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Bacterial infection and pre-treatment with 24-epibrassinolide markedly affect the heat emission and membrane permeability of rape cotyledons

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Abstract

Changes in metabolic activity in spring oilseed rape (*Brassica napus* L.) seedlings during bacterial infection were determined by measuring heat emission with an isothermal calorimeter. Heat emission was markedly increased in cotyledons infected with the incompatible bacterium *Pseudomonas syringae* pv. *syringae.* One day after inoculation, visible signs of necrosis were detected on the infected cotyledons. This indicates that the hypersensitive response had been activated. Heat emission continued to increase as the hypersensitive response progressed, reflecting an increase in the metabolic rate in the infected tissue. With both of the cultivars tested heat emission by infected cotyledons was increased by pre-treatment with BR_{27}. Membrane permeability as measured by ion leakage also rapidly increased during the first 3 days of infection. In uninfected cotyledons pre-treated with BR_{27}, membrane permeability was slightly increased. In infected cotyledons not pre-treated with BR_{27}, both visible necrosis and membrane permeability rapidly increased during the first 2 or 3 days after inoculation. In infected cotyledons pre-treated with BR_{27}, membrane permeability was significantly reduced by the second or third day after inoculation. This confirms that BR_{27} plays a role in preventing damage to plant cell membranes caused by bacterial infection during the hypersensitive response.

Keywords: Bacterial infection; *Brassica napus* L.; Isothermal calorimetry; Stress resistance; 24-Epibrassinolide

1. Introduction

Brassinosteroids are steroidal plant hormones that stimulate plant growth and protect plants against various stressors, including heavy metals, salt, heat shock, cold and pathogens [1–4]. Although they were discovered in the 1970s, little is known about the role brassinosteroids play in combating microbial infections in plants. The hypersensitive response is one of the best characterized defense mechanisms in infected host plants. The hypersensitive response involves programmed cell death, which causes rapid necrosis in infected tissues [5]. During the hypersensitive response, cell permeability rapidly increases as the cell membranes break down. Changes in metabolism also take place in plants during microbial infection. Calorimetric measurement of heat emission has been used to study metabolic changes in plants responding to various other stressors, including salt, drought and herbicides [6–9]. The aim of this study was to determine the effects of 24-epibrassinolide (BR_{27}) on heat emission and cell permeability in oilseed rape seedlings artificially infected with the incompatible pathogenic bacterium *Pseudomonas syringae* pv. *syringae.*

2. Material and methods

2.1. Plant material and pre-treatment with BR_{27}

The study was carried out using two cultivars of spring oilseed rape (*Brassica napus* L.): ‘Licosmos’ and ‘Huzar’. After sowing, the plants were kept in a greenhouse for 14 days, by which time both cotyledons and the first leaf had developed. The cotyledons were then painted with a solution containing 100 nM BR_{27}, prepared from a stock solution containing 2 mM BR_{27} in 96% ethanol. The BR_{27} used in this study was purchased from Sigma (Poznań, Poland). The cotyledons of the control plants were painted with distilled water. A suspension of *P. syringae* pv. *syringae* containing 10^8 cfu cm^{-3} was then injected into the

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cotyledons by applying gentle pressure with the help of a plastic syringe not fitted with a needle. The concentration of the bacterial suspension was determined with the help of a spectrophotometer.

2.2. Measurement of heat emission

Rate of heat flow from the cotyledons was measured with the help of an isothermal LKB-2277 Bioactivity Monitor (Thermometric, Järfälla, Sweden). One day after the plants were treated with BR_{27} and innoculated with bacterial suspension, the cotyledons were cut, placed in an ampule, and allowed to equilabrate for 20 min before analysis. Measurements were carried out for 10 min at 20 °C in four or five replicates. Results were recorded in terms of heat emitted (μW) and recalculated per gram dry weight of sample, thus converted into specific heat production rate. The heat emitted by the bacteria themselves was not a significant factor in this study. Since 1.5 cm^3 of the bacterial suspension emitted 60 μW of heat, and since the amount of suspension used to inoculate each cotyledon was only 20 μl, the amount of heat emitted by the bacteria present in each sample was negligible in comparison to the amount of heat emitted by the plant tissue itself.

2.3. Measurement of membrane permeability

After inoculation, the cotyledons were cut and placed in Petri dishes containing 10 cm^3 of distilled water. Membrane permeability was determined by measuring ion leakage from the cotyledons with an OK-102/10 conductivity meter (Radelkis, Budapest, Hungary) in accordance with the method described by Barna et al. [10]. The first measurement was made 4 h after inoculation. Subsequent measurements were made 1, 2, 3, 6 and 7 days after infection.

