

## CHANGES IN LEAF STRUCTURE IN TWO SOYBEAN LINES INDUCED BY SOIL AND PLANT TREATMENTS

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**Keywords:** *soybean, leaf anatomy, plants reaction, Botrytis infection*

### Abstract

*Different changes in mesophyll and both epidermis tissues and cells dimensions were observed on two soybean line plants when treatments with fungicide and various extracts were applied on soil and plants. These experiments were created to emphasize plants reaction to the Botrytis infection; the used variants were: Botrytis resistant or sensible soybean plants, systemic or contact fungicides and 4 extracts types. Anatomically, the plants from the soybean lines M10 (Botrytis sensible) and B62 (Botrytis resistant) are different one from the other in the mesophyll thickness. When fungicide treatments on the soil were applied, plants from both lines have reacted by slowly increasing thickness at the epidermal cells level and by making up two cell layers palisade tissue. The same reaction was observed when the fungicide treatments were applied on plants. The four kinds of extracts used in this experiment have induced different kinds of reactions in plants either in soil or plant treatments.*

### INTRODUCTION

The leaf structure and morphology reflected the evolutionary adaptations of plants to different habitats [1].

As the vegetative organ with the greatest ecological plasticity, the leaf is a sensitive indicator of any changes in the environmental conditions. Many research papers treat the leaf structure with reference to ecology. The degrees of pollution [5], pathogen attacks [3], applied treatments [4] etc. are reflected in the biometrical characteristics of the epidermis and mesophyll tissues.

On the other hand, these leaves characteristics were used in different study of correlation between them and plants resistance to the pathogen attacks [2].

The results of such a study, conducted on soybean leaves, are presented in this paper, to point up the occurring changes in the structure of the blade when on the plants were applied different stress factors.

## MATERIAL AND METHODS

The plant material consisted of leaves drawing from two lines of soybean plants - M10 (*Botrytis* sensible), B65 (*Botrytis* resistant) on which these treatments were applied:

- contact fungicides - Captan+Teldor, Captan+Batron (fine sprayings on plants);
- systemic fungicides - Topsin M+Rovral, Topsin M (introduced in soil);
- 4 extracts types - 1, 2, 3, 4 (applied on leaves or on soil);
- pathogen inoculation - *Botrytis* (applied on leaves or on soil).

For the microscopic observations and measurements, there were made cross sections in the middle blade zones or in parts of leaf with pathogen attack symptoms.

The sections examination was made with trinocular Novex microscope.

## RESULTS AND DISCUSSION

Anatomically, the plants from the soybean lines M10 and B62 are different one from the other in the mesophyll thickness - B62 plants (*Botrytis* resistant) have higher values of the mesophyll than M10 (*Botrytis* sensible) plants (table 1).

*Table 1*

**Structural characteristics of soybean lines plant blade**

Drawing data	Soybean lines	Upper epidermis ( $\mu$ )	Mesophyll ( $\mu$ )	Lower epidermis ( $\mu$ )
8.VII.08	PR <sub>91</sub> M <sub>10</sub>	10.00	92.00	10.00
8.VII.08	PR <sub>92</sub> B <sub>62</sub>	10.00	103.50	10.00

When fungicide treatments were applied on the soil, plants from both lines reacted by slow increasing in thickness at the epidermal cells level, excepting the lower epidermis of the B62 plants, and at the mesophyll level (table 2).

*Table 2*

**Structural characteristics of the soybean lines plant blade - variants with antifungic treatments introduced in soil**

Drawing data	Soybean lines	Upper epidermis (μ)	Mesophyll (μ)	Lower epidermis (μ)	Difference from untreated plants (μ)
8.VII.08	PR <sub>91</sub> M <sub>10</sub>	11.50	103.50	12.00	✓ +1.5 upper ep. ✓ +11.5 mesophyll ✓ +2.00 lower ep.
8.VII.08	PR <sub>92</sub> B <sub>62</sub>	12.00	117.50	9.50	✓ +2.00 upper ep. ✓ +14.00 mesophyll ✓ -0.50 lower ep.

