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Shikimate Dehydrogenase Expression and Activity in Sunflower Genotypes Susceptible and Resistant to *Sclerotinia sclerotiorum (Lib.)* de Bary

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ABSTRACT

The response of five inbred sunflower seedling lines, including AC 4122, C, HA 89, HA 410, HA 411, to inoculation with *Sclerotinia sclerotiorum* culture filtrate containing endogenous oxalic acid was compared with the exogenous application of synthetic oxalic acid. The reaction of seedlings was evaluated in terms of dry and fresh plant weights and the total chlorophyll concentration relative to untreated controls. The expression of shikimate dehydrogenase in cotyledons was also assessed five days after treatment. The results indicated that exogenous oxalic acid inoculation caused more deleterious effects on stem rot, eliciting photosynthesis reduction and different isoenzyme patterns of shikimate dehydrogenase. A positive correlation was found between increased oxalic acid and shikimate dehydrogenase activity in both treatments. However, the excessive toxicity of the exogenously administrated acid suggests that *Sclerotinia sclerotiorum* infection triggers a more complex metabolic pathway involving oxalic acid secreted by the pathogen. These observations preclude the possibility of using the synthetic acid administration as a method of screening sunflower genotypes for resistance to *Sclerotinia*. In addition to these findings, the reactivation of shikimate dehydrogenase was observed in both treatments. In contrast to synthetic administration, expression during the first phase of growth may serve as a tool for rapid screening and selection of sunflower genotypes resistant to *Sclerotinia sclerotiorum*.

Keywords: *Helianthus annuus*, Oxalic acid, Protein pattern, Sclerotinia sclerotiorum, Shikimate dehydrogenase.

INTRODUCTION

Sclerotinia crown and stem rot caused by *Sclerotinia sclerotiorum* (Lib.) de Bary is a serious fungal disease affecting crop plants such as soybean, bean, sunflower, and canola (Rönicke et al. 2005; Purdy, 1979). The general inability of economically important crops to develop germplasm resistance to this pathogen has focused attention on the need for a more detailed understanding of the pathogenic factors involved in disease development (Cessna et al. 2000). Fungal development in plants is related to high polygalacturonase activities (Bazzalo et al. 1991), for the enzymatic degradation of the host cell wall and membrane and the production of oxalic acid (Lumssden, 1979).

Oxalic acid acts as a toxin and causes pH variations, stem lesions, and complete and irreversible plant wilting (Noyes and Hancock, 1981; Marciano et al. 1983). Some studies

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indicate that progressive acidification of the ambient medium by the fungus is a major strategy for the sequential expression of pathogenicity factors; in certain phytopathogenic species of fungi, OXA further accelerates plant tissue maceration through synergism with polygalacturonase (Cotton et al. 2003). Additional findings indicate that OXA concentration in leaves wilted due to infection are up to fifteen times higher than that in uninfected plants (Noyes and Hancock, 1981). The effect of this increment on chlorophyll (Chl) concentrations is still unknown.

Shikimate dehydrogenase (EC 1.1.1.25) (SKDH) is an important biochemical marker produced by plants which may be used to investigate *S. sclerotiorum* infection. SKDH catalyzes the fourth step in the shikimate pathway, which is essential for biosynthesis of aromatic amino acids and aromatic compounds (Lehninger, 1979).

The increase in SKDH activity occurring after *Sclerotinia* infection affects the biosynthesis of shikimic acid, which is involved in the synthesis of lignin for cell walls (Buiatti, 1993; Carrera and Poverene, 1995) and is considered to be the most promising component in relation to plant resistance to *S. sclerotiorum* (Quillet, 1990).

The aim of the present research was to determine if quantifying SKDH expression could be used as a tool to distinguish resistant sunflower genotypes from susceptible ones. This paper reports the results of studies on the effects of OXA treatment in *Helianthus annuus* L. for OXA produced endogenously by *S. sclerotiorum*, as well as for OXA administered exogenously as a synthetic moiety.

MATERIALS AND METHODS

Plant Material

Five inbred lines of sunflower of different origins were used in these experiments. AC4122 and C are maintainer inbred lines, developed at Udine University from an Italian open pollinated population (ALA). HA89 is a maintainer inbred line, while HA410 (Reg. no. GP-227) and HA411 (Reg. no.GP-228) are inbred lines released by U.S.D.A-ARS, North Dakota. AC4122 and HA410 are resistant to *Sclerotinia*, while C and HA89 are susceptible, with HA411 lying between resistance and susceptibility (Peluffo et al. 2010; Becelaere et al. 2004).

