Phytophthora Resistance of Tomato Plants Grown with EM Bokashi

H.L. Xu, R. Wang, M.A.U. Mridha and U. Umemura

International Nature Farming Research Centre, Nagano – 390-1401, Japan

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Abstract. *An experiment with tomato plants (*Lycopersicum esculentum *L. cv. Momotaro T96) was conducted to examine the effect of EM Bokashi fertilization on phytophthora (*Phytophthora parasitica*) resistance of tomato plants in comparison with chemical fertilization. Tomato plants fertilized with EM Bokashi possessed higher resistance against phytophthora infection compared with plants fertilized with chemicals. However, the photosynthetic activity was not low in leaves of chemical fertilized plants before phytophthora infection. The electrophoresis result showed that there was no difference in leaf proteins among fertilization treatments. This suggested that high phytophthora infection was not related to physiological activity shown by photosynthetic activity and genetic characteristics in proteins. Nitrogen compounds, nitrate and amino acids, in leaves were higher in chemical fertilization plot. Moreover, nitrate reductase activity in the leaf and hydrogenase activity in the soil were lower in chemical fertilized treatment. The integrated results suggested that advantage of nitrogen metabolism in EM Bokashi-fertilized tomato plants accounted for the high phytophthora resistance. Therefore, nature farming with EM technology can be expected in disease control in the plant production systems.*

Introduction

About sixty years ago, chemical farming started to spread in Japan. Many farmers and agricultural scientists had a blind faith in chemical fertilizers and pesticides. Some people even believed that pests would be exterminated by pesticides and starvation would end as chemical fertilizers appeared. Actually, the tragedy of mankind started from this time. Contrary to the opinion of most people, Okada (1941) warned the people that the chemical fertilizers would bear pests and diseases and pesticides would lead to appearances of more stronger pests. The fact has been recognized that as more pesticides are used for disease control, stronger pathogens will appear in plant production systems.

With concerns over environmental pollution by excessive uses of chemicals, many farmers in Japan have adopted nature farming with no or less chemicals for disease control. However, the nature farming practitioners have to seek alternative methods for disease control.

Recently, a technology using a beneficial microbial inoculum called Effective Microorganisms or EM has been proposed and introduced by Higa (1994) to nature farming systems. EM has been used widely for improvements of organic compost and soil quality as well as for foliage spray to control pest, both insects and diseases. However, it is not clear whether EM application together with organic materials to the soil can control diseases and if possible what are the mechanisms. Therefore, an experiment was conducted to examine the effect of EM Bokashi fertilization on phytophthora resistance of tomato plants in comparison with chemical fertilization.

Materials and Methods

Plant Material and Fertilization Treatment

Seedlings of tomato (*L. esculentum* L. cv. Momotaro T96) with five expanded leaves were transplanted into an experimental field in a glasshouse. Four fertilization treatments were made as follows: 1) Chemical fertilization (N-P-K:13-16-17), 2) Half chemicals and half EM Bokashi (100 gm^{-2}), 3) EM Bokashi (200 gm^{-2}), and 4) Aerobically treated EM Bokashi (200 gm^{-2}). EM Bokashi was prepared using organic materials such as oil seed cake, rice bran and fish-processing by-product inoculated with EM (Effective Microorganisms, EM-1, International Nature Farming Research Centre, Atami, Japan). The nitrogen application amount in the chemical fertilization treatment was adjusted to the level of 70 percent (total available fraction) of that for Bokashi treatment for all treatments. Photosynthesis, plant-soil nitrogen nutrition, and soil microbial activity were examined before phytophthora (*Phytophthora parastica*) infection occurred. Phytophthora was induced by increasing air humidity and temperature in the glass house. Phytophthora infection percentage and infection extent was examined.

Phytophthora Inducing Conditions and Examination

Three weeks after the seedlings were transplanted, plants were sufficiently watered and temperature was increased to 30°C and humidity to over 80 percent. Three days later the phytophthora infected the tomato plants. The percentage of the plants infected by phytophthora was defined as infection rate. The ratio of number of infected leaflets against the infected plant number was defined as infection intensity.

Measurement of Photosynthesis

Photosynthetic rate (P_N) of the fifth leaf from the top was measured using L1-6400 photosynthesis measurement system (LI-COR Inc., Lincolin, NE, USA) under different phtosynthetic photon flux (PPF) from 0 to 2000 $\mu mol\ m^{-2}\ s^{-1}$. Photosynthetic capacity (P_C), dark respiration (R_D) and the maximum quantum use efficiency ($Y_Q = KP_C$) were analyzed from the lightresponse curve modeled by an exponential equation as $P_N = P_C(1+e^{-KI}) - R_D$, where K is constant and I is PPF (Xu et al., 1995).

