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INVESTIGATION ON *BRASSICA OLERACEA L.* GENOTYPES IN VIEW OF BREEDING FOR PEST AND DISEASE RESISTANCE. I. ANATOMICAL AND PHYSIOLOGICAL STUDIES ON *BRASSICA OLERACEA VAR. CAPITATA L.*

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ABSTRACT. Ten genotypes of *Brassica oleracea var. capitata* were studied as regards anatomical features of the leaf (leaf-, mesophyll- and cuticle-thickness) and the parameters of chlorophyll fluorescence in view of breeding for resistance to diseases (mildew – *Peronospora parasitica*, alternaria blight - *Alternaria brassicicola* and bacteriosis – *Xanthomonas campestris pv. campestris*) and pests (cabbage aphid – *Bravicoryne brassicae* and lepidopterous pests – *Lepidoptera,* small white butterfly – *Pieris rapae*, cabbage white butterfly - *Pieris brassicae* and mamestra cabbage moth - *Mamestra brassicae*). Differences between the genotypes in relation to the anatomical leaf features were established. The values of the chlorophyll fluorescence parameters, recorded in the studied genotypes differed but they were within the physiological norm. Significant differences were established in the susceptibility to diseases and pests between the studied specimens. Genotypes with the best expressed total resistance were differentiated, which also possessed the highest values for the studied anatomic features.

KEY WORDS: *Brassica oleracea var. capitata,* breeding, chlorophyll fluorescence, disease resistance, leaf anatomy, pest resistance

INTRODUCTION

Breeding of lines, varieties and hybrids resistant to economically important diseases and pests is of great importance in the modern cole crop variety creating programs. Anatomical, morphological and physiological features are used as

important criteria for identification of sources of resistance and mechanisms of susceptibility in breeding of resistance.

Mildew *(Peronospora parasitica)*, alternaria blight (*Alternaria brassicicola)* and bacteriosis (*Xanthomonas campestris pv. campestris)* are economically important cole crop diseases for our country. Specialized literature reports about dependence between the rate of pathogen infestation and anatomical and physiological features of the plants (BRETSCHEIDER ET AL., 1989; IGNATOV ET AL., 1999; GAY AND TUZUN, 2000; ZHAO ET AL., 2000).

Source of resistance from head cabbage to the cabbage aphid (*Bravicoryne brassicae)* and cabbage butterfly (*Pieris brassicae)* are established in the Republic of Bulgaria (АЛИПИЕВА И ДР., 1995; ALIPIEVA & NANKOVA, 1996).

DUNN & KEMPTON (1976), SEKHON & AHMAN (1992) and SINGH & ELLIS (1993) established that the resistance of cruciferous crops to pests is connected with some morphological and anatomical features as leaf lamina and parenchyma colour, parenchyma thickness etc. However, in the world-wide breeding no variety or hybrid resistant to cabbage pests has yet been created and therefore the investigations in this direction still have a priority for many variety breeding and variety improving programs.

The effect of entomogenic and phytopathogenic biotic stress factors on plants is studied by measuring the chlorophyll fluorescence parameters.

Chlorophyll *a* fluorescence analysis is a non-invasive screening method for the effects of many biotic and abiotic factors on photosynthesis in plants (YORDANOV ET AL., 1997; PASTENESM AND HORTON, 1999; YAMANE ET AL., 2000, SCHMITZ & STRASSER, 2001). Chlorophyll fluorescence is one of the forms, under which the energy excess, unused in the photosynthesis, is dissipated. At ambient temperature Chl *a* fluorescence is basically emitted by PSII. The functional changes in the photosynthetic apparatus caused by stress factors lead to corresponding change in the fluorescent emission (GOLTSEV ET AL., 1994; BRIANTAIS ET AL., 1996). Chlorophyll fluorescence has been used recently as a sensitive, *in vivo* probe of photosynthetic function, both in the field and in the laboratories (BOLHAR-NORDENKAMPF & OQUIST, 1993; PETKOVA ET AL., 2003).

The purpose of the present work is to study some anatomical leaf features and chlorophyll fluorescence parameters in cabbage genotypes. The physiological and anatomical analyses, subject of the present work, are an initial stage of a series of investigations concerning the explanation of mechanisms of different susceptibility to economically important diseases and pests.

