ANATOMICAL COMPARISONS AMONG *HELIANTHUS ANNUUS* L., *H. TUBEROSUS* L. AND THEIR PROGENIES

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Abstract

Anatomical differences among the inbred line HA-89 of *Helianthus annuus*, which is susceptible to the fungal pathogen *Phomopsis/Diaporthe helianthi* Munt.Cvet. et al. and the resistant wild populations of *H. tuberosus* were analysed. The anatomical structure of their progenies of the interspecific hybridization was also studied and compared to the progenitors.

The material was collected in the field during summer 1992 and 1993 at the anthesis stage of the plants and consisted of small fragments of the 5th, 10th, 20th and the apical leaves. Nearly 200 histological preparations have been measured. The obtained results were analysed using SYSTAT statistical package.

Key words: Plant anatomy, *Helianthus annuus* L., *Helianthus tuberosus* L., interspecific hybrids

1. Introduction

The fungal disease of sunflower caused by *Phomopsis/Diaporthe helianthi* Munt.-Cvet. et al., first found and described in Yugoslavia in 1980 (Muntañola-Cvetković et al., 1981), spread quickly and it was found in several countries in Europe, in USA and Argentina (Mihaljčević et Vukojević, 1994).

The study of pathogenesis of the disease caused by *P. helianthi* showed that the first symptoms always appeared at the edge of the leaf lamina. The invasion route leaf-petiole-stem of the host invasion by the fungus, was demonstrated through histological studies (Muntañola-Cvetković et al., 1989, 1991).

The analysis of all tissues of lamina, main vein and petiole was done (Duletić, 1995), but in this paper the special attention was given to the lamina and petiole epidermal and vascular tissues, presuming that the differences in those tissues between different genotypes could be responsible for various reactions of genotypes towards pathogen *P. helianthi*.

2. Materials and Methods

The investigation comprised the following sunflower genotypes: *H. annuus*, inbred line HA-89 (susceptible genotype), the wild population of *H. tuberosus* (PHS-2059), and their interspecific F1 hybrid.

The crossing and cultivation were carried out at Rimski Šančevi experimental field during the 1991, 1992 and 1993 growing seasons. The crop was grown under irrigated conditions with recommended production practices.

Small segments of leaves (main vein, lateral veins, margin) and petioles were collected at the beginning of the anthesis stage of each genotype, at the four levels (5th, 10th, 20th and apical leaf). Several pieces of the plant material were immediately placed in formalin-aldehyde conservance. Selected pieces of each collection were afterwards placed in FAA fixing solution where they remained until they sank. Samples were embedded in paraffin for further processing of the fixed material according to the usual techniques. Microtome sections 10-15 μm thick were stained with Safranin O and counterstained with Light Green SF Yellowish and mounted in Canada balsam. The sections were observed, cells measured and photographed using Reichert microscope Diastar^{tm}.

Processing of obtained data was done using statistical package Systat (moduls Stats, MGLH and Sygraph).

3. Results

3.1. Leaf

At the cross-sections of the lamina on the adaxial and abaxial surface the one-layered epidermis is present, consisted of tabular cells with thickened outer walls and covered with a thin cuticle. The dimensions of leaf epidermal cells of parental components and interspecies hybrids were measured.

Progenies from crossing between susceptible *H. annuus* HA-89 and resistant *H. tuberosus* showed lower values than the parental components, except in width of abaxial epidermal cells (the value is between the parental) (Tab. 1). Among obtained values there is not statistically significant differences.

PC analysis of data obtained from measurements of the adaxial epidermal cells height and width showed the greatest differences in variability between *H. tuberosus* and F1, and *H. annuus* was closer to *H. tuberosus*. Variability of abaxial epidermal cells height and width in progenies of crossing wild species and *H. annuus* was significantly higher comparing to hexaploid *H. tuberosus*.

The highest variability of investigated parameters was stated in hexaploid *H. tuberosus* (Tab. 1), which is near the interspecies hybrid. The significant differences in variability between diploid *H. annuus* and hexaploid *H. tuberosus* were not stated.

3.2. Main vein

At the transection on the surface is the one-layered epidermis, consisted of rectangular cells with thickened outer walls and cuticle above. In the parenhimatous tissue the collateral vascular bundles are present. The dimensions of epidermal cells and xylem elements in vascular bundles were measured.

