THE *VICIA FABA* DIAMINE OXIDASE SYSTEM AND ITS ROLE IN RESPONSE TO *ASCOCHYTA FABAE* AND TO WOUNDING

O. Métoui[1], A. Porta-Puglia[1], M. Marrakchi[2] , M. Kharrat[3] and R. Angelini[4]

[1]*Istituto Sperimentale per la Patologia Vegetale (ISPaVe), Via C.G. Bertero 22, I-00156 Roma, Italy*
[2]*Faculté des Sciences de Tunis, Université de Tunis II, El Manar I, Tunis, Tunisia*
[3]*Institut National de la Recherche Agronomique de Tunisie, Rue Hédi Karray 2049 Ariana, Tunisia*
[4]*Dipartimento di Biologia, Università degli Studi "Roma Tre", Viale G. Marconi 446, I-00146 Roma, Italy*

Dedicated to Prof. Antonio Graniti on the occasion of his 75th birthday

SUMMARY

Diamine oxidase (DAO) activity was determined in internodes and leaves of faba bean (*Vicia faba* L.) cultivars susceptible and resistant to *Ascochyta* blight. DAO activity was measured in stems and leaves of whole plants and on detached leaves, untreated or inoculated with a highly virulent *Ascochyta fabae* isolate. DAO activity was higher in leaves than in stems, and decreased acropetally along the plant. No increase in activity was detected as a response to inoculation. The time course of DAO activity was analysed during *A. fabae* infection of the third leaf of *V. faba* seedlings. In fact, DAO activity with different inoculum concentrations showed no significant differences between inoculated plants and controls. Moreover, no increase was detected either in the susceptible line 14-12 or in the resistant line 29H. The time courses of DAO activity was also analysed during wound healing of the third internodes and leaves of the faba bean cultivar 'Lunga delle Cascine' and line 29H. Enzyme activity was enhanced during the days following wounding, showing that this enzyme may have an important role in tissue repair.

Key words: Ascochyta blight, *Vicia faba,* diamine oxidase, wound healing.

INTRODUCTION

Ascochyta fabae Speg. (teleomorph: *Didymella fabae* Jellis *et* Punith.) is the causal agent of *Ascochyta* blight, also known as leaf, stem and pod spot disease of *Vicia faba* L. (Maurin *et al.,* 1990; Jellis *et al.,* 1997). Differences in resistance to artificial inoculation among faba bean genotypes were demonstrated in field trials (Jellis *et al*., 1985; Maurin, 1989). Much effort has been devoted to selecting cultivars resistant to *A. fabae*, and the genetics of this resistance was studied under controlled and natural conditions on F1 and F2 generations (Kharrat, 1999). The histopathology of *V. faba-A. fabae* interaction was also described. In susceptible interactions, the disease spreads throughout the canopy as lesions consisting of a central necrotic region where the fungus has invaded intercellular spaces, whereas, in resistant interactions, the fungus fails to develop after penetration (Maurin and Tivoli, 1992; Maurin *et al*., 1993). The host cells underlying germinating fungal spores become orange-brown and a hypersensitive reaction causes flecks. Molecular mechanisms underlying incompatible interactions and defence responses of *V. faba* to *A. fabae* infection were not investigated. However, previous experiments revealed a key role for diamine oxidases (DAOs) also known as copper containing amine-oxidases (CuAOs) in the defence responses of *Cicer arietinum* L. to *Ascochyta rabiei* (Pass.) Lab. infection. Acting on polyamines present in the apoplast, plant DAOs catalyse the formation of hydrogen peroxide (H_2O_2), which may be utilised by cell wall peroxidases in the lignification process (Federico and Angelini, 1986; Angelini and Federico, 1989; Angelini *et al.*, 1993). Moreover, H_2O_2 functions as a local trigger for programmed death in challenged cells and as a diffusible signal for the induction in adjacent cells of protective genes such as those coding for glutathione peroxidase (Levine *et al*., 1994). DAO activity is constitutively higher, and it increases to a higher extent upon *A. rabiei* infection, in chickpea cultivars resistant to the fungus as compared to susceptible ones (Angelini *et al*., 1993) and upon wound-healing of chickpea seedlings (Angelini and Federico, 1990).

