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The Effect of Methyl Jasmonate on Harpin-induced Hypersensitive Reaction in Tobacco Plant

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Abstract

The hypersensitive reaction (HR) in plants is a plant defense system against pathogen attack. HR is characterized by the rapid death of infected cells accompanying the formation of necrotic lessions in which pathogens are thought to be enclosed. Following the early signaling events activated by pathogen attack, the elicitor signals are often amplified through the generation of secondary signal molecules such as salicylic acid (SA) and jasmonate acid (JA). Some studies have shown that SA- and JA-signaling pathways are interconnected in complicated ways and it is still not clear how defense response is modulated by SA and JA. To understand better the involvement of JA. the action of MeJA on harpin-induced HR was investigated by using tobacco plant (wildtype and transgenic carrying a chimeric gene of *PSPAL2* promoter and GUS gene. Leaves of of tobacco were infiltrated with harpin and MeJA. The results from HR assay and GUS histochemical staining showed that induction of HR by harpin (250 μg/ml) seems to be modulated by 100 μM MeJA. These results suggest that exogenenously application of MeJA will affect the endogenous concentration in plant that will become a switching point for MeJA to lead the potentiation or suppression of defense response.

Keywords: *harpin, hypersensitive reaction, methyl jasmonate,* Nicotiana tabacum, *plant defense response*

1. INTRODUCTION

Higher plants protect themselves from pathogen invasion such as fungi, bacteria, and viruses through the activation of defense responses. These reactions are initiated by the recognition of the pathogen early in the infection process. The incompatible interaction between pathogens and plants often produce the hypersensitive reaction/response (HR), a defensive suicide of plant cells at the site of infection that is believed to limit the multiplication and spreading of pathogens [1-2].

Local HR occurred at the site of infection is often associated with activation of other plant defense responses including expression of many defense related genes in the infected and even uninfected parts of the plants leading to the development of systemic acquired resistance (SAR) [2-3]. Salicylic acid (SA) is one of the most widely studied stress-signaling molecule; its role in influencing the oxidative burst, defense responses and subsequent cell death that lead to the development of a HR and SAR is well documented [4-5]. Beside SA, the plant hormones, Jasmonic acid (JA) and its methyl ester, methyl jasmonate (MeJA) are known as another signal molecule in plant defense responses. JA are produced from linolenic acid, a major lipid in plant plasma membrane via the octadecanoic biosynthesis pathway [6]. JA accumulates rapidly and transiently when plants are wounded or treated with elicitors [7-8]. Emerging evidences regarding the involvement of jasmonate-signaling in defense response toward host-pathogen interactions had been reported. For example, studies by using mutants of Arabidopsis have demonstrated that pretreatment with MeJA was efficient in protecting against the soil-borne pathogenic fungus *Phytium* spp. and resulted in the reduction of disease development by necrotrophic fungi *Alternaria brassicicola, Botrytis cinerea* or *Plectosphaerella cucumerina* [9-10]. Interestingly, Ishiga [1] found that suppressor, a pathogenicity factor isolated from pea pathogen, *Mycosphaerella pinodes*

[12] induced an expression of pea gene encoding oxophytodienoic acid reductase (OPDAR), which is known as a precursor of JA in the octadecanoic pathway. Based on this result, Ishiga (2000) speculated that JA might be involved in the suppression of defense response and/or in the establishment of a compatible interaction between pathogens and host. Furthermore, Rao et al. [13], reported that JA appears to attenuate ozone-induced hypersensitive cell death in *Arabidopsis thaliana*. These contradictory observations on the role of jasmonate in plant defense responses prompt us to further investigate the effect of MeJA in other plant such as tobacco.

Harpin, a proteineous bacterial elicitor isolated from different plant pathogens had been reported to induce HR in plants and cell cultures [3, 14-18]. Harpin-induced resistance could result from the activation of a variety of defense pathways, including the involvement of secondary signaling molecules such as SA and MeJA. Dong et al. [19] reported that harpin elicits disease resistance through the *NIM*1-mediated SAR signal transduction pathway in an SA-dependent fashion. Since MeJA known to antagonize the effect of SA, it is interesting to investigate the action of MeJA in harpin-induced HR.

