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Response of brassinosteroid-treated oilseed rape cotyledons to infection with the wild type and HR-mutant of *Pseudomonas syringae* or with *P. fluorescence*

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Abstract The aim of the study has been to determine the protection effect of brassinosteroid (BR_{27}) in oilseed rape cotyledons against infection by an incompatible wild type of, a hypersensitive response mutant of and saprophytic *Pseudomonas* bacteria. In this paper, membrane permeability, PSII efficiency and metabolic activity were analysed. The following strains of *Pseudomonans* were used: *P. syringae* pv. *syringae* (*Ps*), *P. syringae* pv. *syringae hrcC* mutant (*Pm*) and *P. fluorescence* (*Pf*). The study was carried out using two cultivars of spring oilseed rape (*Brassica napus* L.): 'Licosmos' and 'Huzar'. Pre-treatment of cotyledons with BR_{27} caused about 50–70% increase in ion leakage for both cultivars. However, BR_{27} significantly decreased ion leakage from cotyledons inoculated with *Ps* in both cultivars. Infection with *Ps* and *Pf* caused disturbances of energy flow in PSII by lowering its efficiency in rape cotyledons. We noted insignificant impact of 24-epibrassinolide on PSII efficiency if compared to absolute control, but generally it had a positive effect in plants infected with bacteria. The values of heat flow in all treatments, except for cotyledons infected with *Ps*, decreased during 20 h after inoculation. However, the curves of heat flow for *Ps*-infected cotyledons showed a completely different pattern with at least two peaks. BR_{27} pre-treated cotyledons infected with *Ps* had higher heat flow in comparison to *Ps* infected ones. BR_{27} treatment did not change specific enthalpy of cotyledon growth (Δgh) for both cultivars if compared with absolute control. However, infection with *Ps* markedly increased Δgh values by about 200% for both cultivars. We suggested protective action of BR_{27} in oilseed rape cotyledons after bacterial infection with *Pseudomonas.*

Keywords *Brassica napus* · 24-Epibrassinolide · Isothermal calorimetry · Membrane permeability · Pathogenesis · PSII efficiency

Abbreviations

AC	Absolute control
ABS	Energy absorption
BRs	Brassinosteroids
BR_{27}	24-Epibrassinolide
cfu	Colony forming unit
DIo	Energy dissipation
ETo	Energy flux for electron transport
F_v/F_m	Maximum quantum yield of PSII
HR	Hypersensitive response
Ps	*Pseudomonas syringae* pv. *syringae*
Pm	*Pseudomonas syringae* pv. *syringae hrcC* mutant
Pf	*Pseudomonas fluorescence*
RCs	PSII reaction centres
TRo	Energy flux for trapping
Δgh	Specific enthalpy of growth

Introduction

The discovery of a new naturally occurring plant growth regulator, a polyhydroxylated steroid lactone with high

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growth-promoting activity, called brassinolide, from rape pollen (*Brassica napus* L.) was reported in 1979 [1]. Since then about 70 similar steroid compounds have been found in plants and this group has been called brassinosteroids (BRs) [2]. The discovery of BRs shows that steroids are signalling molecules in both animals and plants. While mammalian steroid hormones are recognised mainly by nuclear receptors, according to current knowledge, brassinosteroids in plants are recognised by a cell surface receptor kinase, BRI1 [3]. BRs occur at low levels [from <0.05 to 1600 ng g^{-1} fresh weight (FW)] throughout the plant kingdom in pollen, seeds, fruits, shoots and roots [4–6]. Examples of brassinosteroids are brassinolide, 24-epibrassinolide (BR_{27}), castasterone, homobrassinolide, etc. [7]. BRs regulate the expression of numerous genes, influencing the activity of complex metabolic pathways, and contribute to the regulation of cell division and differentiation, plant growth and crop production, as well as plant response to stress [5, 8–13].

