Brazilian Journal of Microbiology (2022) 53:595–604
https://doi.org/10.1007/s42770-022-00722-4

BACTERIAL AND FUNGAL PATHOGENESIS - RESEARCH PAPER

Priming of defense-related genes in *Brassica oleracea* var. *capitata* using concentrated metabolites produced by *Rhizobium tropici* CIAT 899

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Received: 31 August 2021 / Accepted: 18 February 2022 / Published online: 22 March 2022

Abstract

To verify the potential of metabolites extracted from *Rhizobium tropici* to trigger the priming of defense responses in cruciferous plants, we analyzed the expression of defense-related genes by qRT-PCR. *Brassica oleracea* var. *capitata*, susceptible to *Xanthomonas campestris* pv. *campestris*, were grown in greenhouse conditions. At 18 days after sowing, plants were inoculated with 1 mL of 1% concentrated metabolites produced by *R. tropici* (CM-RT) in the root. In a second experiment, leaves were sprayed with 1 mL of a solution containing 1% CM-RT. Aerial and root tissue were collected separately at 0 (non-treated control condition), 24, and 48 h after application, submitted to RNA extraction and gene expression analysis by qRT-PCR. The results showed that, after root treatment with CM-RT, most evaluated genes were upregulated at 24 h after application and downregulated at 48 h after application in roots, while in leaves, genes were downregulated both at 24 and 48 h after application. On the other hand, leaf treatment with CM-RT showed that most evaluated genes in leaves and roots were upregulated at 24 and 48 h after application. These results indicate that the effect of CM-RT applied in roots seems restricted to the applied region and is not sustained, while the application in leaves results in a more systemic response and maintenance of the effect of CM-RT for a longer period. The results obtained in this study emphasize the biotechnological potential of using metabolites of *R. tropici* as an elicitor of active defense responses in plants.

Keywords Black rot · *Xanthomonas campestris* pv. *campestris* · Defense response · Concentrated metabolites

Introduction

Xanthomonas campestris pv. *campestris* (*Xcc*), responsible for the black rot disease, is an important bacterium that affects all cruciferous plants cultivated for food and spices and used as ornamental plants. The prevention of black rot disease has been of paramount importance for infection control, especially since it is transmitted through infected seeds [1]. This disease has been partially controlled by the use of chemical applications, which are hazardous for human and animal health, as well as to the environment.

Currently, the search for disease control strategies focused on green technologies is becoming increasingly important in integrated pest management programs, especially when discussing integrated production towards sustainable agriculture. In this context, an important strategy for pest control is the use of metabolites from microorganisms as plant defense inducers. Extracts from different living organisms have been investigated as priming agents of plant resistance and have been able to control phytopathogens [2–6]. Primed plants exhibited faster and more robust activation of defense responses against subsequent challenges by insects, microbes, or abiotic stress [6, 7] and displayed a considerable upregulation in the expression of defense response-associated genes, such as those encoding peroxidase enzymes, chitinases, and beta-1,3-glucanase, known as pathogenesis-related proteins (PR proteins) [8, 9].

Responsible Editor: Luc F.M. Rouws

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In studies carried out by Conrad et al. [10], for example, extracts of *Colletotrichum acutatum* were able to induce a defense response in *Arabidopsis thaliana* against *Botrytis cinerea*, which causes gray rot in several species. Similarly, studies carried out by Munhoz et al. [11] showed that extracellular bioactive compounds produced by *Pseudomonas aeruginosa* were able to stimulate the defense response of tomato plants by activating the activity of the enzymes peroxidases, polyphenol oxidase, and phenylalanine ammonia lyase.

In this study, we evaluated the influence of metabolites produced by *Rhizobium tropici* (CIAT 899), a nitrogen-fixing bacteria associated with legumes, in the resistance to *Xanthomonas campestris* pv. *campestris* and verified the induction of defense-related genes by qRT-PCR in *Brassica oleracea*. The results obtained can help optimize the use of concentrated metabolites produced by *R. tropici* (CM-RT) in biocontrol strategies aiming at the reduction of pesticide use in crop production.