Preparation of *S. sclerotiorum* Culture Filtrate

Sclerotinia sclerotiorum collected from the stems of infected plants was germinated and grown on potato dextrose agar at 25°C (Safari Sinegani et al. 2006). After 14 days, three discs (12 mm diameter) of mycelia were transferred to Erlenmeyer flasks containing 1000 mL of liquid medium (DIFCO® and BBL® CZAPEK-DOX without agar) at a constant temperature of 27°C. After 40 days (to obtain a toxin saturated medium), the mycelia were initially separated from the broth medium containing the toxin and extracellular metabolites using a sterile gaze. The solution was then filtered through a sterile 0.45 µm pore-size filter (Sarstedt Ltd., Germany). Culture filtrate (10 µL) was used for measuring toxin (OXA) concentration by an enzymatic-colorimetric method using an Endpoint Sigma Oxalate kit (591C, Sigma Chemical Co. St. Louis, MO, USA) and spectrophotometric absorption measurement at 590 nm (Cary 1E UV-Vis, Varian Inc., CA, USA).

Conditions of Plant Growth, Synthetic Oxalic Acid Preparation, and Inoculation Test

Seeds of all genotypes were surface sterilized as described by (Burrus et al. 1991) and germinated in sterile test tubes (130 x 25 mm) on a solid MS medium (Murashige and Skoog, 1962).

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A stock solution of 1 M oxalic acid (Sigma Chemical Co.) was prepared and diluted to match the culture filtrate concentration.

Both treatment regimes, consisting of exposure to 25 mL of *S. sclerotiorum* culture filtrate or the addition of the same concentration of synthetic OXA, were applied at the cotyledon stage (20 days after germination) on the roots and middle stalk parts of the seedlings for 24 h as described by (Tahmasebi Enferadi et al. 1998 a; Huang and Dorrel 1978). The treated regions were then washed with sterilized water and were transferred to Hoagland solution (H2395, Sigma Chemical Co. autoclaved and stored at room temperature) where they were maintained at 20-25°C, with a relative humidity of 40-50% and light intensity of 500 mM m^{-2} s^{-1}. Controls consisted of untreated plants of each genotype prepared in the same manner, with the omission of treatment with OXA or culture filtrate.

Chlorophyll Concentration Determination

Total chlorophyll (CHL) concentration was determined spectrophotometrically according to the Standard Operating Procedure (EPA-ERT 1994) and expressed as an areal concentration per square centimeter. Two 0.8 cm^2 discs of photosynthetic leaf tissue were excised from each plant 5 days after treatment with *S. sclerotiorum* culture filtrate and synthetic OXA. The samples were ground with a pestle while being chilled on ice. CHL extraction was performed using 3 mL of extraction solution containing one part 0.1 N NH_4OH solution to nine parts acetone (v/v), with samples placed on ice in the dark for two hours. The slurry was centrifuged (5000 rpm per 10 min) and the supernatant was diluted to give absorbance readings in the range of 0.2-0.8 at wavelengths of 645 nm and 663 nm.

Recovery of Vegetal Extraction and Shikimate Dehydrogenase Activity Assays

Five days after exposure to culture filtrate and synthetic OXA, the plant tissue above the cotyledons was collected from each treated and control plant and was individually homogenized in a mortar and placed in sealed tubes containing 50 mM Tris-HCl buffer (pH 7.4), 0.25 M sucrose, 1 mM EDTA (ethylenediaminetetraacetic acid), 1 mM PMSF (phenylmethanesulfonyl fluoride), and 2.5% v/v β-mercaptoethanol. After homogenization and centrifugation at 2000 RPM for 5 min, the supernatants were used to assess OXA, total protein, and SKDH activity.

Total protein content was assessed using the Bio-Rad protein assay kit, with BSA as a standard (Bradford 1976). Samples were analyzed spectrophotometrically at 595 nm. Shikimate activity was colorimetrically determined using the method described by Pearse (1972) and Glenner (1977). Twenty µL of extracted plant material was incubated at 37°C for 30 min in extraction buffer solution to obtain a total volume of 1 mL, as described by (Tanksley and Rick 1980). The buffer solution contained 0.1 M Tris-HCl (pH 8.5), 1 mg shikimic acid, 0.15 mg $NADP^+$, 0.2 mg methylthiazoletetrazolium (MTT), 0.04 mg phenazine methosulfate (PMS), and 2.5% (v/v) β-mercaptoethanol. The reaction correlates with SKDH activity, producing tetrazolium salt that is measured by absorbance at 570 nm (Ponce et al. 1994).

