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A COMPARISON OF THE ANATOMICAL STRUCTURE OF SUSCEPTIBLE *Helianthus annuus* L., RESISTANT *H. Argophyllus* L. AND THEIR PROGENY

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SUMMARY

Anatomical differences were analyzed between the inbred line HA-89 of *Helianthus annuus*, which is susceptible to the fungal pathogen *Phomopsis/ Diaporthe helianthi* Munt. Cvet. *et al.*, and a resistant wild population of *H. argophyllus*. The anatomical structure of the interspecific hybrid was also studied and compared against the progenitors. Plant materials, especially leaves, were collected in the field in the period 1991-1993 at the anthesis stage of the plants. The results obtained after the analysis of the epidermal and vascular tissues were further processed using SYSTAT statistical package. The results showed that the characteristics of the interspecific hybrid were closer to the susceptible than the resistant parent component. Considering the initial assumption that differences in the dimension of the epidermal and vascular tissues could have significant influence on variability in resistance among genotypes, a negative conclusion can be drawn.

Key words: Anatomy, epidermal tissue, vascular tissue, *Helianthus annuus* L., *Helianthus argophyllus* L., interspecific hybrid.

INTRODUCTION

The fungal disease of sunflower caused by *Phomopsis/Diaporthe helianthi* Munt. Cvet. et al., first found and described in Yugoslavia in 1980 (Muntanola-Cvetković *et al.*, 1981), spread quickly and was found in the neighbouring countries (Vranceanu *et al.*, 1983; Nemeth *et al.*, 1981), then in USA (Yang *et al.*, 1984) and France (Regnault, 1985). In Australia and USA other members of the same genus were described as well (Allen *et al.*, 1980; Herr & Lipps, 1983; Hajdu *et al.*, 1984).

Since the pathogen cannot be always efficiently controlled by chemicals, breeding for resistance has to be employed to offset the disease. Selection within available populations may serve to improve specific characteristic, but it is usually a slow process. To introduce new characters such as resistance to various diseases into adapted cultivars, it is necessary to make crosses with appropriate sources of resistance. Since the genus *Helianthus* is composed of diverse species which represent considerable genetic variability, wild species could be used for the improvement of the cultivated sunflower. Long-term experiments have shown that some diploid, tetraploid, and especially hexaploid wild *Helianthus* species could be used as donors of resistance to the fungal pathogen *P. helianthi.*

Škorić (1985) reported, after several years of field experiments, that only four lines out of the entire breeding material that consisted of a few thousand inbred lines showed high tolerance to *P. helianthi*. One of them was derived from an interspecific cross with *H. argophyllus.* Resistance was not monogenic since crosses between field resistant and susceptible lines exhibited intermediate reaction.

Resistance to *P. helianthi* was correlated with resistance to *Macrophomina phaseoli* and drought (Škorić, 1985). Phenotypic resistance was associated with the "stay green" character of sunflower stems (Vranceanu *et al.*, 1983). Interspecific hybridization is an exceedingly slow process and sometimes it fails or the obtained progenies are sterile or have inferior agronomic characteristics. In order to expedite the production of resistant genotypes, some laboratories use an *in vitro* method with the fungal filtrate or the phytotoxin phomosin to differentiate the levels of resistance (Mazars *et al.*, 1990; Dozet *et al.*, 1992). The level of resistance may also be measured by means of marker genes. Furthermore, some researchers suppose that some morphological and anatomical characteristics could influence the expression of resistance to pathogens.

A long-term investigation of *P. helianthi* pathogenesis in field conditions has shown that first symptoms of the disease appear only on the edges of the leaves at the specific development stage of the 6th pair of leaves (4-5 - week old plant). After penetration into the sunflower leaf, hyphae first appear in the foliar vascular elements, spread through the vascular bundles disintegrating in the process the phloem and adacent parenchymal tissues, reach the midrib through larger strands of the conducting system, and in subsequent stages advance downward along the petiole. The hyphae spread down the leaf axil and invade the cortical tissues of the stem. The invasion route leaf-petiole-stem of the host invasion by the fungus was demonstrated in a histological study (Muntanola-Cvetković *et al.*, 1991).

