PLANT NUTRITION DIAGNOSIS IN JAPAN, WITH A SPECIAL FOCUS ON CROP QUALITY AND THE ENVIRONMENT

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ABSTRACT

This Bulletin reviews recent improvements in the real-time diagnosis of plant nutritional status in Japan, and its extension to farmers. It discusses the benefits from plant diagnosis, including reducing the load of nutrients in the environment while maintaining adequate yields, improving the crop quality, and aiding in the early detection of physiological disorders.

INTRODUCTION

In the 1980's, a lot of work was done in Japan to investigate the relationship between the dosage and timing of nitrogen (N) fertilizer applications and the quality of the rice. It became evident that excess application of N, or applying N fertilizer later than the heading stage, damaged the eating quality of rice. Although Japanese farmers often do not like to reduce their fertilizer applications, these findings made a persuasive case for decreasing the amount of fertilizer applied. Over the past two decades, N input to lowland fields was kept at lower levels than before. Furthermore, the application of slow-releases fertilizers and improved cultivation methods increased N availability, resulting in lower N losses from rice fields.

In the case of vegetables and fruit, fertilizer application levels in Japan are still high. They often leave residual nutrient salts in the soil after the crops are harvested. Two major causes are the sensitive response of vegetable yields to fertilizer levels, and the good appearance of leafy vegetables which have a high N status. Excess N is necessary to maintain high quality in flowers and tea bushes. However, many researchers are currently working to develop minimum pollution agriculture.

This Bulletin reviews the research on the real-time diagnosis of plant nutrition in Japan, and its future role and development.

REAL-TIME DIAGNOSIS OF PLANT NUTRITIONAL STATUS

Improvement in diagnostic techniques

Real-time analysis provides a constant stream of data which reflects a changing situation. In the case of plant nutrition, real-time analysis monitors the nutritional status (adequate, deficient, excess) of the plant. The samples of plant tissue used for analysis are taken from the living plant.

The establishment of efficient and environmentally friendly techniques of fertilizer management based on real-time diagnosis of plant nutrition has been the goal of plant physiologists for a long time, especially for those who attach importance to field work. It was Roppongi (1991) who first used real-time diagnosis in Japan, using the fluid from plant tissues. He showed the relationship between the level of fertilizer applications and the nitrate concentration in the fluid of cucumbers, which have a long harvest period. The ideal N concentration was determined according to the fruit yield.

Keywords: early detection, food quality, Japan, N availability, nitrate, physiological disorders, plant diagnosis

He tested the accuracy of convenient analytical tools such as test strips (Merckquant) and a green meter, and suggested that test strips could be used to test for nitrate in liquid. Roppongi reported critical values as a guide to whether fertilizer should be applied to strawberry (1992) and eggplant (1993). These studies demonstrated the process for determining critical values and developing methods of plant diagnosis (Fig. 1).

Yamada *et al*. (1995) used a simple reflection photometer (RQ-flex) and compact ion meter (Cardy) to analyze levels of nitrate and potassium in the petiole fluids of tomato, and in water extracted from soils. Both measurements of nitrate levels showed a high correlation with the values measured by the ion chromatograph.

Further work has since been done to validate the accuracy of convenient analytical tools, and their use to measure nutrient levels in the fluids of living crop plants. In addition to nitrate and potassium, the applicability of these tools to phosphate, borate and organic components as ascorbate or oxalate have been examined, and the results reported (Hiraoka *et al.* 1990, Takebe and Yoneyama 1995, Morita *et al.* 2001, Kamiyama *et al.* 2000).

Studies based on the real-time diagnosis of plant nutrition have shown great progress in recent years, with improved methods of diagnosis and improved analysis of plant components. By now, critical values for nutrients in plant fluids have been set in several prefectures in Japan, and many trials are being conducted at prefectural institutes.

