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Resting Spore of *Plasmodiophora brassicae* Proliferates Only in the Callus of Clubroot Disease-Susceptible Turnip but Increases the PAL Activity in the Callus of Clubroot Disease-Resistant Turnip

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Abstract

The calluses were induced from turnips, *Brassicae campestris* L. which were susceptible and resistant to *Plasmodiophora brassicae* in Murashige-Skoog's agar medium supplemented with 1.0 ppm 6-benzylaminopurine and 0.5 ppm α-naphthalene acetic acid. When a 10 μl water suspension containing 10^4 resting spores of *P. brassicae* was placed on the surface of the calluses, about 3 x 10^5 zoosporangium-like spheroids (SLS) were recovered from the susceptible calluses after 24 h of the treatment but no SLS was found in the resistant callus. On 6th day after the treatment, the SLS increased to about 4 x 10^6 in the susceptible callus. Upon inoculation of resistant callus with 10^4 resting spores, the phenylalanine ammonia lyase (PAL) activity increased about 4-fold after 20 h, however, such increase was not observed in the susceptible callus during the same period. The constitutive PAL activity of susceptible callus was roughly one 6th of that of resistant callus.

Keywords: *Brassicae campestris* L, callus, clubroot disease resistance and susceptible, PAL, phenylalanine ammonia lyase, *Plasmodiophora brassicae*, resting spore, turnip.

Introduction

Plasmodiophora brassicae is an obligate plant root pathogenic fungus causing clubroot disease in economically important crops of the family *brassicae* (Woronin, 1878). Resting spores have an astonishing toughness (Macfarlene and Last, 1959), therefore, serious damages last for a longer period in the infected area. Infection of this parasite has been shown to consist of two phases. The first phase is the infection of root hair by primary zoospores and the second one is the infection of cortex of main root by secondary zoospores. In the second phase, the invaded zoospores generate secondary plasmodia which develop resting spores inside their structure.

During the development of secondary plasmodia, concentration of several metabolites such as amino acids, sugars, lipids and auxin increase (Keen and Williams, 1969; Williams *et al.*, 1969; Nomoto and Tamura, 1970; Chiang and Nip, 1973; Grisic-Rausch *et al.*, 2000). The increase of these substances has been thought to be associated with the formation of galls, i.e., in the hypertrophied roots, since the phases of both gall formation and secondary plasmodial development are coinciding with each other. Although, phytopathological data for the later events of the disease have been accumulated, very little information is available on the biochemical aspects of early events in the interaction between the host plants and fungi.

Generation of callus from the host plant provides a powerful tool for studying the plant pathogen interaction (Ingram, 1969; Williams *et al.*, 1969; Dekhuijzen, 1975). Ikegami and Mukobata used callus induced from Chinese cabbage to proliferate the fungi. When the fungal infected callus was grown for a month, it turned brown colored and attained rough surface. The rough surface of the callus was thought to correspond to the hypertrophy symptoms of roots during clubroot disease (Ikegami and Mukobata, 1990). Therefore, despite its disadvantage with the polyploidy problem, the calluses

must be a good system to study both earlier and late events in the interaction between host plants and the fungus.

Phenylalanine ammonia lyase (PAL) is known to be a key enzyme involved in the defense reaction of many plants against various agents such as pathogens, fungal elicitors, wounding and UV radiation (Dong *et al.*, 1991; Fukasawa-Akada *et al.*, 1996; Long and Jenkins, 1998; Miura *et al.*, 1999). PAL catalyzes the first step in phenylpropanoid synthesis and the subsequent products are involved in the pathogenesis related reactions of plants. Therefore, it is necessary to determine the level of PAL activity in response to the infection by *P. brassicae*.

We found that the proliferation of the zoospores was quickly occurring in the callus from only the susceptible turnip, beyond our expectation. Interestingly, we also found that the inoculation of resting spores of *P. brassicae*, resulted in the increase PAL activity in the resistant callus. As far as we know, this is the first report to show the increase in PAL activity of host plant callus upon contact with *P. brassicae* spores.

Materials and Methods

Plasmodiophora brassicae

The resting spores of *P. brassicae* were isolated and purified from infected Chinese cabbage root and determined as type IV by Williams' method (1966).

