings, the spectrophotometric observation was carried out to ascertain the destruction of the

IAA-oxidase inhibitor by the enzyme solution. As a result (Fig. 2), the maximum absorbing spectrum of the ferulic acid decreased gradually and disappeared after 30 minutes of incubation with H_2O_2 and the dialysed enzyme solution.

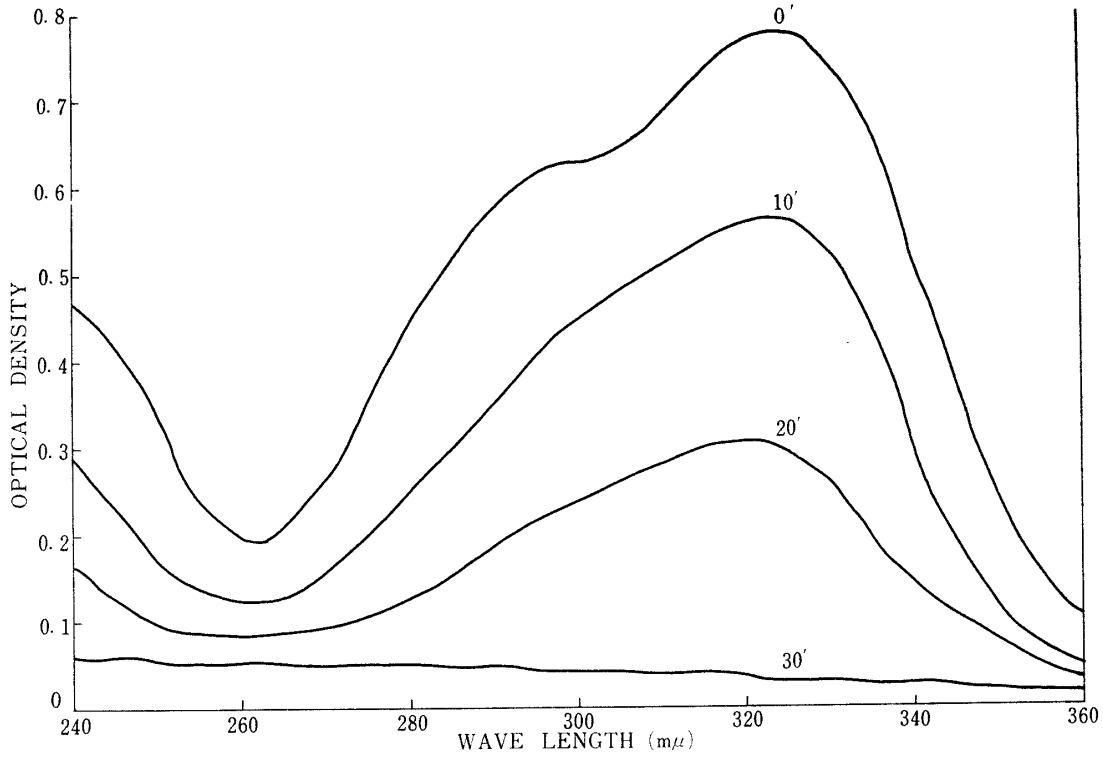

FIG. 2. Changes in UV-absorption spectrum of the reaction mixture, containing 10^{-4}M of ferulic acid, 10^{-4}M of H_2O_2 and the dialysed homogenate in Michaelis' buffer solution (pH 5.2).

It has been reported that H_2O_2 is produced in plant tissues under light conditions (7, 8), and from the results obtained, it may be thought that this H_2O_2 will not be used directly to oxidize IAA (as hydrogen acceptor) but will be used to oxidize IAA-oxidase inhibitors by the H_2O_2-peroxidase system. From then on, IAA will be oxidized by the "oxidase action" of the peroxidase which appears due to the diminution of the inhibitors.

It has been known that the growth of wheat seedling is extremely inhibited by light, especially blue light (9), and this phenomenon may also be closely related to such IAA-destruction.

IAA-destruction by Flavin and Light

It has been reported by Morgan et al (10) that the lag-phase of IAA-oxidation due to the addition of IAA-oxidase inhibitors to the dialysed enzyme from cotton plants is removed by riboflavin (FR), flavin mononucleotide (FMN) or flavin adenine dinucleotide (FAD), and light. The authors have undertaken the same