In this paper, special attention is given to the analysis of the leaf and petiole tissues assuming that differences in epidermal and vascular systems between susceptible and resistant sunflower genotypes could be responsible for the different reactions the genotypes exhibit in the presence of the pathogen *P. helianthi*.

MATERIAL AND METHODS

Plant material and conditions of growth

The investigation comprised the following sunflower genotypes: *H. annuus*, inbred line HA-89 (susceptible genotype), a wild population of *H. argophyllus*, and their F_1 hybrid.

The experiments were carried out at the Rimski Šančevi experiment field during the growing season of 1991, 1992 and 1993. The crop was grown under irrigated conditions with the conventional cultivation practices.

Meteorological data, i.e., temperature and precipitation, for the period from germination to anthesis of sunflower plants (May-August) were obtained from the meteorological station Rimski Šančevi, Institute of Field and Vegetable Crops, Novi Sad.

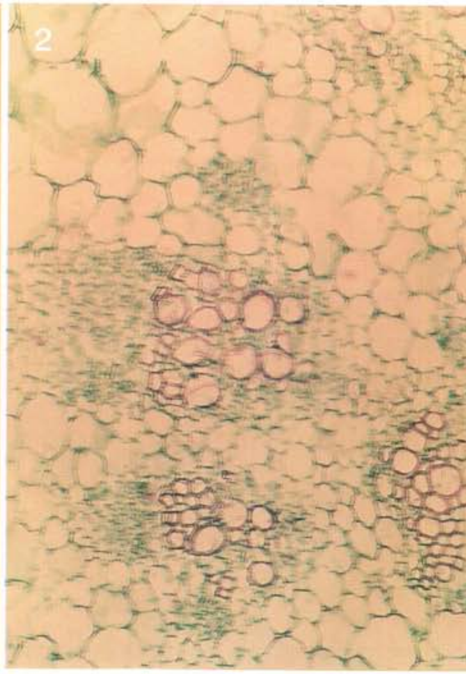
Collecting plant material

Small segments of leaves (main vein, lateral veins, margin) and petioles (Figure 1a) were collected at the beginning of the anthesis stage of each genotype, at four levels (5th, 10th, 20th and apical leaf) (Figure 1b).

Processing plant material

Several pieces of plant material were immediately placed in formalin-aldehyde. Selected pieces of each collection were subsequently placed in FAA-fixing solution (formalin 40%, 5 ml; acetic acid, glacial, 5 ml; ethyl alcohol 70%, 90 ml) and were kept there until they sank. Samples were embedded in paraffin for further processing of the fixed material according to the conventional techniques (Dring, 1971). Microtome sections 10-15 μm thick were stained with Safranin O, counterstained with Light Green SF Yellowish and mounted in Canada balsam. The sections were observed, cells measured and photographed using a Reichert microscope Diastar^{tm}.

Data processing was done using the statistical package Systat (modules Stats, MGLH and Sygraph).



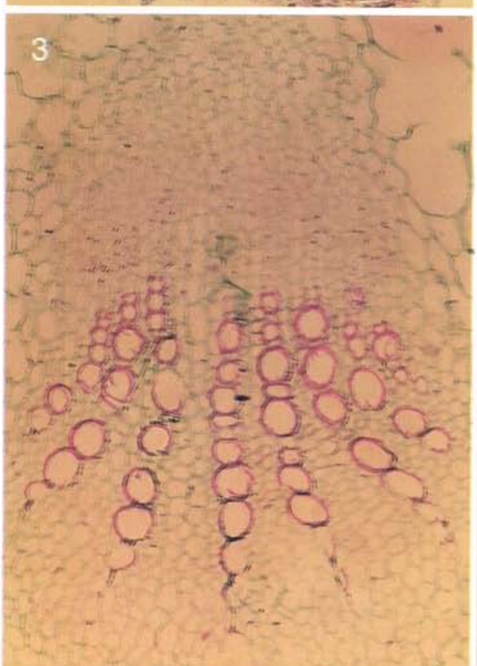


Figure 1 H. annuus x H. argophyllus
5^{th} leaf - edge of the lamina

Figure 2 H. annuus x H. argophyllus
5^{th} leaf - main vein

Figure 3 H. annuus x H. argophyllus
5^{th} leaf - petiole

Figure 4 H. annuus x H. argophyllus
10^{th} leaf - edge of the lamina

RESULTS

Cross sections of the leaf edge, main vein and petiole of the F_1 progeny at 4 leaves of the plant during anthesis stage are shown in the photographs 1 to 12 (Figures 1-12). The results of the measurements of the epidermal and vascular tissues are given in Table 1.