Biochemical and histochemical approaches were used in the present work to define the role of this enzyme in the defence response of *V. faba* to *A. fabae*. In parallel, the constitutive activity of different lines of *V. faba* was analysed and involvement of DAO in wound healing of faba bean tissues following mechanical injury was also studied.

Corresponding author: O. Métoui
Fax: +39.06.86802296
E-mail: epid&resist@ispave.it

MATERIALS AND METHODS

Chemicals. Putrescine (1,4-diaminobutane), o-aminobenzaldehyde, 3,3'-diaminobenzidine, phenylmethylsulfonylfluoride (PMSF), polyvinylpolypyrrolidone (PVPP), aminoguanidine and 2-bromoethylamine were obtained from Sigma, St. Louis, U.S.A.

Plant material. The faba bean cultivar 'Lunga delle Cascine' and four lines with different degrees of resistance to *A. fabae* (29H, 14-12, 19TB, LPF120) were used (Table 1). Shortage of seed of resistant material, which is not commercially available, necessitated inclusion of different lines in our experiments. Seeds were soaked for 12 h in aerated tap water and grown for 15 days in 1 l plastic pots containing a mixture of sand, peat and loam (1: 1: 1), in a greenhouse at 20 ± 3°C where natural illumination was supplemented by artificial light (horticultural lighting group SGR 140, equipped with Philips SONT-T 400W lamps) to give a 12 h photoperiod.

Table 1. Plant material used.

Cultivar or line	Origin	Reaction to *Ascochyta* blight
29H	INRA, France	resistant
19TB	INRA, France	moderately-resistant
14-12	INRA, France	susceptible
LPF 120	Tunisia	susceptible
Lunga delle Cascine	Italy	susceptible

Plant inoculation and sampling methods. Two *A. fabae* isolates belonging to different virulence groups, determined by differential behaviour on the French line 14-12 (Kharrat *et al.,* 1998) were used (Table 2). Spore concentrations were estimated using a haemocytometer and adjusted to final concentrations of 5×10^3, 5×10^4 and 5×10^5 spores ml^{-1} by dilution in 0.4%, w/v water-agar. Localised inoculations (one 50 µl- or six 5 µl-drops) were performed on faba bean leaves and stems at different node levels and on detached leaves. Detached leaves were cut with a razor blade and kept in plastic trays with the petioles in water. Control plants were all treated with agar-water. After inoculation, plants and detached leaves were maintained in a greenhouse at 20 ± 3°C, covered with polythene bags to assure RH near saturation, with lighting as described above. Inoculated leaves and stem portions (with homogeneous and localised necroses, the latter having no visible influence on plant development) and controls were collected at different times, maintained at –80°C and subsequently tested for DAO activity. In order to investigate whether the DAO response to *A. fabae* infection varied according to the development of the plant, plants of different age were used; for example, to inoculate the first leaf, younger plants at the first leaf stage were inoculated. After 15 days, when symptoms became well defined, plants were at the three leaves stage. DAO response was measured in the first leaf (inoculated) to check localised induction of DAO response and in leaves at different distances from the inoculated leaf to check possible systemic induction. Enzyme activities were determined in at least three independent experiments, each with three replicates.

Wounding treatments. Fifteen-day-old seedlings of the resistant line 29H and of the cultivar 'Lunga delle Cascine' were cut lengthwise along the third internode and leaf lamina, then excised at different times and stored at –80°C until processed. DAO was analysed for up to 5 days after wounding. Enzyme activities were determined in at least three independent experiments, each with three replicates.

Preparation of crude extracts. Frozen stem segments and leaves were ground in a mortar at 4°C using 0.1 M K-phosphate buffer (KPi), pH 7.0, (3 ml g^{-1} fresh wt). The homogenate was centrifuged at 13,500 *g* for 15 min and the supernatant was used for determination of DAO activity.

Determination of enzyme activity. DAO activity was estimated spectrophotometrically by following the formation of the yellow adduct (A_{max} = 430 nm, ε = 1.86 mM^{-1} cm^{-1}) arising from the condensation of Δ^1-pyrroline with o-aminobenzaldehyde as previously reported (Federico *et al*., 1985). One unit of enzyme (U) represents the amount of enzyme catalysing the oxidation of 1 mmol of substrate min^{-1}.

Table 2. Isolates used.

