Kor. J. Pl. Prot., Vol. 13, No. 1, p.33~39, 1974 한국식물보호학회지 제13권 제 1 호

Studies on the Relationship Between Chemical Contents of Rice Plants and Resistance to Rice Blast Disease

Kwon, Shin Han* · Oh, Jeung Haing* · Song, Hi Sup*

水稻體의 含有成分과 稻熱病 抵抗性에 關한 硏究

權 臣 漢* · 吳 正 行* · 宋 禧 燮*

접수일자 : 4月 13日

Abstract

For the investigations on the mechanism of blast disease resistance in the rice variety "Tong-il", the chemical contents such as silicate, nitrogen and flavonoid in the leaf blades of the variety were determined as compared to moderately susceptible variety "Pung-kwang". A correlation between disease severity of rice blast and the chemical contents in the variety "Pung-kwang" were also studied.

The results showed that silicate and flavonoid contents were decreased inversely with application of nitrogen fertilizer, and negative correlation between silicate and flavonoid contents and disease severity in the field were observed. In the resistant variety "Tong-il", these chemical contents and silicified cells were significantly higher than in the variety "Pung-kwang". From these results, it seemed that the high contents of silicate and flavonoid in the leaf blades were related closely to the resistance of the variety "Tong-il".

Introduction

As known by many investigators, resistance to rice blast disease is related with chemical contents in rice plants. Adyanthya[1] and Miyake[10] noted a higher silicate content in the leaf blades of the resistant variety than in those of susceptible. It was also referred that increase in silicate application resulted in blast resistance by accumulation of silicate content in the rice plants. Akimoto[3] reported, it had a close relations with SiO_2/N ratio which was influenced by application of nitrogen and silicate fertilizer. While physiological role of silicate related to blast resistance has not been cleared, Volk et al[17] suggested that organo-silicon components of the cell wall might exert inhibiting effects to the activity of certain enzymes participating in blast invasion. Whereas Yoshida[20] concluded that it was attributable to preventing host cells from fungal invasion by mechanical effects of the silicified cells formed on epidermal tissues by accumulation of silicate in state of silicagel.

Meanwhile, it has been shown in many experiments [4,6,7,9,15,18] that a correlation exist between the degree of resistance and phenol level in various plants. In the case of rice plant, Tamari[15] found that the content of polyphenol compounds in the leaves of Indica type rice varieties was good enough for inhibition of blast spore germination. Recently, polyphenols

* Applied Genetics Lab., Korea Atomic Energy Research Institute, Seoul, Korea(韓國原子力硏究所, 應用遺傳學硏究室)
This investigation was supported in part by a grant from IAEA and SIDA.

containing orthodihydroxy phenyl radical were higher in leaves of resistant variety than those of susceptible was reported by Wakimoto and Yoshii[18].

Therefore, the present study was attempted to find effect of fertilizer applications on the chemical contents in the rice plant and to reveal mechanism of blast resistance in the variety "Tong-il" in relation to the chemical contents.

Materials and Methods

A leading rice variety "Pung-kwang" was transplanted in 32 factorial design at the paddy field of Kimpo where blast disease has habitually occurred. Three levels of fertilizers were applied in 10, 15, 20 kg/ha of nitrogen and 0, 300, 600kg/ha of silicate. Nitrogen fertilizer was applied in three times during the growing period in ratio of 4:3:3 and silicate was treated as a basal fertilizer. For a comparative study on the resistance, varieties "Pung-kwang" and "Tong-il" were planted in both conditions of upland and paddy field. The third leaf blade from the top of the plant was used for determination of chemical contents and 10 hills of each treatment were sampled at random at different growing stages. Every test was carried out in three replicates.

1) Analysis of nitrogen and silicate contents in the rice plant

The sampled leaves were dried at 90°C overnight and crushed finely in mortar and pestle. The crushed were dried again for 2 hours at 60-70°C and kept in desiccator until they were put to use for chemical analysis. Total nitrogen and silicate contents were determined by Micro-kjeldahl and gravimetric method, respectively.

2) Determination of flavonoid content in the leaf blades

Two grams of chopped leaves were boiled with 20 ml of 70% methyl alcohol for 3 minutes. After macerate it again in a homogenizer, it was filtered through filter paper No. 2 and adjusted to 100ml with 70% methyl alcohol. One ml of the filtrate were mixed with 1ml of 70% methyl alcohol saturated with Na_2CO_3 and then the flavonoid content was determined with Beckman Spectrophotometer adjusted in a 400mμ wavelength.