Table 1: The investigated characteristics of *Helianthus annuus*, *H. argophyllus* and their interspecific hybrid

Plant organ	Plant tissue	Genotype	Characteristic		Range		Principal components	
			Height	Width	Height	Width	Y1	Y2
leaf	adaxial epidermis	*H. annuus*	19.53	25.44	B	BC	0.456	0.806
		H. argophyllus	18.70	24.40	B	C	0.235	0.949
		F_1	19.62	27.34	B	BC	0.883	0.469
	abaxial epidermis	*H. annuus*	16. 15	22.77	C	BC	0.793	0.512
		H. argophyllus	14.03	21.08	C	BC	0.578	0.815
		F_1	14.95	22.67	C	BC	0.938	0.347
main vein	epidermal cells	*H. annuus*	23.30	26.40	BCD	AB	0.783	0.576
		H. argophyllus	22.10	24.05	BCD	BC	0.977	-0.175
		F_1	24.67	27.34	B	AB	0.964	0.007
	large vascular bundles	*H. annuus*	52.11	41.80	AB	A	0.986	0.150
		H. argophyllus	44.58	36.33	BCD	AB	0.271	0.959
		F_1	53.24	42.10	A	A	0.999	0.013
	small vascular bundles	*H. annuus*	34.56	25.04	AB	B	0.997	0.073
		H. argophyllus	34.04	27.22	AB	B	0.841	-0.517
		F_1	40.96	34.00	A	A	0.990	0.137
petiole	epidermal cells	*H. annuus*	20.79	23.62	B	BC	0.671	0.628
		H. argophyllus	21.42	25.12	B	BC	0.189	0.963
		F_1	30.29	31.94	A	A	0.987	0.154
	large vascular bundles	*H. annuus*	52.14	40.55	ABC	AB	0.826	0.562
		H. argophyllus	49.83	40.22	BC	B	0.997	-0.037
		F_1	33.15	27.53	D	C	0.980	0.197
	small vascular bundles	*H. annuus*	38.10	28.67	A	AB	0.953	0.262
		H. argophyllus	37.34	29.86	A	A	0.400	0.913
		F_1	43.24	34.29	A	A	0.994	0.109

Lamina

Epidermis. At the cross-sections of the lamina, on both the adaxial and abaxial surface, single-layered epidermis is present, consisting of tabular cells with thickened outer walls and covered with a thin cuticle. Dimensions of leaf epidermal cells of the parent components and interspecific hybrid were measured.