MATERIAL AND METHODS

The following ten head-cabbage genotypes (provided by IPGR-Sadovo) were studied during the period July – November 2004 in the Maritsa Vegetable Crops Research Institute, Plovdiv, grown by a technology for late field production:

1. *Brassica oleracea var. capitata* Golden 2003
2. *Brassica oleracea var. capitata* Budericher 2003
3. *Brassica oleracea var. capitata* Kobenhavus

4. *Brassica oleracea var. capitata* Totve – Bira
5. *Brassica oleracea var. capitata* Gewood – Group1
6. *Brassica oleracea var. capitata* Extra Taoi Group1
7. *Brassica oleracea var. capitata* Taoi Group1
8. *Brassica oleracea var. capitata* Brunswijner
9. *Brassica oleracea var. capitata* Brun-swijner Group1
10. *Brassica oleracea var. capitata* Durol.

Samples from 10 plants per each genotype of adjoining rosette leaves (rosette leaves close to the head) during the phase "heading of the cabbage" were taken and fixed in 70 % ethyl alcohol for anatomical analysis. Cross sections of the leaf lamina (100 per genotype) were plated in glycerin medium and observed with Ergaval Carl Zeiss-Jena light microscope (x95.75;x750). The thicknesses of the leaf, of the mesophyll and the cuticle were analyzed with helical eyepiece micrometer. The values of the leaf characteristics were averaged on the basis of 100 measurements per genotype. The differences between the studied genotypes found in anatomical data were assessed by Student's t-test (ЛАКИН, 1990).

Light-microscope microphotographs of the leaf lamina were taken.

Chlorophyll fluorescence was measured in dark-adapted (30 min), fully developed, adjoining rosette leaves, illuminated with actinic light ($\lambda > 650$ nm) with photo flux 1500 $\mu mol.m^{-2}.sec^{-1}$ for 5 s. The initial (Fo), the variable (Fv) and the maximal (Fm) chlorophyll fluorescence parameters and their ratios were recorded using a portable fluorimeter (Plant Efficiency Analyzer, MK2, non-modulated system - Hansatech Instruments Ltd., GB). Ten leaves per genotype were measured.

The experiment was performed without insecticide and fungicide application and the disease and pest infestation (mildew- *Peronospora parasitica,* alternaria blight - *Alternaria brassicicola,* bacteriosis – *Xanthomonas campestris pv. campestris*, cabbage aphid - *Bravicoryne brassicae*, lepidopterous pest *Lepidoptera* from species small white butterfly – *Pieris rapae*, cabbage butterfly - *Pieris brassicae* and mamestra cabbage moth - *Mamestra brassicae)* was recorded at natural infection conditions.

Recording of the rate of disease and pest infestation was performed using the following scale: at 0 – 25 % index of infestation – resistant (R); at 26 – 50 % infestation index – partially resistant, tolerant (PR); 51 – 100 % - sensitive (S).

RESULTS AND DISCUSSION

Anatomical analyses of leaf lamina showed quantitative differences between the studied *Brassica oleracea var. capitata* genotypes.

The leaf lamina thickness varied between 407 μm and 693 μm. It was thinnest in genotype 1 and thickest in genotypes 5 and 6 (Tab. 1, Fig. 1). The following classification could be made on the basis of the mean values for the leaf lamina thickness (Tab. 1) and on the basis of the data for Student's t-criterion for statistical differences between studied genotypes (Tab. 2):

- First group of genotype 1- leaf lamina thickness about 400 μm (407 μm);

- Second group of genotypes 2, 3 and 9- leaf lamina thickness about 500 μm (492 – 538 μm);
- Third group of genotypes 4, 7, 8 and 10- leaf lamina thickness about 600 μm (586 – 599 μm);
- Fourth group of genotypes 5 and 6- leaf lamina thickness about 700 μm (678 – 693 μm).

Mesophyll thickness varied between 365 μm and 647 μm (Tabs. 1, 3). The assimilating parenchyma was the thinnest in genotype 1 and thickest – in genotypes 5 and 6. The trend of genotype classification in ascending line stays the same: 1 < 2, 3, 9 < 4, 7, 8, 10 < 5, 6.