An important aspect of their activity is the protection of plants against different stressors, such as pathogens [14–17]. Programmed cell death is part of plant defence response typical of an incompatible pathogen–host interaction called hypersensitive response [18]. During hypersensitive response cell permeability rapidly increases as cell membranes break down. Pathogen infections not only affect defence reactions but also cause changes in photosynthesis [19, 20]. Bonfig et al. [21] found that infection of *Arabidopsis thaliana* with virulent and avirulent *Pseudomonas syringae* strains differentially decreased photosynthesis and invertase activity in leaves. On the other hand, data on the influence of brassinosteroids on photosynthesis and especially on changes induced by bacterial infection of plants are very limited. In addition, the impact of bacterial infection and BR_{27} on overall metabolic efficiency of plants is only partly recognised [22].

The aim of the investigation was to determine the effect of an incompatible wild type of, a hypersensitive response (HR) mutant of and saprophytic *Pseudomonas* bacteria on membrane permeability, PSII efficiency and metabolic activity of oilseed rape cotyledons with and without brassinosteroid pre-treatment. The following *Pseudomonans* strains were used: *P. syringae* pv. *syringae*, *P. syringae* pv. *syringae hrcC* mutant and *P. fluorescence*. The *P. syringae* species encompasses plant pathogens with differing host specificities and corresponding pathovar designations. Mutant analysis showed that *P. syringae* requires the Hrp (type III protein secretion) system encoded by a 25-kb cluster of *hrp* and *hrc* genes in order to elicit the hypersensitive response in nonhosts or to be pathogenic in hosts [23]. Mutation in *hrc* gene (in *P. syringae* pv. *syringae hrcC* mutant) causes lack of HR reaction in infected plants. *P. fluorescence* is common saprophytic bacteria without pathogenic activity in plants.

Experimental

Plant material and treatment

The study was carried out using two cultivars of spring oilseed rape (*B. napus* L.): 'Licosmos' and 'Huzar'. Seeds were sown into pots and after 14 days of growth in field conditions (September, Latitude: 50°03′ North, Longitude: 19°55′ East, with a day length of ~12 h) until cotyledons and the first leaf developed. Cotyledons were then brushed with solution containing 200 nM 24-epibrassinolide. BR_{27} was purchased from Sigma–Aldrich (Poznań, Poland). Cotyledons of the control plants were brushed with distilled water. After 2 h suspensions of *P. syringae* pv. *syringae* (*Ps*) (10^8 cfu cm^{-3}), *P. syringae* pv. *syringae hrcC* mutant (*Pm*) and *P. fluorescence* (*Pf*) were injected into the whole cotyledons using a plastic syringe not fitted with a needle [24].

The following treatments were tested:

AC	cotyledons brushed with water (absolute control);
BR_{27}	cotyledons brushed with 24-epibrassinolide;
Ps	cotyledons injected with *Ps*;
BR_{27} Ps	cotyledons brushed with 24-epibrassinolide and injected with *Ps*;
Pf	cotyledons injected with *Pf*;
BR_{27} Pf	cotyledons brushed with 24-epibrassinolide and injected with *Pf*;
Pm	cotyledons injected with *Pm*;
BR_{27} Pm	cotyledons brushed with 24-epibrassinolide and injected with *Pm*

Membrane permeability measurements

After inoculation, when water evaporated from infiltrated cotyledons (about 1 h), they were cut and placed on the surface of 10 cm^3 of distilled water in Petri dishes. Membrane permeability was determined by measuring ion leakage from cotyledons with an OK-102/10 conductivity meter (Radelkis, Budapest, Hungary) in accordance with the method described by Barna et al. [25]. The first measurement was made 2 h after inoculation. Subsequent measurements were made 24, 48 and 72 h after inoculation.