Electrophoresis for Proteins

Electrophoresis was conducted for protein analyses using a double slab gel electrophoretic apparatus (NA-1121, Nihon Eido Co., Ltd., Tokyo, Japan).

Measurement of Nitrate Reductase Activity

Nitrate reductase activity in the fifth leaf was assayed by the method of continuous spectrophotometric rate determination according to Gilliam et al. (1993).

Measurement of Nitrate Concentration in Leaves

Nitrate concentration in the fifth leaf was measured using a reflectometer (RQflex, Mereck, Kanto Kagaku Co., Ltd., Tokyo, Japan).

Analysis of Amino Acids in Leaves

Amino acids were measured by HPLC (Jasco) with UV-970 Detector and coloum of Shodex RSPark KC-811.

Determination of Dehydrogenase Activity in the Soil

Dehydrogenase activity in soil was measured by the method of Casida et al. (1964).

Results

High Phytophthora Resistance in EM Bokashi Fertilized Tomato Plants

In this study, the phytophthora infection was shown by two indicators: 1) percentage infection, which showed the resistance of the plant to phytophthora, i.e., the ability not to be infected; and 2) infection intensity, which showed the tolerance to the phytophthora, i.e., the survival ability even if the plant was infected. Results showed that tomato plants fertilized with EM Bokashi possessed higher resistance against phytophthora infection compared with plants fertilized with chemicals. Not only the percentage infection of the plants but also the infection intensity was lower for organic fertilized plants (Table 1). The fertilization treatment of half chemical fertilizers showed the infection percentage and infection intesity between the organic fertilized and chemical fertilized plants. Most of the plants in chemical fertilized plot died completely two weeks after the phytophthora was induced. However, in the organic fertilized plots, some plants remained uninfected and some still alive with partial green leaves. The results indicated clearly that organic fertilized tomato plants showed both higher resistance and tolerance to phytophthora compared with those fertilized with chemical fertilization.

Inconsistent Physiological Activity

The question is what factors account for the higher phytophthora resistance in the organic fertilized tomato plants. First one may consider the physiological activity associated with plant growth. The common indication of the plant physiological activity is the photosynthetic capacity. However, the phtosynthetic activity was not lower but a little higher in leaves of chemical fertilized plants before phytophthora infected the plants, compared with the organic fertilized plants. This suggested that high phytophthora infection was not related to physiological activity shown by photosynthetic activity. Growth and appearance of chemical fertilized plant did not show any disadvantage compared with organic fertilized plants. There must be other factors that account for the improved phytophthora resistance.

Protein Fraction

Electrophoresis results showed no difference in proteins between plants fertilized with EM-Bokashi and chemical fertilizer. If there are some proteins or enzymes missing in chemical fertilized plants, the protein fraction would show some differences on the electrophoretic gel plate. Actually no difference was found. This suggested that the plants with different treatments possess the same kinds of proteins and enzymes. No genetic changes in aspects of proteins and enzymes were caused by fertilization treatment.

Leaf Nitrate and Nitrogen Compounds

The nitrate concentration and the total concentration of amino acids were higher in chemical fertilized plants. However, the concentration of proline, an amino acid related with stress resistance, was higher in EM Bokashi-fertilized plants than those fertilized with chemical fertilizer. Nitrate concentration in the soil was also higher in chemical fertilization plot (Table 2).

Leaf Nitrate Reductase Activity and Soil Dehydrogenase Activity

Nitrate reductase activity was higher in EM Bokashi fertilized plants than in chemical fertilized plants. This was also confirmed by the result of leaf nitrate concentration as mentioned earlier. The dehydrogenase is an enzyme associated with nitrate reduction by microorganisms in soil. The activity of dehydrogenase was also higher in EM Bokashi-fertilized plants than in chemical fertilized plants. This was consistent with the nitrate concentration in the soil. The integrated results suggested that advantage of nitrogen metabolism in EM Bokashi fertilized tomato plants accounted for the high phytophthora resistance. Therefore, nature farming with EM technology can be expected in disease control in the plant production systems.

Discussion and Conclusions

Phytophthora is a dangerous disease of tomato plants caused by a pathogenic fungus (*Phytophthora parasitica*). The stems, leaves, leaf petioles and even the root will rot very fast when plants are infected by phytophthora. Usually, scientists consider that the easiness of the infection is associated with cultivars (Yamada, 1997). There is no report showing whether tomato phytophthora infection is associated with soil-plant nutrition or fertilization regimes.