There has been theoretical organization and validation for diagnosis with plant fluid samples (Yoneyama *et al.* 1995, Takebe *et al.* 2000). Yoneyama *et al.* reviewed characteristic levels of the various nutrient elements in each type of tissue, such as vessels, xylem, leaves and petioles. The constituents of the cytosol and vacuole were also discussed. Takebe (2000) and Takebe *et al.* (2001) compared the levels of nitrate and phosphate both in fluids extruded from the crushed petiole, and water extract from crushed petioles of potato. The nitrate concentrations was similar in samples from both extraction methods, except when the concentrations were very low. However, phosphate levels in the fluid were lower by 5-40% than in the water extracts. In spite of this, the level of inorganic P in the fluid showed a high correlation with the total P in the above-ground portion of the plant. These findings suggest that N and P levels in the fluid reflect the status of the whole plant. This is convenient, since such fluid is readily available for analysis.

Table 1 shows some available methods of practical real-time analysis of fluid from important crops. There are also a few methods which utilize non-destructive analysis, such as SPAD and NIR, but these are not included in this Bulletin.

Nitrate is a common element for diagnosis, because it reflects the nutrient status of the whole plant and the probable yield of almost all crops. In addition, the nitrate level, especially in leafy vegetables, is an important indicator of quality. Although

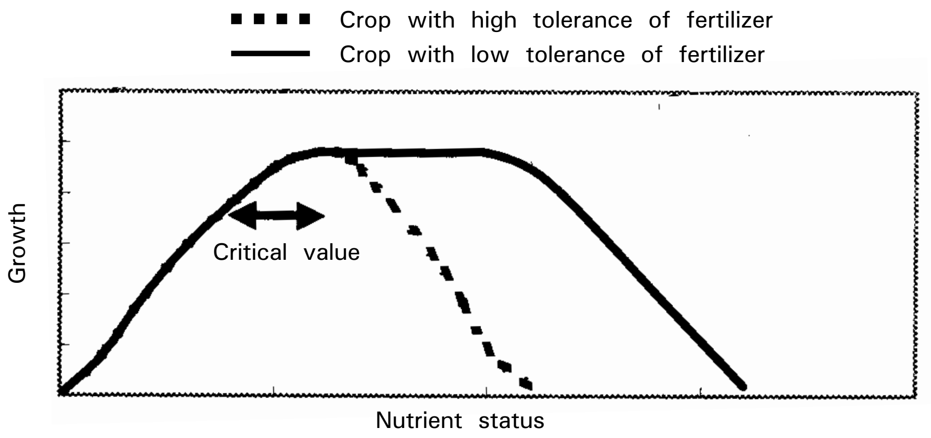

Fig. 1. How to set the critical value

Source: Redrawn after Roppongi (2000)

Table 1

phosphous (P) tends to be deficient in Japanese volcanic-ash soils, excessive applications of P fertilizer can cause eutrophication (i.e. algae blooms because of high nutrient levels) in lakes and rivers.

Selection of the sampling site should be based on avoiding damage to the plant, as well as reflecting the nutrient status of the whole plant. The petiole (i.e. the stalk of the leaf) is a tissue which stores nitrate. It is an effective site for diagnosis because the concentration changes according to the N status of the plant. Takebe (2000) pointed out that the range in nitrate levels was wider in the petiole than in the plant, because of the sensitivity of the petiole. She advised that in periods during which the plant is assimilating a high level of nitrates, sampling should be carried out carefully in order to avoid a large sampling error because the nitrate levels often fall rapidly.

Studies have been also made of which petioles are best for sampling. Yamada *et al.* (1997) reported that petioles which are just beneath fruits 2-3 cm in diameter are the best sample site for tomato. Hikasa (2000) proposed utilizing the petiole of the top leaf below the first truss as the sample. Takebe *et al.* (2001) reported that the petioles of several leaves, all more than 10cm long, should be taken for potato because the nitrate level in the petioles of potato fluctuates according to the location of the leaves. Takebe (2000) suggested that when a potato stem is removed by thinning, the whole petiole of the stem can be used as a sampling material. It has been reported that N content in the fluid taken from vines of cucumber generated from tendrils has a high correlation with N status (Table 1).

A garlic crusher is generally used to collect the fluid. A mortar or food processor can be used to grind the sample. If the sample tissues are hard or lacking in water, a sample can be cut off with scissors or clippers, and prepared as a water extraction.

Sampling after irrigation or rain is not appropriate, because the nitrate level in the fluid will be unstable. Although some reports recommend that sampling should be done during the daytime in fine weather, reports on changes in nitrate levels during the day, or in different weather, are not always consistent. The optimum sampling time should be determined based on scientific evidence. For this, systematic studies are required on the relationship between nitrate levels and environmental conditions such as climate, solar radiation, temperature, the type of tissue and its location on the plant, and cultivation methods.