Table1. *Anatomical characteristics of Brassica oleracea var. capitata leaves (μm)*

Genotype	Leaf lamina thickness	Mesophyll thickness	Cuticle thickness	
			Upper surface	Lower surface
	X ± Sx	X ± Sx	X ± Sx	X ± Sx
1	407.24±6.67	365.04±6.48	3.55±0.08	3.56±0.10
2	492.44±13.63	436.54±12.59	5.56±0.16	5.29±0.17
3	522.83±7.57	477.65±7.02	5.51±0.13	5.62±0.13
4	590.88±11.73	547.92±11.37	4.26±0.17	5.01±0.21
5	693.03±16.81	647.71±16.50	6.52±0.15	6.46±0.16
6	678.16±15.83	632.45±16.64	6.61±0.14	6.79±0.17
7	599.51±8.23	553.93±7.95	6.61±0.18	6.69±0.16
8	586.07±6.69	538.90±7.05	5.27±0.12	5.68±0.12
9	538.33±8.33	486.88±8.22	5.40±0.14	5.81±0.14
10	586.22±11.20	536.30±11.36	6.16±0.14	6.26±0.13

Cuticle thickness also varied between the different genotypes (Tab. 1, Fig. 2). The cuticle is thinnest in genotype 1 (3.5 μm) and thickest – in genotypes 5, 6 and 7 (6.5 – 6.8 μm). Genotype 10 did not differ statistically from genotype 5 ($P > 0.05^{ns}$) and it had very close values to those of genotypes 6 and 7 (P = 0.5). The remaining genotypes were within the above limits. On the basis of the mean values and on the data for the Student's criterion (Tab.4), genotypes could be arranged in the following ascending line: 1 < 4 < 2, 3, 8, 9 < 10 ≤ 5, 6, 7. As seen from table 1, the cuticle from the upper and the lower surface was of equal thickness with most genotypes. Some differences were established in genotypes 4 (P = 0.01), 8 and 9 (P=0.05), where the cuticle from the lower surface was thicker then the upper one.

Morphological and anatomical investigations of different plant genotypes have also been done by other authors with the purpose of searching for a breeding criterion for increasing plant adaptive capabilities. ORTEGA ET AL. (1988) established differences in the studied indices (number and density, leaf mass, leaf surface) between different bean genotypes at different environmental conditions. We also

established differences between the studied head cabbage genotypes regarding the amount of assimilating parenchyma which determines the differences in the leaf lamina thickness. Differences with respect to the cuticle thickness were also found. It is known that waxes are between the main cuticle components. STONER (1990) found that the major component of plant resistance to insects in *Brassica oleracea* was the presence of leaf wax. The same author (STONER, 1997) has observed *Pieris rapae* larva movement and development on upper and lower leaf surface of cabbage plants with video camera in laboratory conditions. He has established a difference in larva behaviour on leaves with normal and smooth wax film.

Table 2. *Data for Student's t-criterion for statistical differences in relation to leaf lamina thickness*

No Geno-type	2	3	4	5	6	7	8	9	10
1	5.62***	11.46***	13.61***	15.81***	15.77***	22.67***	18.92***	12.29***	13.73***
2		1.95 ns	5.47***	9.27***	8.89***	6.73***	6.17***	2.56**	5.32***
3			4.87***	9.23***	8.85***	6.86***	6.26***	1.38 ns	4.69***
4				4.98***	4.43***	0.60 ns	0.36 ns	3.65***	0.29 ns
5					0.64 ns	5.0 ***	5.91***	8,25***	5.29***
6						4.41***	5.36***	7.82***	4.74***
7							1.27 ns	5.92***	0.96 ns
8								4.47***	0.01 ns
9									3.43***

*** Probability- p=0.001; ** p=0.01; * p=0.05; ns - non-significant

Table3. *Data for Student's t-criterion for statistical differences in relation to mesophyll thickness*

No Geno-type	2	3	4	5	6	7	8	9	10
1	5.05***	11.79***	13.97***	15.94***	14.97***	18.41***	18.15***	11.65***	13.09***
2		2.85**	6.57***	10.18***	9.39***	7.88***	7.09***	3.35**	5.88***
3			5.26***	9.48***	8.57***	7.19***	6.16***	0.85 ns	4.39***
4				3.51***	4.19***	0.43 ns	0.67 ns	4.35***	0.72 ns
5					0.65 ns	5.12 ***	6.07***	8,73***	5.56***
6						4.26***	5.18***	7.84***	4.77***
7							1.41 ns	5.86***	1.27 ns
8								4.80***	0.19 ns
9									3.52***