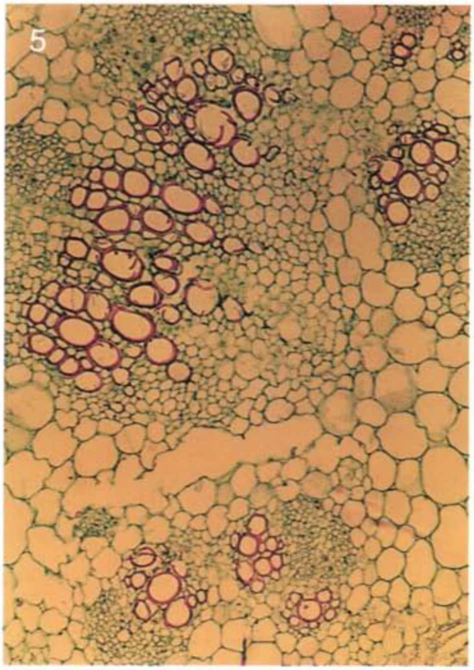


Figure 5 H. annuus x H. argophyllus
10^{th} leaf - main vein

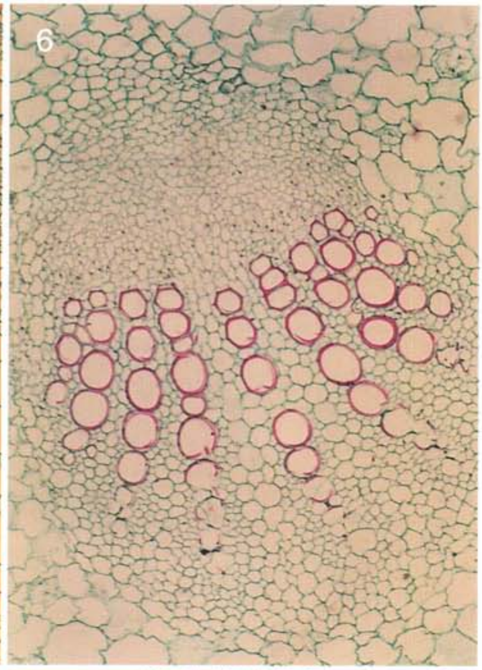


Figure 6 H. annuus x H. argophyllus
10^{th} leaf - petiole

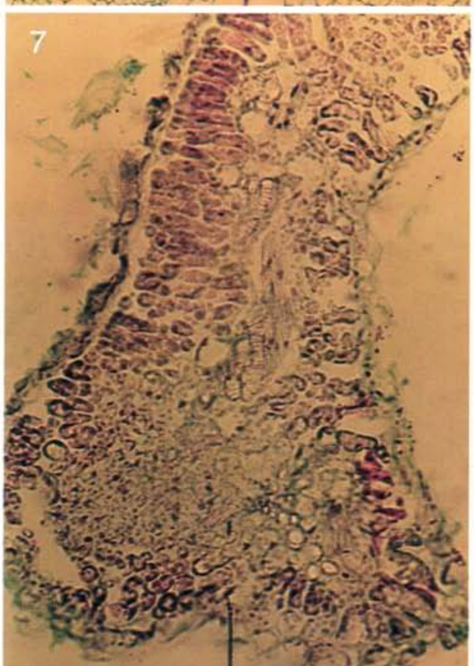


Figure 7 H. annuus x H. argophyllus
20^{th} leaf - edge of the lamina

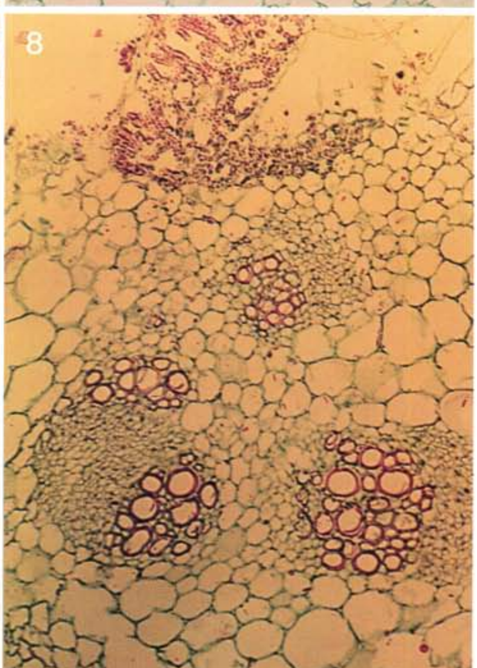


Figure 8 H. annuus x H. argophyllus
20^{th} leaf - main vein

Figure 9 H. annuus x H. argophyllus 20th leaf - petiole

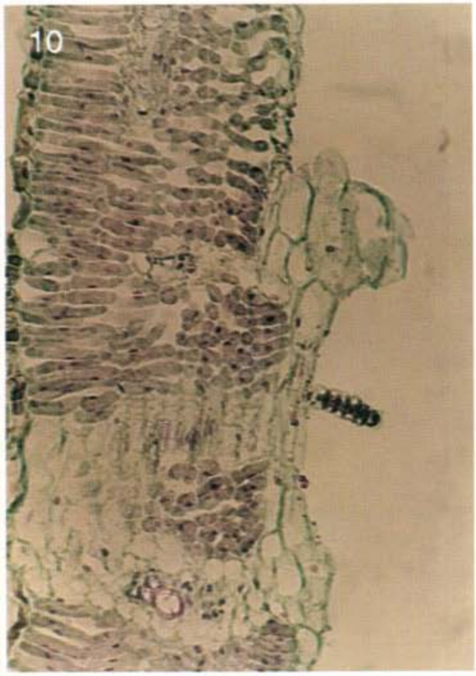


Figure 10 H. annuus x H. argophyllus apical leaf - leaf

Figure 11 H. annuus x H. argophyllus apical leaf - main vein

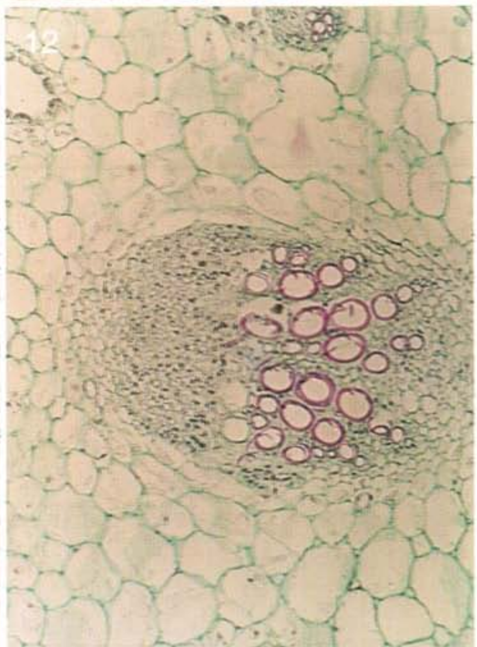


Figure 12 H. annuus x H. argophyllus apical leaf - petiole

The progenies of the susceptible *H. annuus* HA-89 and the resistant *H. argophyllus* showed slightly higher values for the investigated characteristic than the parent components (Table 1). No statisticaly significant differences were found.

The PC analysis of the data obtained by measuring the adaxial epidermal cells showed the greatest variability between F_1 and its parent components; the variability within F_1 was considerably higher than that in *H. argophyllus*. Considering the dimensions of the abaxial epidermal cells, no significant differences were observed.

MAIN VEIN

At the transsection, single-layer epidermis was found on the surface, consisting of rectangular cells with thickened outer walls and a cuticle above them. Collateral vascular bundles were present in the parenchymatous tissue. Dimensions of epidermal cells and xylem elements in vascular bundles were measured.

Epidermis. The progeny of the susceptible *H. annuus* HA-89 and resistant *H. argophyllus* showed slightly higher values for the epidermal cells' dimensions than the parent components (Table 1). No statistically significant differences were found.

