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Methyl jasmonate induces extracellular pathogenesis-related proteins in cell cultures of *Capsicum chinense*

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Suspension cultured cells of *Capsicum chinense* secrete proteins to the culture medium in both control conditions and under methyl jasmonate treatment. The exogenous application of methyl jasmonate induced the accumulation of putative pathogenesis-related proteins, class I chitinase, leucin-rich repeat protein, NtPRp27-like protein and pectinesterase which were also found in suspension cultured cells of *C. annuum* elicited with methyl jasmonate. However, a germin-like protein, which has never been described in methyl jasmonate-elicited *C. chinense* suspension cultured cells, was found. The different effects described as being the result of exogenous application of signalling molecules like methyl jasmonate on the expression of germin-like protein suggest that germin-like proteins may play a variety of roles in protecting plants against pathogen attacks and different stresses. Further studies will be necessary to characterize the differential expression of these pathogenesis-related proteins and to throw light on the complexity of their regulation.

Key words: capsicum, cell cultures, methyl jasmonate, pathogenesis-related proteins

Abbreviations: CaGLP1, *C. annuum* germin-like protein; ET, ethylene; GLP, germin-like proteins; LRR, leucin-rich repeat protein; MJ, methyl jasmonate; NtPRp27, NtPRp27-like proteins; PR-proteins, pathogenesis-related proteins; SA, salicylic acid; SCCs, suspension cultured cells

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When plants are attacked by pathogens, they defend themselves with an arsenal of both passive and active defence mechanisms. The passive or pre-existing defence mechanisms involve structural barriers or strategically positioned reservoirs of antimicrobial compounds which prevent colonization of the tissue. The active or induced defence responses include the hypersensitive response, the production of phytoalexins, lignification and the reinforcement of the cell wall, as well as the biosynthesis of pathogenesis-related proteins (PR-proteins).[1] PR-proteins have been described in plant species from at least 13 families and new members of PR-proteins have been recognized and classified into 17 structurally and functionally distinct families.[2] Some of them, such as β-1,3-glucanases, chitinases, endochitinases and peroxidases possess antifungal or antibacterial activity.[2] In addition to the well-known PR-proteins induced by biotic molecules, some other naturally occurring substances are also involved in the induction of these proteins.[3] Among these, jasmonates and its more active derivative, methyl jasmonate (MJ), coordinate the activation of a large set of defence responses and their exogenous application induces resistance in plants. In this way, the addition of MJ to tomato, tobacco and pepper induces genes that encode PR-proteins, such as chitinases, β-1,3-glucanases and peroxidases.[4-6] Likewise, although PR-proteins are considered inducible proteins by elicitors, the number of data indicating their occurrence as constitutive proteins in both seeds and different plant organs, irrespective of stress conditions, is suggesting a possible role in passive defence mechanisms.[6]

In a previous work, we analyzed the inductor effect of MJ on PR-proteins in *C. annuum* suspension cultured cells (SCCs).[6] Analysis of the extracellular proteome showed the presence of amino acid sequences homologous to PR1 and 4, NtPRp27-like proteins (NtPRp27), Class I chitinases, peroxidases, the hydrolytic enzymes LEXYL1 and 2, arabinosidases, pectinases, nectarin IV and leucin-rich

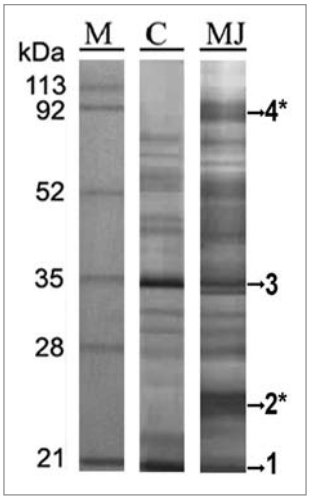

Figure 1. SDS-PAGE followed by Coomassie protein staining of the elicited spent media of *C. chinense*. Lane M, Molecular weight markers in kDa; lane C, control spent media; lane MJ, spent media from treated cells with MJ. *MJ-induced protein bands.

repeat protein (LRR), which suggested that MJ plays a role in mediating defense-related gene product expression in *C. annuum*. Apart from these MJ-induced proteins, other PR-proteins were found in both the control and elicited cell cultures of *C. annuum*. These included class IV chitinases, β-1,3-glucanases, thaumatin-like proteins and peroxidases, suggesting that their expression is mainly constitutive since they are involved in growth, development and defence processes.