*** Probability- p=0.001; ** p=0.01; * p=0.05; ns - non-significant

Table 4. *Data for Student's t-criterion for statistical differences in relation to cuticle thickness*
(data in bold are for the upper cuticle)

No Genot.	2	3	4	5	6	7	8	9	10
1	**9.16***** 10.53***	**13.07***** 12.88***	**3.74***** 6.30***	**17.47***** 15.26***	**19.13***** 16.39***	**15.30***** 16.47***	**12.29***** 13.25***	**11.56***** 13.24***	**16.31***** 16.88***
2		**1.05** ^{ns} 0.29^{ns}	**4.29***** 2.12*	**5.35***** 3.91***	**6.0***** 5.35***	**5.28***** 4.91***	**0.04** ^{ns} 0.6^{ns}	**0.5** ^{ns} 0.43^{ns}	**3.95***** 3.33***
3			**5.95***** 2.44*	**5.05***** 4.0***	**5.79***** 5.57***	**5.0***** 5.09***	**1.35** ^{ns} 0.33^{ns}	**0.59** ^{ns} 1.0^{ns}	**3.42***** 3.56***
4				**9.83***** 5.58***	**8.10***** 6.59***	**9.40***** 6.46***	**4.81***** 2.79**	**5.18***** 3.20**	**8.64***** 5.0***
5					**0.43**^{ns} 1.43^{ns}	**0.39** ^{ns} 1.0^{ns}	**6.58***** 3.90***	**5.33***** 3.10**	**1.71** ^{ns} 0.95^{ns}
6						**0** ^{ns} 0.43^{ns}	**7.44***** 5.29***	**6.14***** 4.45***	**2.28**** 2.52**
7							**6.09***** 6.31***	**5.26***** 4.19***	**1.95** ^{ns} 2.52*
8								**0.72** ^{ns} 0.72 ^{ns}	**4.94***** 3.22**
9									**3.86***** 2.37*

*** Probability - p=0.001; ** p=0.01; * p=0.05; ^{ns} - non-significant.

The summarized results concerning Chl fluorescence parameters are shown in Table 5. The initial fluorescence Fo describes a loss of excitation energy during its transfer from the pigment bed to RC of PS2 (YORDANOV ET AL., 1997). The Fo values varied between 288 (genotype 8) and 404 (genotype 2).The greatest differences were established in the values of the Fv/Fo ratio, describing the electron transport efficiency. The ratio had the lowest values in genotype 2 and the highest one in genotype 4. This parameter had high values also in genotypes 5, 6 and 8. A minimal value (3.918) was established in genotype 2 that showed susceptibility to the three studied diseases.

The ratio Fv/Fm describes the potential effectiveness of the PS2. The lowest value (0.782) was established in genotype 2. This value together with those of the other chlorophyll fluorescence parameters described the genotype as having the lowest photosynthetic activity. However, it is necessary to point out that in all genotypes the Fv/Fm ratio values were within the established physiological norm (BOLHAR-NORDENKAMPF ET AL., 1989). It is obvious that the rate of infestation did not have a stress effect on the cabbage plants and they maintained a normal physiological status.

Table 5. *Chorophylll fluorescence parameters in Brassica oleracea var. capitata genotypes. Values represent the means of six series of measurements x ± sd, n = 60.*

N	Fo	Fv/Fo	Fv/Fm
1	340 ± 19.67	5.009 ± 0.265	0.833 ± 0.007
2	404 ± 11.63	3.918 ± 0.191	0.782 ± 0.007
3	334 ± 8.72	5.020 ± 0.393	0.833 ± 0.011
4	303 ± 23.07	5.747 ± 0.305	0.850 ± 0.018
5	348 ± 9.86	4.715 ± 0.396	0.821 ± 0.016
6	309 ± 8.82	5.076 ± 0.450	0.830 ± 0.032
7	309 ± 7.76	4.345 ± 0.254	0.813 ± 0.009
8	288 ± 1.53	5.318 ± 0.258	0.837 ± 0.019
9	331 ± 3.60	4.520 ± 0.165	0.818 ± 0.011
10	338 ± 10.11	4.916 ± 0.244	0.819 ± 0.008

Results for the infestation rate are shown in Table 6. The difference in the reaction of the genotypes regarding pest infestation was observed. All studied genotypes showed resistance to cabbage aphid except genotype 1, for which a partial resistance was recorded. The genotype reaction towards the lepidopterous pests was as follows: sensitive (9 and 10), partially resistant (3, 5, 7 and 8) and resistant (1, 2, 4 and 6).