Native-PAGE

Native polyacrylamide gel electrophoresis was performed using 12% (W/V) polyacrylamide slab gels in 0.2 M Tris, 2 mM EDTA, and 0.15 M boric acid (pH 8.5) as an electrode buffer (Guries and Ledig 1978). Staining of SKDH was done by fixation for an hour in buffer solution containing tetrazolium salt as described by Tanksley and Rick (1980).

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Experimental Design and Statistical Analysis

The experiment was carried out following a bifactorial completely randomized block design with three replicates and four plants for each replication. The first factor, genotypes, consisted of the five inbred lines, while the second factor, toxin treatments, consisted of endogenous or exogenous OXA treatment. Determinations of OXA contents and enzymatic activity of SKDH from each genotype were subjected to Analysis of Variance. Significant differences were expressed as $P<0.01$, and the least significant difference procedure (LSD) was used to compare means of treatments. Correlation coefficients were calculated and regression analyses were carried out between the variables having significant differences between genotype means.

RESULTS AND DISCUSSION

The effect of OXA administration on plant metabolism was evidenced by a reduction in chlorophyll concentration. Chlorophyll concentration decreased significantly in plants treated with culture filtrate of *S. sclerotiorum* and exogenous OXA. (Figure 1).

OXA weakens pectic polymers and improves access for degrading enzymes to their substrates by chelating calcium (Dutton and Evans 1996; Kurian and Stelzig, 1979). The extracellular pathogen filtrate caused a decrease in CHL concentration that is clearly associated with other symptoms of phytopathogenicity, i.e. stem rot. In samples treated with synthetic OXA, there was a similar reduction in CHL without any signs of stem or basal stalk rot. This implies a different mechanism of phytopathogenicity (Figure 2).The effects of each treatment on plant growth was compared by measuring fresh weight and dry weight (plants were dried at 60ºC in an oven for 3 days); these were the only growth parameters that could be calculated during the early growth phase. In samples treated with culture filtrate, the fresh weights (when considered as a percentage of controls) were significantly high in HA410, intermediate in HA411 and AC4122, and low in C and HA89 (Table 1).

These differences revealed a genotype based individual variability in response to toxin penetrating the cells, confirming the polygenic nature of this disease (Mestries et al. 1998). The dry matter of these samples did not show significant differences between genotypes (Table 1). These data indirectly

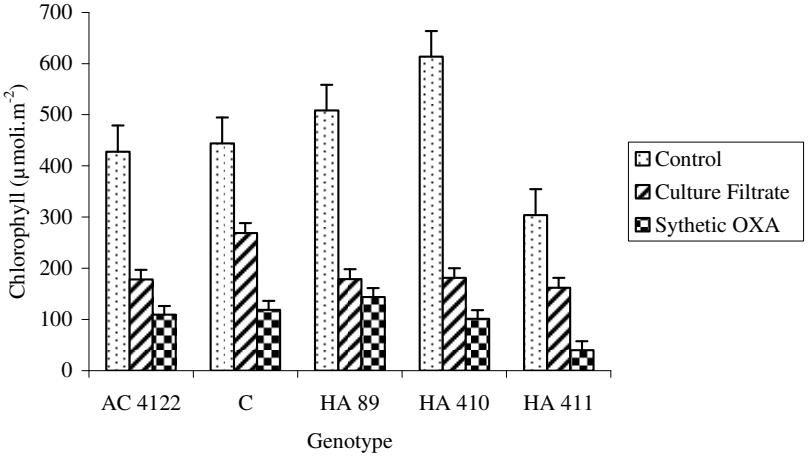

Figure 1. Chlorophyll concentration in different sunflower genotypes 5 days after exposure to toxic metabolites of *Sclerotinia sclerotiorum* (culture filtrate) or synthetic oxalic acid. Bars represent LSD for $P < 0.01$.

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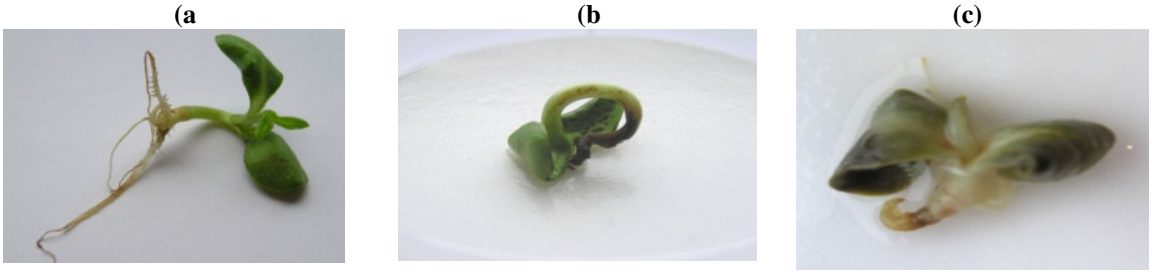

Figure 2. Comparison of growth in the genotype C: a) un-inoculated control plants; b) plants treated with toxic metabolites of *Sclerotinia sclerotiorum* (culture filtrate); and c) treated with synthetic oxalic acid.

suggest that *S. sclerotiorum* manipulates the metabolism of host-derived carbohydrates and consequently increases cell water content.