Measurements of PSII efficiency

PSII efficiency was estimated based on measurements of parameters of chlorophyll *a* fast fluorescence using a Plant Efficiency Analyzer (PEA; Hansatech Ltd. King's Lynn, Norfolk, England). Excitation light intensity was 3 mmol m^{-2} s^{-1} (650 nm peak). Cotyledons were measured after 30 min of adaptation to darkness (clips with a

4 mm diameter hole) at 20 °C. Fluorescence measurements were performed 2, 5 and 20 h after bacterial infection in 15 repetitions. Changes in fast fluorescence were registered during illumination within a period of 10 µs to 1 s. During the initial 2 ms data were collected every 10 µs. After this period, the frequency of measurements was reduced automatically. The data were analysed using the JIP test [26]. The following technical fluorescence parameters were measured: F_o—fluorescence intensity at 50 µs (it is assumed that at that time all PSII reaction centres (RCs) are open); F_m—maximum fluorescence (all RCs closed); F_{300}—fluorescence intensity at 300 µs (point *K*); F_{2ms} (F_J)—fluorescence intensity at 2 ms (point *J*); $V_J = (F_{2ms} - F_o)/(F_m - F_o)$; V_J corresponds to relative variable fluorescence at point *J*; $V_K = (F_{300} - F_o)/(F_m - F_o)$, V_K corresponds to relative variable fluorescence at point *K*. Based on technical fluorescence parameters the following ones were calculated: F_v/F_m—maximum quantum yield of PSII; phenomenological fluxes (or phenomenological activities): energy absorption ABS/CS = F_m; energy flux for trapping TRo/CS = $F_v/F_m \cdot$ (ABS/CS); energy flux for electron transport ETo/CS = $(F_v/F_m) \cdot (1 - V_J) \cdot F_m$; energy dissipation DIo/CS = (ABS/CS) − (TRo/CS); where CS is the sample cross section. The parameters describe plant PSII efficiency.

Calorimetric measurements

Metabolic activity of cotyledons (heat flow/mW g_{DW}^{-1}) (sometimes called specific thermal power) was recorded at 20 °C using TAMIII isothermal calorimeters with TAM Assistant software (Thermometric, Järfälla, Sweden). 20 cm^3 ampoules with lids which enable natural air exchange were used. Cotyledons of oilseed rape, cut off from plants 2 h after bacterial injection, were put into ampoules on filter paper moistened with 300 µL of water. The reference ampoule contained filter paper with water. After thermal equilibration for 30 min, heat flow from cotyledons (thermal power–time curves) was continuously recorded for 18 h. Heat flow and enthalpy values were expressed as per the dry weight of cotyledons. The enthalpy values were determined from heat flow curves as area under the calorimetric curve for the monitoring period (18 h).

Results

During the hypersensitive response, cell membrane permeability rapidly increases as cell membranes break down. Thus, the rate of ion leakage from the infected plant tissue correlates with the strength of HR. Pre-treatment of cotyledons with BR_{27} caused about 50–70% increase in ion leakage for both cultivars (Fig. 1). In accordance with the strong HR, the wild type of *P. syringe* pv. *syringae* induced rapid leakage of ions from cotyledons. However, BR_{27} pre-treatment significantly reduced ion leakage from *Ps* inoculated cotyledons of both 'Huzar' and 'Licosmos' cultivars (Fig. 1). As regards the mutant bacteria, inoculation with *Pm*, in accordance with the absence of HR, caused a slight increase in ion leakage, which was significant only in 'Huzar' cotyledons. Similarly, saprophytic *Pf* bacteria did not cause HR but a small, although significant increase in ion leakage from infected tissues. Brassinosteroid pre-treatment generally reduced ion leakage induced by *Pm* and *Pf*, but the difference was significant only in the case of 'Huzar' cotyledons injected with *Pm* when relatively strong leakage was induced.

