Conclusion

Young roots and young parts of a root hold a large quantity of water and potassi um, and they actively perform the respiration which contains active TCA cycle→cytochome-cytochrome c oxidase system, which may be coupled most effectively with oxidative phosphorylation. All of the active physiological functions of roots requiring energy, particularly active absorption of ions, will probably be dependent upon the activity of this respiratory pathway.

During the growth period, the average age of roots on a plant is relatively young up to the young panicle formation stage, and young roots perform respiration at a high rate absorbing a large amount of essential nutrients including nitrogen, potassium and phosphorus. The active growth of plant during this period seems to be supported by the respiration of TCA cycle→cytochrome-cytochrome c oxidase system in the root.

After that stage, however, the root becomes older in the average age, and the respiratory rate goes down. In this period, considerable respiration via cytochrome-cytochrome c oxidase system is still maintained mainly in numerous, young rootlets bearing on older roots, which be supporting an active absorption of nutrients during the reproductive stage. Ascorbic acid oxidase and or peroxidase, which is activated with the aging of root, may have a possibility to participate in the maintenance of such activity of roots and rootlets through the oxidizing action against the reduced soil condition.

References

1) Baba, I.: Nutritional studies on the occurrence of Helminthosporum leaf spot and "akiochi" of the rice plant. Bull. Nat. Inst. Agr. Sci., **D7**, 1-157, 1958 (in Japanese with English summary).
2) Baba, I. and K. Inada: Physiological studies on the root of crop plants. I. Some characteristics of rice roots clarsified as to their age in relation to nutrient-absorption. Proc. Crop Sci. Soc. Japan, **27**: 151-154, 1958. (in Japanese with English summary)
3) Inada, K.: Phyiological studies on the root of crop plants. II. Role of cytochrome exidase in respiration and nutrient absorption of rice plants. Proc. Crop Sci. Soc. Japan, **28**: 347-350, 1960.
4) Inada, K.: Physiological characteristics of rice roots, especially with the viewpoint of plant growth atage and root age. Bull. Nat. Inst. Agr. Sci., **D 6**, 19-156. 1967. (in Japanese with English summary)
5) Okuda, A. and E. Takahashi: Studies on the plant-physiological role of silicon for crop-plant (part 8). Some examinations on the specific behavior of lowland rice in silicon uptake. J. Sci. Soil and Manure, Japan, **33**: 217-221, 1962. (in Japanese)
6) Yamada, N.: Physiological basis of resistance of rice plant against overhead flooding. Bull. Nat. Inst. Agr. Sci., **D8**, 1-110, 1959. (in Japanese with English summary)
7) Yoshida, T. and J. Takahashi: Studies on the metabolism in roots of lowland rice (part 6). Respiratory and enzymatic gradient in rice root. J. Sci. Soil and Manure, Japan, **31**: 423-426, 1960. (in Japanese)

Identification of Seed Corn by Immuno-chemical Reaction Method

T. MACHIDA* AND S. SAKAGUCHI**

Chief,* and Researcher,** Kikyogahara Branch Station,
Nagano Prefecture Agricultural Experiment Station.

Recent developments in livestock industry have increased demands for corn so rapidly that needs for breeding improved variety and producing quality seeds of corn are now a matter of urgent concern.

In recent years corn is mostly bred as F_1

with the application of heterosis. Accordingly, an instant Know-how is sought for eagerly to identify highly qualified characters and capacity to combine them among those varieties which are introduced from overseas. In practice, however, much time and energy are required for test crossing between any two varieties or for attesting characters and capacity to combine them which is endowed in a specific variety thus produced. Capacity or difference of capacities of newly introduced breeding materials from overseas is also to be assessed before they are tested.

Difficulties also lie in seed production. By applying heterosis for seed production, admixing of non-hybrid seeds with F_1 seed must be prevented because it may deteriorate the quality of seed to be produced critically. Here, too, a simple method is anticipated in which both hybrid seeds and non-hybrid seeds are easily identified.

In producing quality F_1 seeds of corn, registered stock seed farms must be so situated to be free from pollination from other varieties, because corn is mostly pollinated allogamous.

No matter how far the farm is isolated admixture of pollen is eventually resulted and the characterisric of seed is sudjected to change. Here again, purity of registered stock seeds should be attested by a simple method.

These are reasons why we take up immunochemical reaction as a highly dependable yet simple method for analyzing factors innate to newly introduced varieties, for discriminating hybrid from non-hybrid seed and for testing the purity of registered stock seed.

Identification of F_1 seed by Ouchterlony method

Antiserum is produced from the antigen of protein in F_1 seed of corn, and protein liquid is extracted as an antigen from respective kernel of corn which are drawn by random sampling for the purpose of identification. By reacting the antiserum against the protein liquid, hybrid seed is easily distinguished from non-hybrid seed because proteins in the former are of nature innate both to father variety and to mother variety, while those in the latter are not of nature innate to father variety.