Samples are generally taken at an interval of one to two weeks. Samples are collected from more than five plants. These samples are then combined for analysis. A simple reflection photometer, test strips, and a compact ion-meter, were used to analyze plant fluid in the field, and the results were compared. So far as nitrate was concerned, the simple reflection photometer and the compact ion meter had good linearity and accuracy (Yamada 1995). The dynamic range was widest when the compact ion-meter was used.

In the case of test strips, the nitrate level is determined by comparing the strip with a color chart. This method has the advantage of being the cheapest (US$0.3 per strip) of the three, and free of maintenance. However, it is not as accurate as the other two. Yamamoto *et al.* (2002) compared a simple reflection photometer with the test strip method (Fig. 2). Color changes in the strips are much more sensitive at low concentrations, but the test strip method sometimes failed at high nitrate concentrations. The critical level was 2000 ppm. However, results of both analytical methods were in agreement as to whether additional N should be applied. The use of test strips can be optimized by diluting the fluid to the most sensitive range, even with higher critical values.

A manual on the integrated culture of greenhouse tomato using plant diagnosis was published by the Hokkaido Prefectural Dhonan Experiment Station in 2003. It describes how and why to analyze plant tissues, and how to use the results as the basis for fertilizer applications. It also provides several case studies of the N status in tomato and its effect on yield.

BENEFITS OF PLANT DIAGNOSIS

Hoshina and Yoneyama (1992) carried out a survey of plant diagnosis procedures, targeting researchers working at prefectural agricultural institutes and extension specialists. The survey was based on a questionnaire, and was

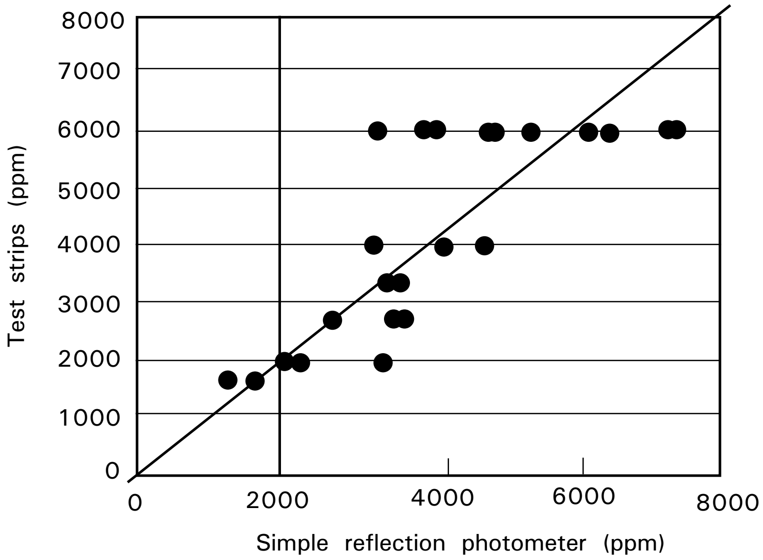

Fig. 2. Accuracy of nitrate measurements

done in 1990, at a time when there were not yet any reports on real-time diagnosis using plant fluids. The results of the survey revealed that those interviewed felt that the purpose of plant diagnosis was:

- To improve crop quality;
- To prevent physiological disorders in the crop; *and*,
- To minimize fertilizer loading from farmland to the environment.

They also felt that in order to carry out diagnosis, they needed an easy method of analysis which could be used under field conditions, and a better understanding of plant nutrition.

Crop quality is an important factor. Although by the end of last century we had achieved a better understanding of how to improve the eating quality of rice, and have developed an easy method of diagnosis, we have not yet made the same advances for vegetables and fruits. The following section discusses the diagnosis of plant nutrition to help farmers improve their fertilizer management, as well as decreasing the nutritional load on the environment.

Avoiding excess fertilizer applications while maintaining yields

One of the main aims of plant nutrient diagnosis is to adapt fertilizer applications so that they match plant demand. This can give a high level of fertilizer availability, and helps reduce the leaching of fertilizer elements, especially nitrate. It is well known that nitrate is a common contaminant of groundwater (Maeda *et al.* 2003). It is also generally believed that nitrous oxide, a gas which is emitted by vegetable and/or fruit gardens when too much nitrogen fertilizer is added, has a strong effect on global warming.