For samples treated with synthetic OXA, fresh weights of HA411, AC4122 and C had higher values. The other genotypes had lower values but maintained significant differences. Concerning dry weights, there were no significant differences except in the resistant AC4122 line, which had the lowest dry weight. These results provide an alternative explanation for oxalate-induced wilting. Synthetic OXA induces an equally destructive effect on all genotypes and initially causes a reduction in plant growth. This is then followed by self-reconstruction and the plant continues its growth.

The intracellular OXA content in sunflower seedlings was determined five days after culture filtrate or synthetic acid administration to verify whether the metabolic response to OXA may be correlated to disease resistance. Differences in OXA values were observed when control plants were grouped according to their genotypic resistance to *Sclerotinia* infection (Figure 3).

Line C (1.10 mM) and HA89 (1.21 mM) had higher OXA contents, both of which are more susceptible to fungal disease with respect to the other lines. This confirms previous observations by (Tahmasebi Enferadi et al. 1998 b) on the different thresholds of OXA concentrations among genotypes. Concerning samples treated with *Sclerotinia* culture filtrate, Figure 3 shows that OXA concentration values were the highest in susceptible C (2.6 mM) and HA89 (1.81 mM); values were also generally increased in other genotypes when compared to untreated controls. In contrast, OXA intracellular concentrations in all genotypes except C treated with synthetic acid increased

Table 1. Growth characteristics of five sunflower genotypes analyzed 5 days after exposure to culture filtrate of *Sclerotinia sclerotiorum* or synthetic oxalic acid.

Genotypes	Culture filtrate		Synthetic oxalic acid	
	Fresh Plant Weight (%) [a]	Dry Weight Plant (%)	Fresh Plant Weight (%)	Dry Weight Plant (%)
AC 4122	36.8 b [b]	65.6 c	43.91 a	58.6 d
C	27.7 d	64.9 d	39.3 c	76.4 a
HA 89	28.6 c	64.4 d	30.9 e	71.1 c
HA 410	47.8 a	71.4 a	36.9 d	71.1 c
HA 411	36.2 b	68.4 b	43.4 b	75.5 b

[a] Values are reported as a percentage of the controls.
[b] Means followed by the same letter are not significantly different at 1% level as indicated by Least significant difference (LSD).

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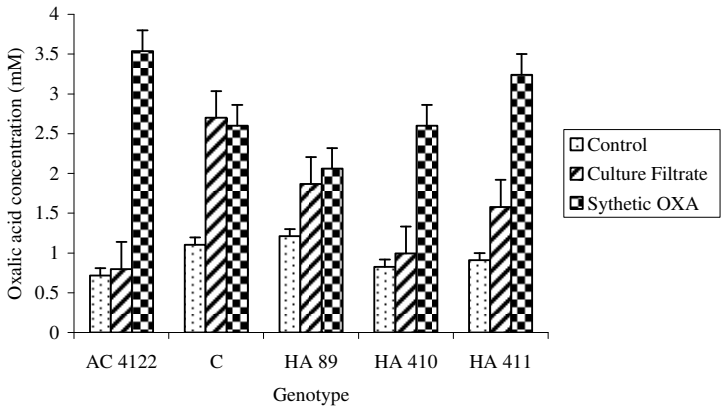

Figure 3. Oxalic acid concentrations of different sunflower genotypes 5 days after exposure to toxic metabolites of *Sclerotinia sclerotiorum* (culture filtrate) and synthetic oxalic acid and untreated plants (control). Bars represent LSD for $P < 0.01$.

much more than the samples treated with culture filtrate. Because OXA concentration increases in pathogen-infected plants, these data demonstrate that more resistant plants are better able to control catabolism of this acid, as found in HA410 and AC4122. This is probably due to an effective intercellular mechanism inhibiting abnormal increases of pH. Specific macromolecules produced by pathogen infection, and elicited by OXA, are recognized by the plant (Buiatti, 1993) and initiate the activation of host defense responses. These signals were absent after synthetic OXA administration, which prevented the plants from internally controlling their OXA levels.