In phytopathological aspect genotypes 2 and 4 were sensitive to the three studied diseases and at the same time they were resistant to both tested pests groups.

Table 6. *Insects and disease infestations of Brassica oleracea var. capitata genotypes, (index, %)*

No genotype	Insects infestation		Diseases infestation		
	Cabbage aphid	Caterpillars *Lepidoptera*	Mildew	Alternaria blight	Bacteriosis
1	45.83 PR	25.00 R	62.50 S	45.00 PR	37.50 PR
2	18.50 R	25.00 R	65.00 S	75.00 S	62.50 S
3	6.88 R	30.00 PR	78.13 S	71.88 S	43.75 PR
4	4.38 R	25.00 R	53.13 S	65.63 S	53.13 S
5	1.43 R	35.00 PR	31.25 PR	31.25 PR	31.25 PR
6	2.78 R	25.00 R	25.00 R	31.25 PR	31.25 PR
7	5.00 R	35.00 PR	35.00 PR	22.50 R	17.50 R
8	8.50 R	50.00 PR	60.00 S	45.00 PR	25.00 R
9	9.11 R	60.00 S	55.00 S	27.50 PR	20.00 R
10	1.50 R	70.00 S	50.00 PR	37.50 PR	20.00 R

Genotypes 6 and 7, followed by 5 are distinguished with the best-expressed total resistance to diseases and pests. These genotypes are also with the thickest cuticle. Genotypes 5 and 6 are also distinguished with the greatest amount of mesophyll and with the thickest leaf lamina, respectively.

The results obtained for genotype 1 which is sensitive only to the mildew were interesting. Towards the remaining pathogens and pests this genotype showed a partial to full resistance. With respect to the chlorophyll fluorescence parameters it

took an intermediate position while its anatomical features were with the lowest values.

CONCLUSION

Differences regarding the anatomical leaf features in ten *Brassica oleracea var. capitata* genotypes were established as a result of the investigations. Values of the chlorophyll fluorescence parameters recorded in the studied genotypes were different but they were within the physiological norm. Significant differences in the susceptibility to diseases and pests between the studied genotypes were established. Genotypes 6 and 7, followed by 5 showed the best-expressed total resistance to diseases and pests. The above genotypes were with the thickest cuticle. Genotypes 5 and 6 were distinguished by the biggest mesophyll quantity and the thickest leaf, respectively.

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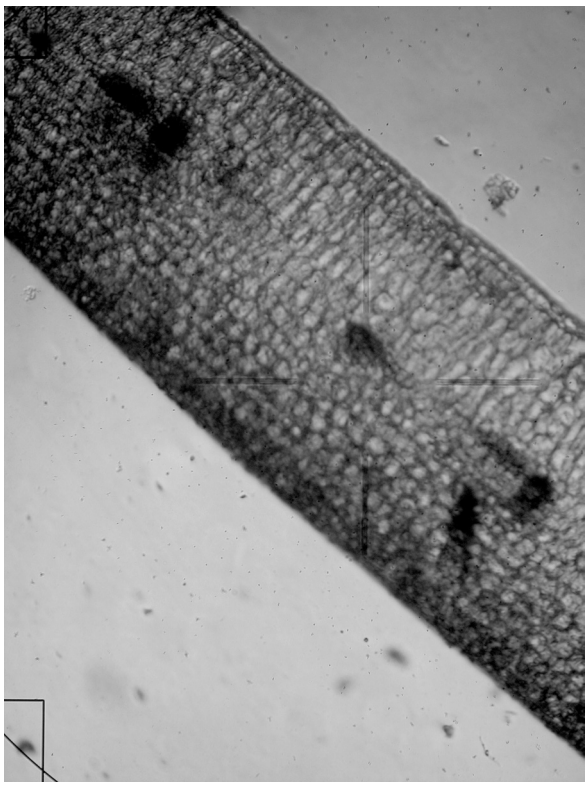