Here, we study the effect of MJ in its role as inducer of PR-proteins on *C. chinense* SCCs. For this, extracellular proteins obtained from elicited *C. chinense* SCCs were separated by SDS-PAGE (**Fig. 1**). **Figure 1** shows the protein pattern of the extracellular medium of *C. chinense* corresponding to the MJ-treatment (lane MJ) that contained two specific protein bands (2 and 4), and whose expression was MJ-dependent. As shown in **Table 1**, microsequencing of protein band 2, which has an apparent molecular weight of 24 kDa, identified peptides with homologous amino acid sequences to a *Solanum tuberosum* NtPRp27-like protein (NtPRp27, accession no. AAO22065) and three *C. annuum* PR-proteins (class I chitinase, LRR and germin-like proteins [GLP] whose accession numbers are AAR90844, AAN62015 and AAR28997, respectively). Tryptic peptides obtained by digestion of MJ-induced protein band 4 (with an apparent molecular weight of 90 kDa) (**Table 1**) contained amino acid sequences that showed homology with a *C. annuum* pectinesterase (accession number ABN08348). These results show that the exogenous application of MJ induces the accumulation of extracellular PR proteins in *C. chinense* SCCs, suggesting a role for MJ in mediating the defense-related gene product expression in Capsicum. Apart from these MJ-inducible proteins, we observed the presence of other PR-proteins in protein bands 1 and 3 of both the control (lane C in **Fig. 1**) and MJ-treated cells of *C. chinense* (lane MJ in **Fig. 1**). These PR-proteins were putative endochitinases and peroxidases (**Table 1**), which suggests that their expression is mainly constitutive. Indeed, others studies have confirmed the constitutive expression of these PR-proteins in the extracellular media of non-treated SCCs from *Zinnia elegans*, *Cycas revolute* and *Taxus baccata* or *C. annuum*.[6,7]

As regards MJ-inducible PR-proteins, class I chitinases, LRR proteins and pectinesterases have been extensively described by various authors.[6,8-10] However, NtPRp27 and GLP have never been described in *C. chinense* SCCs elicited with MJ. The amino acid sequence related to NtPRp27 showed homology with both *Nicotiana tabacum* and *S. tuberosum* NtPRp27, which clearly responded to fungal or virus infection, mechanical wounding, salicylic acid (SA), ethylene (ET) and MJ, which is consistent with many PR-proteins that accumulate transcripts in response to these last signalling molecules.[11-13] As regards GLP, these constitute a large and heterogeneous group of proteins with amino acid identities ranging from 25 to 100%, which have different patterns of expression.[14,15] Noted by Park et al.[4], a *C. annuum* GLP (CaGLP1) was involved in the defense response to a wide range of pathogenic microbes, including

Table 1. Tryptic peptides obtained by the digestion with trypsin of protein bands from *C. chinense* SCCs

Protein bands	Tryptic peptide	Accession number	Protein	Nominal mass	Scores
20 kDa (band 1)	GPI QIS YNY NYG PCG R YCG ILG VSP GDN LDC GNQ R	AAY90154	Endochitinase *Capsicum annuum*	19,591	219
24 kDa (band 2)*	YIQ GYS GDV R	AAO22065	NtPRp27-like protein *Solanum tuberosum*	25,172	62
	NNF YSY NA FIT AAK SFP GFG TTG DTA VR GPI QIS YNY NYG PCG R	AAR90844	Chitinase class I *Capsicum annuum*	13,175	291
	LAG TVP LAN K LSG EIP ISV LK	AAN62015	Leucine-rich repeat protein *Capsicum annuum*	21,557	114
	GEV FAF PR GLV HFQ QN NGD VPA AVV A	AAR28997	Germin-like protein *Capsicum annuum*	23,295	144
33 kDa (band 3)	GFE VIA QAK LGG QTY TVA LGR EMV ALA GAH TVG FAR	CAI48071	Anionic peroxidase *Capsicum chinense*	31,471	114
90 kDa (band 4)*	IDA FQD TLY THT LR TYL GRP WK	ABN08348	Pectinesterase *Capsicum annuum*	60,079	133

*MJ-induced protein bands.

viruses and bacteria. Moreover, when hot pepper plants were treated with SA or ET, they showed a rapid accumulation of CaGLP1 transcripts. In contrast to our results, the expression of CaGLP1 was not affected by MJ.[17] Therefore, the different effects on GLP expression observed as the result of exogenous application of signalling molecules like SA, ET and MJ suggest that GLP may play a variety of roles in protecting plants against pathogen attacks and different stresses. In conclusion, it has been determined that in *C. chinense* SCCs, as well as in *C. annuum* SCCs, the exogenous application of MJ induces a set of putative proteins which are considered PR-proteins. Although a wide range of functions suggests their involvement in defence reactions, further studies are necessary to characterize their differential expression and the complexity of their regulation.

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