Isolate	Origin	Date of collection	Virulence on line 14-12	Virulence on line 29H
AF T 04	Bizerte, Tunisia	1991	high	low
AF T 08	Bizerte, Tunisia	1991	low	low

Histochemical methods. DAO was localised by an indirect histochemical method based on staining due to oxidation of the artificial peroxidase substrate, 3,3'-diaminobenzidine, upon addition of putrescine (Gahan, 1984). Unfixed tissue sections of *V. faba* (100 µm thick) prepared from the third internodes, were thoroughly washed with 50 mM KPi, pH 7.0, containing 0.1 M NaCl, then incubated in the same buffer containing 0.02%, w/v 3,3'-diaminobenzidine. After approximately 10 min, putrescine was added (3 mM final concentration). Upon addition of the diamine, DAO occuring in the cell walls produces H_2O_2, which in turn triggers the peroxidase-mediated oxydation of 3,3'-diaminobenzidine to a brown compound. The addition to control sections of aminoguanidine or 2-bromoethylamine, a strong irreversible inhibitor of amine oxidase, was necessary in order to eliminate H_2O_2 produced in the DAO-catalysed oxidation of endogenous polyamines released at the cut surface.

DAO activity was also analysed along *V. faba* stems after wounding or after *A. fabae* infection. Tissue injury in inoculated *V. faba* seedlings of the resistant line 29H was followed by light microscopy in transverse sections of the third infected internodes.

RESULTS AND DISCUSSION

Constitutive DAO activity in *V. faba* cultivars. DAO activity was significantly higher in susceptible lines LPF 120 and 14-12 than in 29H. Moreover, activity was more abundant in leaves than in stems of different cultivars, the difference being greater between leaves and stems in lines 29H and 19TB than in 14-12 and LPF 120 (Fig. 1). The results also showed that DAO activity decreased acropetally along the stem and among leaves in parallel with cell differentiation, *e.g.*, in the line 29H, DAO activity in the first internode was 7.3 10^{-3} U g^{-1} fresh wt in the second internode it was 5 10^{-3} U g^{-1} fresh wt and in the third one it was 4.4 10^{-3} U g^{-1} fresh wt, indicating its possible role during cell development. The H_2O_2 generated upon oxidation of polyamines can be utilised by peroxidase (POD) isozymes (Federico and Angelini, 1986; 1988), involved in lignification and modulation of cell wall plasticity during plant growth. In the first assays DAO activity was very low, so we tried to optimise tissue homogenisation by using PVPP (1 g g^{-1} fresh matter) in order to eliminate phenols, and PMSF (0.5 mM) as protease inhibitor, but activity remained low. Compared to chickpea cultivars 'Calia' and 'Sultano', respectively susceptible and resistant to *A. rabiei*, *V. faba* DAO activity was almost 100 times lower. Angelini *et al.* (1993) concluded that the higher DAO activity in intact internodes of the resistant chickpea cultivar, as compared with the susceptible one, suggests possible use of DAO activity dosage in screening chickpea for resistance to *A. rabiei*. Our results did not show any correlation between resistance or susceptibility to *A. fabae* and constitutive *V. faba* DAO activity.

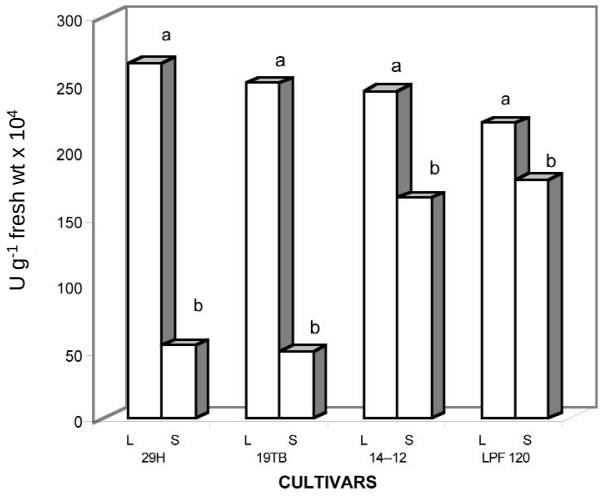

Fig. 1. Constitutive DAO activity variation in leaves and stems of different *V. faba* cultivars. Values followed by the same letters are not significantly different ($P = 0.05$). L: leaf; S: stem.