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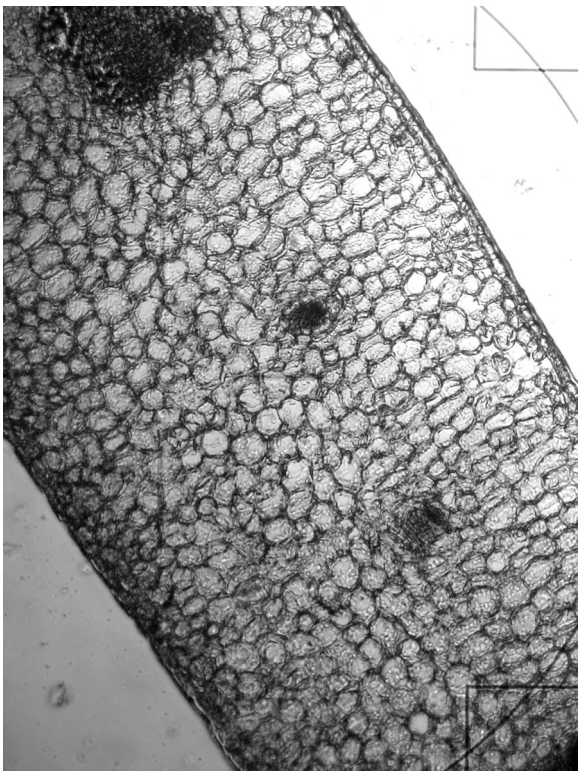

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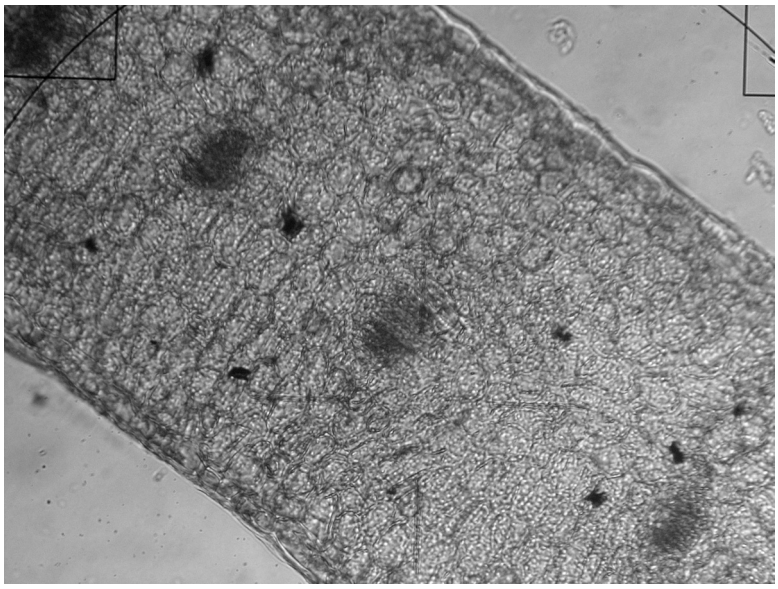

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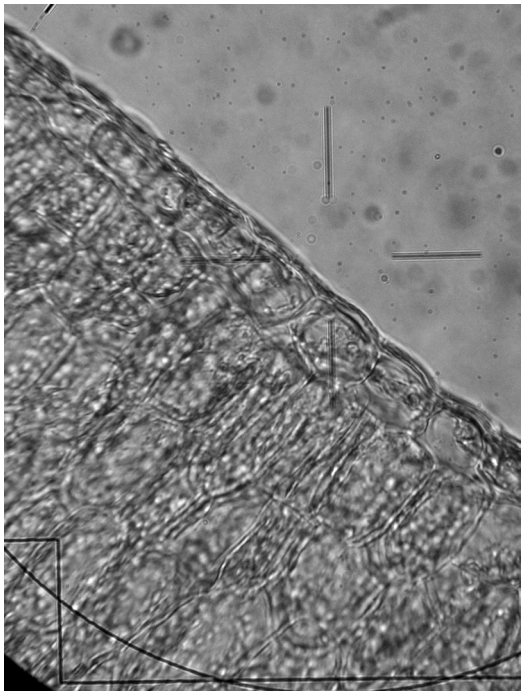
Fig.1. *Cross section of the leaf lamina (x 250):*
***1-** genotype 1**; 2 -** genotype 5; **3-** genotype 6.*