Materials and methods

Growth of *R. tropici* CIAT 899 and extraction of metabolites

For the production of concentrated metabolites (CM), *R. tropici* CIAT 899 was cultivated at 28 °C in TY medium with the following composition in g/L: 5 g tryptone, 3 g yeast extract, and 0.65 g $CaCl_2{\cdot}2H_2O$; pH 6.8–7.0. The vitamins biotin and thiamine were added and dissolved in H_2O then sterilized by filtration (0.22 µm), to a final concentration of 0.2 and 5 µg/mL, respectively. From a pre-inoculum in TY medium, the bacterium *R. tropici* was grown in minimum B-medium constituted by: mannitol (10 g/L), $MgSO_4{\cdot}7H_2O$ (0.55 g/L), KNO_3 (0.55 g/L), $Ca(NO_3)_2{\cdot}4H_2O$ (1.30 g/L), Fe(III)-Na.EDTA (2.5 mL/L), and trace elements (2.5 mL/L). The Fe(III)–Na × EDTA solution (1.3% w/v) was sterilized by filtration. The composition of the trace element solution was as follows: $MnSO_4{\cdot}H_2O$ (0.609 g/L), $ZnSO_4{\cdot}7H_2O$ (0.097 g/L), H_3BO_3 (1.269 g/L), $Na_2MoO_4{\cdot}2H_2O$ (0.398 g/L), and $CuSO_4{\cdot}5H_2O$ (0.035 g/L). The pH was adjusted to 5.0 and autoclaved. Then, 10 mL of sterilized 1 M K_2HPO_4 with pH 7.2 per liter of medium were added. This medium was supplemented with sterilized apigenin (1 mg/mL), except for the negative controls, and was incubated at 28 °C with shaking for 3 to 5 days.

The extraction of the metabolites was performed by removing the microbial cells by centrifugation, followed by the addition of 300 mL of n-butanol per liter of medium. The mixture was kept under stirring for 12 h and left to stand for 24 h until the separation of the aqueous and butanolic phases. Then, the butanolic phase (upper) containing the metabolites was collected and concentrated on a rotary evaporator at low temperature. The concentration of metabolites was performed using a solid-phase extraction cartridge (SPE C_{18} Resprep, Teknokroma). The CM-RT were recovered in methanol, lyophilized, and resuspended in 5 mL of 20% acetonitrile in H_2O (v/v). Then, the CM-RT was diluted in water to a concentration of 1%.

CM-RT treatment of *Brassica oleracea* plants for qRT-PCR analysis

Seeds of *B. oleracea* var. *capitata* Veloce (susceptible to *Xanthomonas campestris* pv. *campestris* isolate 51) were grown in greenhouse conditions without temperature and humidity control at Embrapa Recursos Genéticos e Biotecnologia, Brasilia-DF, Brazil. The seeds were subjected to disinfestation with 3% sodium hypochlorite and washed with distilled water. After disinfestation, the seeds were soaked in a solution containing 1% CM-RT and placed in the cold room at 4 °C for 2 h. Subsequently, the seeds were maintained on Germitest® paper for 4 days and transplanted to plastic cups of 200 mL containing a mixture of sterile soil, manure, and sterile sand (3:1:1 w/w/w).

At 15 days after plants were transplanted, two experiments were conducted. In the first experiment, the plants were inoculated with 1 mL of 1% CM-RT in the root (rhizosphere). Aerial and root tissues were collected in triplicate (each replicate was formed by 3 plants) separately at 0 h (non-treated control), 24, and 48 h after application, totalizing 18 samples. In the second experiment, leaves were sprayed with 1 mL of 1% CM-RT. Aerial and root tissues were collected as described for the first experiment. The treated plants (aerial and root tissues) at 24 and 48 h after application were compared to the non-treated control.