Antigen proteins extracted from respective

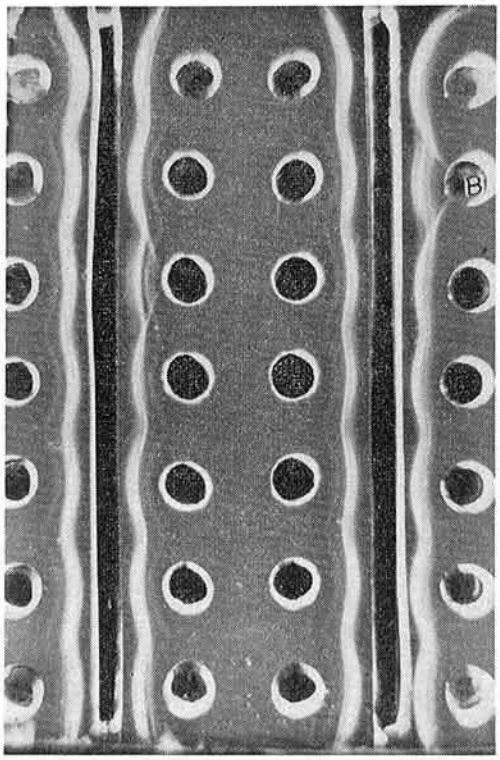

Fig. 1 Photograph of double diffusion analysis of extracts from hybrid and nonhybrid seeds. Detected with an antiserum against extract of hybrid seed.
B: nonhybrid

grain of seeds to be identified are placed in each of the hole as shown in Figure 1. Antiserum is fed along the vertical split. Then both antigens and antibody begin to diffuse on agar gel and form zonal sediments according to relative optimum diffusion of antigens and antibody in reaction. In case when zones are united to the other, the two antigens are assumed homogeneous. Crossed zones of sediments may illustrate thet the two antigens are heterogeneous. When a part of a sediment is united to the other, the components of antigen in the former are considered multiple and are assumed to have some common component with the latter.

In Figure 1, antigen produced from F_1 seed is placed in the topmost and the bottom holes respectively and holes whose sediments contri-

bute to the same pattern as those formed by the said two, signify that hybrid seeds are placed there. Holes developing different patterns is assumably placed with non-hybrid seeds.

Hole B in Figure 1 showes a different pattern as compared with the two extreme hole, while the remaining holes contribute to those formed by the two extremities. Thus, hole B signifies that non-hybrid seed is placed and the remaining hole that hybrid seed is placed. Hybrid and non-hybrid seeds are classified in this way and their qualities, whether excellent or poor, are determined in terms of respective percentage distribution.

Figure 1 also shows that sediment pattern by F_1 antigen has part which is united sediment zone by nonhybrid antigen and other part which is crosed.

Such zonal sediments united signifies that protein common to corn and that peculiar to mother seed both exist. Those sediments developed along antiserum split at A, but not developed at B, suggest that protein peculiar to father seed exists.

Attesting of registered stock seeds by immuno-electrophoresis method

In order to produce qualified seeds, purity of registered stock seed must be maintained at first. We have tried purity attesting of registered stock seed by immuno-electrophoresis method, one of many immuno-chemical approaches.

Antigen from highly purified registered stock seed is placed in the center and antigens to be attested are placed on both sides. Then they are charged with electrophoresis. On finishing electrophoresis, the split is filled with antiserum which is produced from antigen of highly purified registered stock seed. The purity of seed to be attested is determined to the extent that its zonal sediment collate with that of check antigan (Figure 2). Pattern of sediment shown by attesting antigen on the right-hand side of the Figure lacks such A reaction zone as is expected in sediment pattern by checking antigen, whereas sediment pattern by attesting antigen on the left-hand side is short of B reaction zone in sediment pattern by checking antigen. These two examples signify that

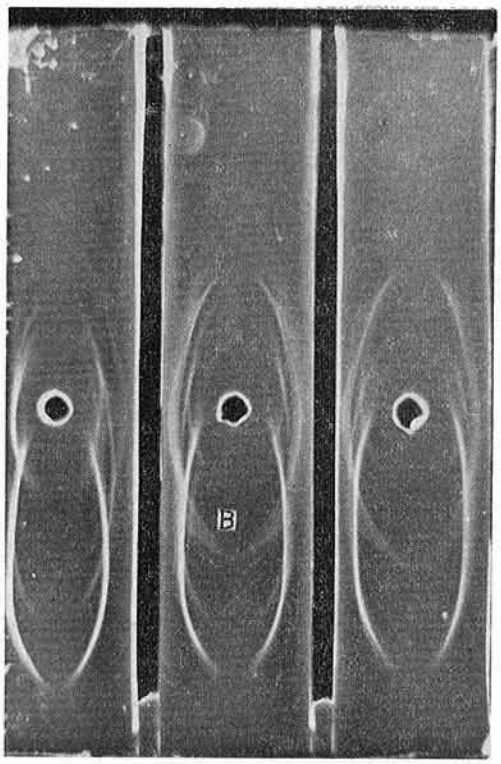

Fig. 2. Immuno-electrophoretograms of extracts from registered stock seeds (pureline-parent) and attesting seed (incressed in an isolated plot). Detected with an antiserum against extract of pureline seed.
R: attesting seed (A arc miss) C: Pureline
L: attesting seed (B arc miss).

purity of seed is low.

