

## THE STUDY OF BIOCHEMICAL INTERACTIONS BETWEEN STRAWBERRY AND VARIOUS FUNGAL EXTRACTS FOR BIOLOGICAL CONTROL OF PATHOGENS

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**Abstract:** *Plants resistance to pathogens attack can be induced by biological control methods including fungal extracts. The main purpose of this study was to obtain chromatographic images of biochemical compounds responsible for biological quality of strawberry intracellular medium, of various culture filtrates of pathogenic or antagonistic fungi and to compare the aspects of interactions between them. Previous research showed that microorganisms such as bacteria and fungi from soil and especially plant rhizosphere can influence plant growth or improve resistance to pathogens. Systemic induced resistance can be also acquired by vaccination with fungal extracts with elicitor effect. Four extracts were obtained from pathogenic isolates of species Botrytis cinerea and from antagonistic species Trichoderma viride, Trichoderma harzianum and Penicillium chrysogenum cultivated on potato-dextrose medium. Fungal extracts E1, E2, E3 and E4, as well as cytosolic extract from strawberry plants cultivar Marmolada were migrated and individual paper chromatograms were obtained for each. The chromatograms of interactions resulted by simultaneous migration of biochemical compounds of strawberry plants and fungal extracts. This is a new approach of paper chromatography we decided to use for the study of plant-fungi interactions, motivated by the biochemical nature of these interactions. The method has the advantage to provide images of the distribution of biochemical compounds from both plant and fungal extracts and of the nature of their interactions. The aspect of contact zones between plant and fungal extracts were different for extracts as a function of the group of fungi involved. E1, E2 and E4 that contained elicitors of immunity from Botrytis isolates induced similar convex zones in plant with low individual variations but E3 from antagonistic isolates of Trichoderma and Penicillium induced a concave margin of plant chromatogram. The method is very usefull for rapid assessment of the answer of plant to a fungal extract and helpfull as a qualitative preliminary test for selecting biological control agents with role in immunologic stimulation of plant.*

**Key words:** *plant resistance, fungal extract, strawberry, Botrytis cinerea, paper chromatography*

### INTRODUCTION

The long term breeding of crops for yield and quality has substantially reduced their natural resistance to pests and pathogens, resulting in a dependency on the use of pesticides for the protection of crops. Biotechnology offers alternative strategies for crop protection. An approach thought to offer the best long term potential is one that improves disease resistance of crops.

As a result of host-pathogen co-evolution, plants have developed mechanisms to protect themselves from disease. Besides preformed physical and chemical barriers that hinder infection, a wide variety of plant defense responses is induced only after pathogen attack (KAMOUN et al., 1993, KOMBRINK and SOMSSICH, 1995, KAZAN et al. 1998). When these induced responses are triggered rapidly and coordinately during a given plant-pathogen interaction, the plant is resistant to disease. Recognition of an invading microorganism, rapid/effective induction of defense responses appears to make essential difference between resistant and susceptible plants (YANG et al., 1997).

The activation of resistance in plants is initiated by host recognition of molecules, which are directly or indirectly released from an invading pathogen. Although elicitors vary widely in their chemical composition and the mechanisms by which plants perceive them may differ (SCOFIELD et al., 1996; TANG et al., 1996), many pathogen elicitors appear to coordinate the overall defence responses of plants. The induced mechanisms frequently manifest themselves as a hypersensitive response (HR), which is characterized by necrotic lesions resulting from localized host cell death at the site of infection. The HR prevents growth and the spread of the pathogen into healthy tissues (GOODMAN and NOVACKY, 1996).

The production of metabolites by pathogenic fungi is important determinant of disease. An appreciable measure of resistance to a pathogen can be related to the ability of the plant to deal with metabolites. This general approach involving the characterization of the secondary metabolites produced by certain fungal pathogens and studying their compatibility relations and phytotoxicity has been applied to the problem associated with the development of "biorational" pesticides from fungal extracts of *Botrytis*, *Trichoderma* and *Penicillium* for control of grey mould to strawberry plants.

Research have been carried out in order to find a preliminary qualitative method for assessing the differences in biological quality of biochemical compounds of fungal extracts, as well as aspects of their interactions on strawberry plants cultivar Marmolada.

#### **MATERIAL AND METHODS**

Four variants of fungal extracts have been obtained: E1- from a *Botrytis cinerea* strain, E2 – from four *Botrytis cinerea* strains, E3- from three *Trichoderma* strains and a *Penicillium* strain and E4- from combined strains of E2 and E3, in order to study their biochemical composition with implications in capacity to elicit immune reactions in strawberry plants cultivar Marmolada for control of grey mould.

Because the eliciting mechanisms are based on interactions of biochemical nature, the study of these phenomena is possible with specific chromatography.

Chromatograms as synthetic images obtained after analytic separation allow interpretation made by differences in color, form of rings or rays as related to corresponding differences in biochemical composition and biological quality (VOITL & GEGGENBERGER, 1986).