In both varieties, oilseed rape infection of cotyledons with *P. syringae* pv. *syringae* and *P. fluorescence* caused disturbances of energy flow in PSII by lowering its efficiency (Table 1). In comparison with absolute control values of parameters F_v/F_m and phenomenological fluxes (energy absorption ABS, energy flux for trapping and flux for electron transport TRo, ETo) were lowered. This effect was observed already 2 h after infection. However, in both varieties changes were stronger in the case of infection with *Ps* than with *Pf*. Energy flux for electron transport 20 h after *Ps* infection was lower than absolute control by about 27% ('Huzar') and 32% ('Licosmos'). Infection with *Pf* caused reduced ETo values by about 8% (Huzar) and 6% ('Licosmos'), compared to absolute control. Dissipation of energy (DIo) was transiently lowered in first hours after infection with *Ps* and *Pf*. After 20 h, however, energy dissipated as heat in cotyledons infected with *Ps* and *Pf* was not different versus 'Licosmos' control. In 'Huzar' plants after 20 h energy loss was a little lower only in cotyledons infected with the *Pf*. *P. syringae* pv. *syringae hrcC* mutant and caused only slight and transient changes in PSII energy flows 2 and 5 h after infection, while after 20 h no significant differences in *Pm* treated cotyledons in comparison to absolute control were detected. The impact of BR_{27} on PSII efficiency was insignificant when compared to absolute control, but generally it had a positive effect in plants infected with bacteria. As noted before, *Ps* lowered PSII efficiency in both cultivars tested. However, in plants cv. 'Licosmos' pre-treated with BR_{27} 2 h after infection mild protective activity of the hormone was already observed. 20 h after inoculation statistically significant differences in PSII activity between *Ps*-infected cotyledons and cotyledons pre-treated with BR_{27} and subsequently infected with *Ps* were noticed. In BR_{27} pre-treated plants an increase in the maximum quantum yield of PSII and phenomenological fluxes was observed. However, values of PSII parameters measured for BR_{27} Ps were still significantly lower than in absolute control. In the case of 'Huzar', *Ps* lowered PSII efficiency, but BR_{27} did not have any improving effect in this cultivar. Moreover, values of some parameters for 'Huzar' cotyledones treated with BR_{27} were even lower than those infected with *Ps*. Infection of *Pf*

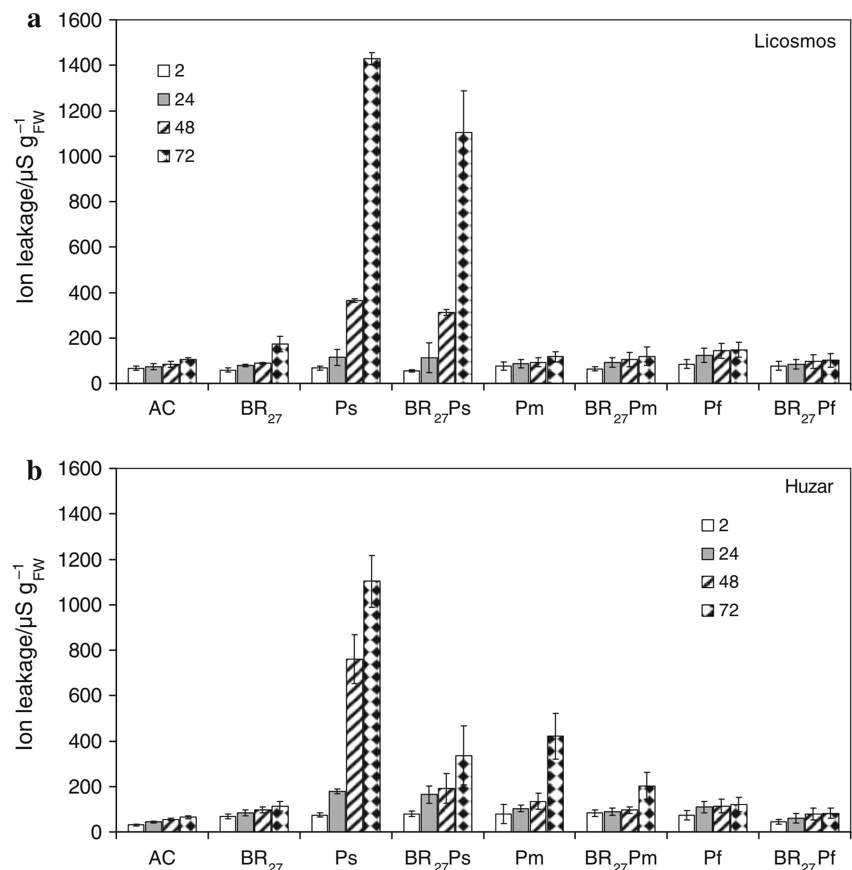

Fig. 1 Ion leakage from cotyledons of oil rape seedlings after treatment with 24-epibrassinolide and infection with *Pseudomonas syringe* pv. *syringae, Pseudomonas fluorescence* and *Pseudomonas syringae* pv. *syringae hrcC* mutant 2, 24, 48 and 72 h after inoculation. **a** cv. 'Licosmos', **b** cv. 'Huzar'. Mean values from 6 repetitions ± SD

