which is bred from customry A and B cultivars.

Furthermore, both antigen from introduced cultivar and that from custmary one are reacted against the antiserum produced from antigen of newly introduced cultivar. In case when an unique pattern, which is never been experienced in the customary cultivar, is seen in the pattern of antigen by introduced one, the introduced cultivar may have a good reason of being endowed with an unique factor which may not be cited in the customary cultiver.

Immuno-chemical methods have proved that they are sound ones, to be applied for breeding of corn with considerable accuracy.

References

1) Grabar, P. and Williams, C. A. 1953. Méthode permettant létude conjuguée proprietés electrophoréti-ques et immunochimiques d'un mékange de protéines application au sérum sanguin. Biochim. Biophys. Acta. 10:193-194.

2) Ouchterlony, Ö. 1948. Antigen-antibody reactions in gels. Acta. Pathonl. Microbiol. Scand. 25:186-191.

Microbial Nitrogen Fixation in Japanese Paddy Soils

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Possible significance of microbial nitrogen fixation in nitrogen economy of paddy field

The total area of cultivated land in Japan is about 7,000,000 ha, 45% of which, about 3,150,000 ha, is paddy field.

In paddy field the decrease of soil fertility is generally less than in upland field. This fact has been pointed out by many research workers for a long time. If no fertilizer would be applied, there would be little harvest in upland field, while in paddy field the harvest might decrease at first, but after that, though not much, yet a constant level of harvest would be kept up year after year.

Many factors can be given as the reasons why paddy soils are more fertile than upland soils. Among them, natural supply of plant nutrients, especially of nitrogen, is considered to be one of the most dominant factors.

Irrigation water, rain or snowfall brings about more or less natural supply of nitrogen, but the amount is usually estimated not to be so much, and we must assume that considerable microbial nitrogen fixation must occur in paddy soils to account for the phenomenon.

This is clearly shown in the results of various kinds of serial field experiments which have been carried out for several decades at many of agricultural experiment stations in Japan.

For instance, in Saitama agricultural experiment station a field experiment regarding the cumulative effect of annual application of calcareous fertilizer on rice crop has been carried out since 1904. Table 1 indicates the result of the experiment.

As is shown in Table 1, crop yield in limed plots was always higher than that in not limed plot throughout the whole period of the experiment. Of course, nitrogen absorbed by rice plant during the period must have been much more in the former plots than in the latter, yet decrease in total nitrogen content of soil in the former is rather too small for the increased yield. Sakai assumed that microbial nitrogen fixation was more vigorous in the former than in the latter. Exactly the same sort of result was obtained in the

Table 1. Result of liming experiment at Saitama Agricultural Experiment Station.

treatment	brown rice yield (Kg/10a)					total nitrogen of soil (1950) %
	1904～1913	1914～1923	1924～1933	1934～1943	1944～1950	
No fertilizer, no lime	225.0	207.0	174.0	150.0	160.5	0.175
No fertilizer, lime 112.5kg/10a	259.5	231.0	202.5	180.0	172.5	0.166
No fertilizer, lime 375.0kg/10a	301.5	285.0	217.5	168.0	180.0	0.757

cases of Ishikawa or Fukui agricultural experiment station, and in these cases annual application of phosphorus and lime effected also more nitrogen gains. In a field experiment on the effect of phosphorous fertilizer which was carried out at Hiroshima agricultural experiment station, Hirano found that the application of phosphorous fertilizer was effective for the maintenance of the nitrogen fertility of paddy soils and deduced that this was attributable to the prevention of nitrogen loss or the promotion of nitrogen fixation by the application of phosphorous fertilizers. Okuda investigated the nitrogen balance in paddy soils for 5 years using lysimeter in his study on the effect of drainage on rice crop. In the series waterlogged all the year round, crop yields were generally higher than that in the series waterlogged during rice crop period only. Quite similar trends were shown in the nitrogen uptake by rice plants. The average nitrogen uptake of the former series through 5 years was higher than that of the latter series. Nevertheless, total nitrogen content of the soil in the former series were higher than that in the latter series. This tendency was observed regardless of the measure or methods of manuring. Considering that the amount of nitrogen carried into the paddy soil by irrigation water was only 7.5kg per ha and that an appreciable part of the soil nitrogen might be lost in water and air, he concluded that no small amount of atmospheric nitrogen must be fixed under the waterlogged condition.