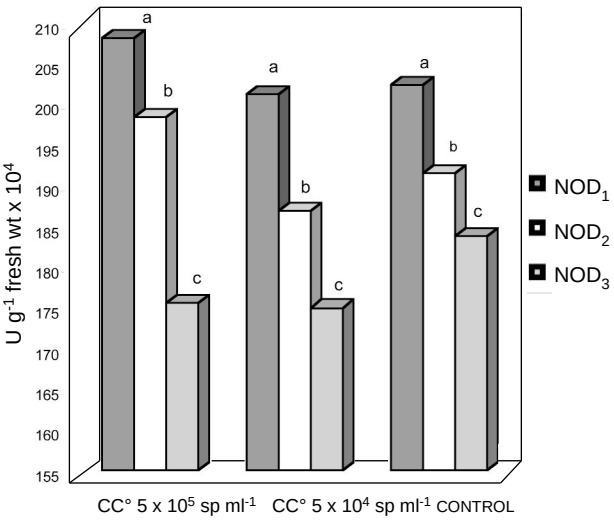

Fig. 2. DAO activity response measured in the crude homogenates of the cultivar 'Lunga delle Cascine' obtained from leaves of the first, second, third node level, 15 days after inoculation of the first leaf with different *A. fabae* inoculum concentrations (5 x 10^5 sp ml^{-1} and 5 x 10^4 sp ml^{-1}). Values followed by the same letters are not significantly different ($P = 0.05$). NOD_1: first node level; NOD_2: second node level; NOD_3: third node level.

DAO activity in *V. faba* cultivars infected by *A. fabae*. The best conditions for symptom development on stems and leaves were obtained with a spore concentration of 5 x 10^5 spores ml^{-1}. On detached leaves, well defined symptoms developed at different node levels, suggesting that this technique could be used in further experiments. As regards DAO response measured after inoculation of detached leaves as well as of leaves and stems of whole plants of the Italian cultivar 'Lunga delle Cascine', analysis of variance of DAO activity showed no significant differences as compared to the control, either using one drop of 50 µl or six drops of 5 µl of inoculum containing different conidium concentrations (Fig. 2).

No increase of DAO activity was detected either in the susceptible line 14-12 or in the resistant 29H, inoculated with both *A. fabae* isolates during the time course at 3, 6, 8, 24 hours after inoculation, 3, 5, 7, 9, 10, 12 and 15 days after inoculation of leaves of the third node (Table 3).

DAO response during wound healing. After wounding, DAO activity increased linearly for five days in wounded organs of *V. faba* 'Lunga delle Cascine' and this response was greater in leaves than in stems. All three experiments (as reported above) confirmed the results (Table 4). Using line 29H, DAO activity in leaves averaged 17.7 10^{-3} U g^{-1} fresh wt 24 hours after wounding, and reached 19.2 10^{-3} U g^{-1} fresh wt five days after wounding. In control leaves DAO activity was 16 10^{-3} U g^{-1} fresh wt. In wounded stems this activity varied from 8.9 10^{-3} U g^{-1} fresh wt 24 hours after wounding to 12 10^{-3} U g^{-1} fresh wt five days after wounding. In the control stems, activity was 7.5 10^{-3} U g^{-1} fresh wt. This result has also been found for other leguminous seedlings. In fact, wounding chickpea stems induces significant increase in DAO activity, suggesting a specific role of DAO in the physiological response of chickpea stem tissue to laceration (Angelini and Federico, 1990; Scalet *et al.,* 1991). DAOs are locally and systemically activated upon injury, with rapid induction both in damaged and distal organs (Rea, 1999).

Histochemical determination of DAO during wound healing. Upon incubation of sections of *V. faba* epicotyl 'Lunga delle Cascine' with 3,3'-diaminobenzidine plus putrescine, staining appeared in cell walls of cortical and vascular parenchyma, sclerenchyma, xylem and epidermis (Fig. 3A). Control sections without added putrescine showed the same activity in 3,3'-diaminobenzidine oxidation. This fact may be due to the oxidation of polyamines diffusing from intracellular compartments after cutting.

In order to avoid this interference, control sections were preincubated with inhibitors of DAO (aminoguanidine, 2-bromoethylamine). Staining was then absent in the epidermis but it persisted in other tissues, suggesting

Table 3. Time course of DAO activity (U g^{-1} fresh wt) 10^3 measured in leaves of the third node level of lines 14-12 and 29H inoculated with *A. fabae* isolates AF T04 and AF T08.

