ANATOMICAL STRUCTURE OF LEAF SECTORS
WITH DIFFERENT RESISTANCE TO POWDERY MILDEW
(*ERISIPHE CRUCIFERARUM* OPIZ EX. L. JUNELL)
IN WINTER RAPESEED CHIMERA

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ABSTRACT

The subject of the study was a sectorial chimera of dihaploid winter rapeseed, obtained with the help of gamma ray treatment (30 Gy) during shoot cloning in vitro. One sector of the plant was infected by *Erisiphe cruciferarum* Opiz ex. L. Junell and the other one was resistant. The anatomical structure of a leaf, divided into the two sectors along the midrib, was studied. The infected part of the leaf blade was thinner and built of a smaller number of palisade and spongy mesophyll cell layers. The size of cells in this sector, both in the epidermis and in the mesophyll, as well as the size of nuclei, chloroplasts and intercellular spaces were bigger than those in the resistant portion. On the other hand, the stomata in the infected segment were smaller but their number was higher than that in the healthy part. The study made it possible to analyse the relation between the anatomical structure of the host plant and the pathogen.

KEY WORDS: anatomical structure, chimera, powdery mildew, rapeseed.

INTRODUCTION

Mutagenic treatment of plant multicellular structures, such as seeds or shoot tips, frequently leads to the creation of chimeras. If particular sectors are distinguishable, they may be separated and used for regeneration of uniform plants in in vitro cultures.

Mutagenic treatment of cell suspensions followed by cell selection in vitro makes it possible to avoid chimeras and to identify mutants in a much shorter time. This method, however, has several limitations, and relatively few agronomic traits, mainly those related to resistance or to production of specific substances, may be selected at the cell level (Widholm 1988). Morphological traits as well as those associated with yield and controlled poligenically are not appropriate for this type of selection. Selection at cell level is not fully reliable because adaptations and epigenic changes occur fairly frequently. Plant regeneration from a mutated cell is not always successful and undesirable changes in the chromosome number in cell suspensions can be often observed. For these reasons, the low radiosensitivity of oilrapeseed also taken in account, the authors decided to mutate young axial meristems of shoots propagated under in vitro conditions.

One plant from among those with changed phenotype, treated with the dose of 30 Gy, had two clearly distinguishable

sectors (Fig. 1). One of them, light green in colour, comprising about 4/5 of the plant, was coated with powdery mildew mycelium, while the other one, vividly green, was free from the fungus. The infected leaves were deeply indented and those with entire edges were healthy. Two leaves were asymmetrical and their midribs were the boundary between the resistant and infected sectors.

The objective of this study was to analyse differences in the anatomy of the leaf blade in the two sectors: the one infected with mildew and the resistant one.

MATERIAL AND METHODS

Flasks with young shoots of dihaploid rapeseed were subjected to irradiation with the help of a cobalt bomb at the rate 166 cGy/min. The initial material was obtained in anther culture of the breeding strain of cv. Górczański. The irradiated shoots had initials of axial meristems composed of a number of cells (from tens to hundreds depending on shoot size) from which a new generation of shoots was regenerated, then rooted in vitro and potted. One plant from among those growing in the greenhouse turned out to be a chimera. It was studied during the vegetative phase, when it reached the height of 20 cm and had several leaves.

Fig. 1. Schematic picture of the chimera; the shaded area represents the infected sector.

Fragments of a leaf composed of the sectors were fixed in Cr-A-F 0.5-0.5-20, then dehydrated with ethyl and n-butyl alcohols, and embedded in paraffin. Microtome preparations 7 μm thick were stained with basic fuchsin of pH=2.6 and with fast green, according to Teleżyńska and Teleżyński's method (1973).

RESULTS

Characterization and localization of the pathogen

The floury coating which covered the stalk and both surfaces of the leaf in the infected sector consisted of mycelium in the conidial phase (Fig. 2). A hypha of mycelium was composed of uninucleate cells with appressoria and globular haustoria which could be found in the epidermal cells but not in the guard cells (Fig. 2A, B). Each cell usually had one haustorium, rarely two. The size of globules ranged from 800 to 3200 μm^2 ; 1832 μm^2 was the average. The globules occupied 13-64% of the cell volume, the average being 33.5%. Their size was correlated with the size of infected cells ($r = +0.27$). The cells of the lower epidermis with their haustoria constituted about 2.6% of the total number of the epidermal cells. About 40% of the cells which contained haustoria were in contact with the guard cells. In all such cases the stomata were closed and their cells were dead, but the cells infected with the fungus remained alive (Fig. 5). The stomata which did not function and which contained the dead guard cells constituted 8.2% of the total number of stomata. Conidiospores, about 30 μm in length and 10 μm in diameter, developed as single ones on short stalks of 2-3 cells (Fig. 2C, D). These traits indicated that the fungus which appeared on the plants was powdery mildew – *Erisiphe cruciferarum* Opiz ex. L. Junell (Sałata 1985).

Epidermis: differences between particular sectors

The results of measurements showing differences between the sector infected by powdery mildew and the resistant one are summarized in Table 1.