Induction of calluses from turnips

The seeds of *Brassicae campestris* L., cv. CR Takamaru Kokabu and Natsumaki 13 Gou Kokabu were respectively resistant and susceptible to clubroot disease. The seeds were generously given by Musashino Shubyo En (Ikebukuro, Toshima-ku, Japan). The resistant cultivar was bred in the company by conventional breeding. The seeds were planted on a pot filled with soil and incubated in a growth chamber at 25℃ under 12 h Dark/12 h Light at 10,000 lux. To obtain infected turnips, soils contaminated with spores of *P. brassicae* at a density of 10^9 spores/g soil, were added to the surface of the pots on 7 or 8th day after seeding. The pot was always kept on a tray containing shallow water for supplying appropriate amount of water to the soil. The turnips grown for 40 days were surface sterilized with 5% sodium hypochlorite and a 5-mm diameter round bar was excised from the roots by a corkscrew. The bar was then cut into 2-mm thick disk and placed on Murashige and Skoog basal medium (1962) supplemented with 3% sucrose, 0.8% agar (MS agar medium) and various concentration of α-naphthalene acetic acid (NAA) and 6-benzylaminopurine (BA). In an induction of primary callus, the MS agar medium containing one half of the basal elements (1/2 MS medium) was also used. These calluses were maintained for about 30 days at 25℃ under a 12 h Dark/12 h Light at 300 lux in fluorescent illumination.

Isolation and Purification of the spores of P. brassicae from the infected turnip

Matured galls of infected turnips were grown in the growth chamber. They were sterilized with 5% sodium hypochlorite and cut into 5 mm cubes. Twenty grams of such cubes were homogenized with a 20 ml of sterilized water by mortar and pestle. The homogenates were passed through 8 layers of cheesecloth and the filtrates were finally centrifuged at 1,500*g*, for 10 min. The precipitate was suspended in distilled water to wash once and finally suspended in a small volume of distilled water.

The spores were purified by a centrifugation at 500*g* for 10 min using 36 and 12% (W/V) discontinuous gradient of Ficoll 400. The spores that appear as a band between 36 and 12% Ficoll were recovered and washed twice with sterilized water. The final precipitates were suspended in a small volume of sterilized distilled water and the number of spores was counted under the microscope using Thoma, hema counting chamber.

Immature spores and secondary plasmodia were also isolated and purified as above, but in the case of former, juvenile galls were used.

An aliquot of the purified spore suspension was placed on MS agar medium for 48 h and only the samples without contaminating microbes were used in the experiments.

Isolation of the spores and sporangium-like spheroids (SLS) from the callus of turnips

Ten μl of water containing 10^4 spores were placed onto surface of the 4-day old calluses with a minimum of 200 mg fresh weight and sub-cultured further on the same MS agar medium supplemented with 0.5 ppm NAA and 1.0 ppm BA for appropriate period. On the designated day, about 1 g of the calluses was collected, washed thoroughly with sterilized water on a glass funnel to remove possible contamination of spores stayed on surface of the calluses and homogenized (20 strokes at 200 rpm) with 5 ml of sterilized water by a glass/Teflon homogenizer. The spores or SLS in the homogenate were purified as described above using Ficoll and counted with the hema counting chamber under a microscope.

Preparation of spore germination-enhancing solution with root-soaking

The seeds of susceptible turnip were germinated in sterilized distilled water. The roots of four one-day old intact turnip seedlings were soaked for 4 days in 1 ml of solution containing 1.0 mM $Ca(NO_3)_2$, 1.0 mM KNO_3, 0.4 mM $MgSO_4$ and 0.4 mM KH_2PO_4. This was then centrifuged at 10,000*g*, for 10 min and supernatant was recovered as germination-enhancing solution (GES) (Ogawa *et al.*, 2001).

Measurement of germination rate of resting spore

Mature resting spores were incubated with germination enhancing solution (GES) for 24 h. The 10^6 spores treated with 10 μl GES were placed onto the surface of four calluses with a minimum of 50 mg fresh weight each. On the designated period after the placement, the surface of calluses was washed off by pipetting with 1-ml water. The wash-off solutions were centrifuged at 1,000*g*, for 10 min and the precipitate was recovered as the spore fraction. The rate of germination was calculated as,

No. of germinated spore recovered/No. of total spore recovered x 100 (%).

Under phase contrast microscope, the germinated resting spores that lack its inner contents were clearly distinguished from the un-germinated one (Ogawa *et al.*, 2001). Standard deviation was calculated from 4 experiments.

Determination of phenylalanine ammonia lyase (PAL) activity

The four-day old calluses with a minimum of 50 mg fresh weight each were inoculated with 10^4 spores in a 10 μl water suspension. On the designated period, the callus-samples were harvested, frozen promptly with liquid nitrogen and stored at -20℃.