With regard to the nitrogen supply alone, this problem is really not so serious in Japan. Because we can supply the element to the soil in the form of chemical fertilizer at any time. Microbial nitrogen fixaton, however, being considered to contribute much to the maintenance and promotion of fertility in paddy soil through not only nitrogen supply but also other ways, much efforts have been made to work out this problem.

Distribution of nitrogen fixing microorganisms in paddy soils

With regard to the distribution of microorganisms which fix atmospheric nitrogen in paddy soils, a number of works have been accumulated. In 1924, Yamagata reported that *Azotobacter* was found in about 30% of cultivated soils in Japan. In paddy soils they were also distributed and *Az. beijerinckii* was dominant. Itano also observed the occurrence of *Azotobacter* in paddy soils. Recently Ishizawa and Toyoda[1] pointed out that *Azotobacter* was often found in paddy soils and the distribution was wider in non-volcanic soils than in volcanic ones, though the bacterial population was generally as sparse as less than 10^3 per g. Suto indicated that an acid tolerant species, *Az. indicum* or *Beijerinckia*, hitherto believed to be tropical inhabitant, was widely distributed in volcanic acid soils in Tohoku district. Supporting Suto's result, Ishizawa et al also suggested that the acid tolerant *Azotobacter* would widely be distributed in temperate regions.

Clostridium seems to be more widely distributed than *Azotobacter*. Yamagata found that *Clostridium* occurred in 95% of Japanese paddy soils and bacterial population was also very high.

Among the autotrophic nitrogen fixers, distribution of blue green algae was investigated in details. For instance, Watanabe [5] tried to isolate nitrogen fixing blue green algae from 851 soil samples collected from south and southeast Asia by repetitive use of nitrogen free culture plates. Purified by dilution method and ultra violet irradiation, the nitrogen fixing ability of these isolates were ascertained and identified. From 33 of 851 soil samples nitrogen fixing blue green algae which belong to *Tolypothrix*. *Cylindrospermum*, *Calothrix*, *Anabaena*, *Nostoc*, *Plectonema*. *Anabaenopsis*, and *Schizothrix* were found. Similar investigations were done by Konishi and Seino [2] and Okuda and Yamaguchi.[3] Konishi et al [2] investigated the distribution of blue green algae in 46 cultivated soils in Hokuriku district, and confirmed that blue green algae were found in every soil and that vigorous growth of the algae was in most cases accompanied with considerable nitrogen gains. Okuda and Yamaguchi [3] also investigated the distribution of nitrogen fixing blue green algae with 117 soil samples collected from all over Japan. According to their data, blue green algae were widely distributed in Japanese soils, and found in paddy soils in especial frequency and abundance. And no small portion of them seemed to be responsible for the nitrogen fixation.

Besides blue green algae, Okuda et al [4] considered the possibility that photosynthetic bacteria may play an important role in the nitrogen fixation in paddy soils. These bacteria had wide distribution in paddy soils and could fix atmospheric nitrogen both independently and in combination with other microorganisms in laboratory experiments, though little information is available about their behaviour under field conditions.

Microorganisms responsible for the nitrogen fixation in paddy soils

Up to the present, following microoganisms have been known as prominent nitrogen fixers.

A) Non-symbiotic microorganisms

1) Heterotrophic bacteria
   a. Aerobic bacteria (*Azotobacter* sp. etc.)
   b. Anaerobic bacteria (*Clostridium* sp. etc.)
2) Autotrophic microorganisms
   a. Blue green algae
   b. Photosynthetic bacteria
   c. Chemoautotrophic bacteria

B) Symbiotic organisms

Symbiotic nitrogen fixation by root nodule bacteria has been widely utilized in agriculture from old times, and the techniques of artificial inoculation has already been established and popularized. It admits of no doubt that the techniques bear an important role in the maintenance and promotion of nitrogen fertility of upland fields. Waterlogged condition, however, being not suited for this symbiotic fixation in general, other agents should be considered for the nitrogen fixation in paddy fields.