RNA extraction and cDNA synthesis

Total RNA was isolated using the TRIzol method according to Simms et al. [12] with modifications for the aerial tissue and CTAB method for roots. The quantification was performed using the NanoDrop® spectrophotometer (ND-1000 UV–Vis-Thermo Fisher). The extracted RNA was treated with Turbo™ DNAse (Applied Biosystems/Ambion) and cDNA synthesis was performed using 2 µg of treated RNA using the Next Generation MMLV RNAse H Minus First-Strand cDNA Synthesis (DNA express). The cDNA obtained was stored at − 20 °C and used for qRT-PCR.

qRT-PCR experiments and data analysis

Genes previously described in the literature with possible involvement in defense response were selected (Table 1). The software Primer3 [13] was used to design all the

Table 1 General information of selected targets for gene expression analysis

Gene	Accession #	Annotation/gene name	PR protein	Forward Primer (5' to 3')	TM (°C)	Reverse primer (5' to 3')	TM (°C)	Amplicon	Primer efficiency (%)
FADS	XM_002302577.2	XP_002302613.1/ fatty acid desaturase/cytochrome b5 fusion protein	-	ATAACA GAATCG CGCAGC TC	53	TGAAGA ACTTGG TGGAGA CG	52	162	85
DEFL	NM_001036559.3	NP_001031636.2/ defensin-like family protein	PR-12	ATCAGC AATGTC TGGTGC AG	53	TTGTCTAGG GATGGG TCCAG	52	179	83
DAO	XM_013745193.1	XP_013600647.1/2-oxoglutarate-dependent dioxygenase DAO-like	-	CAAATC CTCCCA AACCAC AG	51	CCCTTG GAGCAA TCAATC TC	50	160	84
SRG2	NM_001247299.2	XP_001234228.2/ salt responsive protein 2	-	ACGAGG AAAACG GAGACA TC	52	TTAACCCAT AACCGC CTCTG	52	166	88
PIDRP8	XM_008345553.1	XP_008343775.1/ pathogen-induced defense-responsive protein 8	PR-5	AGTCTT GCATGG ACCCAA AC	60	TCTCACAAT CGCGTC TTCAG	60	173	88
SAPX	NM_001340582.1	NP_001319883.1/ stromal ascorbate peroxidase	PR-9	TTCCTCTCT TCGCAC TCAAG	52	GAAACT CCGATT CACCAA CG	52	166	87
ARP	XM_006371764.1	XP_006371826.1/ avirulence-responsive family protein	-	TAACAG ATGCTC AGCTAG AG	49	CGAAAT CCCATT CTCCAT	45	162	81
CYP83B1	NM_119299.3	NP_194878.1/ cytochrome P450	PR-14	CTTAGACTT CACCCA ACCAT	49	CTCTCG GGACAA AACTCG	50	167	83
*UBI**	XM_013778573.1	XP_013634027.1/ ubiquitin-60S ribosomal protein L40	-	ATGTCA AGGCCA AGATCC AG	52	GAGCCA AAGCCA TCAAAG AG	51	186	83
*TBP**	XM_013728490.1	XP_013583944.1/ TATA-box-binding protein 1	-	TCTTGGCTC CAGTAC AAACC	52	ACATTGTGT CGACGG TGAAC	53	177	86

*Reference genes

primers, and the software OligoAnalyzer 3.1 was used to evaluate the potential absence of amplification and nonspecific products [14].