The crude enzyme was prepared as Ozeki *et al.* (1987). Two hundred mg of the frozen callus-samples were ground in 5 ml of 100 mM phosphate buffer, pH 8.0, with mortar and pestle. The homogenate was mixed with 0.2 g of Dowex 1 x 8 (H^+ Type) for 20 min and centrifuged at 20,000*g*, for 15 min to recover supernatants as crude enzyme. The PAL activity was determined using Zucker's method (1965) as slightly modified by Tanaka *et al.* (1974). Five hundred μl of crude enzyme fraction was mixed with 1 ml of 50 mM borate buffer, pH 8.0, pre-incubated for 5 min at 30℃ and the enzyme reaction was started by the addition of 0.5 ml of 40 mM phenylalanine. The reaction was carried out for 60 min and stopped by the addition of 500 μl of 2 M perchloric acid. The reaction mixture was centrifuged at 1,000*g*, for 10 min and the activity was measured by absorbance change at A_{280} nm. In the control, 100 mM phosphate buffer was used instead of the crude enzyme. One unit of the PAL activity (Unit), was expressed as μg *trans*-cinnamic acid formed/μg protein/h. *Trans*-cinnamic acid was quantified with standard calibration curve.

Results

The effects of P. brassicae on the growth of the primary callus on Murashige-Skoog agar medium

The excised disks from four kinds of turnips, i.e., the resistant and susceptible turnips grown in non-contaminated soil (Res-nT and Sus-nT, respectively) and the resistant and susceptible grown in the soil contaminated with *P. brassicae* spores (Res-T and Sus-T, respectively), were placed on the MS agar medium supplemented with 0.5 ppm NAA and 1.0 ppm BA according to the result shown in Ikegami and Mukobata (1990). All four lines exhibited almost the same growth for about 10 days (**Fig. 1**). Beyond this period, however, the growth of the Sus-T was much higher than that of other three samples. The similar results were obtained when 1/2

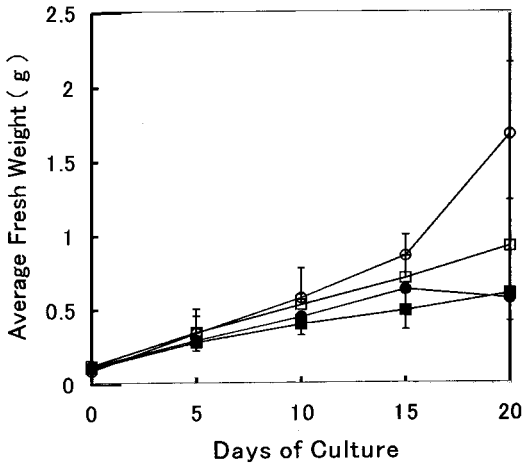

Fig. 1 Growth of the calluses in primary culture originated from the turnips that were susceptible and resistant against *Plasmodiophora brassicae*. Both the turnips, susceptible (○ , ●) and resistant (□ , ■), were grown in the soils contaminated (○ ,□) or non-contaminated (● , ■) with the *P. brassicae* spores. The disks of the tissues were excised and placed on the MS agar medium supplemented with 0.5 and 1.0 ppm NAA and BA. For details, see Materials and Methods. On every 5 days after the placement, five samples from five different flasks were weighed and the average fresh weight in grams was calculated. A bar on the each plot represents SD.

MS agar medium was used (data not shown).

Effects of NAA and BA on the growth of callus from the susceptible turnip

The effects of various concentrations of NAA and BA on the growth of Sus-nT callus on the MS agar medium were checked (**Fig. 2**). At 0.5 ppm NAA and 1.0 ppm BA the maximum growth of calluses was obtained. In this combination, weight of the calluses increased more than 10 times during a period of 20 days. NAA and BA seemed to be necessary for the growth of the callus, since in the medium in which either one of them or the both were lacked, the callus did not grow (data not shown).

The growth of the callus on 1/2 MS agar medium with various concentrations of the hormones was almost the same as that on MS agar medium (data not shown). The growth of calluses in the basal medium of Gamborg B5 (1968) supplemented with 0.5 ppm NAA and 1.0 ppm BA, was not better than MS or 1/2 MS agar medium. Therefore, the calluses were maintained on MS agar medium supplemented with 0.5 ppm NAA and 1.0 ppm BA in all the following experiments.