Kato (2000) categorized plant types into four types, based on their nutrient demand (Fig. 3). Crops belonging to Type III, typical of fruit vegetables, have a long cultivation period, throughout which they demand high levels of nitrogen. Heading vegetables and root vegetables (Type II), such as cabbage or carrot, require most fertilizer during their middle stage, when they are translocating nutrients and assimilated products from their outer to their inner leaves, or from the tops to the roots. At later stages of growth, they do not need as high as a level of nutrients.

One case in which nutrient diagnosis succeeded in reducing N application drastically was reported by Yamamoto *et al.* (2001, 2002). It involved the semi-forced culture of greenhouse tomato. The recommended rate for N fertilizer in Chiba Prefecture for greenhouse tomato was 310 kg/ha (150 kg as a basal application and 160 kg applied as a topdressing). When the amount of fertilizer

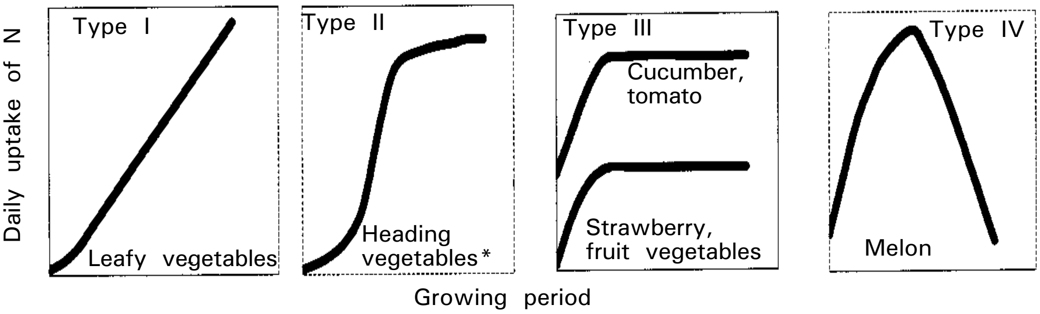

Fig. 3. Classification of types of nutrient uptake by vegetables

* i.e. leaf vegetables which form a head, such as cabbage

Source: Redrawn after Kato (2000)

applied as a topdressing was based on nutrient diagnosis (with a critical value of the petiole fluid of 2000 ppm), total N applications were reduced by 40-70%, irrespective of the soil N level when cultivation began (Table 2). Another use of nutrient diagnosis was shown in farmers' cultivation. One grower (Farmer A) applied abundant N (300 kg/ha as a basal application) while another (Farmer B) applied a normal rate of N (160 kg/ha as a basal dressing), according to their own conventional methods. In the case of Farmer A, no additional topdressing was required, since the petiole nitrate level remained above 2000 ppm during the cultivation period (Fig. 4). Farmer B applied an additional 30 kg/ha N in the form of liquid fertilizer. Both farmers obtained higher yields than their conventional cultivation and saved N fertilizer to the amount of 21% and 14%, respectively. A further reduction in the basal application might even be possible, giving a further saving of N.

IMPROVEMENTS IN CROP QUALITY

As early studies of the diagnosis of plant nutrition show, a very important role is the control of various plant components related to food quality. Japanese consumers tend to emphasize the quality of food, for instance, the taste, freshness, nutritional value, appearance, health effects and safety. If improved nutrient management makes it possible to increase the value of farm products as well as the crop yield, farmers can gain great benefit from plant diagnosis. The diagnosis of components related to quality as well as nutritional status is an important strategy for many farmers.

To achieve this, we need a better understanding of the relationship between cultivation methods and the constituents of crop fluids. Studies are currently being conducted on this.

Takebe (1999) analyzed the relationship between the constituents related to crop quality (rice, leaf vegetables and potato) and nitrogen nutrition. Sugars and ascorbate were treated as favorable constituents, and nitrate and oxalate as unfavorable ones. Although nitrate is an essential component in a wide range of vegetables, levels should be kept low at harvest time because of its harmful effect on human health. Oxalate gives vegetables an acrid taste, and is a possible cause of kidney stones in human being.

