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SOURCES OF RESISTANCE TO *Sclerotinia sclerotiorum* (Lib.) de Bary IN A NATURAL *Helianthus* GENE POOL

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SUMMARY

Sclerotinia sclerotiorum (Lib.) de Bary is one of the major diseases of sunflower (*Helianthus annuus*). The pathogen can attack all parts of the plant at every stage of plant growth, predominantly the capitulum, leaf and stem. In the present study, several perennial *Helianthus* species of diverse origin were evaluated for resistance level to mid-stem and leaf infection using an inoculation method. The evaluation revealed considerable and significant differences among the genotypes in all recorded resistance traits. Three genotypes showed enhanced resistance levels to mid-stem reaction. Two genotypes exhibit resistances to leaf infection. Since all evaluated resistant genotypes in these trials are sexually incompatible with the *Helianthus annuus* genome, newly developed biotechnological methods gain particular importance for the transfer of the discovered levels of resistance into the cultivated sunflower.

Key words: **perennial *Helianthus* ssp., *Sclerotinia sclerotiorum*, mid-stem infection, leaf infection, resistance**

INTRODUCTION

Sunflower is the fourth important oilseed crop in the world. Domestication and selection in breeding programs led to the narrow genetic variability of the cultivars available today, making them susceptible to numerous fungal and insect pests (Seiler, 1992). In contrast, the wild perennial *Helianthus* species are adapted to a wide diversity of habitats and offer a considerable variability for most economic and agronomic characteristics. Hence, the broad genetic diversity in the genus *Helianthus* can be used for variability rescue and introgression of agronomic important traits in the gene pool of cultivated sunflowers. The significance of wild species in

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sunflower breeding is well known (Serieys, 1987), and interspecific hybridization is used as a potential source of cytoplasmatic male sterility (Serieys, 1995), fertility restoration, insect and disease resistance, early ripening and improved oil and protein quality (Seiler, 1992).

White rot caused by the polyphagous fungus *Sclerotinia sclerotiorum* (Lib.) de Bary is one of the major disease of sunflower in countries with humid climate, while in countries with moderate climate it causes yield losses in rainy years. The ascomycetes of *Sclerotinia sclerotiorum* can attack all parts of the plant at every plant growth stage. In the temperate areas of Europe, capitulum and mid-stalk infections induced by airborne ascospores between flowering and maturity are prevalent. In ascospore-infected leaves, the mycelium spreads to the petiole and stem, thus causing stem lesions and finally mid-stalk rot (Maširević and Gulya, 1992). Under favorable conditions, in high *S. sclerotiorum* infested sunflower fields, yield losses can reach up to 100% (Maširević and Gulya, 1992; Rashid, 1993). In general the cultivated sunflower hybrids possess a low level of resistance to this pathogen, although differences in susceptibility do exist (Tourvieille *et al.*, 1996; Degener *et al.*, 1998). Fortunately, a large spectrum of resistance has been detected in wild perennial *Helianthus* species (Škorić, 1987; Seiler, 1992). Thus, wild *Helianthus* species are promising sources of genes for *Sclerotinia* disease resistance (Köhler, 1997).

The main aim of this study was to evaluate different perennial *Helianthus* species in a 3-year field trial for their level of resistance to:

a) *Sclerotinia sclerotiorum* mid-stem infection (field trials)

b) *Sclerotinia* leaf infection (field trials and detached leaf test) and

c) to test if there exist correlations between the recorded traits.

Furthermore, the recorded resistance levels of the genotypes were tested for relationships with symptomatologic characteristics associated with the pathogenesis e.g. occurrence of sclerotia in the stem pith and on the leaf surface.

MATERIAL AND METHODS

Plant materials and field trials

A total set of 21 perennial *Helianthus* species of diverse origin, belonging to the specific A genome of the genera, were evaluated in field trials for resistance to *Sclerotinia sclerotiorum* mid-stalk rot and leaf infection. Field trials were carried out to detect mid-stem and leaf resistance at the star bud stage to full flowering of the genotypes (June - September) and conducted at the experiment station in Bonn (Germany). Several experiments were undertaken which differed with regard to the genotypes tested, test environment, year, aim of experiment and are shown in Tables 1 and 2. Field experiments were performed using complete randomized trials (block design).