cotyledons in the first hours disturbed energy fluxes in PSII but BR_{27} had a recovery effect in both oilseed rape cultivars. 'Huzar' plants pre-treated with BR_{27} demonstrated restored values of photosynthetic parameters of BR_{27} Pf on the AC level 20 h after inoculation. At the same time values of phenomenological fluxes in *Pf*-infected plants still differed from those in AC. As for 'Licosmos', BR_{27} protective activity was observed already in the 2nd and 5th hour. The changes between PSII parameters of BR_{27} Pf and absolute control were not significant. At the same time the efficiency of PSII of *Pf*-infected plants was lower than AC. After 20 h the negative effect of *Pf* was neutralised also in *Pf*-infected 'Licosmos' plants. Then, in this cultivar, BR_{27} enabled faster PSII recovery. Infection of oilseed rape cotyledons with *P. syringae* pv. *syringae hrcC* mutant did not cause severe damage and BR_{27} had no effect in this case. Some transient changes in F_v/F_m values in BR_{27} Pm plants were observed when compared to *Pm* treated ones, but in the 20th hour values of all tested parameters were stable on the absolute control level.

Heat flow–time growth curves are shown in Fig. 2a, b for cotyledons of both cultivars, 'Licosmos' and 'Huzar'. Thermal power of all treatments except for *Ps* infection decreased during 18 h. The curves of heat flow power for *Ps*-infected cotyledons showed completely different patterns in at least 2 peaks. The first peak for both cultivars was observed after 2 h (Fig. 2a, b), in fact 4 h post-inoculation (see 'Experimental'). The second peak appeared in the 7th hour after *Ps* injection (5 h of monitoring) for 'Huzar' cotyledons (Fig. 2b). In the case of 'Licosmos' the second peak was much less visible (Fig. 2a). It is interesting to note that *Ps*-infected and BR_{27} pre-treated cotyledons had higher heat flow in comparison to *Ps*-infected ones (Fig. 2a, b).

Specific enthalpy values for cotyledon growth of AC plants were 673 and 654 for 'Licosmos' and 'Huzar', respectively (Table 2). BR_{27} treatment did not change specific enthalpy of cotyledons in both cultivars tested compared to AC. Bacterial inoculation changed the Δgh of cotyledon growth. *Ps* infection markedly increased Δgh values by about 200% for both cultivars. *Pm* and *Pf* inoculation did not alter Δgh in comparison to AC. BR_{27} pre-treatment of cotyledons which were subsequently *Ps* infected caused a slight tendency of higher Δgh values in both cases (in comparison to *Ps* infected).

Discussion

As expected, incompatible, HR inducing bacteria caused strong leakage of electrolytes from damaged cotyledon tissues. Interestingly, the HR negative mutant and even the

Table 1 Oilseed rape cotyledon efficiency of PSII after treatment with 24-epibrassinolide and infection with *Pseudomonas syringe* pv. *syringae*, *Pseudomonas fluorescence* and *Pseudomonas syringae* pv. *syringae hrcC* mutant 2, 5 and 20 h after inoculation; (**a**) cv. 'Licosmos', (**b**) cv. 'Huzar'