It became clear that for rice, a higher level of nitrogen fertilizer applications results in a lower level of sucrose (Takebe 1999). In studies with leaf vegetables, nitrate levels increased rapidly with increased N application until harvest. The levels of sucrose, glucose and ascorbate were highest in the plot where N applications were from 0 to 100 kg/ha, and were lower in plots given more N. The content of oxalate in spinach could be reduced by supplying N in the form of ammonia-N in a hydroponic system. However, oxalate levels were not influenced by the dosage of N.

A field study revealed that less oxalate accumulated in spinach when slow-release ammonium fertilizer was used than with a basal application of ammonium sulfate. This indicates the possibility of controlling oxalate levels in spinach by N nutrition. Oxalate in

Table 2

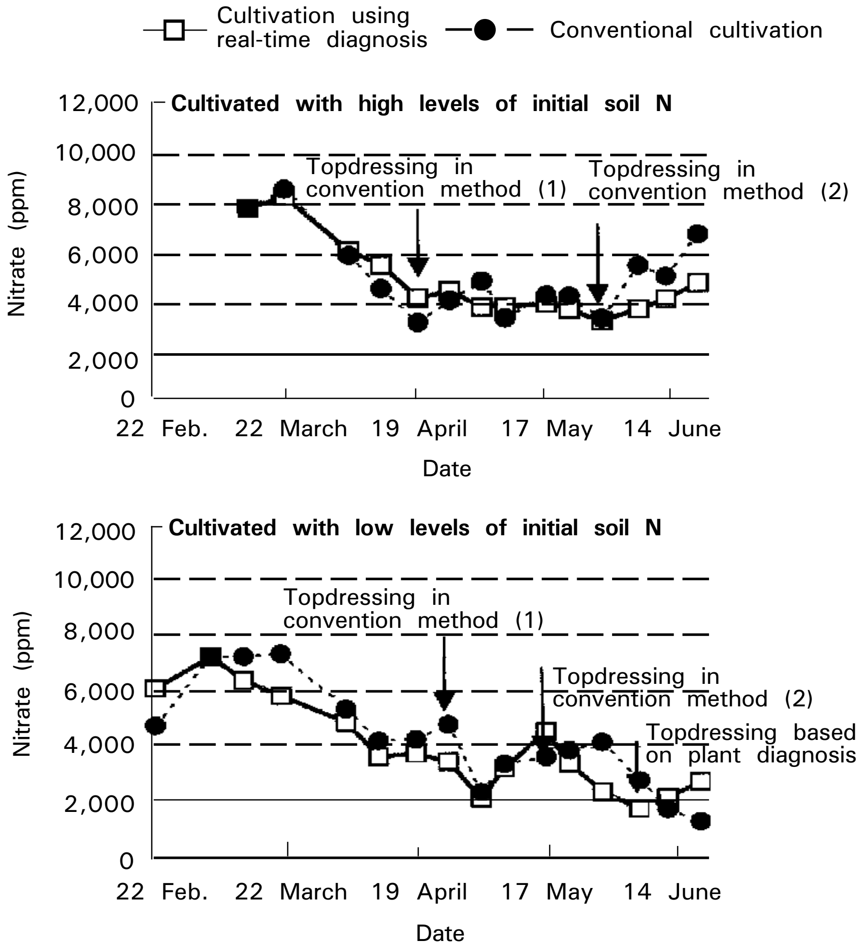

Fig. 4. Changes in nitrate levels over time in petioles of tomato plants cultivated by conventional method and with real-time plant nutrient diagnosis

Source: Yamamoto *et al.* 2002

spinach is supposed to act as a neutralizing agent of alkalized cytosol in the assimilation of nitrate for the synthesis of amino acids (Takebe *et al.* 1995, Takebe 1999). Tanaka *et al.* (2000) showed a high correlation between nitrate reduction and the synthesis of amino acids and oxalate, measured using stable isotopes (^{15}N and ^{13}C). This result supports Takebe's assumption about the function of oxalate in spinach.