The number of the upper epidermal cells per unit of area in the infected sector was almost 50% lower than that in the resistant sector (Figs 3, 4). In the lower epidermis the difference was smaller (Figs 5, 6). Guard cells of rapeseed develop according to the anizohelicocytic type (Payne 1970). In this type of development the size of other epidermal cells varies considerably. The cell surface of the lower epidermis in the infected sector ranged from 21 to 1267 μm^2 . It was larger than that in the resistant sector, with variation of this trait being

Fig. 2. *Erisiphe* mycelium on the leaf.
A – a hypha composed of uninucleate cells with two appressoria.
B – a globular haustorium in a cell of the upper epidermis.
C – a young conidiophore.
D – a mature conidiospore.

TABLE 1. Anatomical traits of the leaf in the resistant and infected sectors of the rapeseed sectorial chimera.

	Sector	
	resistant	infected
Number of cells per mm ²		
in the upper epidermis	3742	1937
in the lower epidermis	4414	2813
Size of cells in the lower epidermis, μm^2	233	289
Volume of the cell nuclei in the lower epidermis, μm^2	28.4	43.2
Correlation coefficient between the cell surface and nucleus volume in the lower epidermis, r	+ 0.45	+ 0.74
Cells developed as guard cells, %		
in the upper epidermis	6	26
in the lower epidermis	5	24
Number of stomata per mm ²		
in the upper epidermis	93	232
in the lower epidermis	139	362
Diameter of guard cells nuclei, μm ,		
in the upper epidermis	25.4	22
in the lower epidermis	24.7	22.4
Volume of guard cells nuclei, μm^3 ,		
in the upper epidermis	38.8	18.8
in the lower epidermis	36.1	20.6
Number of cells per mm ²		
in palisade mesophyll	3547	1513
in spongy mesophyll	1424	734
Diameter of cells in palisade mesophyll, μm , *	22.1	37.0
Surface of intercellular spaces, %, *		
in palisade mesophyll	10.6	17.0
in spongy mesophyll	28.0	46.5
Diameter of chloroplasts, μm		
in palisade mesophyll	4.1	4.4
in spongy mesophyll	3.7	5.2

* on the surface section

much higher in the infected sector than in the resistant one (Fig. 7).

The differences in cell size were associated with differences in the size of their nuclei. The nucleus volume of cells in the infected sector was larger than that in the resistant sector (Fig. 8). Variation of this trait was much higher in the infected sector.

Nuclei of different sizes had a different number and size of chromocenters (Figs 3, 4). The nuclei of 13-16 μm^3 had 5-7 chromocenters; the biggest nuclei, amounting to 100-120 μm^3 , contained up to 19 chromocenters. The size of chromocenters differed markedly and the biggest chromocenters in large nuclei were bigger than the biggest chromocenters in small nuclei.

The two sectors differed considerably in the number of epidermal cells developed as guard cells: the percentage of the guard cells was 4-5 times larger in the infected sector than that in the resistant one. The number of stomata per mm² was

only about 2.5 times larger, because the cell size of the epidermis in the infected sector was bigger. The size of the guard cells and the volume of their nuclei in the infected sector were smaller than those in the resistant one, which was in contrast to the size of other epidermal cells. The surface of the guard cells in this sector ranged from 70 to 100 μm^2 .

Parenchyma: differences between particular sectors

An oilseed rape leaf is distinctly bifacial with a well differentiated palisade mesophyll on the adaxial side and with a spongy mesophyll on the abaxial side. Numerous differences in the leaf structure were observed between the two sectors of the studied chimera. The leaf blade in the resistant sector was about 14% thicker than the one in the infected sector. The difference resulted from a varying number of cells in the palisade and spongy mesophyll. The palisade mesophyll in the infected sector had 3-4 cell layers (Fig. 9) and in the resistant

Figs 3-6. Epidermis of the leaf in the infected (left) and the resistant (right) sectors. Figs 3 and 4 – the upper epidermis, Figs 5 and 6 – the lower epidermis.

one 3-6 layers, usually 4-5 (Fig. 10). The spongy mesophyll had 6 and 7 cell layers in the infected and the resistant sectors respectively. The difference in the thickness of the leaf blade was relatively smaller because the cells in the infected sector were markedly bigger than those in the resistant one, both in the palisade and spongy mesophyll. The cell diameter of the palisade mesophyll in the infected sector was about 67% bigger than that in the other sector. The number of cells per 1 mm² was almost 50% lower in the infected sector in comparison to the resistant one.

Marked differences, amounting to 60-65%, were observed between the two sectors with regard to intercellular spaces. On the section through the palisade and spongy mesophyll the intercellular spaces in the infected sector were larger (Figs 11, 12, 13, 14).

The chloroplasts in the infected sector were bigger than those in the resistant one, with particularly marked differences (about 40%) observed in the spongy mesophyll.