3) Blast disease severity of symptoms in paddy field.

Infection percentage of the leaves was calculated with the percent diseased leaves to total leaves observed. Neck blast index was indicated by following formula.

$$\text{Neck blast index} = \frac{A \times 0.7 + B \times 0.5 + C \times 0.3}{D \times \text{No. of spikes examined}}$$

A: Whole spikes damaged entirely
B: More than 70% damaged
C: More than 30% damaged
D: Healthy spikes wholly

4) Microscopy of silicified cells in leaf blade

Leaf blade was cut in a piece 1cm long. The 15 pieces of each variety were soaked in 70% ethyl alcohol for 3 days or more to remove chlorophyll pigments, and stained in a beaker containing 20ml of safranin-phenol reagent and then boiled it gently for 1 minute. Silicified cells were examined under a microscope with optimal magnification (×150).

Results

Rice plants grown at the low productive field habitually brought about blast disease were affected on nitrogen, silicate and flavonoid contents in the rice plant by different level of fertilizer applications. These chemical contents also appeared to be correlated closely with resistance to blast disease.

Table 1 shows the chemical contents in rice plants of various growth stages applied with different level of fertilizers. Nitrogen content was increased in proportion to application of nitrogen fertilizer regardless of the amount of silicate application. However silicate content was decreased inversely by excess application of nitrogen fertilizer, while it was increased proportionally with application of silicate fertilizer. With advanced in age of the plants, it had a tendency to be increased in silicate content constantly until ear-forming stage, whereas it was rather decreased in nitrogen content.

Table 1. Nitrogen and silicate contents of leaf blades at different levels of nitrogen and silicate fertilizer applications in the variety "Pung-kwang".

N-level[c]	July 13[a]			July 29			August 24			Combined			
	Si_0[b]	Si_1	Si_2	Si_0	Si_1	Si_2	Si_0	Si_1	Si_2	Si_0	Si_1	Si_2	Total
Nitrogen contents (%)													
N_1	4.06	3.90	3.82	2.62	2.63	2.61	2.01	1.85	1.98	8.69	8.38	8.41	25.48
N_2	4.44	4.60	4.44	3.03	2.84	2.93	2.14	2.08	2.08	9.61	9.52	9.45	28.58
N_3	4.48	4.06	4.77	2.89	3.47	3.31	2.33	2.11	2.22	9.70	9.64	10.30	29.64
Total	12.98	12.56	13.03	8.54	8.94	8.85	6.48	6.04	6.68	28.00	27.54	28.16	83.70
Silicate contents (%)													
N_1	5.05	4.59	5.04	2.69	4.31	4.61	4.71	5.52	5.61	12.45	14.42	15.26	42.13
N_2	3.59	3.72	4.06	3.51	3.91	4.01	4.45	4.79	5.34	11.54	12.42	13.41	37.37
N_3	3.45	3.29	3.88	3.23	3.80	3.64	4.28	4.56	4.79	10.96	11.65	12.31	34.92
Total	12.09	11.60	12.98	9.43	12.02	12.26	13.44	14.87	15.94	34.95	38.49	40.98	114.42

a: Date Sampled

b: Silicate fertilizer levels, Si_0: 0, Si_1 300kg/ha, Si_2: 600kg/ha.

c: Nitrogen fertilizer levels, N_1: 10kg/ha, N_2: 15kg/ha, N_3: 20kg/ha.

Leaf blades of 10 hills from each plot were sampled for analysis.

Table 2. SiO_2/N ratios in the leaves of rice plants applied with different level of nitrogen and silicate in the variety "Pung-Kwang".

Si-level	Nitrogen level			
	N_1	N_2	N_3	Avg.
Si_0	1.42	1.20	1.13	1.25
Si_1	1.72	1.30	1.21	1.41
Si_2	1.83	1.42	1.20	1.48
Ave.	1.66	1.33	1.18	

SiO_2/N ratio implies a great significance as a factor relevant to blast disease resistance rather than absolute amount of silicate in the rice plant. Table 2 illustrates results of the experiments in which the ratio appeared to be enhanced with silicate application but reduced by excess nitrogen fertilizer applied. The same value of SiO_2/N ratio between $SiO_2 \times N_2$ plot and $Si_0 \times N_1$ plot was found and this result implies that sufficient application of silicate fertilizer will be able to raise capacity of nitrogen application to a certain extent by means of increase in silicate content of the rice plant and thus to be improved in resistance to blast disease. Meanwhile, Table 3 shows that the flavonoid contents were also increased with age of the rice plant as well as silicate contents. Relationship between the chemical contents in rice plant and blast disease is given in Table 4. Positive correlations between both of leaf and neck blast and nitrogen contents were observed, whereas flavonoid and silicate contents were correlated with blast disease negatively. Nitrogen content appeared negative correlation to flavonoid and silicate contents significantly.