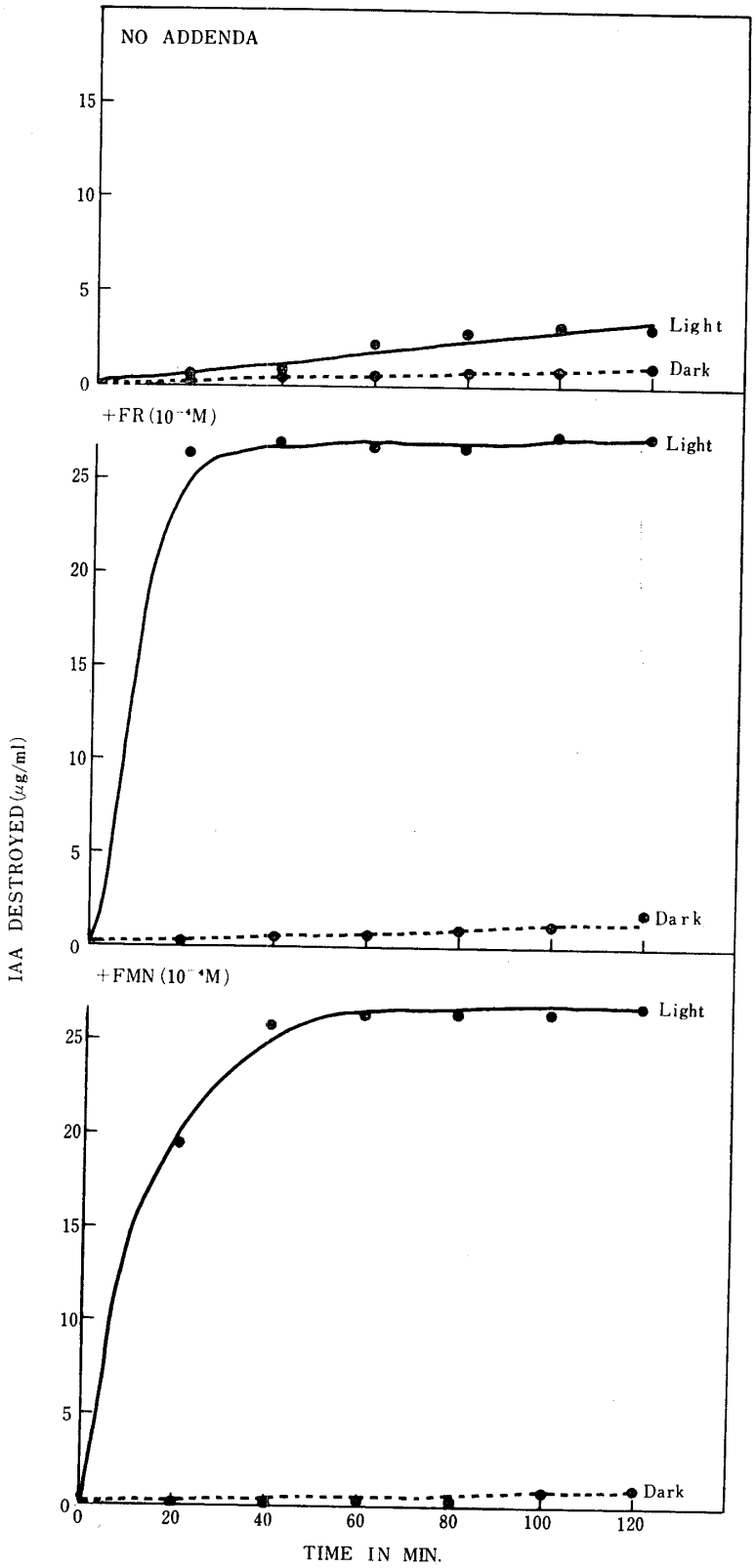

FIG. 3. Effect of riboflavin and flavin mononucleotide on IAA-oxidation by the crude homogenate of wheat coleoptiles under light and dark conditions.
Three thousand lux of white fluorescent light was used for illumination.