DISCUSSION

The sector infected by *Erisiphe cruciferarum* Opiz ex. L. Junell and the resistant one differed markedly in numerous leaf traits. It is difficult to distinguish the differences resulting from the genetic structure of the two sectors from those which were caused by the pathogen. The moment when the infection took place during the leaf development is not clear either. The

Fig. 7. Variability of cell surface in the lower epidermis. A – in the resistant sector. B, C – in the infected sector; B – cells without haustoria, C – cells with haustoria.

Fig. 8. Variability of the cell volume in the lower epidermis.

A – in the resistant sector.

B – in the infected sector.

determination of genetic differences could provide a basis for the selection of resistant genotypes.

The number of cell layers in the palisade and spongy mesophyll was significantly different in the two sectors. This trait is determined in an early developmental phase of the leaf primordium, therefore the conclusion that this difference had a genetic source seems to be justified.

It is proved that haustoria of powdery mildew (*Erisiphales*) develop in the epidermal cells only, but they affect the adjacent mesophyll cells as well (Kochman 1980). Minarič and Paulech (1978) found that *Erisiphe graminis* f. sp. *hordei* had a harmful effect on the organelles of mesophyll cells, not only of those adjacent the infected ones but also of those in the neighbouring layers. According to the two authors, the fungus inhibits chloroplast divisions and it causes their gradual destruction. Pronounced changes of chloroplasts begin to be vi-

sible during the sporulation of the fungus. The chloroplast membrane is then injured and the internal structure of chloroplast is broken. The increased size of chloroplasts in the infected sector of the chimera during the fungus sporulation probably reflected this destructive process.

One of the basic effects of *Erisiphe* infection is its influence on water relations in the host plants, which was investigated by Paulech and Haspelová-Horvatovicová (1970), Paulech et al. (1975) and Majerník (1971). The authors found that the fungus, starting from its early developmental phases, lowers the transpiration by stomata in barley. We observed a necrotization of guard cells and closing of stomata when haustoria appeared in the neighbouring cells. These phenomena undoubtedly resulted in lowering the rate of transpiration. On the other hand, the number of stomata in the infected sector was twice as high as that in the resistant one. In addition, the

Figs 9-10. Leaf cross section through the infected (left) and the resistant (right) sectors.

Figs 11-14. Cells of palisade (above) and spongy mesophyll (below) in the infected (left) and resistant (right) sectors of the leaf.

intercellular spaces in the infected sector were 60-65% bigger than those in the resistant part. On the basis of all these observations it may be concluded that the transpiration in the infected sector of the studied chimera was considerably more intensive than that in the resistant sector.

Substantial differences in the size of nuclei in the two sectors seem to indicate that *Erysiphe* influences the synthesis of DNA. It has been proved that the doubling of the nucleus volume is usually associated with the doubling of the DNA level. The size of the nuclei of the guard cells in the resistant sector was twice as large as that in the infected one. One may conclude, therefore, that the DNA synthesis in the guard cells of the infected sector was blocked. If so, the DNA level in the guard cells in the infected sector would equal to 1C, and in the resistant sector to 2C. The guard cells in the infected sector differed much in their size and the size of their nuclei. The range of size variability indicates that four groups of nuclei can be distinguished with regard to the DNA level: 1C, 2C, 4C and 8C.

It is interesting to note that the guard cells and the remaining epidermal cells responded to the *Erysiphe* in different ways. The fungus caused a necrotization of the guard cells adjacent the cells which contained haustoria, and an inhibition of DNA synthesis in the other guard cells. The epidermal cells containing haustoria and the neighbouring ones remained alive. Some of these cells and their nuclei increased considerably in size, which seemed to correspond to the rise of the DNA level to 4C and 8C.

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BUDOWA ANATOMICZNA SEKTORÓW CHIMERY RZEPAKU OZIMEGO
O RÓŻNEJ ODPORNOŚCI NA MĄCZNIAKA PRAWDZIWEGO
(*ERISIPHE CRUCIFERARUM* OPIZ EX. L. JUNELL)

STRESZCZENIE

Przedmiotem badań była dihaploidalna chimera sektorialna rzepaku ozimego. Materiał uzyskano drogą androgenezy i napromieniowano dawką 30 Gy promieni gamma w trakcie klonowania pędów in vitro. Jeden z sektorów tej chimery był porażony *Erisiphe cruciferarum* Opiz ex. L. Junell, a drugi był odporny na tego patogena. W sektorze porażonym blaszka liściowa była cieńsza, zbudowana z mniejszej liczby warstw komórek miękiszu palisadowego i gąbczastego. Rozmiary komórek obu rodzajów miękiszu i komórek epidermy oraz przestworów międzykomórkowych były większe. Dotyczyło to również rozmiarów jąder i chloroplastów. Sektor porażony charakteryzował się też znacznie większą liczbą aparatów szparkowych, lecz o mniejszych rozmiarach niż sektor odporny. Wyniki badań pozwalają lepiej zrozumieć związek pomiędzy budową anatomiczną rośliny a patogenem.

SŁOWA KLUCZOWE: budowa anatomiczna, chimera, mączniak prawdziwy, rzepak ozimy.