Previously, using tobacco BY-2 cells we found that MeJA suppressed the harpin-induced cell death, H2O2 generation and defense genes expression encoding *PAL* and *β-1-3-glucanase*. To verify what had been observed in tobacco suspension culture, and to better understand the regulatory mechanism of MeJA on harpin-induced HR was investigated by using wildtype and transgenic tobacco plants.

2. MATERIALS AND METHODS

Plant material and transformation

Nicotiana tabacum cv. Xanthi NC (kindly provided by Nihon tobacco Inc.) was used in the transformation experiments and also used as a wild-type plant. Seeds were surface-sterilized in 5% hypochlorite for 10 min, followed by soaking in 70% ethanol for 2 min, and rinsed in sterilized water. Sterilized seeds were germinated in a sterile petri dish containing the solidified basal MS medium [20]. Leaves that grown from seedling seeds were used in leaf disk transformation. Leaves of the sterilized tobacco were cut into 0.5-1.0 cm2 in size and immersed for 10 minutes in solution of *Agrobacterium tumefaciens* LBA 4404 carrying the PsPAL2-FL chimeric genes [21]. After drying on a sterilized whatmann 3MM filter paper to remove the excess bacteria, inoculated leaf disk was placed abaxial surface down on solidified free-antibiotic 1st MS medium. After 2-3 days of incubation, the leaf disk was transferred onto selective 2nd MS medium until shoots were formed. Regenerated shoots that grown at the edge of the disks were re-placed and maintained in free-hormone MS medium containing 100 mg/l kanamycin before transferred to a green house. All material plants were maintained at 22°C under 16 h light (150mE/m2/sec)/8 h dark cycle. The integration of PsPAL2-FL-GUS fusion in the genome of transgenic plants was confirmed by GUS-PCR analysis as described by Hosaka [22].

Harpin and Methyl Jasmonate

The amplified DNA carrying HrpZ*Psg* was subcloned into pET 16b vector for expression in *Escherichia coli* strain BL21 (Novagen, WI USA) cells and harpin was purified from this transformed cells according to Taguchi et al.[17].

Plant hypersensitive response assay

Recombinant harpin protein was prepared using pET 16b vector for expression in *Escherichia coli* strain BL21 cells (Novagen, WI USA) as described by Taguchi et al.(2001). MeJA was obtained from Wako (Tokyo, Japan). The assay was performed in wild-type and transgenic plants. Leaves were infiltrated with 250 μg/ml harpin from P. syringae pv. glycinea, different concentrations of MeJA (20, 50 and 100 μM), combinations of harpin and MeJA, and sodium phosphate buffer (pH 6.5) as control treatment. Infiltration was carried out by pricking the 5th or the 6th leaves with a dissecting needle and then pressing the blunt-end of a tuberculin syringae against the leaf surface while supporting the leaf with a finger [23].

GUS histochemical assay

The activity of reporter gene product β-glucuronidase (GUS) in transgenic plants can be assayed by histochemical assay for the specificity of expression. The histochemical assay uses 5-bromo-4-chloro-3-indolyl-β-D-glucuronide (X-Gluc) as the substrate. Cleavage of this substrate, produces the final insoluble blue precipatate dichloro-dibromoindigo (ClBr-indigo) [24]. Detached leaves of transgenic plants were placed on moist cotton in sterile petri dish, and the infiltration procedures were as described for the HR assay. The leaves were subjected to histochemical assay at 8 h after infiltration. Leaves were fixed by submerging in a buffered 1% formaldehyde solution for 10 min and rinsed three times with buffer assay (50 mM sodium phosphate buffer pH 7.0). Then, the leaves were incubated in staining solution at 37°C for overnight as described by Jefferson et al. [25]. Application of buffer assay, fixative and staining solutions into