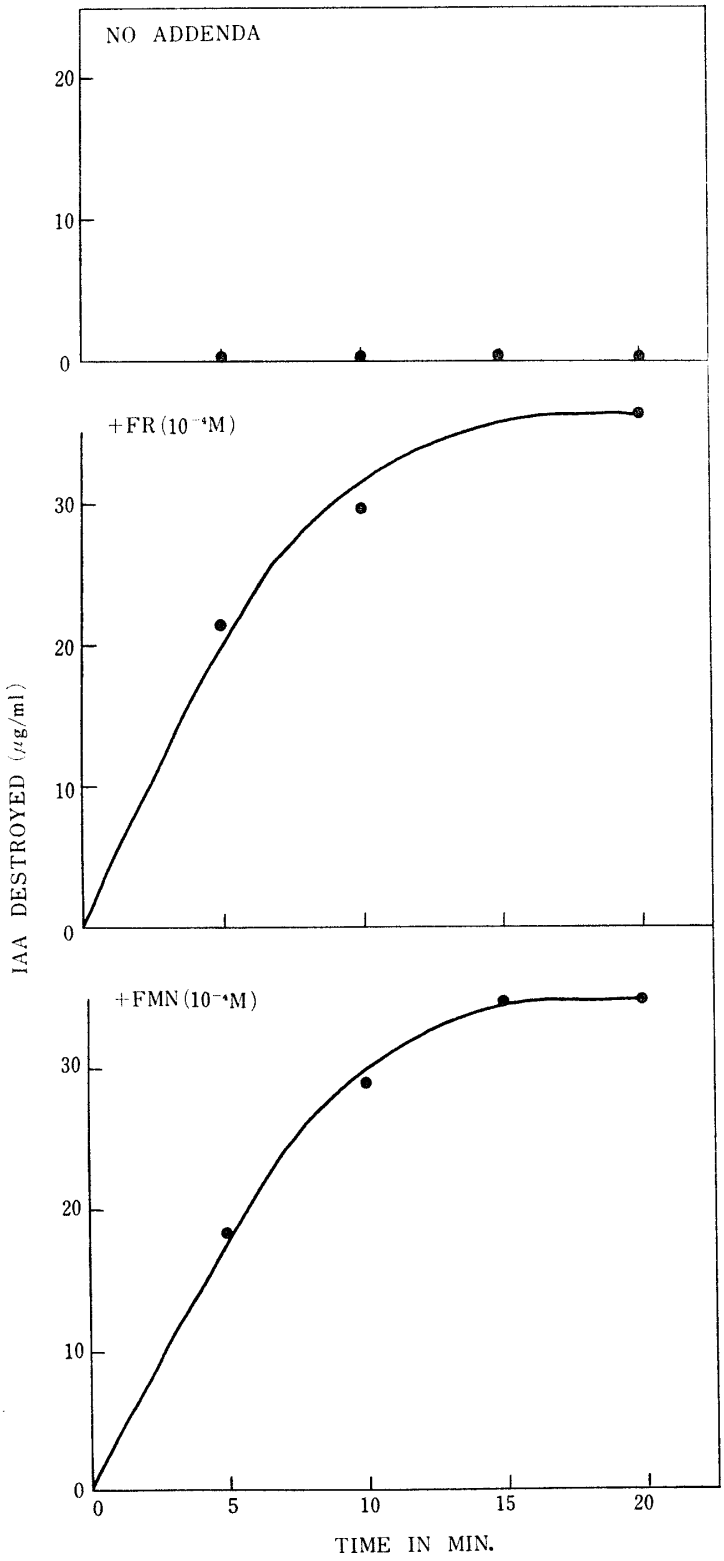

FIG. 4. Non-enzymatic destruction of IAA caused by riboflavin (flavin mononucleotide) and light.
Three thousand lux of white fluorescent light was used for illumination.

experiment using the crude enzyme solution of wheat seedlings and obtained the same results, i.e. that the lag-phase of IAA-destruction in the crude enzyme was removed and a strong IAA-destroying power appeared when FR or FMN was added plus illumination by 3000 lux of white fluorescent light (Fig. 3). This is just like H_2O_2 addition. However, a stronger IAA-destruction by FR (FMN) and light was observed even without any enzyme preparation (Fig. 4). From this finding, it may be considered that this enhancement of IAA-destroying power in the crude enzyme is not due to the removal of inhibitors by H_2O_2 produced through flavin and light but due to the non-enzymatic destruction of IAA accompanied by the photo-degradation of flavin (6). Moreover, from the point of view that this non-enzymatic destruction of IAA is slightly inhibited by the crude enzyme, it is suggested that some inhibitors are contained in the solution.

Effect of IAA-oxidase Inhibitors on the Non-enzymatic Destruction of IAA

It was shown that the phenolics, (IAA-oxidase inhibitors) contained in wheat seedlings, also inhibited the non-enzymatic destruction of IAA by flavin and light (Table 1). Such may be due to the inhibition by the phenolic -OH, of the photo-degradation of the FR to lumichrome in the acidic solution (11).

TABLE 1. *Inhibiting Effect of Phenolics and Boiled Extract of 5-day-old Wheat Seedlings on the Non-enzymatic Destruction of IAA by Riboflavin (Flavin Mononucleotide) and Light*

	Inhibition (%)	
	FR	FMN
Ferulic acid (10^{-3}M)	88.1	97.4
Vanillic acid (10^{-3}M)	77.4	78.2
p-Cumaric acid (10^{-3}M)	56.3	50.5
Boiled extract (20*mg/ml*)	48.2	40.6

Five ml of reaction mixture, which contained 3 ml of Walpole's buffer solution (pH 5.0) and a final concentration, 10^{-4}M of FR (or FMN) with or without various phenolics or boiled extract, was incubated at 30°C, and shaken 100 times per minute under 3000 lux of white fluorescent light.