Table 3. A comparison of nitrogen, silicate and flavonoid contents in the plants of the rice variety "Pung-kwang".

Date sampled	Nitrogen[a]	Silicate[a]	Flavonoid[b]
Aug. 28	2.47	4.01	0.145
Sept. 6	2.21	4.30	0.165
Sept. 16	2.08	4.92	0.352
Avg.	2.25	4.41	0.221

a: Nitrogen and silicate contents (%) were analyzed with the leaf blades of 10 hills from each plot.

b: Flavonoid content (%) was determined with 3rd leaves from the top of the rice plants.

In order to find factors pertinent to blast resistance in variety "Tong-il", the chemical contents analyzed in various stages of growth as compared with those of moderately susceptible variety "Pung-kwang" were presented in Table 5. It was found that flavonoid and

Table 4. Correlation coefficients between rice blast disease and chemical contents in the rice plants of the variety "Pung-kwang".

Items	N-content	Si-content	Flavonoid
Leaf blast (July 13)	0.38	−0.82**	−0.618
Leaf blast (July 27)	0.78**	−0.33	−0.833**
Top leaf blast	0.44	−0.30	−0.506
Neck blast	0.72	−0.50	−0.382
Flavonoid content in leaf blade	−0.72*	0.80**	
Silicate content in leaf blade	−0.84**		

**, *: Refer statistically significant at 1% and 5% levels, respectively.

silicate contents were much higher in "Tong-il" than in "Pung-kwang", while nitrogen content was rather higher in "Pung-kwang" than in "Tong-il". The contents in both varieties had a tendency to increase gradually according as the rice plants were aged. Particulary, the flag leaves of both varieties contained the contents in large quantities but nitrogen content was reduced.

In view of the fact that mechanism of plast resistance by means of silicate content in rice plant is closely related with the number of silicified cells formed in epidermal tissues, the silicified cells in the leaves of both varieties were examined and the

Table 5. Chemical contents in the leaves of varieties "Tong-il" and "Pung-kwang".

Days	Nitrogen[a]		Silicate[a]		Flavonoid	
	Tong-il	Pung-kwang	Tong-il	Pung-kwang	Tong-il	Pung-kwang
35[b]	3.9	3.7	3.5	3.1	0.433	0.398
55	3.9	4.0	3.4	2.4	0.421	0.396
75	3.9	4.2	3.6	2.5	0.448	0.392
95	2.8	3.9	3.7	2.6	0.356	0.399
115	2.6	4.2	3.8	3.6	0.548	0.488
F.L.[c]	2.4	3.9	4.6	4.2	—	—

a: Chemical contents (%) were determined by the same method as before.
b: Denotes days after sowing.
c: Flag leaves only were analyzed for determination of the chemical contents.

Table 6. Number of silicified cells at 5 leaf stage of varieties "Tong-il" and "Pung-kwang".

Sample No.[a]	Tong-il				Pung-kwang			
	Youngest L.[b]	2nd L.	3rd L.	4th L.	Youngest L.	2nd L.	3rd L.	4th L.
1	0	0	80	1275	0	0	23	573
2	0	0	1713	1134	0	0	1	260
3	0	95	2366	1833	0	0	0	115
4	0	693	2832	773	0	0	7	14
5	0	0	370	199	1	11	0	9
6	1	262	65	1521	0	1	95	10
7	0	498	4502	697	0	4	2168	12
8	0	263	351	1353	0	0	1	822
9	1	0	387	1173	0	0	0	16
10	0	288	1693	1394	0	2	0	409
11	0	72	597	1365	0	0	1	0
12	0	63	676	1293	1	3	3	42
13	0	0	239	1411	0	0	54	0
14	0	98	359	178	0	1	0	0
15	0	0	2465	1457	0	5	26	37
Avg.	0.2	155.5	1242.1	1137.0	0.2	1.8	158.6	154.6

a: Silicified cells were examined with a piece of 1cm long leaves. A total of 15 leaves were sampled from each variety.
b: Leaves from the top of the rice plants.