qRT-PCR was performed according to the manufacturer's protocol in a 7300 Real-Time PCR System (Applied Biosystems). Reactions were made in 12 µL containing 5 µL of Fast SYBR® Green Master Mix (Applied Biosystems), 2 µL cDNA (diluted 20×) probably corresponding to approximately 10 ng, and 0.2 µM of each primer (forward and reverse) [15–18]. Reactions were performed with three biological replicates and run in experimental triplicates using the manufacturer's recommended cycling parameters (holding stage: 95 °C for 10 min, cycling stage: 40 cycles of 95 °C for 15 s (denaturation), and 60 °C for 1 min (annealing and extension)). The melting curve was held after the end of the amplification to verify the presence of nonspecific products or primer dimers. The parameters used were 95 °C for 15 s, 60 °C for 60 s, and an increase of 0.3 °C up to 95 °C. The raw *Cq* values from all runs were calculated using the real-time PCR Miner software [19], to determine the *Cq* value and the PCR efficiency. The REST software was used to perform the analyses of expression and statistics [20]. The geNORM algorithm was used to determine the most stable reference genes for normalization. The *t*-test was used to compare the differences in expression among groups, as determined by the REST software.

Xanthomonas campestris pv. *campestris* inoculation in CM-RT treated plants

To verify the symptoms in *Brassica oleracea* var. *capitata* Veloce (susceptible to *Xanthomonas campestris* pv. *campestris* 51), the seeds were subjected to disinfestation with 3% sodium hypochlorite and soaked in a solution containing 1% CM-RT. Subsequently, seeds were placed in a cold room at 4 °C for 2 h and maintained on Germitest® paper for 4 days. After germination plantlets were transplanted to plastic cups of 200 mL containing a mixture of sterile soil, manure, and sterile sand (3:1:1 w/w/w). A total of 3 biological replicates (samples) were used, totalizing 9 plants. To examine disease symptoms, at 15 days after transplanting, the leaves were sprayed with 1 mL of 1% CM-RT. Plants non-treated (control) and treated (CM-RT treated groups) with 1% CM-RT were inoculated with *Xanthomonas campestris* pv. *campestris* isolate 51 at 24 h after application.

For bacterial suspension preparation, *Xanthomonas campestris* pv. *campestris* was grown for 24 h, centrifuged, and suspended in sterile saline solution (NaCl 0.85%). Approximately 0.5 mL of the bacterial solution ($A_{600}=0.3$) was sprayed on the plant leaves. The inoculated plants were kept in a greenhouse, and disease symptoms were visually monitored and also quantified considering the percentage of lesioned leaf area captured in the total plant leaves ($n=9$), at six time points (1–3–5–7–10–15 days after infection—dai), using the software QUANT®, according to Vale et al. [21]. Statistical analysis was performed using one-way ANOVA (p-value <0.05) followed by a post hoc T-student test (p-values $<0.01; <0.0001$) for average comparison between treated and control groups.

Results

Gene expression analysis reveals plant primed defense responses

To evaluate the potential of using CM-RT to prime plant resistance against phytopathogens, we used qRT-PCR to analyze the relative expression of 8 defense-related genes in *B. oleracea* (listed in Table 1) treated with the metabolites extracted from *R. tropici*. When CM-RT was applied in the plant roots, the results showed that all genes analyzed, except for gene *FADS*, were upregulated 24 h after CM-RT application in root parts when compared to the control condition (time point 0 h after application) (Fig. 1(a)). Moreover, it was interesting to observe that, at 48 h after application, the expression of most genes analyzed was downregulated, except for genes *CYP83B1*, *DAO*, and *PIDRP8*, which suggested a short-term induction of defense response in plant root cells by the CM-RT. Notwithstanding, in aerial parts, only part of the set of analyzed genes was downregulated at 24 h after application, when compared to the control condition (time point 0 h after application). At 48 h after application, the results showed that all genes analyzed, except for gene *FADS*, were downregulated, when compared to the control condition.