In some cases, however, similar sediment patterns by attesting antigen and by checking antigen do not necessarily mean purity of seed is high. By adding antiserum further, which is produced from attesting antigen, such a zonal sediment may develop in the pattern of attesting antigen which may not be expected for that of checking antigen (C in Figure 3). Accordingly, these cases show that attesting seed is low in their purity too. Checking antigen is placed on the right-hand side and attesting antigen in the center and on the left-hand side respectively. After charged with electrophoresis the split is filled with antiserum which is produced from the some antigen as that placed on the left-hand side. Such a

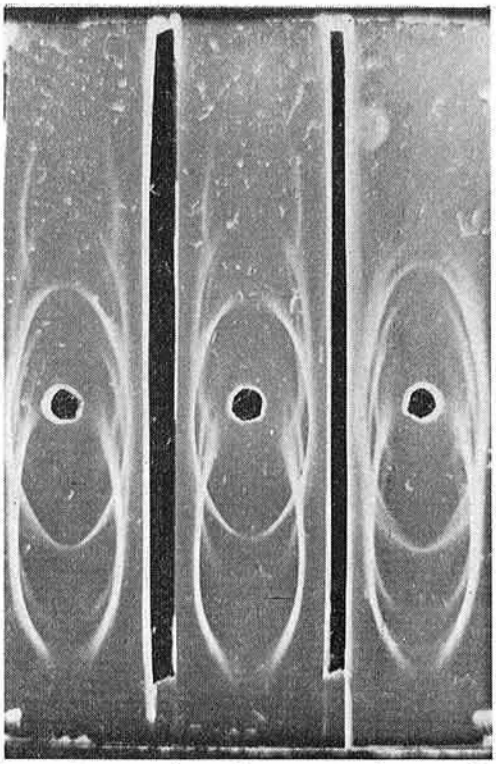

Fig. 3. Immuno-electrophoretograms of extracts from registered stock seed (pureline-parent) and attesting seed (increased in a isolated plot). Detected with an antiserum against extract of attesting seed.
R: Pureline C: attesting seed (A arc miss and C arc add) L: attesting seed (C arc add)

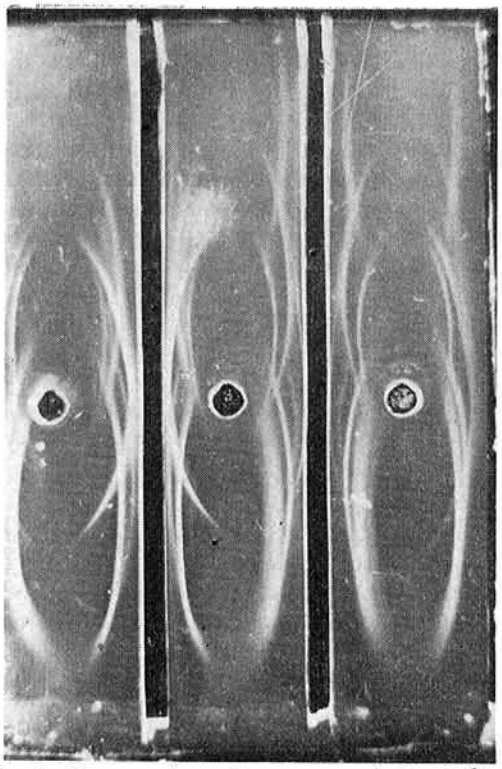

Fig. 4. Immuno-electrophoretograms of extracts from new variety seed introduced and varieties which were already analysed immunologically. Detected with antisera against varieties which were already analysed immunologically.
R: A-variety already analysed (possess specific A arc) C: New variety introduced (possess A and B arc) L: B-variety already analysed (possess specific B arc).

sediment pattern, which is never shown by checking antigen (of registered stock seed) with high purity, is apparently observed in the pattern by attesting antigen.

Thus, a considerablly high purity of registered stock seed may be attested by immuno-electro phoreses method.

Factor analysis for newly introduced varieties

Immuno-electrophoresis method is applied to analyse factor unknown in newly introduced varieties, impromptu.

Antigen and antiserum are prepared respectively from each of customary and introduced cultiver. As shown in Figure 4, antigen of introduced cultivar is placed in the center and that of customary one, A and B, on the both sides of it.

After charging them with electrophoresis, "a" antirerum, which is produced from antigen A, is fed in the A-side split and "b" antiserum, produced from antigen B, is fed in the B-side split. Then the two patterns are compared with each other. The pattern shown by introduced cultivar include both of the entire pattern shown by A cultivar antigen and that shown by B cultivar antigen (Figure 4). Hence, the introduced cultivar is assumed to be F_1

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 electrophorétiques 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

M. YAMAGUCHI

Professor, College of Agriculture, University of Osaka Prefecture

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