Time after inoculation	Lines 14-12 (susceptible)			Lines 29H (resistant)		
	AF T 04	AF T 08	Control	AF T 04	AF T 08	Control
0 hour	8.1 ± 0.3[a]	7.2 ± 0.2	8.4 ± 0.2	8.3 ± 0.3	8.7 ± 0.2	7.9 ± 0.3
3 hour	8.3 ± 0.2	7.8 ± 0.2	8.9 ± 0.5	7.2 ± 0.4	8.0 ± 0.3	7.8 ± 0.2
6 hour	8.8 ± 0.2	8.2 ± 0.2	7.9 ± 0.2	7.8 ± 0.3	9.1 ± 0.2	7.7 ± 0.2
8 hour	8.6 ± 0.0	8.3 ± 0.4	7.8 ± 0.3	7.1 ± 0.3	8.2 ± 0.4	7.8 ± 0.3
24 hour	9.6 ± 0.1	8.9 ± 0.2	8.6 ± 0.2	7.5 ± 0.3	8.9 ± 0.2	8.5 ± 0.2
3 days	12.0 ± 0.2	7.1 ± 0.2	11.2 ± 0.2	8.2 ± 0.4	7.7 ± 0.2	11.1 ± 0.2
5 days	13.5 ± 0.2	9.2 ± 0.2	9.1 ± 0.3	7.9 ± 0.5	7.8 ± 0.3	9.7 ± 0.4
7 days	9.8 ± 0.1	10.6 ± 0.2	8.5 ± 0.2	8.6 ± 0.3	9.9 ± 0.4	10.5 ± 0.2
9 days	9.5 ± 0.1	9.9 ± 0.2	9.3 ± 0.2	9.7 ± 0.4	12.1 ± 0.2	10.6 ± 0.2
10 days	13.6 ± 0.3	11.6 ± 0.3	13.5 ± 0.2	12.5 ± 0.3	12.3 ± 0.2	11.9 ± 0.2
12 days	16.5 ± 0.2	14.5 ± 0.2	14.2 ± 0.4	16.8 ± 0.3	17.4 ± 0.5	14.5 ± 0.3
15 days	20.3 ± 0.2	17.3 ± 0.3	16.6 ± 0.3	18.5 ± 0.3	20.1 ± 0.3	15.8 ± 0.3

[a] Standard error.

Table 4. Effect of wounding on time course of DAO activity (U g^{-1} fresh wt) 10^3 measured in leaves and internodes of the third node level of the susceptible Italian cultivar 'Lunga delle Cascine'.

Time after wounding	Organ			
	Leaf		Stem	
	Wounded	Control	Wounded	Control
24 hours	28.0 ± 0.6[a]	15.1 ± 0.2	8.7 ± 0.2	6.0 ± 0.1
48 hours	29.5 ± 0.4	15.4 ± 0.2	7.5 ± 0.3	5.7 ± 0.1
3 days	32.0 ± 0.2	16.7 ± 0.3	9.6 ± 0.2	6.6 ± 0.2
4 days	41.0 ± 0.1	20.0 ± 0.4	12.0 ± 0.2	8.6 ± 0.3
5 days	52.0 ± 0.2	21.0 ± 0.5	16.0 ± 0.3	9.2 ± 0.4

[a] Standard error.