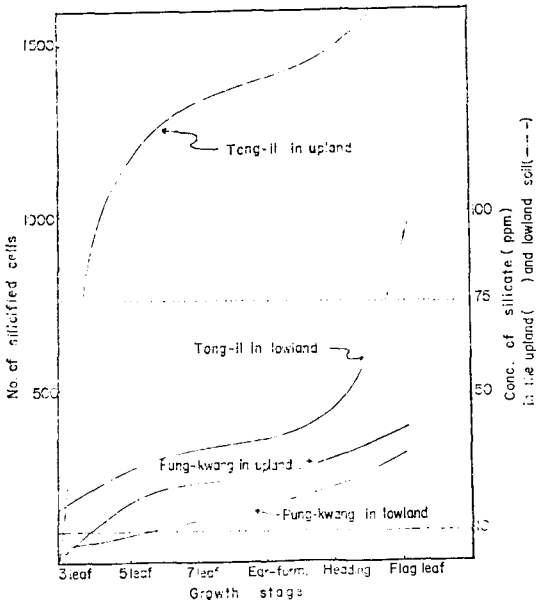

Fig. 1. Number of silicified cells in the 3rd leaves of the varieties "Tong-il" and "Pung-kwang".

results are presented in table 6. In the youngest leaves of both varieties silicified cells were scarecely observed. Although large variance was found among individuals, the lower leaves contained the more silicified cells and large quantity of silicified cells were observed in the resistant variety "Tong-il" (Fig. 2) as compared to the susceptible variety "Pung-kwang" (Fig. 3).

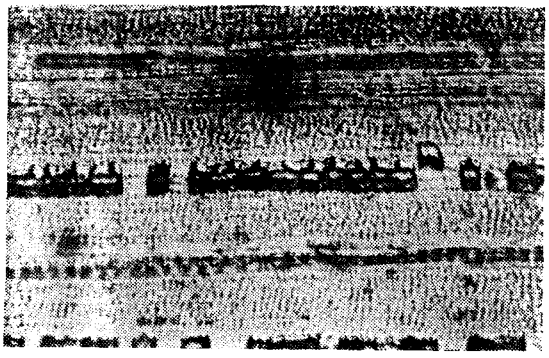

Fig. 2. Silicified cells formed on the epidermal tissues of leaf blades in the variety Tong-il grown at lowland.

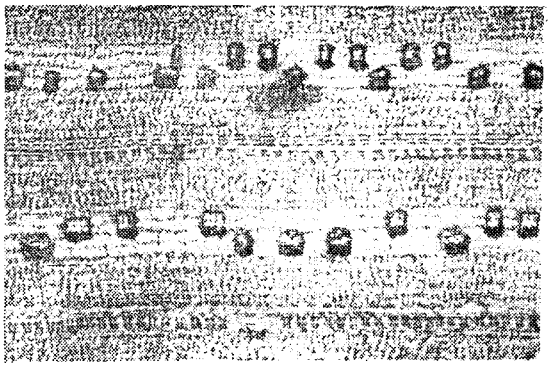

Fig. 3. Silicified cells formed on the epidermal tissues of leaf blades in the variety Pung-kwang grown at lowland.

As shown in the figure 1, much more silicified cells were formed in the rice plants grown at upland soil containing higher level of silicate than at paddy soil, and were formed in the variety "Tong-il" than in the variety "Pung-kwang" through whole term of growth. Silicified cells had a tendency to rise gradually in their number with advanced in age of the plants in both varieties and made conspicuous increase in the flag leaves.

Discussion

Although there are many reports, regarding disease resistance are increased in proportion to silicate content in certain cereal crops, such as barley, rye[11] and wheat[2], the effect of silicate pertaining to disease resistance seems to be remarkable in rice plant which prefer to absorb silicate in large amounts. Results obtained in this experiment are in good agreement with the many reports (10-12, 19) that infection by rice blast disease were influenced with application of nitrogen and silicate fertilizer. This fact might be attributable to reduction of silicate and flavonoid contents relevant to blast resistance by excess application of nitrogen fertilizer and thus result in being susceptible to blast disease.