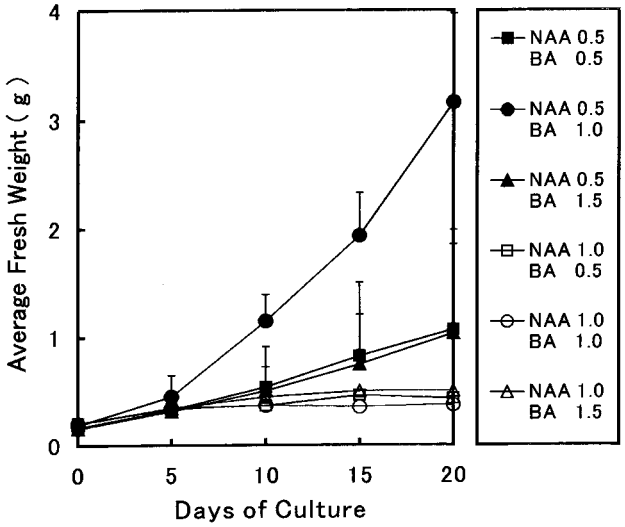

Fig. 2 Growth of the calluses obtained from primary culture of susceptible turnip on the MS agar medium supplemented with various concentrations of NAA and BA. The calluses established in the primary culture shown in **Fig. 1** were maintained for 20 days in the MS agar medium. On every 5 days, three samples in different three flasks were weighed and the average fresh weight in grams was calculated as in **Fig 1**. The SD is represented as a bar on each plot. The MS agar medium was supplemented with various concentrations of NAA and BA as shown in the right side box.

Effects of resting spores on the growth of calluses

The calluses came from turnips contaminated with spores, grew faster than those came from non-contaminated turnip (**Fig. 1**). The effects of resting spores on growth of the callus were quantified by infecting them with 10^6 spores.

The spores at concentration $10^6/10\ \mu l$ water were placed on the susceptible (Sus) and resistant (Res) calluses weighing about 100 mg each and their growth was compared to that of the resistant and susceptible of non-treated calluses (-nT) (**Fig. 3**). During initial 6 days, both the calluses of treated Sus (Sus-T) and Res (Res-T) grew equally as non-treated Sus (Sus-nT) and Res (Res-nT). After 9 days, however, the callus growth of Sus-T was much greater than that of Sus-nT (**Fig. 3A**). The growth rate of the Res-T and the Res-nT was obviously lower than that of Sus-T and also the spore treatment did not affect the growth of resistant lines (**Fig. 3B**).

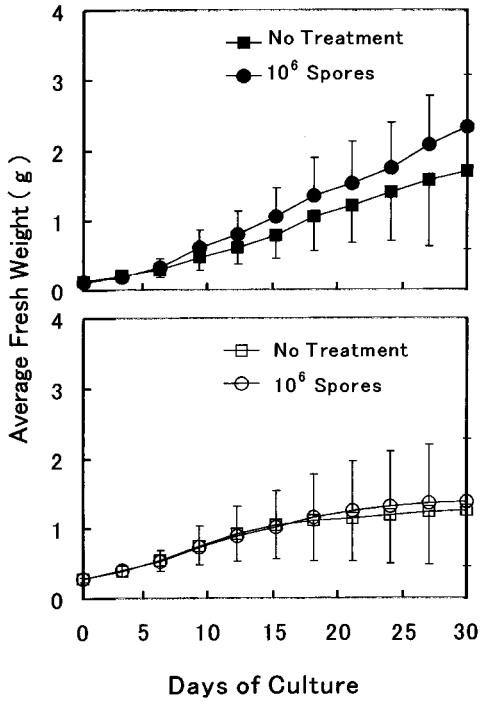

Fig. 3 Effects of the treatment with resting spore of *Plasmodiophora brassicae* on the growth of the calluses of susceptible and resistant turnips. About 100 mg of the susceptible (●) and the resistant calluses (○) were treated with 10^6 spores and weight of the calluses were measured on the designated day. In control experiment with susceptible (■) and resistant calluses (□), 10 μl of water containing no spore was applied to the calluses. The average fresh weight was calculated using three calluses from three different flasks. The SD is represented as a bar on each plot.