3. Results

One day after inoculation, localized tissue necrosis was visible on the cotyledons. Two days after inoculation, the cotyledons were completely withered in the plants not pre-treated with BR_{27}. On the other hand, in infected plants pre-treated with BR_{27}, the spread of necrosis was significantly reduced and remained confined to a small area surrounding the injection site (Fig. 1). One day after inoculation, heat emission was significantly higher in infected cotyledons than in uninfected cotyledons (cultivar 'Huzar'). This indicated that metabolic activity increased in tissue during the hypersensitive response induced by bacterial infection. In uninfected cotyledons, pre-treatment with BR_{27} had no effect on heat emission. On the other hand, in infected cotyledons, pre-treatment with BR_{27} significantly increased heat emission in both of the cultivars tested. This strongly suggests that BR_{27} plays a role in preventing necrosis due to infection by incompatible bacteria in oilseed rape cotyledons (Fig. 2). Membrane permeability rapidly increased from the first to the second or third day after inoculation. In inoculated plants not pre-treated with BR_{27}, membrane permeability gradually increased throughout the 7 day observation period in both of the cultivars tested. In inoculated plants pre-treated with BR_{27}, membrane permeability was significantly reduced starting on the second or third day after inoculation and continuing for several days in both of the cultivars tested, espe-

Fig. 1. Effect of BR_{27} on necrosis caused by infection with *Pseudomonas syringae* pv. *syringae* in the oilseed rape cultivar 'Licosmos' 7 days after inoculation. The healthy cotyledon on the left had been pre-treated with 100 nM BR_{27}, whereas the withered cotyledon on the right had not been pre-treated with BR_{27}.

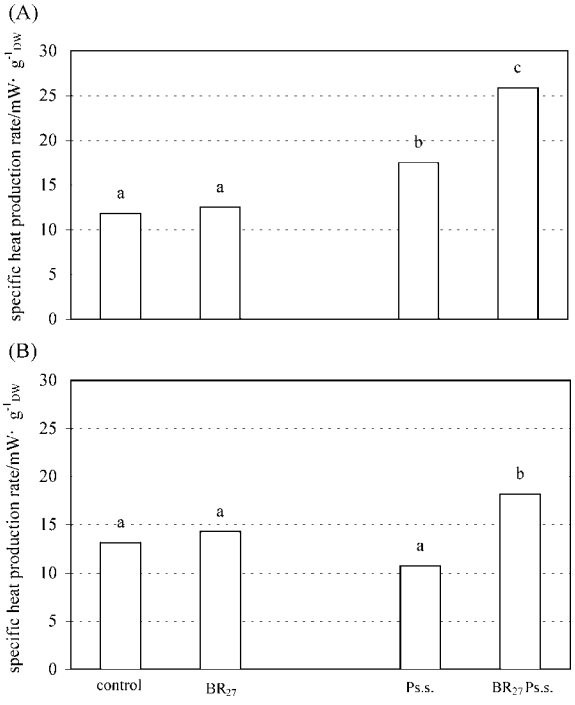

Fig. 2. Specific heat production rate in cotyledons of the oilseed rape cultivars Huzar (A) and Licosmos (B) 1 day after inoculation with *Pseudomonas syringae* pv. *syringae* both with and without pre-treatment with 100 nM BR_{27}. Abbreviations: control, cotyledons pre-treated with water; BR_{27}, cotyledons pre-treated with BR_{27} (100 nM) solution; Ps.s., cotyledons injected with suspension of incompatible bacteria *P. syringae* pv. *syringae* (10^8 cfu cm^{-3}); BR_{27} Ps.s., cotyledons pre-treated with BR_{27} and injected with *P. syringae* pv. *syringae*; DW, dry weight. Data marked with the same letter are not significantly different according to Duncan's multiple-range *t*-test at $P < 0.05$.