Sixty years ago, a Japanese philosopher, Mokichi Okada warned the people that chemical fertilizer would cause diseases and pest insects. Now, scientists have known that more applications of pesticides cause more and stronger new pest insects and the same for pathogens. However, many people have not understood yet about Okada's warning about chemical fertilizer as the cause of diseases.

In this study, it is found that the easiness of tomato phytophthora infection is associated with nitrogen metabolism and nitrogen status and metabolism in the soil. Tomato plants fertilized with chemical fertilizer contain more nitrate nitrogen and nitrogen compounds such as amino acids than plants fertilized with EM Bokashi. The high concentration of nitrogen compounds might be favourable to the infection and development of phytophthora pathogens. On the contrary, low nitrogen compounds in EM Bokashi fertilized plants might account for the phytophthera resistance. The question is why the nitrate and nitrogen compounds were low in concentration in EM Bokashi fertilized tomato plants. Results showed that the high activity of the enzyme, nitrate reductase, accounted for the low concentration in leaves of EM Bokashi-fertilized plants. This might be due to balance, even release and sustainability of nutrients in EM Bokashi. The activity of dehydrogenase in the soil was also higher in EM Bokashi fertilized plots than in chemical fertilized plots. This might enable nitrogen in soil to be supplied evenly and in different types.

Another phenomenon found in this study is that the concentration of proline, an amino acid associated with stress resistance, was higher, although the total concentration of amino acids was lower, in EM Bokashi fertilized plants than in chemical fertilized plants. This might be also associated with the phytophthera resistance in EM Bokashi fertilized plants. However, the detailed mechanism is not known. Because the high nutrient availability of chemical fertilizer, the plant growth and photosynthetic activity at the early growth stage were not low in leaves of chemical fertilized plants before phytophthora infection. Results of electrophoresis also showed no difference in leaf proteins among fertilization treatments. This suggested that high phytophthora infection in EM Bokashi fertilized plants was not related to physiological activity shown by photosynthetic activity and genetic characteristics in proteins. Therefore, advantage of nitrogen metabolism in EM Bokashi-fertilized tomato plants accounted for the high phytophthora resistance. It is concluded that nature farming with EM technology can be expected in disease control in the plant production systems.

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Table 1. Phytophthora Infection on the 5th Day and Plants Dead on the 12th Day the Photosynthetic Parameter before Infection in Plants with Different Fertilizations

Treat	Infection D 5		Dead on	P_C	R_D	Y_Q
	%	Intensity	D 12 (%)	(μmol m^{-2} s^{-1})		(μmol mol^{-1})
Che	88.2 +12.3	2.43 +0.29	88.3 +9.6	22.1 +2.3	0.41 0.06	62.54 +5.928
Hlf-Che	85.7 +7.6	1.83 +1.3	71.4 +6.7	23.2 +1.9	0.46 0.05	61.48 +5.23
EM-B	60.5 +7.2	1.57 +1.7	63.7 +8.2	19.2 +1.4	0.47 0.03+	60.48 +4.93
Aer EM-B	48.6 +5.3	1.61 +15	59.2 +5.4	21.1 +1.6	0.41 0.06	59.71 +4.165

Che, chemical; Hlf-Che, half chemical and half Em Bokashi; EM-B, anaerobically fermented EM-Bokashi; Aer EM-B, Aerobically treated EM-Bokashi.

Table 2. Nitrate Reductase (NRtase) Activity (μmol kg^{-1} FM), Nitrate Concentration (g kg^{-1}DM), and Total Amino Acids and Proline Concentrations (mmol kg^{-1}DM) in the Fifth Leaf as well as the Hydrogenase (H-ase) Activity (μg TPF g^{-1} d^{-1}) and Ammonia and Nitrate Nitrogen Concentration (mg kg^{-1}) in the Soil of Different Fertilization Treatments

Treat.	Leaves				Soil		
	NRtase Activity	NO_3^-	Amino acids	Proline	H-ase Activity	NH_4^+	NO_3^-
Unfert.	-- --	-- --	-- --	-- --	17 ±1.2	44 ±4.5	88 ±5.0
Che	5.47 ±0.51	2.7 ±0.21	2.33 ±0.19	0.43 ±0.037	21 ±1.2	55 ±4.4	224 ±21.5
Hlf-Che	6.17 ±0.43	2.3 ±0.17	2.04 ±0.17	0.47 ±0.021	40 ±4.6	64 ±11.5	169 ±5.6
Ana-Org.	7.34 ±0.53	1.9 ±0.08	1.64 ±0.09	0.57 ±0.042	81 ±5.6	723 ±11.1	174 ±5.2
Aer-Org.	8.26 ±0.49	1.8 ±0.12	1.71 ±0.14	0.55 ±0.039	67 ±5.5	54 ±6.0	186 ±10.4