The PC analysis of the data obtained from the measurements of the epidermal cells showed that the differences in variability between the F_1 hybrid and its parent components were not significant, although increased variability was noticed in *H. argophyllus*.

Xylem elements in large vascular bundles. The highest values for xylem elements' dimensions were obtained in the F_1 hybrid, but the differences were not statistically significant (Table 1).

The PC analysis showed a significant differnce between the resistant diploid species *H. argophyllus* and the susceptible *H. annuus*. In comparison with the parent components, the progeny was very close to the susceptible *H. annuus*.

Xylem elements in small vascular bundles. The highest values for xylem elements' dimensions were obtained in the F_1 hybrid, but the differences between the investigated genotypes were not statistically significant (Table 1).

There were no significant differences in variability between the susceptible inbred line *H. annuus* and the resistant diploid *H. argophyllus*, as well as the interspecific hybrid. The progeny was very close to the susceptible parent component.

PETIOLE

At the cross section of the petiole, single-layered epidermis was found on the surface. The epidermal cells were rectangular in shape, with thickened external walls, covered with a cuticle. The collateral vascular bundles were placed in the parenchyma. The dimensions of epidermal cells and xylem elements in vascular bundles were measured.

Epidermal cells. The highest values for the investigated characteristic were obtained for the hybrid *H. annuus* x *H. argophyllus*, which were statistically significant (Table 1).