Time/h	Treatment	F_v/F_m		ABS/CSm		TRo/CSm		ETo/CSm		DIo/CSm	
(a)											
2	AC	0.841	a	1126	a	916	a	576	a	210	ab
	BR_{27}	0.840	ab	1129	a	918	a	585	a	211	ab
	Ps	0.831	d	963	b	776	b	496	b	187	c
	BR_{27} Ps	0.831	d	1012	b	813	b	514	b	199	bc
	Pf	0.837	c	1009	b	819	b	526	b	190	c
	BR_{27} Pf	0.837	c	1109	a	896	a	568	a	212	ab
	Pm	0.837	bc	1134	a	919	a	584	a	215	a
	BR_{27} Pm	0.837	bc	1119	a	907	a	577	a	212	ab
5	AC	0.840	ab	1140	a	926	a	588	a	214	a
	BR_{27}	0.841	a	1130	a	919	a	584	a	211	a
	Ps	0.825	e	891	c	712	c	449	c	179	c
	BR_{27} Ps	0.827	e	944	bc	754	c	466	c	190	c
	Pf	0.835	cd	1003	b	811	b	511	b	193	bc
	BR_{27} Pf	0.837	bc	1084	a	879	a	566	a	205	ab
	Pm	0.836	cd	1147	a	928	a	587	a	218	a
	BR_{27} Pm	0.834	d	1103	a	890	a	565	a	214	a
20	AC	0.838	ab	1096	ab	889	ab	556	ab	207	abc
	BR_{27}	0.841	a	1088	ab	886	ab	564	ab	203	bc
	Ps	0.808	d	863	d	670	d	380	d	193	c
	BR_{27} Ps	0.823	c	954	c	756	c	444	c	198	bc
	Pf	0.838	ab	1035	b	839	b	524	b	196	bc
	BR_{27} Pf	0.837	ab	1026	b	831	b	527	b	194	bc
	Pm	0.833	b	1067	ab	859	ab	544	ab	209	ab
	BR_{27} Pm	0.835	ab	1133	a	914	a	583	a	219	a
(b)											
2	AC	0.836	a	1094	a	885	a	554	a	209	a
	BR_{27}	0.832	abc	1049	ab	845	ab	535	ab	203	ab
	Ps	0.829	bcd	1044	abc	837	ab	512	bc	207	ab
	BR_{27} Ps	0.821	e	978	cd	774	c	460	d	204	ab
	Pf	0.828	bcd	968	d	775	c	482	cd	193	b
	BR_{27} Pf	0.825	de	1021	bcd	815	bc	505	bc	206	ab
	Pm	0.834	ab	1018	bcd	823	bc	528	ab	195	ab
	BR_{27} Pm	0.827	cd	1036	abc	829	bc	519	abc	208	ab
5	AC	0.837	a	1191	a	963	a	606	a	228	a
	BR_{27}	0.836	a	1178	a	954	a	608	a	224	ab
	Ps	0.824	b	983	c	784	cd	477	c	199	d
	BR_{27} Ps	0.817	c	972	c	767	d	462	c	205	cd
	Pf	0.828	b	1048	bc	838	bc	525	b	210	bcd
	BR_{27} Pf	0.825	b	1075	b	857	b	537	b	217	abc
	Pm	0.834	a	1099	b	886	b	568	ab	213	abcd
	BR_{27} Pm	0.825	b	1087	b	866	b	544	b	221	abc
20	AC	0.838	a	1185	a	958	ab	588	ab	226	a
	BR_{27}	0.840	a	1192	a	969	a	610	a	223	ab
	Ps	0.815	b	1003	c	787	d	428	d	217	ab
	BR_{27} Ps	0.815	b	918	d	720	e	407	d	198	c
	Pf	0.837	a	1098	b	888	c	542	c	209	bc
	BR_{27} Pf	0.836	a	1113	ab	900	bc	553	bc	213	abc

Table 1 continued

Time/h	Treatment	F_v/F_m		ABS/CSm		TRo/CSm		ETo/CSm		DIo/CSm	
	Pm	0.839	a	1113	ab	904	abc	549	bc	209	bc
	BR_{27} Pm	0.838	a	1156	ab	938	abc	576	abc	219	ab

Mean values marked by the *same letters* are not significantly different according to Duncan's multiple range test ($p < 0.05$)