Morita *et al.* (1999) and Morita and Tsuji (2002) also showed a higher level of oxalate in tea bushes fertilized with nitrate than in those fertilized with ammonium. Shading, which is an essential technique in the production of Gyokuro, a high-quality tea, raised the nitrate content of tea bushes. This is the result of reduced assimilation because of a lack of sunlight. They suggested, however, that oxalate in tea bushes not only acted as a neutralizer, but was also involved in other functions, because the oxalate level fluctuated with the nitrate level.

Takebe *et al.* (2001) investigated the effect of nitrate levels in the petiole fluid on the yield of potato, and the starch content of the tubers. She suggested that if the nitrate level in petiole fluid was above a critical value, the tuber would not have the required starch content.

Hikasa (1997) produced various nitrate levels in cabbage by applying different amounts of nitrogen fertilizer, and investigated the effects on yield and sugar content. He showed that the sugar level of cabbage fell with increasing N levels in the leaves.

Takano (2003) made it clear that a higher N content in the leaves of peach trees meant a

Table 3. Influence of powdery mildew infection on polyamine concentration in cucumber leaves

	Polymine concentration (μmol/gDW)		
	Putrescine	Spermidine	Spermine
Experiment 1			
Control	0.074	1.28	0.35
Symptoms* +	0.054	1.13	0.41
Symptoms ++	0.034	0.98	0.63
Experiment 2			
Control	0.115	1.60	0.44
Symptoms +	0.065	1.34	0.46
Symptoms ++	0.031	0.98	0.70

Diseased leaves were obtained from the lower parts of cucumber plants grown with 2 mm Ca (Experiment 1) or 7 mm Ca (Experiment 2).
\+ and ++ indicate the extent of the lesions (+<++)

lower Brix (sugar content) in the fruit. He also suggested that very high applications of N meant a decline in yield, because of shading by the many shoots induced by the N application. He succeeded in increasing the Brix and yield in some orchards which had produced low-Brix fruit by reducing the fertilizer and pruning the shoots.

EARLY DETECTION OF PHYSIOLOGICAL DISORDERS, DISEASES AND STRESS

A physiological disorder from a deficiency of some trace element is not difficult to correct if we know the cause. For this reason, the diagnosis of trace element status is useful, especially in fields which often suffer from micronutrient deficiencies. Few reports are available on real-time diagnosis for trace elements. A small reflecting photometer and test strips have been useful in testing for various elements. It is likely that we shall soon establish real-time diagnosis for a wide range of micronutrients.

It is well known that various stresses such as infection with diseases, attacks by insect pests and ultra-violet absorption influence plant metabolites, especially flavonoids (Whinkel-Shirley 2002). Recently, Fujihara and Yoneyama (2001) described the possibility of using polyamines as a diagnostic marker of plant development and physiological disorders. They reported that leaves infected with powdery mildew showed a lower level of putrescine and an increased spermine content, in keeping with the severity of the fungal infection (Table 3).

They also measured the polyamine composition of various organs from cucumber plants. A high level of polyamines was detected in rapidly growing tissues. Fujihara (2001) reported that levels of nitrous oxide and ethylene, both related to the biosynthesis of polyamines, were also significant. If changes in plant metabolism caused by external stresses can be detected at an early stage, it may be possible to prevent physical symptoms of abnormality.

There is a close relationship between the N status of the crop and disease incidence (Asakawa 1987). For instance, the incidence of infection with rice blast is well known to be related to high N status. However, we do not yet fully understand the mechanism whereby abundant N leads to an outbreak. We need to find some internal marker which varies according to N stress. Early methods of detecting physiological disorders, diseases and stress, using internal markers, might reduce economic risks from abnormal growth or reduced yields. Being able to diagnose plant stress would be a trigger for making real-time diagnosis popular among farmers.

DRIP FERTIGATION, AN INTEGRATED SYSTEM OF CULTIVATION FOR MINIMUM POLLUTION WITH MAXIMUM EFFICIENCY

Drip fertigation is a system which is becoming increasingly popular in the greenhouse cultivation of vegetables and flowers in Japan. The method originated in Israel, as a way of cultivating crops in arid soils. Japan is located in the humid temperate

Table 4. Fertilizer constituents

Fertilizer type	Chemical in constitutents of fertilizer
With accessory elements	$(NH_4)_2SO_4$, Ca (H_2PO_4), KCl
Without accessory elements	NH_4NO_3, KH_2PO_4, KNO_3