If there is that system of non-enzymatic destruction of IAA in plant tissue, as reported by Galston et al (6), the destruction of phenolics must occur in the first place. From this point of view, the destruction of phenolics through the H_2O_2-peroxidase system will also hold a very important role in the destruction of IAA in plant tissues.

From the experimental results mentioned above and including the present findings, the authors have theorized the IAA-destroying system in wheat seedlings as shown in Fig. 5. That is, there are two ways for IAA-destruction in the tissue: first, the non-enzymatic destruction by flavin and light, and the other is the

enzymatic destruction by peroxidase. Both ways are masked by the phenolics, so that it will act only when these phenolics are destroyed by the peroxidase and H_2O_2, the latter of which may be produced by flavin enzyme which are activated by light. It is a very notable point that the phenolics contained in wheat seedlings are phenolcarboxylic acids, and a low concentration of these substances having been decreased by such a system, show, on the contrary, the promotion of IAA-destruction by peroxidase.

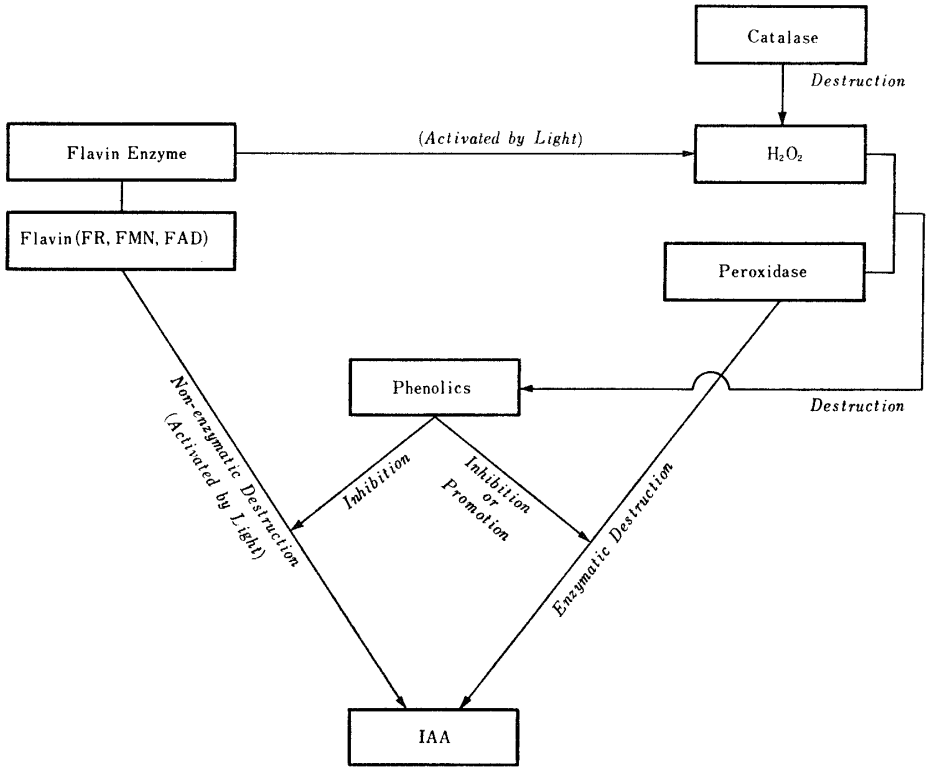

FIG. 5. Scheme of the IAA-destroying system in wheat seedlings.

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References

1) Harada, J. and Wada, K., *Tohoku J. Agr. Res.*, **19**, 8 (1968)
2) Harada, J. and Wada, K., *Tohoku J. Agr. Res.*, **19**, 145 (1968)
3) Galston, A.W., Bonner, J. and Baker, R.S., *Arch. Biochem. Biophys.*, **42**, 456 (1953)
4) Matsunaka, S., *Nogyo-Gijitsu*, **16**, 382 (1961) (in Japanese)
5) Furuya, M., *Kagaku*, **33**, 473 (1963) (in Japanese)
6) Galston, A.W. and Baker, R.S., *Amer. J. Bot.*, **36**, 773 (1949)
7) Pilet, P.E. and Galston, A.W., *Physiol. Plant.*, **8**, 888 (1955)

8) Siegel, S.M. and Galston, A.W., *Arch. Biochem. Biophys.*, **54**, 102 (1955)
9) Roesel, H.A. and Haber, A.H., *Plant Physiol.*, **38**, 523 (1963)
10) Morgan, P.W. and Hall, W.C., *Plant Physiol.*, **38**, 365 (1963)
11) Yagi, K., "*Biochemistry of Flavin*," Kyoritsu Publ. Co. Ltd., Tokyo, p. 296 (1957) (in Japanese)