On the other hand, CM-RT plant leaf treatment resulted in a wide plant defense response, in which most of the analyzed defense genes was upregulated in both root and leaf plant tissues, except for the *SAPX* gene that was downregulated at 24 and 48 h after application in plant roots and leaves, and *PIDRP8* that was downregulated at 24 h after application in the plant leaves (Fig. 1(b)). Interestingly, the APX gene was upregulated in roots at 24 h after application of CM-RT in roots. According to the analysis performed by *Blastn*

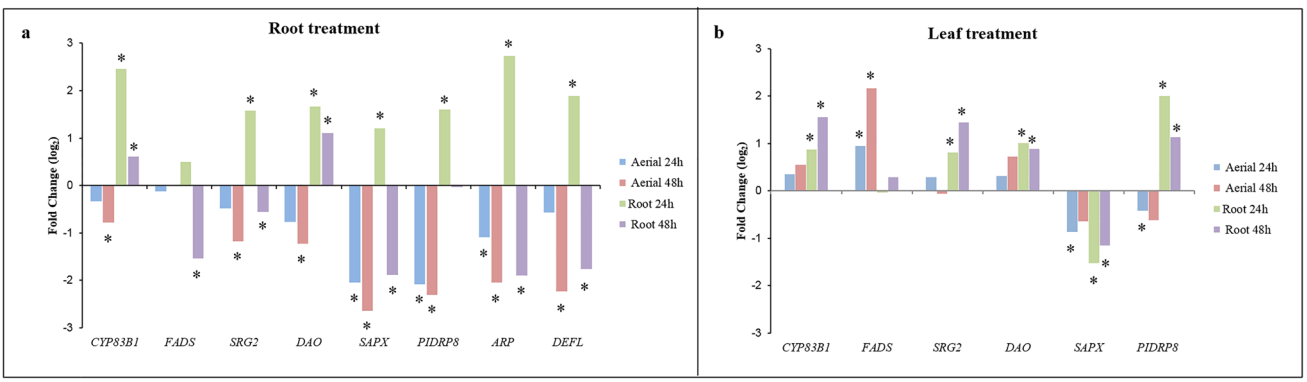

Fig. 1 Analysis of the relative gene expression of the targets associated with defense response, using the time point 0 h after application as a non-treated control condition. (a) Root treatment with CM-RT. (b) Leaf treatment with CM-RT. As indicated, the blue and red bars represent aerial tissue collected at 24 and 48 h after application, whereas the green and purple denote the root tissue collected at 24 and 48 h after application. * symbol indicates statistical significance of gene expression (p-value ≤ 0.05)

(NCBI tool), the primers used can also anneal to L-ascorbate peroxidase S, chloroplastic/mitochondrial isoform present in chloroplasts and mitochondria, which may explain the upregulation observed in roots. The *ARP* and *DEFL* genes did not show a significant regulation in gene expression in CM-RT-treated leaves.

The potential of CM-RT usage for the management of black rot disease

To verify the potential of CM-RT to reduce infection by *Xanthomonas campestris* pv. *campestris*, the leaf treatment was chosen because *Xanthomonas campestris* pv. *campestris* infection mainly occurs by stomata and also by the fact that leaf treatment seems to induce a more systemic response in *B. oleracea* and could potentially provide higher protection to the plant. To examine disease symptoms, *B. oleracea* plants (control and CM-RT treated groups) were inoculated with *Xanthomonas campestris* pv. *campestris* at 24 h after application. At 7 days after infection (dai) with *Xanthomonas campestris* pv. *campestris*, CM-RT treated plants showed only chlorotic spots (Fig. 2—I(b)), whereas control plant leaves showed chlorotic and also necrotic spots (Fig. 2—I(a)). At 10 days after infection, plants in the control condition were still displaying a more affected phenotype. However, at the last time point (15 days after infection), progressive symptoms, such as leaf senescence, were visually remarked on both the control and treated plant groups, suggesting to us the end of CM-RT lifespan, i.e., the degradation/oxidation of the metabolite compounds, due to its natural decay and also the exposure time to the hostile environment of the foliar apoplast.

The observed foliar disease symptoms were also quantitatively assessed using the software Quant® (Vale et al., 2003). The quantification of symptoms (mean ± SE) in terms of the percentage of healthy foliar surface area revealed that brassica leaves at 10 days post-treatment with CM-RT showed the most significant ratio of healthy foliar tissue (post hoc *t*-test; p-value < 0.0001) when compared to the control group (Fig. 2—II), ultimately suggesting effective priming of foliar plant defense responses against *Xanthomonas campestris* pv. *campestris*, due to the application of CM-RT.