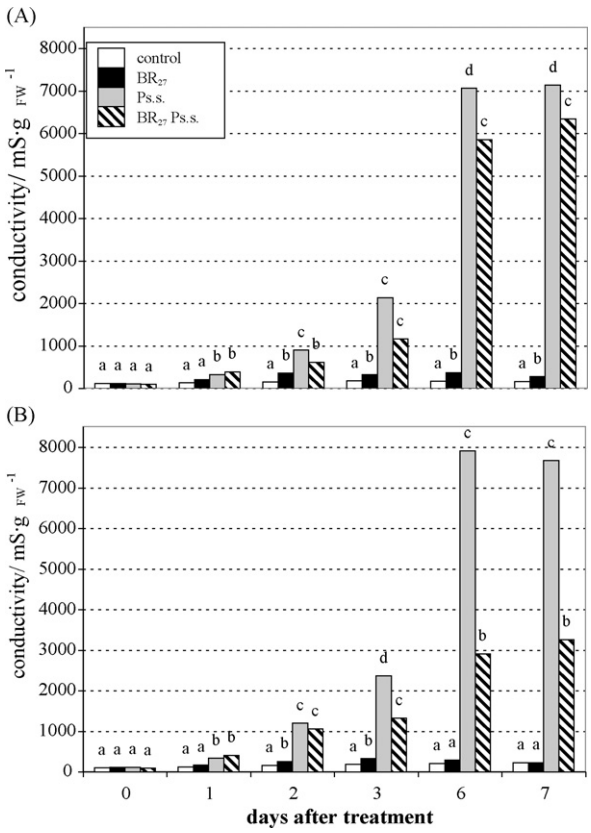

Fig. 3. Ion leakage in cotyledons of the oilseed rape cultivars Huzar (A) and Licosmos (B) after inoculation with *Pseudomonas syringae* pv. *syringae* both with and without pre-treatment with 100 nM BR_{27}. For abbreviations see Fig. 2. Data for the same day marked with the same letter are not significantly different according to Duncan's multiple-range *t*-test at $P < 0.05$.

cially 'Licosmos'. In uninoculated plants that were pre-treated with BR_{27}, membrane permeability was only slightly increased (Fig. 3).

4. Discussion

Brassinosteroids protect plants against biotic and abiotic stressors, including infection by *Phytophtora infestans*, *Pseudomonas syringae* pv. *tabaci*, *Fusarium sulfuricum*, and *Oidium* sp. [1,2,11]. In the present study, BR_{27} significantly reduced the extent of cell membrane breakdown and necrosis in oilseed rape tissue infected with the incompatible bacterium *P. syringae* pv. *syringae.* However, brassinosteroids also suppress bacterial multiplication in the compatible plant–bacterium interaction between tobacco and *P. syringae* pv. *tabaci* [11]. The mechanism by which brassinosteroids activate plant defense systems has not been completely elucidated. The resistance mediated by brassinosteroids does not require the biosynthesis of salicylic acid [11]. Brassinosteroids increase the amount of protective compounds such as phenolics and terpinoids in infected tissues [2]. The amount of heat emitted by plant tissues is affected by changes in various biological processes, including growth, metabolism and biocatalytic activity [6,7,12]. Calorimetric techniques have been used to study the activity of insects and fungi in stored wheat [6]. They have also been used to monitor the growth of bacteria in bio-reactors [13,14]. However, little is known about changes in heat emission from infected plant tissue [15,16]. In the present study, heat emission was increased in oilseed rape cotyledons during bacterial infection. This indicates that the hypersensitive response had been activated by bacterial attack. Furthermore, heat production rate was significantly increased in infected cotyledons of both cultivars which had been pre-treated with BR_{27}. To the best of our knowledge, this is the first time calorimetry was used to demonstrate the activation of plant defense mechanisms by hormones in response to infection with microbial pathogens. Membrane permeability typically increases in response to microbial infection. This reflects the damage to cell membranes caused by the attacking pathogen [17]. In the present study, membrane permeability was slightly increased in uninfected oilseed rape cotyledons pre-treated with BR_{27}, which agrees well with previous reports [18]. On the other hand, membrane permeability was decreased in infected cotyledons that had been pre-treated with BR_{27}. This confirms that BR_{27} plays a role in reducing stress due to bacterial infection during the hypersensitive response. BR_{27} has also been shown to protect oilseed rape plants against tissue damage due to various other stressors, including cadmium and cold [18].

4.1. Conclusions

Heat emission by oilseed rape cotyledons increased in response to infection by the incompatible bacterium *P. syringae* pv. *syringae.* Pre-treatment with BR_{27} markedly increased the metabolic activity (heat production rate) in infected cotyledons, and increased host plant resistance to necrosis caused bacterial infection. Calorimetric measurement of heat emission by cotyledons or leaves can be used to complement other tests in studying plant–pathogen interactions.

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