Effects of the fungicide applied on plants treatments were the same with those when the fungicide was introduced in soil: the increasing in thickness of the epidermal and the mesophyll cells (table 3). The differences from untreated plants were bigger in M10 plant leaves than those in B62 plant leaves.

*Table 3*

**Structural characteristics of the soybean lines plant blade - variants with antifungic treatments applied on plants**

Drawing data	Soybean lines	Upper epidermis (μ)	Mesophyll (μ)	Lower epidermis (μ)	Difference from untreated plants (μ)
8.VII.08	PR <sub>91</sub> M <sub>10</sub>	13,50	112,50	14,50	✓ +2.5 upper ep ✓ +20.5 mesophyll ✓ +2.50 lower ep.
8.VII.08	PR <sub>92</sub> B <sub>62</sub>	14,00	112,50	11,00	✓ +2.00 upper ep. ✓ +9.00 mesophyll ✓ +1.00 lower ep.

The four kinds of extracts used in this experiment induced different kinds of reactions in plants either in soil (table 4) or plant treatments (table 5): increasing in leaf size was observed in the extracts number 2 and number 4 for M10 plants, respectively extracts number 3 and number 4 for B62 plants when these were applied on soil; the same soil treatments but with extracts 1 and 3 for M10 plants, respectively extracts 1 and 2 for B62 plants, have induced the decreasing of the leaves mesophyll size. Plant treatments with the four extracts lead to mesophyll size and epidermal cells increasing at M10 plants and extracts number 1, 2, and 4; mesophyll and lower epidermal cells thickness decreasing at M10 plants and extract no. 3; decreasing of the mesophyll thickness at B62 plants and all extracts, excepting extract no.4 and increasing of the epidermal cells size at all B62 plant variants.

**Table 4**

**Structural characteristics of the soybean lines plant blade – variants with extract treatments applied on soil**

Drawing data	Soybean lines	Upper epidermis (μ)	Meso-phyll (μ)	Lower epidermis (μ)	Difference from untreated plants (μ)
21.VII.08	<b>PR<sub>91</sub>M<sub>10</sub></b> <b>Extract 1</b>	15.00	90.00	12.50	✓ +5,00 upper ep. ✓ -2,00 mesophyll ✓ +2,50 lower ep.
21.VII.08	<b>PR<sub>91</sub>M<sub>10</sub></b> <b>Extract 2</b>	15.00	107.00	13.50	✓ +5.00 upper ep. ✓ +15.00 mesophyll ✓ +3.50 lower ep.
21.VII.08	<b>PR<sub>91</sub>M<sub>10</sub></b> <b>Extract 3</b>	12.50	85.50	12.50	✓ +2.50 upper ep. ✓ -6.50 mesophyll ✓ +2.50 lower ep.
21.VII.08	<b>PR<sub>91</sub>M<sub>10</sub></b> <b>Extract 4</b>	14.50	107.00	13.50	✓ +4.50 upper ep. ✓ +15.00 mesophyll ✓ +3.50 lower ep.
21.VII.08	<b>PR<sub>92</sub>B<sub>62</sub></b> <b>Extract 1</b>	13.00	83.50	11.00	✓ +3,00 upper ep.; ✓ - 20,00 mesophyll ✓ +1,00 lower ep.
21.VII.08	<b>PR<sub>92</sub>B<sub>62</sub></b> <b>Extract 2</b>	13.00	81.50	11.00	✓ +3,00 upper ep. ✓ - 22,00 mesophyll ✓ +1,00 lower ep.
21.VII.08	<b>PR<sub>92</sub>B<sub>62</sub></b> <b>Extract 3</b>	16.00	104.50	12.50	✓ +6.00 upper ep. ✓ + 1.00 mesophyll ✓ +2.50 lower ep.
21.VII.08	<b>PR<sub>92</sub>B<sub>62</sub></b> <b>Extract 4</b>	13.50	105.00	13.00	✓ +3.50 upper ep. ✓ + 1.50 mesophyll ✓ +3.00 lower ep.