In sunflowers, the induction and accumulation of phenolic compounds, their deposition on cell walls and lignifications, is a well-characterized mechanism of disease resistance against *S. Sclerotiorum* (Prats at al. 2003; Rodríguez et al. 2004). Conceivably, resistant plants also have higher associated levels of phenylalanine ammonia–lyase (PAL), which facilitates the biosynthesis of important phenolic derivatives such as lignin. Similar to phenolic compounds and PAL, shikimic acid and the related enzymatic activity of SKDH were considered in this study to identify a biochemical paradigm that provides a clear correlation to disease resistant genotypes. SKDH is an intermediate step in the aromatic amino acid biosynthetic pathway and is essential to lignin production.

SKDH activity showed considerable increases in samples treated with synthetic OXA (Figure 4).

In susceptible genotypes, the inability to elicit an effective metabolic response against pathogen infection by induction of phenolic compounds may imply the activation of an alternative defensive strategy that incorporates the biosynthetic pathway controlled by SKDH activity. This could explain the greater SKDH activity after culture filtrate administration in resistant genotypes than in susceptible ones, and could explain the higher constitutive and induced concentrations of phenolic compounds in resistant genotypes (Prats et al. 2003). The general increase in SKDH detected in samples treated with synthetic OXA, regardless of disease susceptibility, may be due to the lack of intracellular molecular intermediates specifically elicited by fungal infection. This observation rules out the possibility of using the synthetic acid administration for screening sunflower resistance to *Sclerotinia*.

It is worth observing that the isoenzyme pattern of SKDH becomes enzymatically active in sunflowers at seed germination and reaches its maximum during the cotyledon stage, gradually decreasing and disappearing

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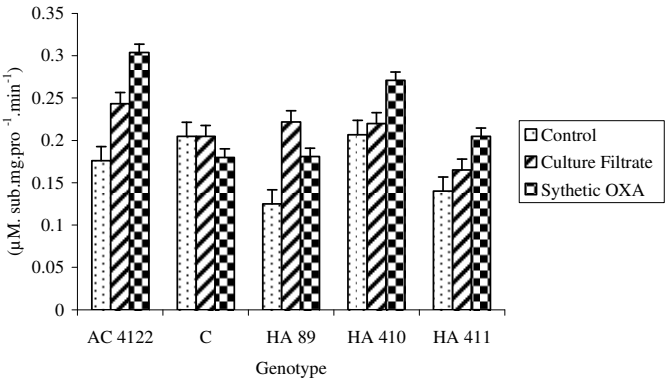

Figure 4. Shikimate dehydrogenase activity of different sunflower genotypes 5 days after exposure to toxic metabolites of *Sclerotinia sclerotiorum* (culture filtrate) and synthetic oxalic acid and untreated plants (control). Bars represent LSD for $P < 0.01$.

after the fourth leaf stage (Diaz et al. 1997). The SKDH enzyme has a monomeric structure encoded by a single gene with two different co-dominant alleles, *skdh*-a and *skdh*-b, in heterozygous plants (Tahmasebi Enferadi et al. 1998 b). The increase in SKDH activity for both homozygous and heterozygous individuals following *S. sclerotiorum* exposure is accompanied by the expression of only *skdh*-b. The lack of *skdh*-a expression in homozygous plants confirms the hypothesis that *skdh*-a is a null allele (Goodman et al. 1980). Both alleles are likely to have the same domains with minor changes in the variable regions, which concerns regions interacting with OXA.

Expression of SKDH on Native-PAGE 48 h after treatment of the studied inbred lines demonstrated that only *skdh*-b was expressed (Figure 5).

Conceivably, SKDH was very faint in control plant protein extracts, with intensity greatly increasing in samples derived from seedlings inoculated with *S. sclerotiorum* and synthetic OXA (Figure 5). In correlating of OXA concentration and SKDH activity, there was an increase in both OXA concentration and SKDH activity in samples treated with both culture filtrate and synthetic OXA (Figure 6).

Samples treated with culture filtrate showed a distribution typical of any individual genotype against this polygenic disease. In contrast, samples treated with synthetic OXA showed a homogenous distribution (oval), which would be expected for a monogenic disease. This indicates that the presence of OXA alone, through the administration of synthetic OXA, is incapable of activating the enzymatic reaction mechanisms of the plants.