Individual chromatograms of fungal extracts E1, E2, E3 and E4, as well as of vegetal extract of strawberry plants cultivar Marmolada have been accomplished.

Specific chromatograms for the study of the aspect of interaction zone of the four fungal extracts with strawberry plants cultivar Marmolada have been obtained by simultaneous migration of biochemical compounds from plant and fungi.

#### **RESULTS AND DISCUSSIONS**

Analysis of specific chromatogram of strawberry plant cultivar Marmolada (Fig. 1) emphasized the biochemical peculiarities with migration of various compounds and distribution on areas with higher colors in the center of chromatogram and darker towards the periphery .

Its good biological quality is given by thick brown rays very well outlined from the center to the dark brown marginal belt.

The chromatogram of fungal extract E1 (Fig. 2) from a single strain of *Botrytis cinerea* is different from both that of E2 (Fig. 3) from the mixture of isolated of the same pathogenic genus and of the extracts E3 (Fig. 4) or E4 (Fig. 5) containing metabolites from antagonistic and respectively antagonistic plus pathogenic genera. Thus, even though in all of them exist a light color central zone, wider than in strawberry chromatogram, the aspect of

darker margins differs. At E1, zone of separation between compounds that confer lighter and darker colors is broad sinuous, with thin dark brown margins finely toothed. In the other extracts chromatograms both separating zones and the margins are sharp and continuous.

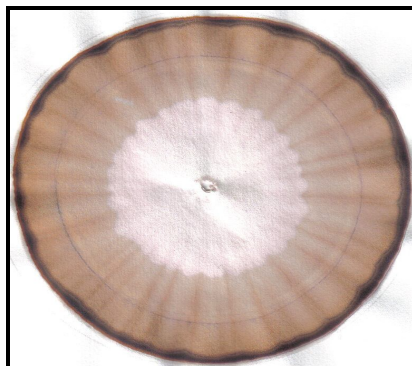


Figure 1: Specific chromatogram of strawberry extract- Marmolada cultivar



Figure 2: Specific chromatogram of fungal extract - E1



Figure 3: Specific chromatogram of fungal extract - E2

At extract E2 from pathogenic isolates, a central grey zone can be observed nearly the first migration circle and the transit towards external black thin zone is done through a zone intermediary as dimensions and color between the two adjacent areas.

At extract E3 with metabolites from antagonistic fungi, zone between the two migration circles is green grey with dark green margins to dark.

The chromatogram of E4 combines the aspects of E2 and E3, reflecting the existence in its composition of biochemical constituents of the fungal isolates that produce it.