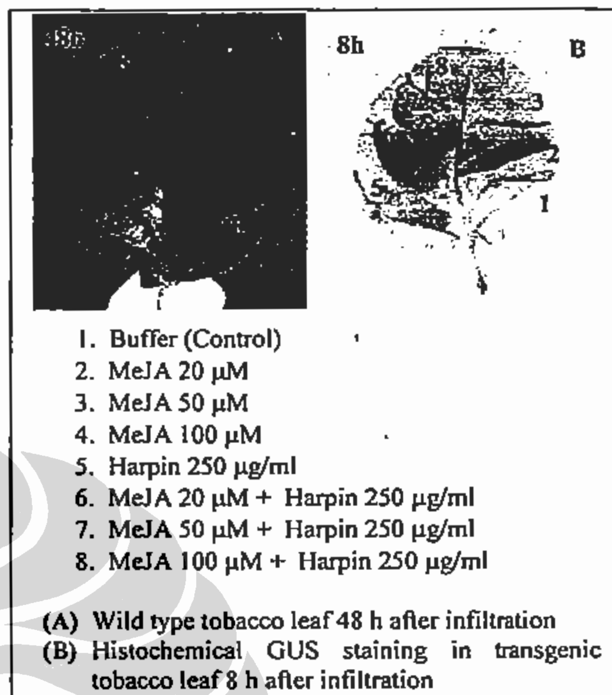
tissues were facilitated by vacuum infiltration. Staining reactions were stopped by transferring the tissues into 70% ethanol which also cleared chlorophyl from the stained tissues.

3. RESULT AND DISCUSSION

Previous study has shown that harpin from P. syringae pv. glycinea induced HR and GUS activity in tobacco plant Taguchi et al. [17].(2001). To investigate whether application of MeJA will modulate the effect of harpin as observed in BY-2 cells, HR assay and histochemical localization GUS activity were performed. Induction of HR by harpin start to be observed within 24 h. Fig. 1A, showed the result from HR assay 48 h after infiltration. MeJA alone had no effect as well as buffer control to the leaves. Application of MeJA together with harpin showed that MeJA modulate the harpin effect. Concentration of MeJA at 20 µM and 50 µM showed the tendency to potentiate the harpin effect, while 100 µM to suppress. Similar observation was observed when transgenic tobacco plants carrying a chimeric gene of *PsPAL2* promoter and *GUS* gene. Fig. 1B, showed that the induction of GUS activity in the leaf areas infiltrated with MeJA (20 µM and 50 µM) plus harpin, seems to be more stronger than that of harpin alone. In contrast, when 100 µM of MeJA was used, the GUS activity became less visible. The results from HR assay and GUS histochemical staining showed that 100 µM of MeJA was sufficient enough to suppres the harpin effect, and this observation is consistent with the results observed in BY-2 cells. However, since 20 µM and 50 µM of MeJA seem to potentiate the HR-inducing effect of harpin in wild-type plant and the GUS-expression in transgenic plant, it could be suggest that exogenously application of MeJA will affect the endogenous concentration in plant that will become a switching point for MeJA to lead the potentiation or suppression regulation.
Interaction between concentration of MeJA and other signal molecule such SA concentration could also to be considered since SA- and JA- signaling pathways are interconnected in complicated ways.

4. CONCLUSION

Our present results demonstrating that MeJA modulate the harpin-induced defense response in tobacco plant. Low concentration of MeJA up to 50 µM seems to potentiate the harpin-induced HR, while high concentration (100 µM) appears to suppress the harpin-induced HR.



1. Buffer (Control)
2. MeJA 20 µM
3. MeJA 50 µM
4. MeJA 100 µM
5. Harpin 250 µg/ml
6. MeJA 20 µM + Harpin 250 µg/ml
7. MeJA 50 µM + Harpin 250 µg/ml
8. MeJA 100 µM + Harpin 250 µg/ml

(A) Wild type tobacco leaf 48 h after infiltration
(B) Histochemical GUS staining in transgenic tobacco leaf 8 h after infiltration

Fig 1. The effect of MeJA on harpin-induced HR in tobacco leaves.

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