CONCLUSION

In conclusion, the differences observed between symptoms generated by pathogenic and synthetic OXA can be related to the different biochemical pathways elicited by each treatment, in both resistant and susceptible inbred lines. Subsequently, this

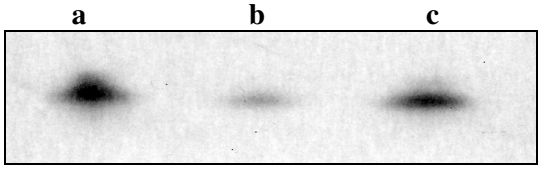

Figure 5. Banding patterns of shikimate dehydrogenase activity in cotyledons of homozygous lines of sunflower on Native-PAGE 48 hours after treating with: a) Synthetic oxalic acid; b) untreated plant as control; and c) culture filtrate.

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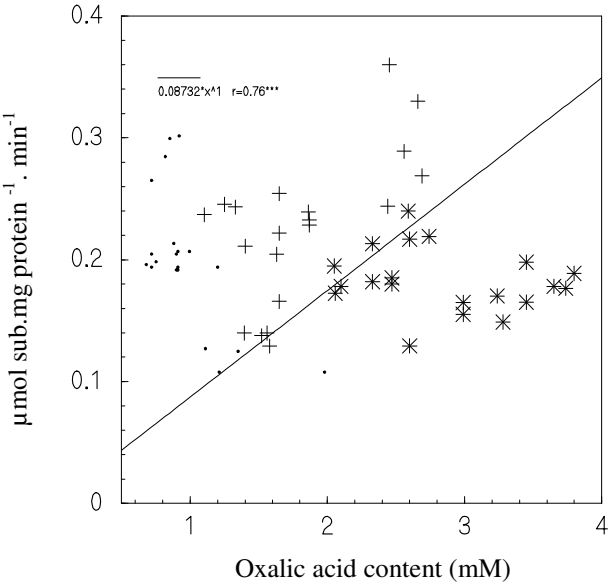

prevents the use of synthetic OXA instead of direct inoculation for rapid screening of genotypes resistant to *S. sclerotiorum*. However, measuring SKDH activity has potential advantages as a rapid and reliable screening method for genotypic resistance to *Sclerotinia*, including earlier discrimination of resistance and cost effectiveness. Although different disease resistance mechanisms can be activated simultaneously, SKDH levels can be directly evaluated to identify resistant lines.

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REFERENCES

1. Becelaere, V., G., Miller, J. F., 2004.Combining Ability for Resistance to Sclerotinia Head Rot in Sunflower. *Crop Sc*i. **44**:1542-1545
2. Bazzalo, M. E., Dimarco, P., Martinez, F., and Daleo G. R. 1991. Indicators of Resistance of Sunflower Plant to Basal Stalk Rot like *Sclerotinia sclerotiorum*: Symptomatological, Biochemical, Anatomical and Morphological Characters of the Host. *Euphytica*, **57**: 195-205.
3. Bradford, M. M. 1976. A Rapid and Sensitive Method of Quantitation of Microgram Quantities of Protein Utilizing the Principle of Protein Binding. *Anal. Biochem*. **72**: 248-254.
4. Buiatti, M. 1993. Basi Biochimiche della Resistenza a Patogeni e Loro Controllo Genetico. In Crinò *et al.*, Eds, Miglioramento Genetico Delle Piante Per La Resistenza a Patogeni e Parassiti. *Edagricole, Bologna* 65-78.
5. Burrus, M., Chanabé, C.**,** Alibert, G.**,** and Bidney, D. 1991. Regeneration of Fertile Plants from Protoplasts of Sunflower (*Helianthus annuus* L.). Plant Cell Rep. **10**: 161-166.
6. Carrera, A. and Poverene, M. 1995. Isozyme Variation in *Helianthus petiolaris* and *H. annuus*. *Euphytica* , **81**: 251-257.
7. Cessna, S. G., Sears V. E., Dickman, M. B., and Low, P. S. 2000. Oxalic Acid, a Pathogenicity Factor for *Sclerotinia sclerotiorum*, Suppresses the Oxidative Burst of the Host plant, *Plant Cell*, **12**: 2191-2200.