Figure 4: Specific cromatogram of fungal extract – E3

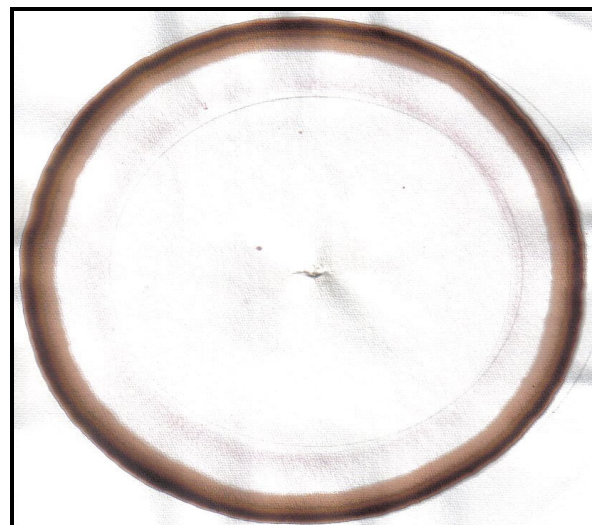


Figure 5: Specific cromatogram of fungal extract – E4

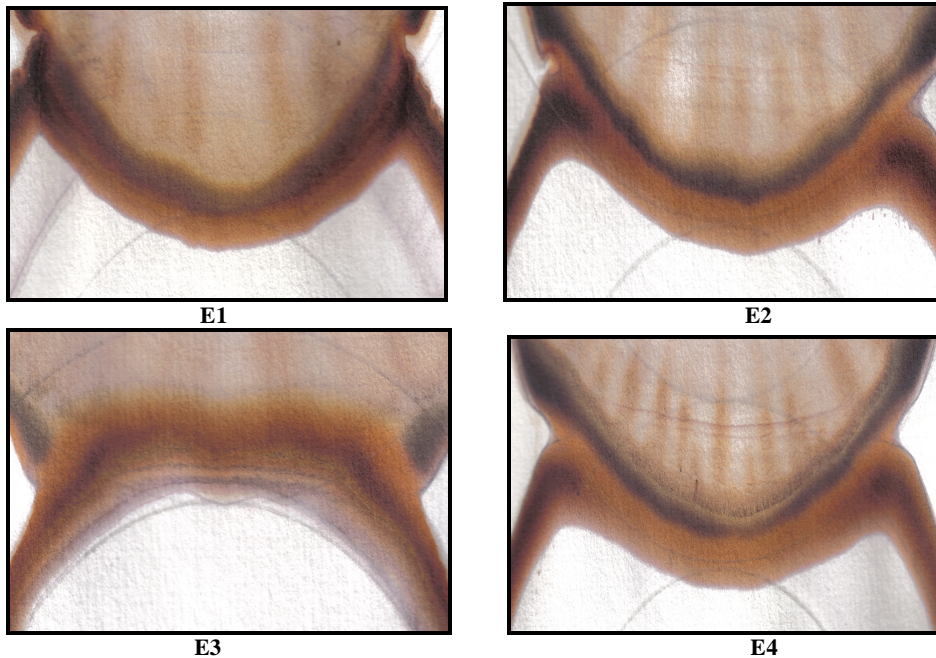


Figure 6: Specific chromatograms of interaction zones between strawberry extract-(Marmolada cultivar) and fungal extracts-E1, E2, E3 and E4

Analysis of the images from figure 6 which represent details of interaction zone between the four types of extracts and strawberry plants cultivar Marmolada shows the existence of similarities of contact zones of plant with fungal extracts E1, E2 and E4 which contain metabolites produced by pathogenic isolates of *Botrytis cinerea*. Thus, all chromatograms have the margin concave, but at extract E3 the phenomenon is inverse: the chromatogram of the antagonists mixture presents the margin convex, well outlined and overlapped with that of plant.

The very different aspect of contact zones illustrates the different nature of biochemical interactions of plant with metabolites provided by plant growth promoting fungi.

Defense reactions in plant were revealed by chromatographic techniques, making this method a useful tool for assessing the nature of relationships between plant species and various biological agents of microbial origin utilizable for eliciting plant resistance to phytopathogens.

### CONCLUSIONS

Individual chromatogram of strawberry plant cultivar Marmolada showed a good biological quality underlined by the presence of thick brown rays from the center to the margins of chromatogram on the background of centric migration zones transient from lighter central zones to darker peripheral zones.

The chromatograms of fungal extracts were different of plant chromatogram, with broader light central zone and without rays.

The chromatograms of E1 from single *Botrytis* strain presented specific characteristics such as external toothed black margin and broadly sinuous zone of contact between light and dark areas.

Extracts E2, E3 and E4 with metabolites from mixed fungal strains presented chromatograms with sharp, entire margins with general aspect relatively similar, reflecting their biochemical composition.

The different aspect of interaction zones between the four fungal extracts and strawberry illustrate the different nature of biochemical relationships of the plant with metabolites from pathogenic and growth promoting fungal species.

Specific chromatogram proved to be a useful tool in revealing the defense reactions of biochemical nature induced in plant by fungal elicitors from extracts E1, E2 and E4.

#### **BIBLIOGRAPHY**

1. KOMBRINK, E., AND SOMSSICH, I.E., 1995 Defense responses of plants to pathogens. *Adv. Bot. Res.* **21**:1-34.
2. KAMOUN, S., YOUNG, M., GLASCOCK, C.B., AND TYLER, B.M., 1993 Extracellular protein elicitors from *Phytophthora*: Host-specificity and induction of resistance to bacterial and fungal pathogens. *Mol. Plant-Microbe Interact.* **6**:15-25.
3. KAZAN, K., MURRAY, F.R., GOULTER, K.C., LLEWELLYN, D.J., AND MANNERS, J.M., 1998 Induction of cell death in transgenic plants expressing a fungal glucose oxidase. *Mol. Plant-Microbe Interact.* **11**:555-562
4. KOMBRINK, E., AND SOMSSICH, I.E., 1995 Defense responses of plants to pathogens. *Adv. Bot. Res.* **21**:1-34
5. SCOFIELD, S.R., TOBIAS, C.M., RATHJEN, J.P., CHANG, J.H., LAVELLE, D.T., MICHELMORE, R.W., AND STASKAWICZ, B.J., 1996 Molecular basis of gene-for-gene specificity in bacterial speck disease of tomato. *Science* **274**:2063-2065
6. TANG, X., FREDERICK, R.D., ZHOU, J., HALTERMAN, D.A., JIA, Y., AND MARTIN, G.B., 1996 Initiation of plant disease resistance by physical interaction of AvrPto and the Pto kinase. *Science* **274**:2060-2063
7. VOITL, H., GEGGENBERGER, E., 1986 *Der Chroma-Boden-Test. Die Bodenqualität bestimmen, bewerten und verbessern. Ein unentbehrlicher Ratgeber für Landwirte, berufs- und Hobbygärtner.* Verlag orac, Wien, Germany, 181p.
8. YANG, Y., SHAH, J., AND KLESSIG, D.F., 1997 Signal perception and transduction in plant defense responses. *Genes Dev.* **11**:1621-1639