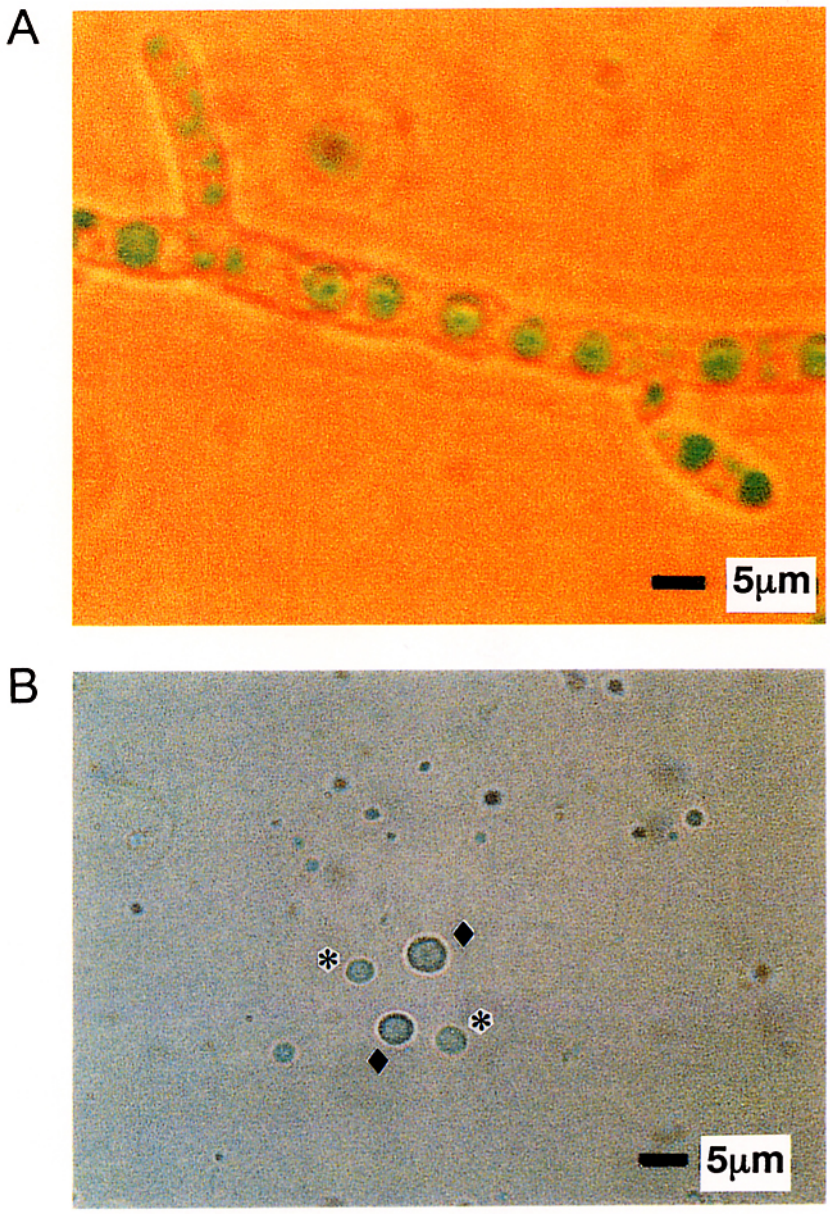

Fig. 4 Light micrographs of sporangium - like spheroids. The susceptible and resistant callus treated with resting spore was homogenized and was subjected to light microscopic observation. (A) Primary plasmodium containing zoosporangium, which was very rarely observed only in the susceptible callus. (B) The large (◆) and small (*) -sized free sporangium- like spheroids (SLS) were also observed only in the susceptible callus.

Germination and proliferation of P. brassicae resting spore in the callus

The growth of the calluses seemed to be affected by the proliferation of *P. brassicae*. Therefore, the proliferation of it in the calluses was evaluated. Both the resistant and susceptible calluses with a minimum of 100 mg fresh weight each, maintained on MS agar medium were inoculated with 10^4 spores in 10 μl water. The callus-samples were harvested at various intervals for 20 days and checked the occurrence of certain peculiar structures associated with the early stages of *P. brassicae* life cycle. Sporangium sack-like structure i.e., primary plasmodium, and two free sporangium-like spheroids (SLS), which were distinguishable by their size, were observed only in the homogenate of the susceptible callus (**Fig. 4**). Thus, a large SLS with 4-6 μm diameter and a small one with 2-3 μm diameter were discriminated by the microscope (**Fig. 4A** as indicated by ◆ and ＊ , respectively). The sporangial sack-like structure (**Fig. 4B**), however, was very rarely seen, which may be due to the fact that the homogenization of the calluses disrupted the original structure of the fragile primary plasmodium.