Yoshida et al [20] clarified the chemical form of silicate in rice plant and its role in mechanical resistance to the blast disease. Silicate absorbed in rice plant deposited mostly in the form of silica gel to the epidermis, vascular system and sclerenchyma,

inasmuch as it is difficult being translocated in the plant. The polymerized silicic acid also fills up interstices of cellulose miscelles, making a silicate cellulose membrane. No enzyme has known to participate in the polymerization of silicic acid. The number of silicified cells is larger in rice plants grown on water-flooded soil than in plants grown on soil than in plants grown on soil with low moisture contents and puncture resistance also increases with increasing soil moisture [14]. Even though the puncture resistance seems not be attributed solely to the silicification of rice leaves, the higher the puncture resistance of tissues, the higher the resistance to blast disease. Accordingly, it seems possible to assume that the characteristics of blast resistance in the variety "Tong-il" is mainly attributed to a remarkable high level of SiO_2/N ratio, which is due to the high silicate, and flavonoid contents in the plant as compared to those in the plant as compared to those in the variety "Pung-kwang", Akai[2] pointed out, toughness, silicification and total amount of silicic acid deposited in the plant tissues increased in susceptible variety with supply of silicate, but showed no definite tendency in resistant variety. In this experiment, however, the high silicification in the resistant variety "Tong-il" was marked and more conspicuous when the rice plant were grown at upland soil abundant in silicate content (Fig. 1). The difference may be due to the absorption capacity of the varieties. In particular that number of silicified cells were much higher in the flag leaves of "Tong-il" implies a great significance in resistance to neck blast of "Tong-il" in view of the numerous reports[8] that number of silicified cells in the flag leaf closely related with resistance to neck blast.

Hypersensitive reaction in the variety "Tong-il" has been observed in a very common form of defense reaction to blast disease. This suggests a significance of flavonoid content related to blast resistance in the variety "Tong-il". Ku'c[9] presented an experiment to increase in disease resistance by infusing young apple plants with phenylalanine. The increase in resistance with increased amounts of phenol compounds which were synthesized from the phenylalanine pool. It seems probable to conclude that the higher level of phenolic compounds in the resistant variety reflects in fact a greater synthetic capacity for these substances. Meanwhile, Szweykowska[9] postulated that aromatic biosynthesis and nitrogen metabolism may stand as a competing pathway. The results that nitrogen fertilization decreased in flavonoid content give assent to the hypothesis. These are also consistant with the observation that application of nitrogen fertilizer in large amounts tends to increase susceptibility of cereals to rust disease and at the same time decrease the total phenol level[6]. The same correlation was apparent in the rice blast disease[18].

Race specificity due to differences of geographic distributions of the pathogenic races accounts for the blast disease resistance in the variety "Tong-il" with the evidence that "Tong-il" which is highly resistant to blast disease in Korea were susceptible in the Philippines[5]. However, plant could be improved genetically inbreeding for blast resistant variety.

摘　　要

統一 品種이 가지는 稻熱病抵抗性의 要因을 究明하기 爲하여 水稻體內에 含有하는 窒素, 硅酸 및 Flavonoid 含量을 分析하고 施肥水準을 달리하여 栽培한 豊光에서 이들 成分含量이 稻熱病 發病率에 미치는 影響을 調査하였다.

1. 水稻體內 硅酸과 Flavonoid 含量은 窒素 施肥量과 反比例하고 硅素 施肥量과는 正比例하여 增加하였다.

2. 稻熱病 常習畓에서의 稻熱病 發病率은 體內 窒素 含量에 正比例하고 硅酸含量에 反比例 하였다.

3. 稻熱病에 對하여 抵抗性인 統一에서는 抵抗性의 要因이 되는 硅酸 및 Flavonoid의 體內含量이 罹病性인 豊光에 比하여 현저히 높았다.

4. 硅化細胞의 數는 古葉일수록 많고 生育의 進展에 따라 增加하였으며 豊光에서 보다는 統一에서 현저히 높았다.

5. 이들 體內 成分의 含量은 統一이 갖는 稻熱病 抵抗性의 한 要因인 것으로 보였다.

Summary

Tests were made for investigation of resistance to rice blast disease in relation with chemical contents existing in the rice plant.

1. Silicate and flavonoid contents in the rice plant

were increased in proportion to silicate application but decreased with nitrogen application.

