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Increase in soil pH due to Ca-rich organic matter application causes suppression of the clubroot disease of crucifers

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Abstract

Clubroot disease of cruciferous plants caused by the soil-borne pathogen *Plasmodiophora brassicae* is difficult to control because the pathogen survives for a long time in soil as resting spores. Disease-suppressive and conducive soils were found during the long-term experiment on the impact of organic matter application to arable fields and have been studied to clarify the biotic and abiotic factors involved in the disease suppression. The fact that a large amount of organic matter, 400 t ha^{-1} yr^{-1} farmyard manure (FYM) or 100 t ha^{-1} yr^{-1} food factory sludge compost (FSC), had been incorporated for more than 15 yr in the suppressive soils and these soils showed higher pH and Ca concentration than the disease conducive soil led us to hypothesize that an increase in soil pH due to the long-term incorporation of Ca-rich organic matter might be the primary cause of the disease suppression. We have designed a highly reproducible bioassay system to examine this hypothesis. The suppressive and conducive soils were mixed with the resting spores of *P. brassicae* at a rate of 10^6 spore g^{-1} soil, and *Brassica campestris* was grown in a growth chamber for 8 d. The number of root hair infections was assessed on a microscope. It was found that the incorporation of FYM and FSC at 2.5% (w/w) to the conducive soil suppressed the infection and that the finer particles ($\leqslant$5 mm) of FSC inhibited the infection and increased soil pH more effectively. Neutralization of the conducive soil by $Ca(OH)_2$, $CaCO_3$ and KOH suppressed the infection, but the effectiveness of KOH was less than those of $Ca(OH)_2$ and $CaCO_3$. Acidification of the suppressive soils by H_2SO_4, promoted the infection. The involvement of soil biota in the disease suppression was investigated using the sterilized (γ-ray irradiation) suppressive soils with respect to soil pH. The γ-ray irradiation promoted the infection at pH 5.5, but no infection was observed at pH 7.4 irrespective of the sterilization status. All these observations suggest that soil pH is a major factor in disease suppression by organic matter application and that Ca and soil biota play certain roles in the suppression under the influence of soil pH.

Keywords: Calcium; Clubroot disease; Organic matter; *Plasmodiophora brassicae*; Soil-borne pathogen; Soil pH; Suppressive soil

1. Introduction

Clubroot of cruciferous plants is a major disease that is widespread throughout the world. It is caused by the soil-borne obligate parasite, *Plasmodiophora brassicae* classified amongst the Protist (Castlebury and Domier, 1998; Ward and Adams, 1998). The life cycle of *P. brassicae* consists of two phases: the primary phase is characterized by germination of the resting spore in the rhizosphere and subsequent infection of the root hair, and the secondary phase is characterized by colonization and proliferation in the root cortex (Ingram and Tommerup, 1972). It is difficult to control the disease because the pathogen survives in soil for a long time as resting spores. The breeding of resistant plant cultivars is one of the strategies to control clubroot, and several genes (Piao et al., 2004) and loci (Suwabe et al., 2003; Hirai et al., 2004; Rocherieux et al., 2004) involved in resistance to the disease have been identified from *Brassica* sp. Biological control using a

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fungal endophyte has also been proposed (Narisawa et al., 1998). Another approach is controlling the soil environment to suppress the disease, and analysis of disease-suppressive soils has been conducted for this purpose (Young et al., 1991; Murakami et al., 2000a). The involvement of soil pH in the occurrence of clubroot has been suggested: liming is a conventional technique to control the disease (Dobson et al., 1983; Campbell et al., 1985; Webster and Dixon, 1991; Murakami et al., 2002a; Tremblay et al., 2005). Young et al. (1991) suggested that gentisic acid, a phenolic compound, found in disease-suppressive soils was involved in disease suppression. In addition to these abiotic factors, it has been suggested that the microbial community plays an important role in the suppression of clubroot (Murakami et al., 2000a).