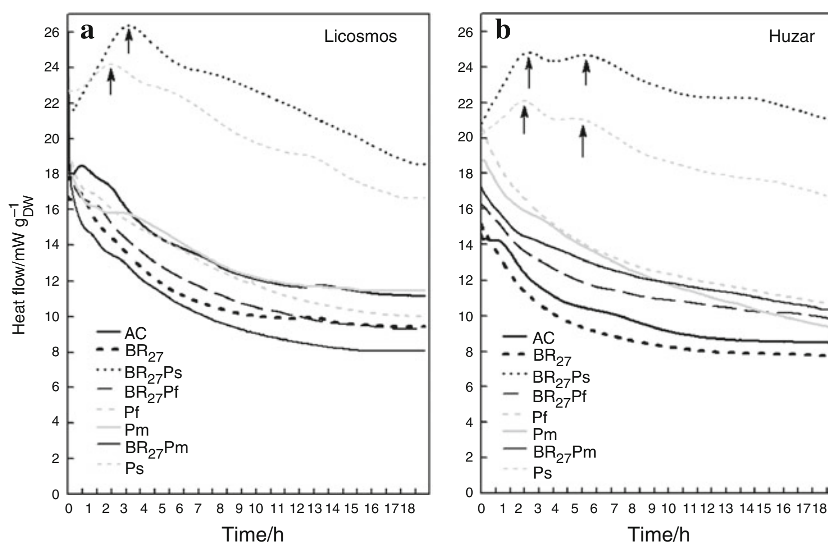

Fig. 2 Heat flow curves for cv. 'Licosmos' and 'Huzar' oilseed rape cotyledons treated with 24-epibrassinolide and infected with *Pseudomonas syringe* pv. *syringae, Pseudomonas fluorescence* and *Pseudomonas syringae* pv. *syringae hrcC* mutant during 18 h (monitoring started 2 h after inoculation)

saprophytic bacteria caused some increase in ion leakage for both cultivars, although it was significant only when mutant bacteria were injected into 'Huzar' cotyledons (Fig. 1b), suggesting some stress in the plant tissue.

Another interesting point is that, in accordance with our earlier findings, BR_{27} treatment alone caused a significant elevation of ion leakage from rape cotyledons [22, 27]. Zhang et al. [28] reported that brassinosteroid-initiated cell expansion was correlated with plasma membrane hyperpolarisation and regulation of anion channels and proton pumps. On the other hand, BR_{27} pre-treatment decreased bacteria-induced leakage from cells of both cultivars not only in the case of HR but also when the HR negative mutant or saprophytic bacteria were applied (Fig. 1). We found a similar opposite effect of BRs on control and cold-treated rape cotyledons [27] which is probably due to the general stress tolerance effect of BRs [13, 16, 29].

As regards the mechanism, Xia et al. [30] suggested that reactive oxygen species are involved in brassinosteroid-induced stress tolerance, and Ali et al. [31] claimed that antioxidant enzymes play an important role in brassinosteroid-induced aluminium stress tolerance of the mung bean. In addition, Vlašánková et al. [32] showed that BRs have cytokinin- and auxin-like effects on pea and flax, which correlates with our finding that cytokinins elevate antioxidant enzyme activities [33, 34]. Another interesting point is that effectors of *P. syringae* bind *Arabidopsis* receptor-like kinase BAK1, the shared signalling partner of both flagellin receptor FLS2 and brassinosteroid receptor BRI 1 [35].

Brassinosteroids influence varied developmental processes, such as growth or flowering [36, 37], and improve efficiency of photosynthesis [38], but especially interesting is their protective activity in stress conditions [16, 39]. In these cases activation of the antioxidant enzyme system and production of heat-shock proteins caused by brassinosteroids may lead to protection of photosynthetic pathways, increased viability of cells and better plant recovery [39–45]. As stated before, photosynthetic processes are generally very sensitive to many kinds of stressors, such as drought, heavy metals or thermal stressors. Protection of photosynthetic pathways has great importance for the minimisation of stressor effects and maintenance of crop yield. Our previous results revealed that 24-epibrassinolide protects PSII under cadmium stress [39]. BR_{27} reduces the toxic effect of Cd on photochemical processes by diminishing the damage of photochemical active reaction centres and oxygen-evolving centres as well as maintaining efficient photosynthetic electron transport. BR_{27} also favours PSII recovery in barley damaged at high temperature