Discussion

Plants have inducible or constitutive defense mechanisms to block the invasion of pathogens [22]. The induction of defense response in plants is typically triggered by conserved components of the pathogen's cell wall, such as

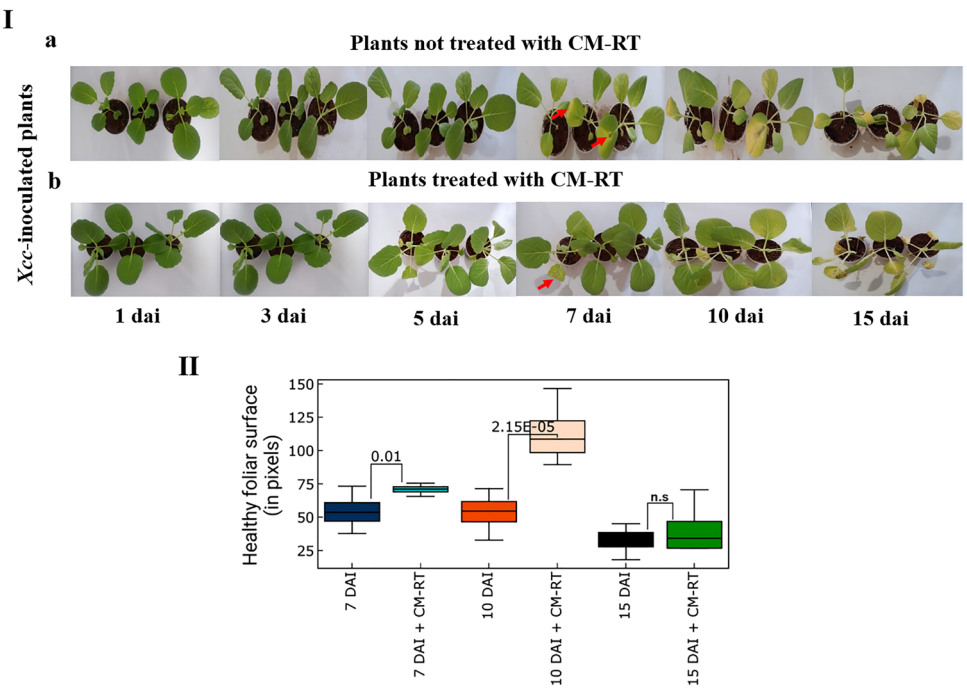

Fig. 2 Phenotyping post-treatment of brassica plant leaves with CM-RT. Photographs were taken at six time points 1, 3, 5, 7, 10, and 15 days after infection (dai), and the severity of symptoms was analyzed using the software Quant®. (I) Black rot disease symptoms in *Brassica oleracea* var. *capitata* inoculated with *Xanthomonas campestris* pv. *campestris*, in (a) non-treated control plants and (b) CM-RT-treated plants. (II) Significance for the ratio of healthy foliar surface between treated and control group was calculated using one-way ANOVA (p-value < 0.05) followed by post hoc *T*-student test (p-values < 0.01, < 0.0001). Each colored boxplot represents the average (± SE) of three biological replicates (n = 9 leaves). ns, non-significant; dai, days after inoculation; ANOVA, analysis of variance

lipo-polysaccharides, known as pathogen-associated molecular patterns (PAMPS). These plant-defense inducing molecules are known as elicitors, and, their recognition by cognate-specific receptors, known as pattern recognition receptors (PRRs), located on the plasma membrane of plants, leads to a PAMP-triggered immunity response or PTI [23]. PTI and ETI (effector-triggered immunity) are two different overlapping pathways, that are defined according to the types of pathogen molecules recognized by the plants [23, 24]. In PTI, host defense signaling events include the production of phytohormones, phenolic compounds, phytoalexins, and a large transcriptional response that increases the expression of defense-related genes, including pathogenesis-related (PR) genes [25]. It is well known that pathogenesis-related proteins are encoded by a complex group of gene families related to the regulation of physiological processes and play a crucial role in the defense response against abiotic and biotic stresses [26].