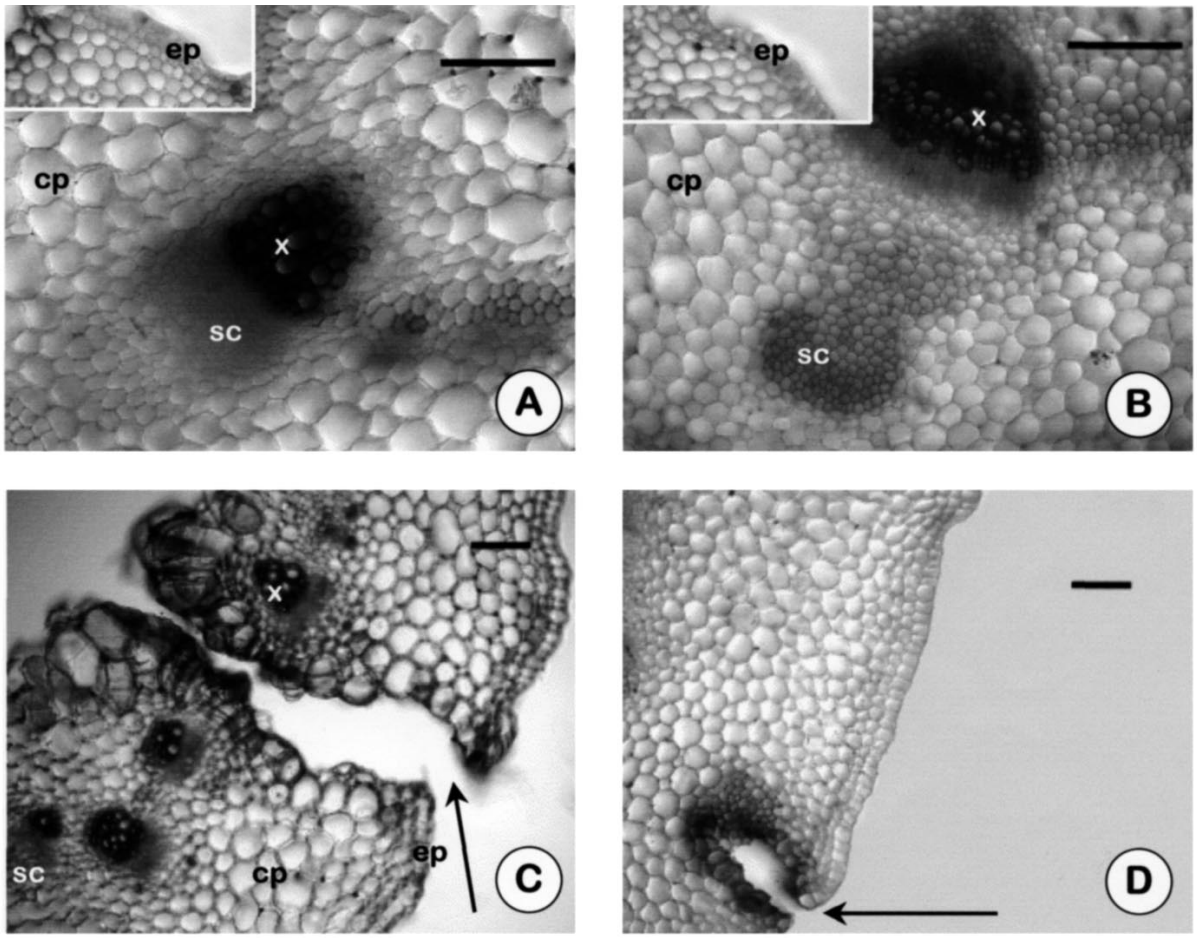

Fig. 3. Histochemical detection of DAO activity in *V. faba* stems of the cultivar 'Lunga delle Cascine'. Fresh transverse sections (100 µm thick) obtained from the third internode were treated as described in Materials and Methods for histochemical determination of DAO activity. **A**: transection incubated with 3,3'-diaminobenzidine and putrescine; **B**: control section incubated with 3,3'-diaminobenzidine and 2-bromoethylamine, a strong specific DAO inhibitor, in the absence of putrescine; **C**: transection obtained 5 days after wounding, incubated with 3,3'-diaminobenzidine and putrescine; **D**: control section obtained 5 days after wounding incubated with 3,3'-diaminobenzidine and 2-bromoethylamine, in the absence of putrescine. cp: cortical parenchyma; sc: sclerenchyma; x : xylem; ep: epidermis. Bars = 70 µm. The arrows show the wound healing.

a role for DAO in H_2O_2 synthesis in the epidermis and the contribution of other oxidative systems which produce H_2O_2 in other tissues (Fig. 3B). Possible diffusion of the DAO inhibitor could be limited by suberin and lignin deposition in the cell walls, providing an alternative explanation. Staining was particularly intense in the cut zone five days after wounding, and was diffuse in cortical and vascular parenchyma, sclerenchyma, xylem and epidermis (Fig. 3C).

In control sections, staining persisted in the cut zone suggesting the contribution of alternative oxidative systems producing H_2O_2 in the wound-healing periderm or a limited spread of the DAO inhibitor (Fig. 3D). This result was confimed using the resistant line 29H (data not shown). Angelini *et al.* (1993) showed that lignosuberized periderms, which possess high histochemical DAO activity, are produced in chickpea seedlings as a result of both mechanical injury and pathogen infection, and suggested a close relationship between lignosuberisation catalysed by peroxidase and polyamine oxidation catalysed by DAO.