Recently, a large amount of organic waste from sewage sludge, farming practices and the food industry has caused environmental pressures such as eutrophication of fresh water due to nitrogen leaching and greenhouse gas emissions. It is becoming necessary for industries producing organic waste to organize recycling systems. The incorporation of organic waste into arable fields is one option for dealing with the problem. In particular, organic waste from farming and food factories is usually safe (i.e. free from toxic elements such as heavy metals) and thus suitable for incorporation into arable fields. Enrichment of soil organic matter improves soil aeration, physical structure, drainage, water-holding capacity, nutrient availability and microbial activity. There are many examples showing that organic matter application can effect soil-borne diseases. Chicken litter decreased the population densities of the root-knot nematode *Meloidogyne incognita* (Riegel et al., 1996; Riegel and Noe, 2000) due to an increase in soil microbial activity (Riegel and Noe, 2000). The incidence of verticillium wilt of potato caused by the fungal pathogen, *Verticillium dahliae*, was reduced by animal manure application (Conn and Lazarovits, 1999). Incorporation of composted sewage sludge also significantly reduced the lettuce drop caused by the fungus *Sclerotinia minor* (Lumsden et al., 1986). An excess application of organics to the field, however, can cause eutrophication of terrestrial freshwater systems. The influence of long-term application of organic matter to soil on crop productivity and nitrogen-leaching has been investigated in an experimental field of the Nagoya University since 1987 to define an appropriate level of organic matter application. The clubroot disease was first observed on cabbage in 1997 in the field, but little or no symptom was found in the plots in which a large amount of organic matter had been incorporated. It has been confirmed through this field experiment that the suppressive effect in the plots is not temporary and that the soils of the plots can be designated as 'suppressive soils'.

The occurrence of clubroot disease in the field, however, is not constant year by year. Therefore, it is required to establish a reproducible experimental system under controlled environmental conditions to improve our knowledge of this disease caused by an unculturable pathogen. The objectives of this study were to identify the biotic and abiotic factors involved in disease suppression by organic matter application in a model bioassay system.

2. Materials and methods

2.1. Experimental field, assessment of disease incidence and soil sampling

Seven treatments have been designed for the field experiment on the influence of long-term application of organic matter on crop productivity, soil chemical and physical properties and nitrogen-leaching in Nagoya University, Aichi, Japan since 1987. The following three treatments (plots) in which clear difference in the disease incidence were observed were chosen from seven original treatments. The conventional treatment plot has been amended with chemical fertilizer (270–520 kg N, 200–520 kg P_2O_5, 240–520 kg K_2O ha^{-1} yr^{-1}) and 40 t ha^{-1} yr^{-1} farmyard manure (FYM) since 1987. The FYM treatment plot has been amended with 400 t ha^{-1} yr^{-1} FYM since 1987. The food factory sludge compost (FSC) treatment plot has been amended with 100 t ha^{-1} yr^{-1} of the FSC since 1993. The FYM was compost of cattle feces and rice straw. The FSC was compost of dehydrated activated sludge and corn gluten feed discharged from a cornstarch factory. The properties of the FYM and the FSC are shown in Table 1. The pH of FYM and FSC were 9.8 and 7.3, respectively, and both of the composts showed high levels of base content. The size of each plot was 3×17 m ($n = 1$), and each plot consisted of two rows. Melon (*Cucumis melo* L. cv. Prince Melon) and cabbage (*Brassica oleracea* L. var. *capitata* cv. Hukamidori) were cultivated in 1997, and sweet corn (*Zea mays* L. cv. Peter Corn) and chinese cabbage (*B. rapa* L. var. *pekinensis* cvs. Satokaze and Tomikaze) have been cultivated since 1998. Crop residue was incorporated by a rotary cultivator after each harvest.

The incidence of clubroot disease has been assessed annually after the crucifer crop cultivation since 1997. Twenty cabbages were chosen randomly in each plot, and the presence or absence of clubroot galls was assessed visually. Disease incidence was expressed as percentages of clubbed plants.

About 3 kg of soil samples were taken after the harvest of cabbage in 2003 from 5 cm below the surface (20 cm in depth) from three randomly chosen spots in each row (6×3 kg soil from each plot) of the conventional, FYM and FSC plots, combined, mixed thoroughly, air-dried, passed through a 5 mm sieve and stored at room temperature for chemical analyses and bioassay.

2.2. Soil chemical properties and resting spore density

A subsample was taken from each of the stored soils for the following chemical analyses ($n = 1$). Soil pH and