ACCUMULATION OF SOLUBLE PHENOLIC COMPOUNDS IN SUNFLOWER CAPITULA CORRELATES WITH TOLERANCE TO *SCLEROTINIA SCLEROTIORUM*

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Abstract

Phenolic content has been analysed in four sunflower (*Helianthus annuus* L.) lines with different tolerance (from susceptible to highly tolerant) to head rot caused by *Sclerotinia sclerotiorum* (Lib.) de Bary. Capitula at the beginning of the flowering stage were inoculated by spraying with a water suspension of ascospores, and disease symptoms were evaluated 6 to 14 days later. At day 14 the most susceptible plants presented many lesions on the capitulum, which were watery, pale brown and with no defined boundaries, whereas, the most tolerant lines had smaller, dry, dark brown and clearly localised lesions. Mycelium was observed on the anthers in all lines. Differences between genotypes were due to greater or lesser fungal invasion of the different organs. The most susceptible genotypes showed that the ovaries were completely necrosed and there were abundant lesions in the corollas, bracts and receptacle. Conversely, in the highly tolerant line, the ovary and corolla was only partially necrosed with no symptoms in the bracts or the receptacle. An analysis of total soluble phenolics was carried out on bracts and corollas from capitula of both inoculated and non-inoculated plants. The amount of phenolic compounds depended on sunflower line, time after inoculation, and tissue. The most tolerant line had higher constitutive and induced phenolic content, this difference being greatest for bracts. This differential accumulation is correlated with the absence of disease symptoms and can be interpreted as being a defence mechanism to prevent fungal spread and development. A similar interpretation was possible from analysis of individual sunflower phenolic compounds such as 7-hydroxylated simple coumarins. Work is in progress on the structure of specific compounds which discriminate between sunflower lines with different degrees of tolerance to *Sclerotinia.*

INTRODUCTION

Sclerotinia sclerotiorum (Lib.) de Bary is a world-wide distributed sunflower pathogen attacking most plant parts, including root, stem, capitulum, leaf and terminal bud at any developmental stage. As chemical control is not practical, genetical control appears to be the best crop protection strategy. A wide range of susceptibility to attacks under field conditions has been described among sunflower inbred lines, varieties and hybrids (Thompson *et al*., 1978; Bazzalo *et al*., 1991). Molecular markers as a selection criterion would be very useful in directing plant breeding programmes.

Phenols have long been associated with passive (preinfectional) and active (postinfectional) defense responses of plants against a number of pest and pathogens. Some previous biochemical studies of the *Sclerotinia*-sunflower interaction under controlled conditions with both inbred lines and wild relatives have evaluated the presence of phenolic compounds in both inoculated and non-inoculated tissues. Accumulation of soluble phenolic compounds, their deposition on cell walls, melanization and lignification are typical sunflower responses to *Sclerotinia* infection which takes place in stem and leaves in both infected and healthy surrounding tissue, this accumulation being lower in *Sclerotinia* susceptible varieties (Orellana, 1975; Marciano and Di Lenna, 1983; Bazzalo *et al*., 1985, 1987; Hemery-Tardin *et al*., 1998). Although qualitative differences in phenolic profiles between sunflower genotypes may not be associated with differences in susceptibility, the presence of flavonoids in leaf epidermis has been claimed to be a preinfectional resistance factor in *H. resinosus* (Mondolot-Cosson *et al*., 1997). Under field conditions, a negative correlation between postinfectional phenolic accumulation and wilting range of different genotypes has been reported (Bazzalo *et al*., 1991), although with considerable environmental effects (Jorrín and Prats, 1999). Compounds such as coumarins and caffeoyl-quinic derivatives, inhibit mycelium growth and are induced in response to infection (Urdangarín *et al*., 1999).

Research is in progress to characterise phenolic metabolism and accumulation in sunflower genotypes with different level of tolerance to *Sclerotinia* both at the pre and postinfectional levels in order to understand how these compounds can contribute to the tolerant character and to identify markers for breeding programmes. Although postinfectional phenolic content is usually correlated with tolerance, such a correlation has not been found while analysing preinfectional levels. Thus, a caffeoyl-quinic derivative analysis in leaves of wild *Helianthus* spp. and interspecific hybrids showed no correlation with resistance to *Sclerotinia* (Tourvieille de Labrouhe *et al.,* 1997). Here we present preliminary data on total soluble phenolic compounds in inoculated and non-inoculated separate parts of the capitulum

MATERIAL AND METHODS

Plant and fungal material The sunflower lines 30302, 45103, FRTS and 0148V1 used in this study were provided by Advanta Seeds S:A.I.C. (Balcarce, Argentina). Seeds were sown in 20 litre pots containing a methyl bromide sterilised peat:perlite (3:1) mixture. Plants grew in a greenhouse with a 20 ºC average temperature and 80%

HR. Sclerotia obtained from field infected plants were induced to carpogenical germination (15 days at -20 ºC treatment) and incubated in a humid chamber on wet soil with a 13h photoperiod (60mE m2s-1) at 18ºC. After a month, ascospores were harvested from apothecia and stored at -20 ºC in dry conditions until use.