Table 1. The investigated characteristics of *Helianthus annuus* L., *H. tuberosus* L. and their progenies

plant organ	plant tissue	genotype	characteristics		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. tuberosus	22.06	28.57	B	BC	0.153	0.967
		F1	19.34	24.29	B	BC	0.903	0.427
	abaxial epidermis	*H. annuus*	16.15	22.77	C	BC	0.793	0.512
		H. tuberosus	14.13	21.05	C	BC	0.923	0.314
		F1	12.95	22.38	C	BC	0.951	0.306
main vein	epidermal cells	*H. annuus*	23.30	26.40	BCD	AB	0.783	0.576
		H. tuberosus	19.28	16.76	D	D	0.314	-0.900
		F1	21.05	25.24	BCD	ABC	0.985	0.114
	large vascular bundles	*H. annuus*	52.11	41.80	AB	A	0.986	0.150
		H. tuberosus	30.94	26.06	EF	CD	0.995	-0.096
		F1	46.29	40.39	ABC	A	0.994	0.011
	small vascular bundles	*H. annuus*	34.56	25.04	AB	B	0.997	0.073
		H. tuberosus	37.03	29.41	AB	AB	0.985	-0.092
		F1	32.19	25.11	B	B	0.996	0.075
petiole	epidermal cells	*H. annuus*	20.79	23.62	B	BC	0.671	0.628
		H. tuberosus	19.74	22.48	B	BC	-0.299	0.954
		F1	20.84	22.89	B	BC	0.986	0.109
	large vascular bundles	*H. annuus*	52.14	40.55	ABC	AB	0.826	0.562
		H. tuberosus	47.40	39.40	C	B	0.554	0.816
		F1	47.33	39.07	C	B	0.980	0.196
	small vascular bundles	*H. annuus*	38.10	28.67	A	AB	0.953	0.262
		H. tuberosus	23.34	18.95	BC	C	0.576	0.817
		F1	38.39	31.43	A	A	0.994	0.109

Epidermal cells

The smallest epidermal cells were measured in *H. tuberosus*, slightly bigger in the progenies after crossing with *H. annuus* HA-89 (Tab. 1). Although the smallest values were noted in *H. tuberosus*, the statistically significant differences were not stated, considering the investigated characteristics, between *H. tuberosus*, *H. annuus* and their interspecies hybrid.

The analysis of height and width of epidermal cells of main vein using PC analysis showed that the hexaploid *H. tuberosus* was very different regarding to diploid *H. annuus* and their interspecies hybrid. In comparison with the parental components progenies are much closer to the diploid *H. annuus* HA-89 than to the hexaploid parental component.

Xylem elements in large vascular bundles

In *H. tuberosus* relatively small elements were measured, but in hybrids with *H. annuus* dimensions were considerably increased (Tab. 1). Test for the significance of differences between mean values of lenght and width of xylem elements in the main vein showed absence of statistically significant differences between diploid *H. annuus* and hexaploid *H. tuberosus*.

Between susceptible inbred line *H. annuus* and resistant hexaploid *H. tuberosus*, as well as interspecies hybrids, there is no statistically significant differences in variability.

Xylem elements in small vascular bundles

In F1 generation obtained after crossing *H. annuus* HA-89 and *H. tuberosus* the xylem elements are smaller than in the parental components (Tab. 1).

No significant differences in variability were noticed between investigated genotypes. Interspecies hybrid was very close to diploid susceptible parent.

3.3 Petiole

At the cross sections of the petiole the one-layered epidermis is on the surface. The epidermal cells are rectangular in shape, with thick external walls, and covered with a thin cuticle. The collateral vascular bundles are placed in the parenchyma. The dimensions of epidermal cells and xylem elements in vascular bundles were measured.

Epidermal cells

The values obtained from measurements of epidermal cells dimensions were rather uniformed.

Significant smaller variability was obtained in the case of hexaploid *H. tuberosus* in relation to *H. annuus*. Interspecies hybrids were diverse from their parents, but much closer to susceptible *H. annuus*, than to the resistant hexaploid parental component.

Xylem elements in large vascular bundles

Almost identical values from measuring xylem elements were obtained for *H. tuberosus* and interspecies hybrid with *H. annuus* (Tab. 1).

Between *H. tuberosus* and *H. annuus*, as well as progenies from their crossing, statistically significant differences in variability were not established. F1 generation was closer to susceptible parental component *H. annuus*.

Xylem elements in small vascular bundles

In *H. tuberosus* considerably smaller elements were measured than in *H. annuus* HA-89 (Tab. 1). The elements were considerably larger in F1 generation (Tab.1).

Hexaploid *H. tuberosus*, with considerably lower variability, was significantly different from the other genotypes. Between *H. annuus* and interspecies hybrid *H. annuus* x *H. tuberosus* difference was very small.

4. Discussion

Considering the aims of the study, the size of the lamina epidermal cells is not the characteristic which could differentiate the investigated material.

Analysis of the dimensions of the epidermal cells and xylem elements in vascular bundles of the main vein showed that connection between size and resistance can not be confirmed.

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

Interspecies hybrid is much closer to the susceptible parental component, than to the resistant hexaploid parental component.

It could be concluded that different reactions of investigated genotypes to the presence of the pathogen are the result of its possibility or impossibility to penetrate in the host tissue, not the consequence of the various ways of colonisation of fungus caused by the differences in dimensions of the investigated structures.

5. References

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