Histochemical studies of DAO activity after *A. fabae* infection. Brown staining was observed in epidermis, cortical and vascular parenchyma, sclerenchyma and xylem (Fig. 4A) but the difference with control sections (Fig. 4B) incubated without putrescine and treated with specific DAO inhibitors, was not great, indicating as reported above that DAO activity in *V. faba* is very low and that other oxidative systems producing H_2O_2 are probably involved. This result was confimed using the cultivar 'Lunga delle Cascine' (data not shown). Experiments with specific DAO inhibitors have shown that CuAO activation in chickpea is essential for H_2O_2 production in defence against fungal infection (G. Rea *et al.*, unpublished results). Our data indicate that *V. faba* DAO may play an important role during plant development and wounding response; its role in resistance to *A. fabae* infection may be secondary and in any case less important compared to the role played by DAO in chickpea resistance to *Ascochyta* blight.

ACKNOWLEDGEMENTS

This paper is part of research supported by EC, DG XII, Project 'FRYMED', Contract no. 1C18-CT98-0300. O. Métoui, supported by a FRYMED grant, is preparing her doctoral thesis in collaboration with Italian (ISPaVe and University 'Roma Tre') and Tunisian (University of Tunis and INRAT) institutions. We wish to thank Dr. A. Infantino (Istituto Sperimentale per la Patologia Vegetale), Prof. R. Federico and Dr. G. Rea (Università degli Studi 'Roma Tre') for helpful discussion and Dr. S. Moreno (Università degli Studi 'Roma Tre') for technical assistance with microscopy.

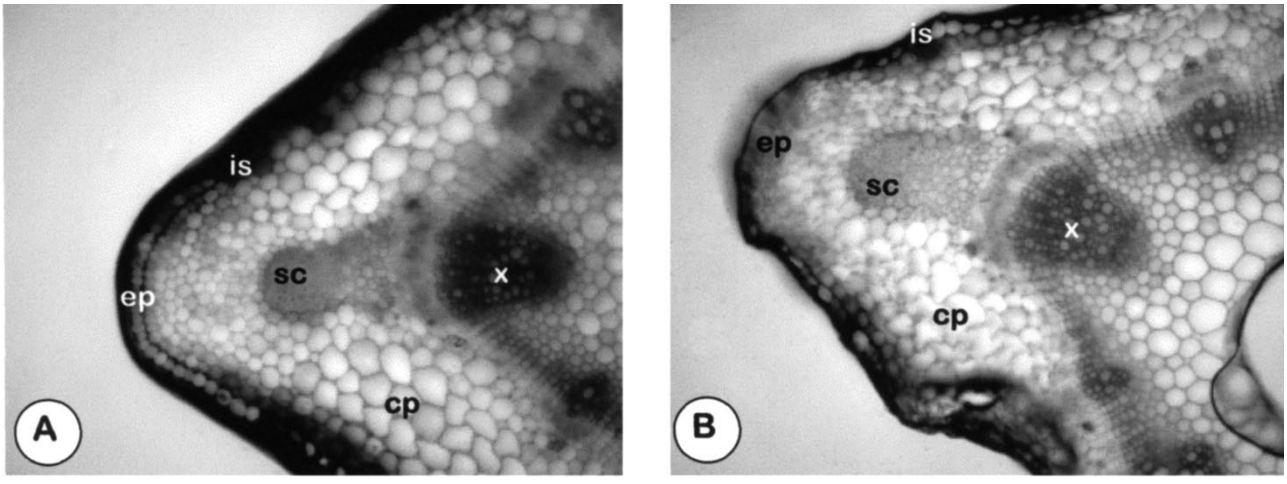

Fig. 4. Histochemical detection of DAO activity in *V. faba* stems of the resistant cultivar 29H after inoculation with *A. fabae* conidia. Fresh transverse sections (100 µm thick) obtained from the third internode (15 days after inoculation) were treated as described in Materials and Methods for histochemical determination of DAO activity. Fungal infection resulted in death of epidermal cells. **A**: transection from the third internode of inoculated 29H incubated with 3,3'-diaminobenzidine and putrescine; **B**: control section from the third internode of inoculated 29H incubated with 3,3'-diaminobenzidine and 2-bromoethylamine, in the absence of putrescine. is: inoculation site; cp: cortical parenchyma; sc: sclerenchyma; x : xylem; ep: epidermis.