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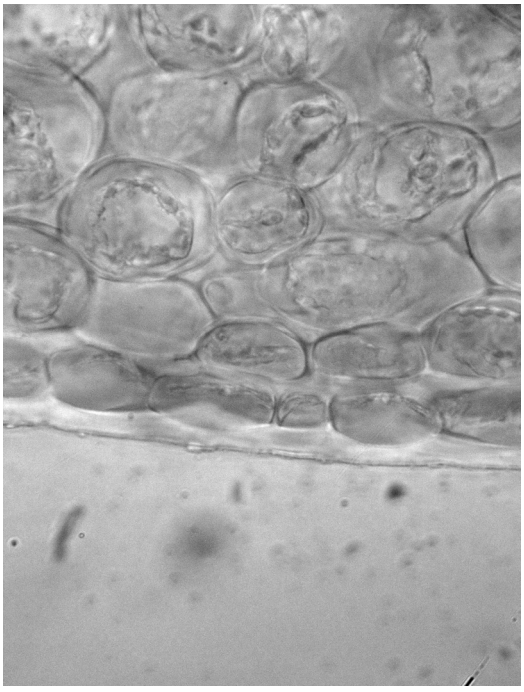

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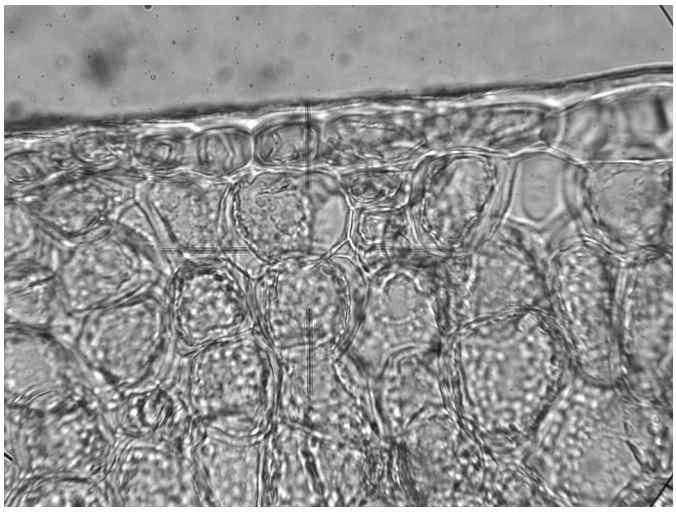

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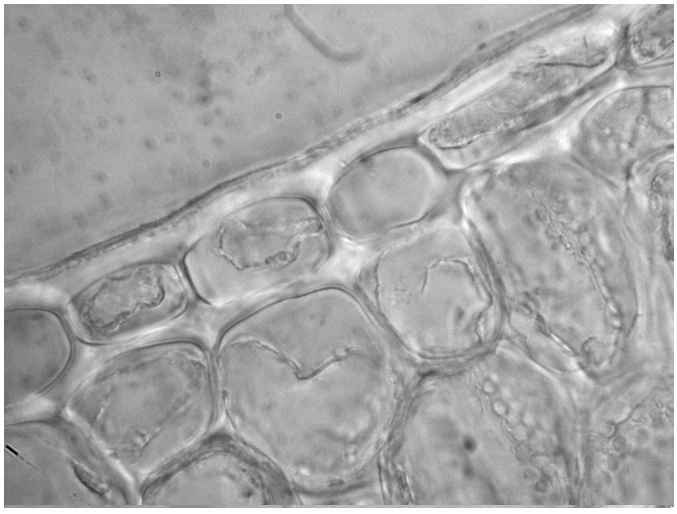

4

Fig.2. *Cross section of the leaf lamina (x 2000):*
1 *- upper surface of genotype 1;*
2 *-lower surface of genotype 6;*
3 *- upper surface of genotype 4;*
4 *- upper surface of genotype 5*