Source: Ono and Mori (1996)

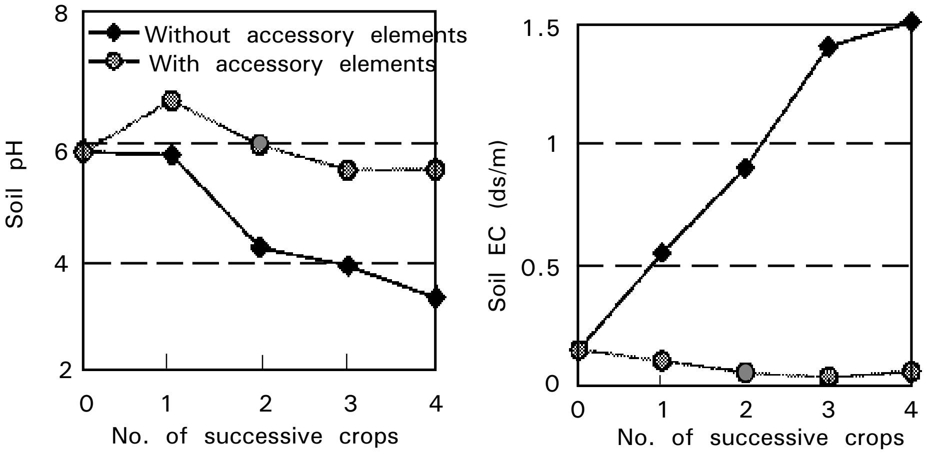

Fig. 5. Effect of fertilizer constituents on soil pH and EC during the cultivation period

Source: From Ono and Mori 1996

zone, but the soil conditions inside a greenhouse are similar to those of arid lands.

In place of a basal application of fertilizer, plants in this system are irrigated with a weak fertilizer solution. The volume of irrigation water and the amount of fertilizer are regulated according to crop demand. The duration and the concentration of each nutritional element are both controlled. In a well-designed system, nearly all the elements supplied are recovered. There is no leaching of nutritional elements or accumulation of salts. Plant diagnosis is required to match the fertilizer supply to the nutrient status of the crop.

Ono and Mori (1996) reported that ordinary commercial fertilizer contains accessory elements as counter ions of major components, for instance, SO_4 of ammonium sulphate and Cl of potassium chloride. Absorption of these elements by plants is limited. The soils to which such fertilizers are applied over a long period accumulate salts and become acid. Ono and Mori designed a low-stress fertilizer which does not contain SO_4 or Cl^- (in Table 4). The EC value and pH of soil in a pots without these accessory elements remained favorable. In contrast, soil in a pot of ordinary fertilizer developed a high EC value and a low pH after four consecutive crops of vegetables (Fig. 5). An integrated system using drip fertigation and low-stress fertilizer is a good method of greenhouse cultivation. Sustainability may be improved if fertilizer without accessory elements may be used.

Tamai and Onishi (2001) reported that drip fertigation gave a higher yield for less applied N than conventional cultivation in farmers' fields. On the basis of a series of field trials, they estimated the saving of fertilizer to be around 30%, compared to conventional cultivation. It was shown that high availability of fertilizer was achieved in drip fertigation, as reflected in the higher nitrate level in the petiole.

Nakano *et al.* (2001) reported that the incidence of blossom-end rot in tomato fruits was reduced by using fertigation to amend the topsoil. They noted that the conventional basal application of fertilizer caused chemical stress in the soil. The water flux accompanied by the plants' calcium absorbance was reduced by this stress. When fertigation was used, most of the plant roots were confined in areas of soil which received the fertilized irrigation water. This reduced chemical stress and also reduced the incidence of blossom-end rot due to calcium uptake deficiency.

CONCLUSION

Considering the current status of real-time diagnosis, it does not seem enough to provide critical values for particular areas. Specific critical values must be determined for different crops, localities (according to climate and soil), cultivation methods and seasons. A basic understanding of crop physiology is needed to detect disorders or diagnose the relationship between nutrient status and crop quality.

In age of world population growth, agriculture has the responsibility to grow sufficient food without ruining arable land and damaging the global environment. Agriculture which has maximum efficiency, and a minimum output of nutritional elements from arable land, cannot be achieved without the diagnosis of soil or plant nutrition.

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