**Table 5**

**Structural characteristics of soybean lines plant blade – variants with extract treatments applied on plants**

Drawing data	Soybean lines	Upper epidermis (μ)	Meso-phyll (μ)	Lower epidermis (μ)	Difference from untreated plants (μ)
21.VII.08	<b>PR<sub>91</sub>M<sub>10</sub></b> <b>Extract 1</b>	13.50	121.50	13.50	✓ +3.50 upper ep. ✓ +29.50 mesophyll. ✓ +3.50 lower ep.
21.VII.08	<b>PR<sub>91</sub>M<sub>10</sub></b> <b>Extract 2</b>	14.00	127.00	13.00	✓ +4.00 upper ep. ✓ +35.00 mesophyll. ✓ +3.00 lower ep.
21.VII.08	<b>PR<sub>91</sub>M<sub>10</sub></b> <b>Extract 3</b>	12.50	78.00	9.75	✓ +2.50 upper ep. ✓ -14.00 mesophyll ✓ -0.25 lower ep.
21.VII.08	<b>PR<sub>91</sub>M<sub>10</sub></b> <b>Extract 4</b>	14.50	124.00	12.00	✓ +4.50 upper ep. ✓ +32.00 mesophyll ✓ +2.00 lower ep.

Drawing data	Soybean lines	Upper epidermis ( $\mu$ )	Meso-phyll ( $\mu$ )	Lower epidermis ( $\mu$ )	Difference from untreated plants ( $\mu$ )
21.VII.08	<b>PR<sub>92</sub>B<sub>62</sub></b> <b>Extract 1</b>	12.50	96.50	13.50	✓ +2.50 upper ep. ✓ - 7.00 mesophyll ✓ +3.50 lower ep.
21.VII.08	<b>PR<sub>92</sub>B<sub>62</sub></b> <b>Extract 2</b>	13.00	96.50	12.50	✓ +3.00 upper ep. ✓ - 7.00 mesophyll ✓ +2.50 lower ep.
21.VII.08	<b>PR<sub>92</sub>B<sub>62</sub></b> <b>Extract 3</b>	15.50	101.00	12.00	✓ +5.5 upper ep. ✓ - 2.50 mesophyll ✓ +2.00 lower ep.
21.VII.08	<b>PR<sub>92</sub>B<sub>62</sub></b> <b>Extract 4</b>	13.0	115.00	12.50	✓ +3,00 upper ep. ✓ + 11,50mesophyll ✓ +2,50 lower ep.

**Table 6**

**Structural characteristics of soybean lines plant blade – variants with *Botrytis* infection applied on soil**

Drawing data	Soybean lines	Upper epidermis ( $\mu$ )	Mesophyll ( $\mu$ )	Lower epidermis ( $\mu$ )	Difference from untreated plants ( $\mu$ )
21.VII.08	<b>PR<sub>91</sub>M<sub>10</sub></b> <b>Extract 1</b>	9.05	92.00	11.00	✓ -0.50 upper ep. ✓ 0.00 mesophyll ✓ +1.00 lower ep.
21.VII.08	<b>PR<sub>91</sub>M<sub>10</sub></b> <b>Extract 2</b>	<b>13.00</b>	<b>86.50</b>	<b>12.00</b>	✓ <b>+3.00 upper ep.1</b> ✓ <b>- 5.5 mesophyll</b> ✓ <b>+2.00 lower ep.</b>
21.VII.08	<b>PR<sub>91</sub>M<sub>10</sub></b> <b>Extract 3</b>	<b>13.00</b>	<b>97.00</b>	<b>12.50</b>	✓ <b>+3.00 upper ep.1</b> ✓ <b>+5.00 mesophyll</b> ✓ <b>+2.50 lower ep.</b>
21.VII.08	<b>PR<sub>91</sub>M<sub>10</sub></b> <b>Extract 4</b>	11.50	90.5	11.00	✓ +1.50 upper ep.1 ✓ -1.50 mesophyll ✓ +1.00 lower ep.
21.VII.08	<b>PR<sub>92</sub>B<sub>62</sub></b> <b>Extract 1</b>	13.00	85.00	11.50	✓ +3.00 upper ep.1 ✓ - 18.50 mesophyll ✓ +1.50 lower ep.
21.VII.08	<b>PR<sub>92</sub>B<sub>62</sub></b> <b>Extract 2</b>	12.00	86.00	11.00	✓ +2.00 upper ep.1 ✓ - 17.50 mesophyll ✓ +1.00 lower ep.
21.VII.08	<b>PR<sub>92</sub>B<sub>62</sub></b> <b>Extract 3</b>	12.50	109.50	10.00	✓ +2.50 upper ep.1 ✓ + 6.00 mesophyll ✓ 0.00 lower ep.
21.VII.08	<b>PR<sub>92</sub>B<sub>62</sub></b> <b>Extract 4</b>	13.00	92.50	13.00	✓ +3.00 upper ep.1 ✓ -1 1.00 mesophyll ✓ +3.00 lower ep.