With regard to the practical significance and utilization of non-symbiotic nitrogen fixation, many studies have been carried out for years, too. *Azotobacter* is well-known to distribute widely in paddy soils, but the population is generally so sparse that soil sample which contains over 10^3 *Azotobacter* cell per g is scarcely found. Judging from the population, amount of nitrogen fixation by *Azotobacter* is not considered so large however high its nitrogen fixing efficiency is. *Clostridium* has far wider distribution and denser population than *Azotobacter*, but about its behaviour in soil, little information is available at present. Anyway, these heterotrophs need organic matter as substrate for their proliferation as well as nitrogen fixation. It may be possible for heterotrophs to fix atmospheric nitrogen in uncultivated soil or forest soil which has high carbon and low nitrogen contents, but in cultivated soil they may be unable to fix nitrogen unless an enormous amount of organic matter with fairly wide C/N ratio was supplied for them. But this in turn causes severe nitrogen starvation of crops due to the locking up of available soil nitrogen, and therefore is practically impossible. Because of these reasons, heterotrophic nitrogen fixation seems to be herdly expected in cultivated soil.

As to autotrophs, the situation is quite different from heterotrophic process. These nitrogen fixing agents need no carbonaceous materials as substrate. As nitrogen fixing autotrophs, photoautotrophs such as some kinds of blue green algae or photosynthetic bacteria, and chemoautotrophs such as methane bacteria or sulfate reducing bacteria are known. But considering their characters and environmental requirements for growth, it seems that photoautotrophs, especially blue green algae are the most promising agent in paddy soils.

Artificial inoculation of blue green algae to paddy soils

Attempts to inoculate nitrogen fixing blue green algae to promote the fertility of paddy soils or increase the yield of rice crop have been made by some research workers. Watanabe, Nishigaki and Konishi[6] carried out an experiment on the effect of inoculation of nitrogen fixing algae, *Tolypothrix tenuis*, *Calothrix brevissima*, and *Anabaenopsis* sp. on rice crop. Among these algae, *Tolypothrix* was most effective and appreciable increase of rice yield was effected by inoculation of the algae (Table 2).

Encouraged by this result, a large scale joint field experiment on the effect of inoculation of *Tolypothrix tenuis* was conducted at 9 agricultural experiment stations and 3 universities for 5 years, supported by the grant from the Ministry of Agriculture and Forestry.[7] Effect of inoculation varied with localities, but when the algae inoculated proliferates successfully, appreciable increase were observed in ammonification activity of soil as well as crop yields. This tendency was strengthened year after year, and average increase in crop yield in the fifth year came up to 10.6%.

Konishi and Seino[2] reported in details that nitrogen fixation by blue green algae contributed much to the nitrogen fertility of paddy soil, and the inoculation of nitrogen fixing algae brought about appreciable increase of rice yield, although much efforts were needed to secure the proliferation of the inoculum.

The author also conducted a field experiment to investigate the effect of inoculation for 5 years But the statistical analysis of the result indicated no significant effect of inoculation except a slight increase of nitrogen absorption by the rice plant. As one of the main reasons why the inoculation was not successful in this case, the presence of autochthonous algae can be given. In soils, varieties of microorganisms live, restricted by the climatic conditions, geographical features and physical or chemical characters of soil, giving influences to each other, and compose soil microflora specific to each soil. Alteration of soil microflora, therefore, is not readily accomplished. A small quantity of algal inoculum must have struggled for existence and proliferation with autochthonous organisms, some of which have nitrogen fixing ability. In such a case additional treatments favourable to the inoculum seem to be essential. This is true in the case where the inoculum succeeded to grow. But in this case, autochthonous nitrogen fixer in turn was depressed by the inoculated algae, and the effect of inoculation might be cancelled. Anyway, much more studies on soil ecology seem to be needed to secure the constant success in inoculation.