Inoculation and sample collection Capitula at the beginning of the flowering stage were inoculated by spraying with a 1.5 ml of a 20 000 ascospores ml-1 water suspension with control plants only receiving water treatment. In order to ensure high humidity conditions with no thermal stress plants were covered with a black paper bag. Capitula were visually observed during 14 days and samples collected 6, 10 and 14 days after inoculation. Inner bracts and corollas from the external rays were separated, the necroses zones removed and healthy surrounding tissue stored at -20 ºC until biochemical analysis. Similar plant tissue was obtained from non-inoculated controls at the same moment.

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Disease symptom evaluation A factorial experiment was performed with an incomplete block design with 8 replicates. The three factors were: sunflower line, time after beginning of flowering and inoculation. Tolerance/susceptibility was evaluated either visually or microscopically by observing the presence of mycelium in the anthers and necrotic lesions in the receptacle, bracts and corollas. Disease incidence is indicated as percentage of plants showing mycelium in the anthers. Necrotic lesions in corollas, bracts and receptacle were quantified by using a discontinuous scale from 0 (no necrotic lesions observed) to 100% (tissue totally necrosed) with intermediate values of 10, 25, 50 and 75 indicating the percentage of the organ affected.

Extraction and analysis of soluble phenolic compounds Phenolics were extracted from frozen tissue and spectrophotometrically determined by using the Folin-Ciocalteu reagent as reported by Prats-Pérez, (1998).

RESULTS AND DISCUSSION

Evaluation of tolerance/susceptibility Disease symptoms appeared 6 days after inoculation and afterl 14 days the genotypes tested showed big differences. At day 14 the most susceptible plants presented many lesions on the capitulum which were watery, pale brown and with no defined boundaries, whereas the most tolerant lines had fewer, dry, dark brown and clearly localised lesions. Table 1 presents the data, the most tolerant line being 1V8410 > FRTS > 30145 > 20303, the most susceptible. This data confirms previous evaluations under field conditions.

The presence of mycelium on the anthers was observed in all the lines independently of the phenotype, although there were small quantitative differences between them. This indicates active germination of the fungal ascospores once they have reached the anthers, as reported by Says-Lesage and Tourvieille (1988). At day 14 the most susceptible genotype (20303) showed all the ovary necrosed and abundant lesions in corollas, bracts and receptacle, whereas 1V8410 only showed a partially necrosed ovary and corolla with no symptoms in bracts or receptacle. The intermediate

lines showed invasion of the ovaries and some lesions in the corollas, bracts and receptacle. This data indicates the existence of preformed or active defences which prevent fungal invasion from the anthers to other parts of the capitulum. Such defences are manifested in the resistant genotypes in corollas, bracts and receptacle, although there may be a time-dependent induction in the ovary.

Table 1. Disease symptoms in *Sclerotinia* infected sunflower plants. Disease incidence indicates percentage of the plants presenting mycelium in the anthers. Necrotic lesions in corollas, bracts and receptacle were quantified by using a discontinuous scale from 0 (no necrotic lesions observed) to 100% (tissue totally necrosed).

Sunflower lines	20303	30145	FRTS	1V8410
days after inoc.		incidence		
6	1	12.5	12.5	14.2
10	100	57.1	28.6	75
14	100	100	71.4	87.5
		necroses in anthers		
6	0	5	5	5
10	15.6	36.3	10	10
14	57.5	32.5	17	11.3
		necroses in bracts		
6	0	0	0	0
10	16	10	10	1
14	63.6	18	5	1
		necroses in receptacle		
6	0	0	0	0
10	22.7	10	2	1
14	36.4	20	1	1

It is well known that phenolic compounds can play an important role in disease resistance as they have fungitoxic or antibiotic activity or act as a physical barrier, thus preventing plant tissue colonisation (Nicholson and Hammerschmidt, 1992; Friend, 1977). In the case of sunflower phenolics, either coumarins or caffeic derivatives inhibit *in vitro* fungal spore germination and mycelium growth (Urdangarín *et al*., 1999). The analysis of total soluble phenolics in different parts of the capitulum in both inoculated and non inoculated plants helped to confirm the hypothesis of the defensive role of these compounds. Data corresponding to corollas and bracts extracts are presented in figure 1. Higher constitutive levels were detected in corollas than in bracts in all cases, as corresponds to cells rich in flavonoid pigments. Significant differences in soluble phenolics at days 10 and 14 in bracts and at day 14 in corollas were found between genotypes, with the highest value being for 1V8410. This difference is not only

quantitative but also qualitative, with the presence of 7-hydroxylated simple coumarins, supporting the idea of using of phenolics as molecular markers to assist breeding programmes for tolerance to *Sclerotinia*.