The occurrence of small SLS was least compared to that of the large SLS and furthermore, the difference between cellular organelles and small SLS was less clear under microscope. Therefore, in the present analyses only the large SLS was counted (**Fig. 5**). After the inoculation, surprisingly, the large SLS increased by several 10 times within first 24 h and increased further by 1,000 times during subsequent 6 days, after the inoculation (**Fig. 5**). After the 6 days, the numbers were retained around 10^7 for further 14 days.

Effects of the spore inoculation on phenylalanine ammonia lyase (PAL) activity

P. brassicae proliferated in the Sus callus but did not grow in Res callus. These results prompted us to check the defense reactions in the Res callus. First, the level of constitutively expressed PAL activity was determined in the susceptible callus to be 1 to 2 U. On the other hand, in resistant callus, it was as high as 5 to 6 U (**Fig. 6**).

Both the calluses were inoculated with 10^6 mature resting spores as described above. In the susceptible callus, no change of the activity was observed during 24 h of subculture (**Fig. 7**). On the other hand, in resistant callus, the activity increased by 3 fold on the same period (**Fig. 7A**). The magnitude and timing of increase in PAL activity was highly reproducible in several experiments with different sample. Beyond this time point, PAL activity decreased to the basal level i.e. 5 μg cinnamic acid

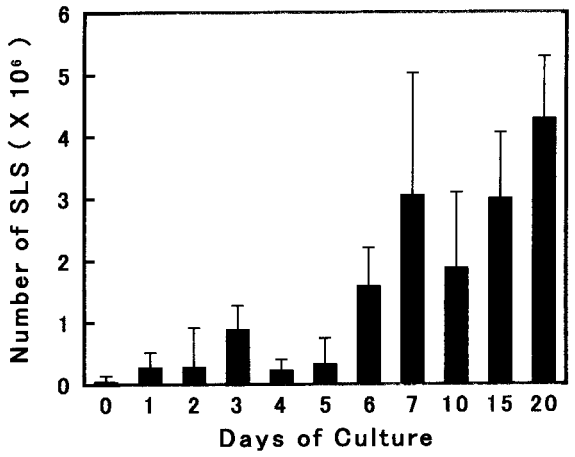

Fig. 5 Number of sporangium-like spheroid (SLS) recovered from the susceptible callus treated with the spores of *Plasmodiophora brassicae*. Ten μl of water containing 10^4 spores were placed on surface of about 100 mg of calluses that had been maintained on MS agar medium. After grinding the calluses, SLS was recovered by centrifugation and counted under the microscope using a Thoma, hema counting chamber. Column represents number of the spheroids (SLS) with 4-6 μm in diameter. SD was calculated with five samples from different flasks and shown with a bar on each plot.

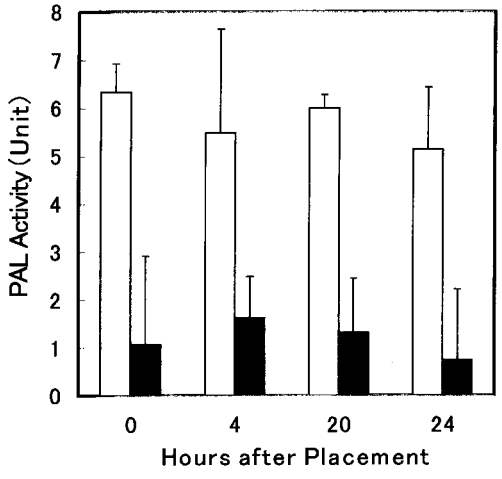

Fig. 6 Constitutively expressed PAL activity in resistant and susceptible calluses of turnips. The resistant (□) and susceptible (■) calluses were being maintained on MS agar medium for 4 days and about 50 mg of calluses were placed on MS agar medium. On designated period, after the placement, each callus was homogenized and PAL activity was determined as shown in Materials and methods. Activity was expressed as Unit equal to 1 μg *trans*-cinnamic acid formed/μg protein/h. The SD was calculated from four experiments in different days and expressed with a bar on each plot.

