Recently, plant activators that induce disease resistance in plants, either locally or systemically, have attracted much attention with the development of new environmentally friendly disease control technologies. Plant activators of this nature include artificial compounds such as acibenzolar-S-methyl (ASM) or probenazole, and also natural substances of biological origin. Reported examples of the latter include low molecular chitin (Kadota et al., 2000) and cucumber leaf extract (Negishi et al., 2011).

Alternatively, Obara et al. (2007) reported that pathogenesis-related genes were induced in tobacco leaves treated using yeast extract solution. Yoshida (2007) reported reduced sizes of anthracnose lesions on the upper leaves of tea plants that were inoculated with the pathogen two days after spraying yeast extract solution on the lower leaves. Nakaho et al. (2007) reported that bacterial wilt of tomatoes decreased markedly when the roots of tomato seedlings were soaked in yeast extract solution before pathogen inoculation. Here, we examined the resistance induced against cucumber anthracnose by yeast extract, emphasizing on the time required for transfer of the signals that result in the systemic acquired resistance (SAR).

**Materials and Methods**

Tsu yatarou (Takii & Co., Ltd.) cucumber (Cucumis sativus L.) cultivars were used for the inoculation tests. The 104-T strain of Colletotrichum orbiculare (Berk. & Mont.) Arx from the Laboratory of Plant Pathology, Graduate School of Agriculture, Kyoto University, was used as the pathogen. Cucumber plants were grown in clay pots (diameter, 15 cm) with the commercial gardening soil for about one and a half months. The soil was slightly acidic and its components were peat moss, vermiculite and perlite. It contained fertilizer, nitrogen (300 mg), phosphoric acid (500 mg) and potassium (450 mg) per liter of soil. A 1,000-fold solution of liquid fertilizer (commercial name; HYPONeX) was applied during cultivation.

The plants were used for experiments when the fifth leaf was half expanded and the 6th leaf was one-third expanded. The upper part of the cucumber plant, from the fourth leaf to the top of a plant, was covered using a polyethylene bag (size, 46 cm×60 cm), as shown in Figure 1, to prevent the splash of spray. Then 50 mL of aqueous solution of yeast extract powder (made from baker’s yeast by Oriental Yeast Co., Ltd.) at a concentration of 2,000 μg·mL⁻¹ was sprayed on both surfaces of the first (true leaf), second, and third leaves. After the leaf surface had dried, the polyethylene bag was removed. As the control plant, 50 mL of distilled water was sprayed in the same way. The three leaves sprayed with yeast extract were detached from the base of the petiole at 8 h, 1 day, 3 days, 5 days, and 7 days after being treated with yeast extract spray. The plants with intact leaves were used as the control plants. At seven days after the spray treatment and leaf detachment, conidial suspension of the pathogen was sprayed onto whole cucumber plants, at a rate of 50 mL per plant. The pathogen was cultures on potato dextrose agar medium and conidial suspensions were adjusted to 3.0-3.5×10³ conidia per mL. The inoculated plants were placed in a polyethylene bag (size, 90 cm×100 cm) and maintained in high humidity for 24 h, in an illuminated room with a temperature of 25°C. Thereafter, the inoculated plants were maintained in a greenhouse. At eight or nine days after inoculation, we counted the anthracnose lesions on the upper leaves that were not sprayed with yeast extract, which were 9 or 10 leaves. Since the examined leaves included ones that emerged after inoculation, no lesions were found on the top leaf, regardless of the treatments. Three cucumber plants

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**Disease Resistance against Anthracnose Induced in Cucumber Leaves Treated Using Yeast Extract**

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were used for each treatment, and the experiment was repeated thrice at one-month intervals.

**Results and Discussion**

To examine the time required to induce SAR, we removed the first, second, and third leaves at various time points after part of the plant was treated with yeast extract and the entire plant was inoculated with *C. orbiculare* conidial suspension. The mean number of lesions on the upper leaves in the three experiments and standard errors (SE; n=3) were shown in Figure 2. Bars labeled with the same letters did not differ significantly (p<0.01, Tukey-Kramer test). Compared with the control plants (those sprayed with distilled water and with intact leaves), plants in which the leaves were removed showed significantly lesser number of lesions at three days after spraying the yeast extract. The number of lesions decreased with increase in the time elapsed after treatment with the yeast extract spray to detachment of the lower leaves. There was no significant difference between the treatments detached after 3 days. The least number of lesions were found on the leaves of the plants from which the treated leaves were not detached. These data showed that the leaves sprayed with yeast extract needed to remain attached for at least three days for inducing SAR. On the other hand, leaf removal did not reduce the number of lesions in plants sprayed using distilled water (control), regardless of the time when the leaves were detached.

In the experiments reported by Obara *et al.* (2007), Yoshida (2007), and Nakaho *et al.* (2007), yeast extract solution (the type of yeast was undisclosed) was sold as a fertilizer, Agrevo Ex, was used. It contained potassium chloride and citric acid in addition to the yeast extract. However, we used pure yeast extract powder. Thus, it can be concluded that the induction of resistance is due to the
yeast extract.

The period between plant activator treatment and SAR occurrence might depend on the type of plant activators used. Ishii et al. (2002) reported that when the first leaf of a cucumber seedling was soaked in the ASM solution (100 μg·mL⁻¹) and detached 2 or 24 h later, and the upper leaves were inoculated with *C. orbiculare* 3 h after the ASM treatment, the number of anthracnose lesions on the upper leaves moderately decreased in the case of the detachment 2 h later or markedly decreased 24 h later. Further, Narusaka et al. (1999) reported that the signals inducing resistance against cucumber scab were transferred as early as 4–6 h after ASM treatment in experiments following the same procedure as that by Ishii et al. (2002). According to Ishii et al. (1999), ASM can trigger induced resistance in an exceptionally rapid manner after treatment. Further, Ishii et al. (1999) reviewed some previous studies and showed that the general assumption was that the induction of disease resistance in plants occurred several days after treatment with the chemical or biological agents. Our results showed that the leaves sprayed with yeast extract needed to remain attached for at least three days before the signals were transferred to the upper leaves. On the control plants, shown in the right side of Figure 2, the number of lesions on the upper leaves tended to decrease with an increase of periods before the lower leaves were detached, although significant difference was not detected. It is possible that the upper leaves might grow to a slightly larger size because of some compensatory effect after detachment of the lower leaves.

It is necessary to clarify the substances that might induce disease resistance. Resistance against cucumber anthracnose could also be induced by a solution of yeast that had been sterilized in an autoclave at 121°C (data not shown). Thus, substances resistant to heat may be involved. Although this is the case in bacterial wilt of tomato, Nakaho et al. (2012) showed that the l-type amino acids in Agrevo Ex, such as l-histidine, l-arginine, and l-ricin, were effective in inducing resistance.

Métraux et al. (1990) reported an increase in salicylic acid concentration at the onset of SAR in cucumber inoculated with *C. orbiculare*. In addition, Kubota and Nishi (2006) reported the accumulation of salicylic acid in the second leaf after inoculating the first leaf with *C. orbiculare*. Thus, salicylic acid could play a central role in SAR-signal transduction after infection with a necrogenic pathogen. However, further research is necessary on the signal transduction mechanism induced by yeast extract.

Considering practical purposes, research of such nature should be conducted through field trials to determine whether the level of disease control of yeast extract is acceptable to farmers, mainly because, although synthetic, many excellent fungicides are available that confer resistance against the anthracnose of cucumber and other foliage diseases.

Reference