As a result of the inoculation there was an increase in the content of phenolic compounds in bracts but not in corollas and only in the most tolerant plants;, comparable with that observed by. Hemery-Tardin *et al.* (1998) in other sunflower varieties and tissues. Although we can not discard the existence of other defense reactions involved, the correlation beween phenolic accumulation and absence of disease symptoms in the tolerant sunflower lines allow us to establish that either constitutive or induced phenolics may contribute to head rot resistance.

REFERENCES

BAZZALO ME, HERBER EM, CASO, OH. 1987. Factores fisicos y localizacion anatomica de compuestos fenólicos en relacion con la tolerancia del tallo del girasol (*Helianthus annus*) frente a *Sclerotinia sclerotiorum*, causal de la podredumbre basal. Bol. Soc. Bot. Arg. **25**, 197-212.

BAZZALO, ME, HEBER EM, DE PERO MARTINEZ, MA, CASO, OH 1985. Phenolics compounds in stems of sunflower plants inoculated with *Sclerotinia sclerotiorum* and their inhibitory effects on the fungus. Phytopatol. Z. **112**, 322-332

BAZZALO, ME, DIMARCO P, MARTINEZ F, DALEO GR 1991. Indicators of resistance of sunflower plant to basalt stalk rot (*Sclerotinia sclerotiorum)*: Symptomatological, biochemical, anatomical, and morphological characters of the host. Euphitica **57**, 195-205

FRIEND J. 1977. Phenolic substances and plant disease. En: Recent Advances in Phytochemistry, Vol.12 Biochemistry of Plants Phenolics, pp 557-588. Plenum Press, New York

HEMERY-TARDIN M.C, TOURVIEILLE de LABROUCHE D, JAY M, LEDOIGT G, VEAR F. 1998. Effect of infection by *Sclerotinia* spp. on the phenolic metabolism of sunflower capitula and leaves. Helia

JORRIN J, PRATS E. 1999. Allelochemicals, phytoalexins and insect-feeding deterrents: different definitions for 7-hydroxylated coumarins. Recent Advances in Allelopathy- Volume I- A Science for the future. pp 179-192. Servicio de Publicaciones de la Universidad de Cádiz,.

MARCIANO P, DI LENNA P. 1983. Oxalic acid, cell wall-degradating enzymes and pH in pathogenesis and their significance in the virulence of two *Sclerotinia sclerotiorum* isolates in sunflower. Physiol. Plant Pathol, **22,** 339-345

MONDOLOT-COSSON L, ANDARY C, DAY G, ROUSSEL J. 1997. Histolocalisation de subtances phenoliques intervenant lor d'interaction plante-pathogène chez le tournesol et la vigne. Acta Bot. Gallica **144,** 353-362

NICHOLSON R, HAMMERSCHMIDT R. 1992. Phenolic compounds and their role in disease resistance. Annu. Rev. Phytopathol. **30,** 369-389

ORELLANA RG, 1975. Photoperiod influence on the susceptibility of sunflower to *Sclerotinia* stalk rot. Phytopathology, **65**, 1293-1298

PRATS-PEREZ E. 1998. Inducción de metabolitos de defensa de naturaleza fenólica en girasol mediada por estreses abióticos, azúcares e inductores de fenómenos de resistencia sistémica adquirida. Ms. Thesis, ETSIAM, Universidad de Córdoba.

SAYS-LESAGE V, TOURVIEILLE D. 1988. Reserche des sites de pollution et d´infection des fleurons de tournesol, in situ, par les spores de *Sclerotinia sclerotiorum.* Inf. Techniques C.E.T.I.O.M. **102**, 3-13

THOMPSOM TE, ROGERS CE, ZIMMERMAN DC, HUANG HC, WHELAN ED, MILLER J.F. 1978. Evaluation of *helianthus* species for disease resistance and oil content and quality. Pp 501-509 En Proc. 8 th int. Sunflower Conf., Minneapolis, MN, 23-27 July 1978. Int. Sunflower Assoc., Paris. France.

TOURVIEILLE de LABROUHE D, MONDOLOT-COSSON L, WALSER P, ANDARY C, SERIEYS H. 1997. Relation entre teneurs en derives cafeolyquiniques des feuilles et la resistance de *Helianthus* spp. a S*clerotinia sclerotiorum.* Helia **20,** 39-50

URDANGARIN C, REGENTE MC, JORRIN J, de la CANAL L 1999 Sunflower coumarin phytoalexins inhibit the growth of the virulent pathogen *Sclerotinia sclerotiorum*. J. Phytopathol, **147**, 441-443.

Figure 1. Phenolic content in corollas (A) and bracts (B) of control (non-inoculated, left) and inoculated (right) sunflower capitula. Data correspond to the four lines tested at 6, 10 and 14 after inoculation. Values, expressed as µg equvalent of chlorogenic acid/g fresh weight, are mean of 8 replicates $\pm$ S.E.

(Figure à rajouter)