References

1) Ishizawa, S. and Toyoda, H.: Microflora of Japanese soils. Bull. Natl. Inst. Agric. Sci. (Japan) Series B, No. 14, 204-284 (1964). (in Japanese with English summary)

2) Konishi, C. and Seino, K.: Studies on the maintenance mechanism of paddy soil fertility in nature. Bull. Hokuriku Agric. Exp. Sta. 2, 41-136 (1961).

3) Okuda, A. and Yamaguchi, M.: Algae and atmospheric nitrogen fixation in paddy soils. Mem. Res. Inst. Food Sci., Kyoto Univ. 2, 1-14, 4, 1-11 (1952), Nitrogen-fixing microorganisms in paddy soils. Soil and Plant Food 1, 102-104 (1955), 2, 4-7 (1956).

4) Okuda, A. et al: Nitrogen-fixing microorganisms in paddy soils. Soil and Plant Food 3, 131-133 (1957), 5, 73-76 (1959), 6, 35-39 (1960), Soil Sci. and Plant Nutrition 7, 146-151 (1961).

5) Watanabe, A.: Distribution of nitrogen fixing blue green algae in various areas of south and east Asia. J. Gen. Appl. Microbiol. 5, 21-29 (1959).

Table 2. Effect of inoculation of *Tolypothrix* on rice yield

	brown rice yield (Kg/10a)		% increase
well drained paddy field	not inoculated	333.6	
	Tolypothrix inoculated	384.9	15
badly drained paddy field	not inoculated	225.1	
	Tolypothrix inoculated	280.9	25

6) Watanabe, A., Nishigaki, S. and Konishi, C.: Effect of nitrogen-fixing blue-green algae on the growth of rice plant. Nature **168**, 748 (1951).
7) Watanabe, A.: Effect of nitrogen fixing blue green alga, ***Tolypothrix tenuis*** on the nitrogenous fertility of paddy soil and on the crop yield of rice plant. J. Gen. Appl. Microbiol. **8**, 85-91 (1962).

Breeding of Seedless Citrus Variety

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The seedlessness is considered to be quite useful for citrus fruits. Almost all of the leading varieties of citrus in the world are either seedless or nearly seedless. Thus, new varieties bred in the future could not secure its entry into the group of important commercial varieties without possessing this character. In relation to the seedlessness in citrus, various kinds of sterility have been reported. Recently, new types of the sterility were found in some of seedless varieties and hybrids, offering a particular interest for the future citrus breeding.

Reciprocal translocation in the Valencia orange

The Valencia orange is the most important variety among the late maturing citrus, and is grown more widely in the world and in larger acreage than any other variety. In Japan, however, this variety cannot grow successfully because of the unfavorable climate. Thus, the Japanese citriculture is eagerly demanding some other superior late variety well adapted to their climatic conditions.

It was made clear that the very poor seediness of Valencia orange, at least in part, is caused by the formation of a large number of inviable gametes resulted mostly from the segmental interchange of chromosomes (Iwamasa, 1963).

Cytological examinations were carried out on two trees of each of two strains of the Valencia. In pollen mother cells of each of these trees, a quadrivalent was observed frequently at metaphase I (Table 1). Out of 252 quadrivalents, 238 were non-disjunctional closed ring, 7 disjunctional ring, and remaining 7 chain-of-four (Fig. 1.). In the Lue Gim

Table 1. Chromosome configurations at metaphase I in PMCs of the Valencia and Lue Gim Gong Orange

Variety aud Strain	Frequency of PMCs								Total number of PMCs
	$8_{II}+2_{I}$	9_{II}	$1_{III}+7_{II}+1_{I}$	$1_{IV}+6_{II}+2_{I}$		$1_{IV}+7_{II}$			
				Ring[a]	Chain	Ring[a]	Ring[b]	Chain	
Valencia A-I	1	16	—	2	—	74	1	4	98
〃 A-II	2	7	1	—	—	54	1	1	66
〃 B-I	5	14	—	5	—	31	2	0	57
〃 B-II	1	3	1	2	—	70	3	2	82
(Total)	9	40	2	9	—	229	7	7	303
Lue Gim Gong	2	42	—	4	1	151	5	10	215

[a] Non-disjunctional and [b] disjunctional orientation of the quadrivalents.