The *Botrytis* soil infection has induced similar effects in M10 and B62 plants:

- cell dimensions reduction: in the upper epidermis - extract 1 plants variant;  
in the mesophyll - extract 2, 4 plant variants;

- cell dimensions increase: in the epidermis - extract 2, 3, 4 plant variants; in the mesophyll - extract 3 plants variant (table 6).

Applied on plants, the *Botrytis* infection has induced in M10 plants cells increasing in the mesophyll for the extract 2 and 4 variants and mesophyll cells reductions for the two others variants.

The same treatment has induced in B62 plants a reduction of the mesophyll cell dimensions for all four extract variants.

**Table 7**

**Structural characteristics of soybean lines plant blade – variants with *Botrytis* infection applied on plants**

Drawing data	Soybean lines	Upper epidermis (μ)	Meso-phyll (μ)	Lower epidermis (μ)	Difference from untreated plants (μ)
21.VII.08	<b>PR<sub>91</sub>M<sub>10</sub></b> <b>Extract 1</b>	13.50	95.50	11.00	✓ +3.50 upper ep. ✓ +3.50 mesophyll; ✓ +1.00 lower ep.
21.VII.08	<b>PR<sub>91</sub>M<sub>10</sub></b> <b>Extract 2</b>	15.00	90.00	12.50	✓ +5.00 upper ep. ✓ -2.00 mesophyll ✓ +2.50 lower ep.
21.VII.08	<b>PR<sub>91</sub>M<sub>10</sub></b> <b>Extract 3</b>	14.00	84.00	13.50	✓ +4.00 upper ep. ✓ -8.00 mesophyll ✓ +3.50 lower ep.
21.VII.08	<b>PR<sub>91</sub>M<sub>10</sub></b> <b>Extract 4</b>	12.50	100.00	12.50	✓ +2.50 upper ep. ✓ +8.00 mesophyll ✓ +2.50 lower ep.
21.VII.08	<b>PR<sub>92</sub>B<sub>62</sub></b> <b>Extract 1</b>	13	85	11.5	✓ +3.00 upper ep. ✓ - 18.50 mesophyll ✓ +1.50 lower ep.
21.VII.08	<b>PR<sub>92</sub>B<sub>62</sub></b> <b>Extract 2</b>	11.50	99.00	12.50	✓ +1.50 upper ep. ✓ - 4.50 mesophyll ✓ +2.50 lower ep.
21.VII.08	<b>PR<sub>92</sub>B<sub>62</sub></b> <b>Extract 3</b>	14.00	84.00	13.50	✓ +4.00 upper ep. ✓ - 19.5 mesophyll ✓ +3.50 lower ep.
21.VII.08	<b>PR<sub>92</sub>B<sub>62</sub></b> <b>Extract 4</b>	13.50	92.50	13.00	✓ +3.50 upper ep. ✓ -11.00 mesophyll ✓ +3.00 lower ep.

## CONCLUSIONS

1. The *Botrytis* sensibility is associated with thin mesophyll.
2. Treatments with fungicides increase the mesophyll dimensions in both soybean plant lines.
3. In the two soybeans plant lines, the effects on mesophyll dimensions of the four extract treatments are various.

4. The *Botrytis* infection applied on B62 plants induced a decreasing of the mesophyll dimensions in all four extract variants.

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