2. Negative correlations between blast disease and contents of silicate and flavonoid were found, whereas blast disease was directly proportional to increase of nitrogen application.

3. Silicate and flavonoid contents in the rice plant was higher conspicuously in blast resistant variety "Tong-il" than in susceptible variety "Pung-Kwang".

4. Number of silicified cells in the leaf tissues was higher remarkably in "Tong-il" than in "Pung-kwang".

5. More silicified cells were formed in aged leaves than in young leaves and increased with advanced age of the plant.

6. It was known that the chemical contents were one of the factors relevant to blast disease resistance in the variety"Tong-il".

References

1. Adyanthya, N. R. and G. Rangaswami, 1952. Distribution of silica in relation to resistance to blast disease in rice. Madras Agr. J. 39: 198-204.
2. Akai, S. *et al.*, 1958. Effect of silicon supply on the resistance of wheat to powdery mildew. Report of Research Investigations supported by Min. of Education Jap. 48:42-50.
3. Akimoto, S., 1939. Physiological studies on the susceptibility of paddy rice to the blast disease. Proc. Crop Sci. Soc. Jap. 11: 165-167.
4. Barnes, E.H. and E.B. Williams, 1960. A biochemical response of apple tissues to fungus infection. Phytopath. 50: 855-856.
5. Chung, H.S., 1972. Reactions of some IR lines of rice to *Pyricularia oryzae* in Korea and IRRI. Kor. J. Pl. Prot. 11: 15-18.
6. Farkas, G.L. and Z. Kiraly, 1962. Role of phenolic compounds in the physiology of plant diseases and disease resistance. Phytopathol. Z. 44: 105-150.
7. Holowczak, J., J. Ku'c, and E.B. Williams, 1962. Metabolism of DL-and L-phenylalanine in malus related to susceptibility and resistance to *Venturia inaqualies*. Phytopath. 52: 699-703.
8. Hori, M., K. Uchino, and Y. Kijima, 1957. Correlation between the number of silicified cells in uppermost leaves of rice plants and neck blast. Plant Protect. Jap. 11: 333-336.
9. Ku'c, J. 1964. Phenolic compounds and disease resistance in plants. In V. C. Runeckles (Ed.) Phenolics in normal and diseased fruits and vegetables. pp. 63-81.
10. Miyake, K. and M.Ikeda, 1932. Influence of silica application on rice blast. J. Sci. Soil and Manure Jap. 6.53-76.
11. Ou, S. H., 1963. The Rice Blast Disease, Proc. Symp. at IRRI, Johns Hopkins Press, Baltimore, Md·
12. 白壽鳳, 1971. 水稻品種들에서 P.K.Si의 施肥量이 苗形質과 稻熱病侵入抵抗 및 擴大抵抗에 미치는 영향. 건대학술지 12 875-883.
13. 朴天緖, 1967, 窒素와 硅素의 相互作用이 水稻增收에 미치는 영향. 植環研報. 1-14.
14. Suzuki, H., 1935. Studies on the susceptibility of the rice plant to blast and Helminthosporium diseases and on the anatomical characters of the plants (III). J. Coll. Agr. Imperial Univ. Tokyo 13 277-331.
15. Tamari, K. and J. Kaji, 1957. Biochemical studies on the blast disease of the rice plant (IX). The stimulating action of piricularin on the rice plants respiration and studies on the polyphenols of rice plant. Jap. Agr. Chem. 31: 538-544.
16. Tasugi, H. and K. Yoshida, 1958. Effects of silicon supply on the resistance of rice plant to blast disease. Report of Research Investigation supported by Min. of Education Jap. 48: 31-36.
17. Volk, R.J., R.P. Kahn and R.L. Weintraub, 1958. Silicon content of the rice plant as a factor influencing its resistance to infection by the blast fungus, *Piricularia oryzae*. phytopath. 48: 179-184.
18. Wakimoto, S. and H. Yoshii, 1958. Relation between polyphenols contained in plants and phytopathogenic fungi. (1) polyphenols contained in rice plants. Ann. phytopathol. Soc. Jap. 23: 79-84.
19. Yoshida, S., 1961. Some aspects of the role of silicon in plants. J. Sci. Soil and Manure Jap. 31: 42-48.
20. Yoshida, S., Y. Ohnish, and K. Kitagishi, 1962. Histochemistry of silicon in rice plants. Soil. Sci. Plant Nutrition 8 30-41.