PR proteins were first reported by van Loon, van Kammen [27], in tobacco plants infected with the tobacco mosaic virus. These proteins have been associated with the active defense of plants, restricting the development and spread of diseases [25]. Currently, there are 17 PR protein families (PR-1 to PR-17) that carry out various antimicrobial properties involved in plant defense through different mechanisms [28]. Other genes involved in the defense response to pathogens have also been reported, such as fatty acid desaturase/cytochrome b5 fusion protein, 2-oxoglutarate-dependent dioxygenase DAO-like, salt responsive protein 2, and avirulence-responsive family protein [29].

The results obtained in this study indicate that plant root application of CM-RT does not seem to trigger a systemic defense response in treated plants, which was limited to the root cells. Moreover, the downregulation of these genes in leaves (after root treatment) may have occurred to save energy for a more pronounced and active defense response in roots. However, ours results suggested that CM-RT application in plant leaves triggers a systemic defense response, even though a lower gene expression level was observed when compared to the root application.

In this study, the application of CM-RT in *B. oleracea* was able to alter the expression of several defense-related genes, including *ARP*, *FADS*, *DAO*, *SRG2*, as well as known PR genes, such as *SAPX*, *DEFL*, *CYP83B1*, and *PIDRP8*, reported to be induced upon pathogen attack [29]. These genes are important in the plant defense process, for example, the *SAPX* gene controls the content of hydrogen peroxide (H_2O_2) generated during abiotic and biotic stresses [30], while *FADS* has the ability to adjust the levels of unsaturated fatty acids, introducing double bonds in the acyl chain of fatty acids, which leads to increased resistance of plants against various stressor agents including heat, cold, drought, and pathogens [31–34]. The genes of the P450 family (*CYP*) have different functions and are involved in several metabolic pathways playing important roles in a wide range of reactions, such as in the biosynthetic pathway of the major phytoalexins (defense molecules) [35, 36].

The ability of microorganism extracts to elicit defense responses in plants has been reported. Studies carried out by Yu et al. [37] showed that an elicitor of *Trichothecium roseuma* was able to positively regulate the expression of resistance-related genes, as well as increase the activity of resistance-related enzymes and antifungal compounds, such as PRs β-1,3-glucanase (*GLU*) and chitinase (*CHT*). Recently, bacterial extracts have also been investigated as plant defense elicitors and studies have shown their ability to induce defense-related genes. For example, studies carried out by Faillace et al. [38] showed that an extract of *Xanthomonas axonopodis* was able to induce a defense response in *Solanum tuberosum* against *Pectobacterium atrosepticum*, activating antioxidant enzymes such as superoxide dismutase, catalase, and ascorbate peroxidase, and modulating the activity of defense-related enzymes such as phenylalanine ammonia oxidase lyase, polyphenol, β-1,3-glucanase, chitinase, and peroxidase.

The fact that CM-RT is derived from rhizobacteria is particularly interesting from a biotechnological point of view. It has been reported that beneficial rhizobacteria produce several secondary metabolites such as surfactin, fengicin, bacillomycin, bacillen, macrolactin, difficidin, bacillisin, indole-3-acetic acid (IAA), and 2, 3-butanediol [39, 40]. Some of these metabolites have already been reported to elicit plant defense responses, exhibiting a protective effect mediated by systemic resistance, such as surfactin and fengicin produced by *Bacillus subtilis* strains [41]. Elicitors produced by plant growth-promoting rhizobacteria (PGPR) have been identified and characterized such as 2,4-diacetylphloroglucinol and *N*-acylated-l-homoserine lactones of *Pseudomonas* [42, 43].