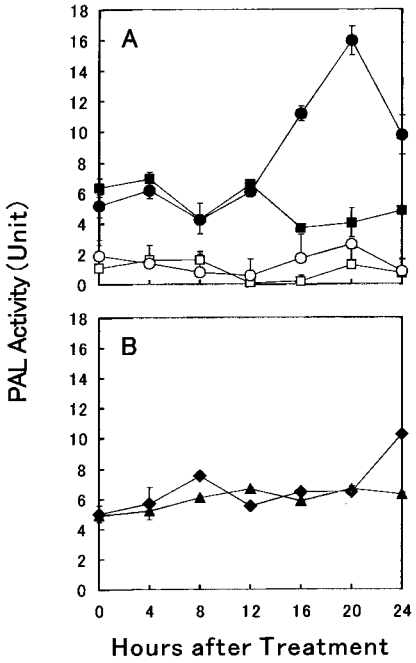

Fig. 7 Effect of *Plasmodiophora brassicae* spores on PAL activity in resistant and susceptible calluses of turnips. (A) Ten μl of water containing 10^4 spores were placed on surface of the resistant (●) and susceptible callus (○) and PAL activity was measured on every 4 h after the treatment. In control experiments, water alone was placed instead of spore suspension onto the resistant (■) and susceptible callus (□). (B) The same number of pre-matured spores prepared from young galls (◆) or secondary plasmodium from juvenile galls of turnips (△) were also applied onto the resistant calluses and the PAL activity was measured as (A).

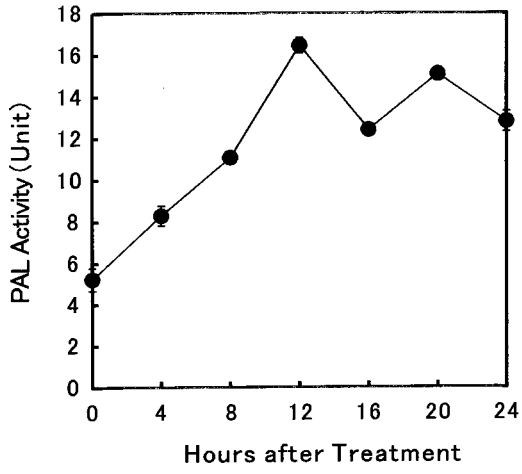

Fig. 8 Effects of the germination of the resting spores on the timing of activation of PAL activity. The resting spore was incubated with germination enhancing solution (GES) for 24 h and then the spore at $10^4/\mu$l was applied on to the resistant callus as **Fig. 7**. SD was calculated from five experiments with five samples in different flasks and expressed as a bar on each plot.

production. The magnitude of increase in PAL activity was almost similar in treatments with 10^3, 10^4 and 10^5 spores (data not shown).

The young spores that were prepared from the immature gall were shown to produce no primary zoospores (Williams and McNabola, 1970; Yukawa and Tanaka, 1979). Therefore, it is of interest to check if spore maturation and/or capability of sprouting are necessary for the enhancement of PAL activity. Then, immature resting spores and secondary plasmodium were checked for their capability to enhance the PAL activity upon their inoculation to calluses (**Fig. 7B**). Neither immature spores nor the plasmodium increased the PAL activity. The slight increase shown at 24 h after the treatment with plasmodium might be due to the effect of small number of mature resting spores contaminating the preparation.

Effects of the spore soaked with GES on PAL activity

Germination-enhancing suspension (GES) was prepared and was incubated with spores for 24 h. The GES-treated spores were placed on the surface of the calluses and PAL activity was checked on every 4 h for 24 h (**Fig. 8**). The increase in PAL activity was not observed in the susceptible callus (data not shown). On the other hand, two peaks of PAL activity induced were observed in resistant callus that was inoculated with the GES-treated spores. One peak corresponded to the 20-h's peak that was observed in treatment with GES untreated-spores (**Fig. 7A**), and another peak came 8 h earlier than the original peak (20-h peak) (**Fig. 8**).

The spores treated for 24 h with GES was shown to germinate on the surface of the callus during 24 h after the placement. Within first 8 h, about 8% of the GES treated spores were germinated (**Table 1**).

Table 1 Germination rate of mature GES-treated spores on surface of the resistant callus

Time after Placing (h)	Rate of Germination (%)
0	3.9 ± 0.5
4	6.3 ± 0.9
8	7.7 ± 3.5
12	17.8 ± 7.1
16	16.3 ± 1.7
20	12.8 ± 2.9
24	22.5 ± 16.8

The mature resting spores were incubated with GES for 24 h and placed on the surface of resistant calluses. On the designated time, germinated spores were counted. For the detail see Materials and Methods.

These germinated spores, i.e., primary zoospores, seemed to be responsible for the first peak of PAL activity observed in **Fig. 8**. On the other hand, the spore that was not subjected to GES treatment did not germinate during 16 h (data not shown). Therefore, the PAL activity was also not enhanced till 20 h after the treatment. These results clearly suggested that the zoospores germinated on the callus surface trigger the acceleration of PAL activity.