The interspecific hybrid differed from the progenitors in variability, but it was much closer to the susceptible *H. annuus*. The variability in *H. argophyllus* was significantly lower.

Xylem elements in large vascular bundles. Significantly smaller values were obtained for the dimensions of the xylem elements in the progeny.

The PC analysis showed that *H.argophyllus* had the highest variability. No significant differences were noticed between *H. argophyllus* and the progeny.

Xylem elements in small vascular bundles. The xylem elements were larger in the hybrid than in the parent components, but the differences in dimensions were not statistically significant.

Significantly lower variability was shown by *H. argophyllus*. Little difference was found between *H. annuus* and the progeny.

DISCUSSION

Considering the aim of the study, primarily the relationship of the resistant and the susceptible genotype, the size of the epidermal cells of the lamina was not the characteristic applicable to differentiate the investigated material.

The analysis of the dimensions of the epidermal cells and xylem elements in vascular bundles of the main vein failed to confirm the connection between size and resistance.

Differences in dimensions of the epidermal cells and xylem elements in vascular bundles of the petiole can not be used as a reliable criterion for differentiating resistant and susceptible genotypes of sunflower.

Considering the initial assumption that the differences in dimensions of the epidermal and vascular tissues of the lamina, main vein and petioles could have significant influence on the variability of resistance among certain genotypes, a negative conclusion could be drawn.

The interspecific hybrid was much closer to the susceptible parent component than to the resistant hexaploid parent component.

It could be concluded that the different reactions of the investigated genotypes to the presence of the pathogen are the result of the pathogen's capacity or incapacity to penetrate the host tissue and not the consequence of the various ways of colonization of the fungus because of the differences in dimensions of the investigated structures.

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UNA COMPARACIÓN ENTRE LA ESTRUCTURA ANATÓMICA DEL *Helianthus annuus* L. SUSCEPTIBLE Y *Helianthus argophylus* L. RESISTENTE Y SU DESCENDENCIA

RESUMEN

Se analizaron diferencias anatómicas entre la línea HA-89 de *Helianthus annuus* que es susceptible al hongo patógeno *Phomopsis/Diaporthe helianthi* Munt.-Cvet. et al., y una población silvestre resistente de *H. argophyllus*. La estructura anatómica del híbrido interespecífico fué también estudiada y comparada con sus progenitores. Materiales de la planta, especialmente hojas, fueron recolectados en el campo en el periodo 1991-93 en el estado de antesis de las plantas. Los resultados obtenidos después del análisis de los tejidos vasculares y epidérmicos fueron procesados utilizando el paquete estadístico SYSTAT. Los resultados mostraron que las características del híbrido interespecífico estuvieron más cerca del parental susceptible que del resistente. Considerando la asunción inicial de que las diferencias en la dimensión de los tejidos vascular y epidermal podrían tener influencia significativa sobre la variabilidad en resistencia entre los genotipos, se puede extraer una conclusión negativa.

COMPARAISON DE LA STRUCTURE ANATOMIQUE *d'Helianthus annuus* L., SENSIBLE; D'H. *argophyllus* RÉSISTANT, ET DE LEURS DESCENDANCES

RÉSUMÉ

On a analysé les différences anatomiques entre la lignée fixée HA-89 *d'Helianthus annuus*, sensible au pathogène fongique *Phomopsis /Diaporthe helianthi* Munt.-Cvet. et al., et une population sauvage résistante d'*H. argophyllus*. La structure anatomique de l'hybride interspécifique a été aussi étudiée et comparée à ses parents. Le matériel végétal, particulièrement les feuilles, a été collecté au champ entre 1991 et 1993, au moment de l'anthèse des plantes. Les résultats obtenus après l'étude des tissus épidermiques et vasculaires ont été analysés plus en détail avec le logiciel statistique SYSTAT. Les résultats montrent que les caractéristiques de l'hybride interspécifique vent plus proches du parent sensible que du parent résistant. Compte tenu de l'hypothèse de départ selon laquelle les différences de dimension des tissus épidermiques et vasculaires pourraient avoir une influence significative sur la variabilité de la résistance des génotypes, on en tire une conclusion négative.