In our study, we could clearly observe that leaves treated with CM-RT led to a reduction of the lesions caused by *Xanthomonas campestris* pv. *campestris*, resulting in increased protection. This effect is probably due to the elicitor properties present in CM-RT, which seems to result in a systemic resistance, since the effects of leaves treatment, such as the induction of defense-related genes, was also observed in roots. According to Marks et al. [44], the main components of the extract of *R. tropici* are lipo-chitooligosaccharides (LCOs), exopolysaccharides (EPSs), and plant hormones. Many plant-associated bacteria, including rhizobia, synthesize plant hormones, such as auxins, cytokinins, ethylene, and gibberellins [45–48]. Therefore, the combination of these compounds may have contributed to the effects produced by the extract of *R. tropici* in brassica.

Studies showed that chemicals from PGPR, such as lipochitooligosaccharides or LCOs (Nod factors), in addition to

increasing the growth of plants, leguminous and non-leguminous stimulate the defense response [49]. The symbiotic relationship begins with an exchange of chemical signals between the host and the N_2-fixing rhizobia. In conditions of low nitrogen, roots of legumes release (iso)flavonoids that activate the bacterial production of specific signaling compounds (LCOs) and induce the nodulation genes of rhizobia [50, 51]. The LCOs induce several genes related to the defense response in non-leguminous plants such as pathogenesis-related proteins chitinase and peroxidase [52, 53] and enzymes of the phenylpropanoid pathway, such as L-phenylalanine ammonia-lyase [54].

In the present study, a protective effect of CM-RT was remarkably noticed in *B. oleracea*, especially in the treated leaves up to 10 days after infection. These results demonstrate the capacity of the bioactive compounds, such as the LCOs, present in the CM-RT to prime plant defense by a prompt and systemic gene expression modulation of a specific set of defense-related genes, which reflected in a significant reduction of disease symptoms and also in the delay of disease progression.

However, the precise molecular mechanisms involved in defense response during *Xanthomonas campestris* pv. *campestris* infection in *B. oleracea* leaves treated with CM-RT are complex and remain unclear. Our results are consistent with the general observation and emphasize the biotechnological potential of using metabolites of *R. tropici* as an efficient elicitor of defense in plants. Ultimately, these results indicate, at least for the present pathosystem, that CM-RT achieved its higher potential as an elicitor of plant defense responses when applied to the aerial parts of the plant.

Conclusion

The genes evaluated in this study have important roles related to defense response. CM-RT treatment in leaves showed a more systemic and prolonged effect than treatment in roots. Although CM-RT application in leaves induced lower expression levels of the defense-related genes, it was able to reduce the incidence of *Xanthomonas campestris* pv. *campestris* in *B. olearea* plants. The results obtained in our study indicate a high biotechnological potential for the use of concentrated metabolites of *R. tropici* (CM-RT) to induce resistance in *B. oleracea.*

Moreover, we show that brassica leaves treated with concentrated metabolites of *R. tropici* (CR-MT) and inoculated with *Xantomonas campestris* pv. *campestris* (*Xcc*) significantly delayed the progression of the disease and decreased the symptoms caused by the phytopathogen. However, it is important to emphasize that this effect is temporary and can be corrected with subsequent applications.

The results obtained here suggest that CM-RT acts as an elicitor of active defense responses in plants and has a high biotechnological potential for disease control. This study was the first step to understand the potential role of CM-RT in inducing defense response in brassica and may be useful in future studies for generating a product with high efficacy against *Xanthomonas* ssp. or other species of pathogens. Such advances favor agricultural sustainability by bringing economic and environmental benefits, contributing to a more sustainable production.

Funding This study was financed by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior — Brasil (CAPES) — Finance Code 001″, Embrapa, Conselho Nacional de Desenvolvimento Científico e Tecnológico-CNPq, and Fundação de Amparo Pesquisa do Distrito Federal FAPDF.

Data availability Not applicable.

Declarations

Ethics approval Not applicable.

Consent to participate Not applicable.

Consent for publication Not applicable.

Conflict of interest The authors declare no competing interest.

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