Discussion

Induction and culture of turnip callus

Various combinations of NAA and BA were used for growth of the calluses and 0.5 ppm NAA and 1.0 ppm BA was shown to be the best combination. The result was consistent with the results of Ikegamai and Mukobata (1990). Infected callus grew faster than non-infected callus. Although, the enhancement in growth was not high in the present experiment when compared to the 3 fold increase on 12 days as observed by Williams *et al.* (1969), the overall pattern of our findings corresponded to theirs. In resistant calluses, as expected, the spore-treatment did not accelerate the growth (**Fig. 3B**).

Proliferation of P. brassicae in the callus

The primary zoospores have been thought to penetrate into the host plants via root hair. Although we could not observe the primary zoospores in the calluses, they must have penetrated at least into the susceptible callus after the germination, since they proliferated in the callus and resting spores obviously could not invade into the callus. The established callus had no morphological resemblance to root hair, suggesting that the structure similar to root hair may not be necessary for the zoospore-penetration. At present, however, it is still possible that there are specific sites for the penetration of primary zoospores on the surface of callus, which are similar to those in root hairs.

Any way, resting spores must have germinated on the surface of the calluses and the primary zoospores must have penetrated at least into susceptible callus. Then, it rapidly proliferated and/or developed to sporangium-like spheroids (SLS) with 4-6 μm diameter during 24 h after the treatment. On the other hand, absolutely no SLS, not even small sized one, was shown in resistant calluses. Surprisingly, this suggested that the proliferation of sporangium could take place within 24 h after placing spores onto surface of the susceptible callus. The number of small sized SLS became constant on 6 days after the treatment (data not shown), however, large-sized SLS slightly increased for the subsequent 14 days. Zoosporangia of *P. brassicae* were observed in the root hair on 4-6 days after inoculation with resting spores (Matsumiya *et al.*, 1992). Thus, compared to natural occurrence of zoosporangia, in the callus at least of turnips, the proliferation and development seemed to be much faster.

Dekhuijzen (1975) observed plasmodia in the callus of *Brassicae campestris* L. and grouped them into three groups by the diameter ranging 5-20, 5-30 and 7-90 μm, respectively. Indeed in the photographs presented by Ingram (1969), primary and secondary plasmodia could be around 10 μm and 15-20 μm in diameter, respectively. Contrary to these observations, size of SLS was 1-6 μm in diameter in our study. Ingram (1969) presented the pictures showing the primary zoosporangia with the minimum of 5 μm diameter. Thus, the SLS we observed was most likely to be zoosporangium. Indeed, the antiserum raised against the resting spore did not react with SLS (data not shown). The callus was homogenized on every day for 6 days but no plasmodia-like spheroids having a diameter of 10-30 μm or more could be observed.

Activation of PAL activity with the resting spores

The PAL activity is thought to be expressed constitutively for the synthesis of various phenolic compounds. The level of the PAL activity, however, was different between the susceptible and resistant calluses (**Fig. 6**). The resistant strain of *B. campestris* used in this study, had been selected by conventional breeding technique in Musashino Shubyo En. Thus, the breeding of the cultivar resistant to clubroot disease must have led to the selection of plants with enhanced PAL activity.

Although, at this time it is difficult to conclude the involvement of PAL activity in the mechanism of the resistant to clubroot disease, the observation by Dekhuijzen (1979) is worth to be noted. Using electron microscope, he observed that the zoospores were prevented to grow further in resistant turnips and insisted that a hypersensitive reaction was taking place. In our case, however, neither development of zoospore nor proliferation of secondary plasmodium happened in the resistant callus. Thus, the turnip we used should be so resistant compared to that of Dekhuijzen's that ours did not even allow the zoospore to invade or grow for a second.

The inoculation of immature resting spores and secondary plasmodia did not increase the level of PAL activity. In addition, when the resistant callus was incubated with GES-treated resting spores, the first peak of PAL activity occurred 8 h earlier compared to the resistant callus that was inoculated with GES-untreated resting spores. These results

indicated that PAL activity was elevated due to a signal from the primary zoospores sprouted from resting spores. All results shown in this paper, however, could neither suggest the penetration of primary zoospore of *P. brassicae* into the resistant callus nor their growth after the invasion. Further work should be carried out to check whether turnip develops resistance to *P. brassicae* at the level of penetration of primary zoospore or by increasing the activities of enzyme involved in defense reactions like PAL after the zoospore invaded into the cell.

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