[DOR: 20.1001.1.16807073.2011.13.6.5.8]

8. Cotton, P., Kasza Z., Bruel, C., and Rascle, C. 2003. Ambient pH Control the Expression of Endopolygalacturonase Genes in the Necrotrophic Fungus *Sclerotinia sclerotiorum*. *FEMS Microb.i Let.* **277**: 163-169.
9. Dìaz, J. Rosbrcelò, A. and Merino de caceres, F. 1997. Changes in Shikmate Dehydrogebìnase and End Products of the Shikimate Pathway , Chlorogenic Acid and Lignin's During the Early Development to Seedlings of *(Capsicum annumm L.) New phytol,136, 183-188*
10. Dutton, M. V., and Evans, C. S. 1996. Oxalate Production by Fungi: Its Role in Pathogenicity and Ecology in the Environment. *Can. J. Microbiol.* **42**: 881-895.
11. [EPA-ERT] Environmental Protection Agency, Environmental Response Team. 1994. Chlorophyll Determination, Standard Operating Procedure #2030. Edison, NJ: Environmental Protection Agency.
12. Glenner, G. 1977. Formazans and Tetrazolium Salts. In: R. D. Lillie (ed.), H. J. Conn's Biological Stains. Williams and Wilkins Company, Baltimore pp: 225-235.
13. Goodman, M. M., C. W. Stuber, C. N. Lee, and F. M. Johnson. 1980. Genetic Control of Malate Dehydro Genase Isozymes in Maize. Genetics **94**:153-168.
14. Guries, R. P., and Ledig, F. T. 1978 Inheritance of some Polymorphic Isoenzymes in Pitch Pine (*Pinus rigida Mill*). *Heredity* , **40**: 27-32.
15. Huang, H. C., and Dorrel, D. G. 1978. Screening Sunflower Seedlings for Resistance to Toxic Metabolites Produced by *Sclerotinia sclerotiorum*. *Can. J. Plant Sci.,* **58**: 1107-1110.
16. Kurian, P., Stelzig, and D. A. 1979. The Synergistic Role of Oxalic Acid and Endopolygalacturonase in Bean Leaves Infected by *Cristulariella pyramidalis. Phytopathology,* **69**: 1301-1304.
17. Lehninger.A. L. 1979 Biochimica Secondo Edizione Biosintesi degli Amino Acidi Aromatici Fenilalanina e Triptofano, pp: 550 and 639-641
18. Lumssden, R. D. 1979. Histology and Physiology of Pathogenesis in Plant Diseases Caused by *Sclerotinia* Species. *Phytopathology,* **69**: 890-896.
19. Marciano, P., Di Lenna, P. D., and Magro, P. 1983. Oxalic Acid, Cell Wall-Degrading Enzymes and pH in Pathogenesis and Their Significance in the Virulence of Two *Sclerotinia sclerotiorum* Isolates on Sunflower. *Physiol. Plant Pathol.,* **22**: 339-345.
20. Mestries, E., Getzbittel, L., Tourvieille de Labrouhe, D., Nicolas, P., and Vear, F. 1998. Analyses of Quantitative Trait Loci Associated with Resistance to *Sclerotinia sclerotiorum* in Sunflowers (*Helianthus annuus* L.) Using Molecular Markers. *Molecul. Breed.,* **4**: 215 – 226.
21. Murashige, T., and Skoog, F. 1962. A Revised Medium for Rapid Growth and Bioassays with Tobacco Tissue Culture. *Physiol. Plant*., **15**: 473-497.
22. Noyes, R. D., and Hancock, J. G. 1981. Role of Oxalic Acid in the *Sclerotinia* Wilt of Sunflower Physiol. *Plant Pathol*., **18**: 123-132.
23. Pearse, A. G. E. 1972. Histochemistry. Theoretical and Applied. 3rd ed., Vol. 2. Churchill Livingstone, Ltd. Edinburgh, pp: 760-1518.
24. Peluffo, L., Lia, V., Troglia, C., Maringolo, C., Norma, P., Escande, A., Hopp, H. E., Lytovchenko, A, Fernie A .R., Heinz, R., and Carrari, F. 2010. Metabolic Profiles of Sunflower Genotypes with Contrasting Response to *Sclerotinia sclerotiorum* Infection. *Phytochemistry*, **71:** 70-80
25. Ponce, M. M., Navarro, A. I., Martìnez, G. M. N., and Alvares, C. R. 1994. Effect Antigiardiàsico *in vitro* de 14 Extractos de Plantas. *Rev. De Inv. Clin.,* **46**: 343-347.
26. Prats, E., Bazzalo, M. E., Leon, A., and Jorrin, J.V. 2003. Accumulation of Soluble Phenolic Compounds in Sunflower Capitula Correlates with Resistance to *Sclerotinia sclerotiorum. Euphytica,* **132**: 321-329.
27. Purdy, L. M. 1979. *Sclerotinia sclerotiorum*: History, Diseases and Symptomatology, Host range, Geographic Distribution, and Impact. *Phytopathol*., **69**: 875-880.
28. Quillet, M. C., 1990. Etude du Polymorphisme Enzymatique et Proteique Chez le Tounesol, Application à la Charactérisation de Ligneés. Stage Realizé à la Station d'Ameliorement des plantes de l'INRA de Clermond-Ferrand pp: 30-33.
29. Rodríguez, M. A.**,** Venedikian,N.**,** Bazzalo, M. E.**,** and Godeas, A. 2004. Histopathology of *Sclerotinia sclerotiorum* Attack on Flower Parts of *Helianthus annuus* Heads in Tolerant and Susceptible Varieties. *Mycopathologia.,* **157**: 291-302.
30. Rönicke, S., Hahn V., Vogler, A., and Friedt, W. 2005. Quantitative Trait Loci Analysis of