REFERENCES

Angelini R., Federico R., 1989. Histochemical evidence of polyamine oxidation and generation of hydrogen peroxide in the cell wall. *Journal of Plant Physiology* **135**: 212-217.

Angelini R., Federico R., 1990. Spatial and functional correlation between diamine oxidase and peroxidase activities and their dependence upon de-etiolation and wounding in chick-pea stems. *Planta* **182**: 89-96.

Angelini R., Bragaloni M., Federico R., Infantino A., Porta-Puglia A., 1993. Involvement of polyamines, diamine oxidase and peroxidase in resistance of chickpea to *Ascochyta rabiei*. *Journal of Plant Physiology* **142**: 704-709.

Barham D., Trinder P., 1972. Improved colour reagent for the determination of blood glucose by the oxidase system. *Analyst* **97**: 142-145.

Federico R., Angelini R., Cesta A., Pini C., 1985. Determination of diamine oxidase in lentil seedlings by enzyme activity and immunoreactivity. *Plant Physiology* **79**: 62-65.

Federico R., Angelini R., 1986. Occurence of diamine oxidase in the apoplast of pea epicotyls. *Planta* **167**: 300-303.

Federico R., Angelini R., 1988. Distibution of polyamines and their related catabolic enzyme in epicotyls of etiolated and light-grown Leguminosae seedlings. *Planta* **173**: 317-321.

Gahan P.B., 1984. Plant histochemistry and cytochemistry. Academic Press, London.

Jellis G.J., Bond D.A., Button R.E., 1997. Diseases of faba bean. In: Allen D.J., Lenné J.M. (eds.). The pathology of food and pasture legumes, pp. 371-422. CAB International, Wallingford.

Jellis G.J., Lockwood G., Aubury R.G., 1985. Phenotypic influences of the incidence of infection by *Ascochyta fabae* in spring varieties of faba beans (*Vicia faba*). *Plant Pathology* **34**: 347-352.

Kharrat M., Onfroy C., Tivoli B., Halila H., 1998. Caractérisation morphologique et biologique des souches tunisiennes d'*Ascochyta fabae*, responsable de l'anthracnose des fèves. Les légumineuses alimentaires méditerranéennes, Rennes (France), 20-22 février 1997. INRA, Paris (Les Colloques, no. 88).

Kharrat M., 1999. Analyse génétique de la résistance de *Vicia faba* L. à différents pathotypes d'*Ascochyta fabae* Speg. Conséquences pour la mise en oeuvre de stratégies de sélection. Ph.D. Thesis. Ecole Nationale Supérieure Agronomique de Rennes, France.

Levine A., Tenhaken R., Dixon R., Lamb C., 1994. H_2O_2 from the oxidative burst orchestrates the plant hypersensitive disease resistance response. *Cell* **79**: 583-593.

Maurin N., 1989. Biologie d'*Ascochyta fabae* Speg. et études des relations hôte-parasite en vue de l'appréciation de la résistance de la féverole à l'anthracnose. Ph.D. Thesis. University Rennes 1, France.

Maurin N., Gourret J.P., Tivoli B., 1993. Histopathology of the interaction between *Ascochyta fabae* and *Vicia faba*: comparison of susceptible and resistant reactions. *Agronomie* **13**: 921-927.

Maurin N., Tivoli B., 1992. Variations among cultivars of *Vicia faba* infected by *Ascochyta fabae* in relation with the disease evolution in field trials. *Plant Pathology* **41**: 737-744.

Maurin N., Tivoli B., Onfroy C., 1990. Mieux connaître les maladies et leurs agents pathogènes pour mieux les combattre. *Perspectives Agricoles* **146**: 36-45.

Rea G., 1999. Le amino ossidasi a rame: coinvolgimento nei processi di sviluppo e nella risposta al ferimento in *Cicer arietinum* L. Ph.D. Thesis. Università degli Studi "Roma Tre" Italy.

Scalet M., Federico R., Angelini R., 1991. Time courses of diamine oxidase and peroxidase activities, and polyamine changes after mechanical injury of chickpea seedlings. *Journal of Plant Physiology* **137**: 571-575.

Received 2 April 2001
Accepted 12 October 2001