Table 2 Specific enthalpy of growth, Δgh (area under each heat flow curve during 18 h-long monitoring started 2 h after inoculation), for oilseed rape cv. 'Licosmos' and 'Huzar' cotyledons treated with 24-epibrassinolide and infected with *Pseudomonas syringe* pv. *syringae*, *Pseudomonas fluorescence* and *Pseudomonas syringae* pv. *syringae hrcC* mutant

Treatment	$-\Delta gh$/J g_{DW}^{-1}	% of AC
LICOSMOS		
AC	673 ± 123^{bc}	100
BR_{27}	598 ± 85^{c}	89
Ps	1397 ± 45^{a}	207
BR_{27} Ps	1554 ± 63^{a}	231
Pf	761 ± 81^{bc}	113
BR_{27} Pf	741 ± 31^{bc}	110
Pm	699 ± 13^{bc}	103
BR_{27} Pm	933 ± 51^{b}	139
HUZAR		
AC	654 ± 11^{bc}	100
BR_{27}	585 ± 20^{c}	89
Ps	1196 ± 77^{a}	183
BR_{27} Ps	1313 ± 213^{a}	201
Pf	802 ± 81^{bc}	123
BR_{27} Pf	778 ± 12^{bc}	119
Pm	769 ± 66^{bc}	117
BR_{27} Pm	967 ± 143^{ab}	148

Mean values (±SD) marked by the *same letters* do not significantly differ according to Duncan's multiple range test ($p < 0.05$)

(Janeczko, unpublished data). In the present experiment, protective action of BR_{27} is noted after bacterial infection. The 'Licosmos' cultivar is more susceptible to BR_{27} and positive effects of this hormone were observed already 2 h after infection with saprophytic bacteria (*Pf*) and after 20 h in the case of invasive bacteria (*Ps*). As in the case of 'Huzar' improvement of energy flow in PSII in cotyledons treated with BR_{27} is observed after 20 h in the case of *Pf* infection and it is not observed after infection with invasive *Ps* bacteria in the time period tested. The mechanism of brassinosteroid activity in this case is unknown; however, one possible way of their action in photosynthetic pathway protection may be the control of heat-shock protein (HSP) synthesis observed by Dhaubhadel et al. [29, 41] in the case of heat shock in *B. napus*.

The resistance mechanisms require high energy expenditure; thus the increase in metabolic activity of the infected tissue can be expected [46–48]. Specific enthalpy values showed a slight tendency to increase in cotyledons of both cultivars infected with *Ps* pre-treated with BR_{27} in comparison to *Ps* infected. These observations are consistent with our earlier findings [22]. It is interesting to note that BR_{27} pre-treatment of cotyledons which were subsequently *Pm*-infected significantly increased Δgh values when compared to those obtained for cotyledons treated only with BR_{27}. This phenomenon remains unexplained.

The results presented in our paper showed that pathogenic processes could be monitored using calorimetry. This technique has an advantage in that measurements are continuous and do not interfere with the processes investigated. Moreover, calorimetric changes in plant metabolism caused by external stressors are visible very rapidly. As shown, the first effects of pathogenesis occurred 4 h after inoculation. The large exothermic peaks observed in *Ps* and BR_{27} Ps calorimetric curves (Fig. 2a, b) were an indicator of tissue response to an incompatible pathogen (*Ps*). Similarly, endo- or exothermic peaks were observed on calorimetric curves describing germination of seeds [49], response of weeds to herbicides [50] or allelopathic effects [51, 52].

Conclusions

The pre-treatment of cotyledons in two cultivars of oilseed rape suggests protective action of BR_{27} after bacterial infection with *Pseudomonas*. The cultivars tested pointed out different susceptibility to BR_{27}. Positive effects of this hormone were observed at different time points after infection. Our results show that pathogenesis processes can be monitored by calorimetry. With calorimetry, changes in plant metabolism caused by external stressors are visible very rapidly. Thus, calorimetry can be a timer which indicates the precise moment when specific analytical measurements should be carried out in order to clarify the details of complex physiological processes [49], including pathogenesis. Alternatively, it could be desirable to combine isothermal calorimetry with specific analytical techniques. This trend in this analytical field was proposed earlier by Wadsö [53].

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