[DOR: 20.1001.1.16807073.2011.13.6.5.8]

Resistance to *Sclerotinia sclerotiorum* in Sunflower. *Phytopathol*., **95**: 834–839.
31. Safari Sinegani,A. A., Emtiazi, G., and Hajrasuliha , S. 2006.Comparative Studies of Extra Cellular Fungal Laccases under Different Condition. *J. Agric. Sci. Technol.* **9**: 69-76
32. Tanksley, S. D., Rick, C. M. 1980. Isozymic Gene Linkage Map of the Tomato: Applications in Genetics and Breeding. *Theor. Appl. Genet.*, **57**: 161-170.
33. Tahmasebi Enferadi, S., Vannozzi, G. p., Gomez-Sanchez, D., and Baldini, M. 1998 a. Results of Screening Sunflower for Resistance to Toxic Metabolites Produced by *Sclerotinia sclerotiorum* (Lib) de Bary and Prospects for the Use of Biochemical Markers in Breeding. *Agr. Med.,* **128**: 47-58.
34. Tahmasebi Enferadi, S., Gomez-Sanchez, D., Baldini, M., and Vannozzi, G. P. 1998 b. Effect *of Sclerotinia sclerotiorum* (Lib) de Bary Culture Filtrate on Sunflower Characters, Oxalic Acid Content and Shikimate Dehydrogenase Activity. *Helia,* **28**: 81-96.

بیان فعالیت آنزیمی شیکیمات دهیدروژناز در ژنوتیپ های آفتابگردان حساس و مقاوم به بیماری اسکروتینا اسکروتیوروم

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چکیده

در این مطالعه پاسخ ۵ اینبرید لاین آفتابگردان شامل AC 4122, C, HA 89, HA 410, HA 411، نسبت به مایه زنی با دو روش مختلف شامل عصاره کشت میسلیوم اسکروتینا اسکروتیوروم که محتوی اسید اگزالیک اندوژن بوده و همچنین اسید اگزالیک سنتزی که محتوی اسید اگزالیک اگزوژن بوده، مورد مطالعه و بررسی قرار گرفت. واکنش گیاهچه ها با بررسی وزن خشک و تر، غلظت نسبی کلروفیل بین نمونه های مایه زده شده نسبت به گیاهچه های کنترل همان ژنوتیپها ارزیابی گردید. بیان شیکیمات دهیدروژناز در فاز دو برگی ۵ روز پس از مایه زنی مورد بررسی قرار گرفت. مایه زنی با اسید اگزالیک اگزوژن تاثیرات تخریب کننده بیشتری از نظر پوسیدگی ساقه، تحریک در جهت کاهش فتوسنتز و ارایه الگوی متفاوت ایزوانزیمی شیکیمات دهیدروژناز نسبت به مایه زنی با عصاره کشت نشان داد. بین افزایش مقدار اسید اگزالیک و واکنش آنزیماتیک شیکیمات دهیدروژناز در هر دو روش مایه زنی همبستگی مثبتی مشاهده شده است. به هرحال سمیت شدیدتر اسید اگزالیک اگزوژن، به طور ضمنی نشان می دهد که عفونت به بیماری قارچی اسکروتنیا در رابطه با مسیرهای بسیار پیچیده متابولیتکی درگیر با تولید اسید اگزالیک است که کاملاً از مایه زنی با اسید اگزالیک اگزوژن متفاوت می باشد. این مشاهدات، احتمال کاربرد مایه زنی با اسید اگزالیک اگزوژن را به عنوان روشی برای غربالگری ژنوتیپهای آفتابگردان مقاوم به اسکلروتینیا نفی می کند. علاوه بر این یافته ها، دوباره فعال شدن شیکیمات دهیدروژناز به میزان متفاوت در هر دو روش مایه زنی مشاهده شده است که تعقیب این بیان در طی فاز اول رشد آفتابگردان می تواند به عنوان ابزاری سریع جهت غربالگری و انتخاب ژنوتیپهای آفتابگردان مقاوم به اسکروتینیا مورد استفاده قرار گیرد.

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