Table 1: Characteristics of the utilized genotypes, recorded resistance levels to *Sclerotinia* infection, and their crossability with cultivated sunflower

Species	Scored resistance level[a]	Set of chromosomes[b] n =	Interspecific crossability with *H. annuus*[c]
H. atrorubens	ms, lsf, lsh	17	n.a.
H. divaricatus	ms, lsf, lsh	17	–
H. decapetalus	ms	34	±
H. giganteus	ms, lsf, lsh	17	–
H. grosseserratus	ms, lsf, lsh	17	–
H. xlaetiflorus	ms, lsf, lsh	51	–
H. maximiliani AC M	ms, lsf, lsh	17	–
H. maximiliani AC 1	ms, lsf, lsh	17	–
H. maximiliani AC 4	ms, lsf, lsh	17	–
H. maximiliani AC 5	ms, lsf, lsh	17	–
H. maximiliani AC 7	ms, lsf, lsh	17	–
H. microcephalus	ms, lsf, lsh	17	±
H. nuttallii	ms, lsf, lsh	17	–
H. pauciflorus (*rigidus*)	ms, lsf, lsh	51	±
H. rigidus ssp. *rigidus*	ms, lsf, lsh	51	±
H. rigidus AC 1745	ms, lsf, lsh	51	±
H. rigidus AC 1747	ms, lsf, lsh	51	±
H. rigidus	ms	51	±
H. salicifolius	ms, lsf, lsh	17	–
H. strumosus	ms, lsf, lsh	51	–
H. tuberosus	ms, lsf, lsh	51	±

[a] ms = mid-stem scoring,
lsf = leaf resistance in field trials,
lsh = leaf resistance conducted in a humid chamber.

[b c] according to Georgieva-Todarova, 1984,
n.a. = information not available,
± = crosses are difficult but possible,
– = complete sexual incompatibility.

Table 2: General survey of experiments

Aim of experiment	Location	No. of repl./ genotype	Month/year of performance	No. of independent repl./year	No. of tested genotypes	Data recording: days after inoculation
Mid-stem scoring	Field	4	07-08/1998	2	21	4d
		4	07-08/1999	2	21	4d
		4	07-09/2000	2	13	4d
Leaf scoring	Field	4	07/1999	2	19	4d
		4	07/2001	2	19	4d
Detached leaf test	Humid Chamber	4	08/1998	1	19	4d
		4	07/1999	2	19	4d

Fungal isolate

The *Sclerotinia* isolate used in this study was collected in 1997 from naturally infected sunflowers at Sindelfingen (southwest Germany). The isolate was chosen for its high mycelial growth in culture. The sclerotia were surface sterilized with 70% ethanol prior to placement on agar medium (1.5%) containing 40% V 8 vegetable juice. The inoculum was cultured at 20°C in the dark. After two days mycelial germination occurred from the initially placed sclerotia.

Stem infection

Stem inoculation was conducted as previously described by Henn *et al.* (1997). Mycelial disks of 0.5 cm in diameter were cut from the edge of *Sclerotinia* culture and placed on the 4^{th} – 6^{th} internodal stem-section of field grown plants. The mycelium was in direct contact with the epidermis of the stems. The explants were fixed with Parafilm and covered by a transparent plastic film to prevent drying of the inoculum.

Leaf infection

***Field trials*:**

The field leaf tests were conducted as described by Degener *et al.* (1998), and slightly modified with regard to the smaller size of the perennial *Helianthus* leaves. One leaf of the 10^{th} - 12^{th} fully grown leaf pair (corresponding to the fifth to sixth internodal segment) of four plants in a plot was infected by *Sclerotinia* mycelium. Mycelial disks of 0.5 cm in diameter were cut from the edge of *Sclerotinia* culture and placed at the edge of the leaf main vein. The disks were fixed with Parafilm. The fungal explants and leaves were covered by a transparent plastic bag and filled with 2 ml water to maintain a humid atmosphere.

***Detached leaf test*:**

Four fully expanded leaves of the 10^{th} - 12^{th} leaf pair of each genotype were harvested, placed in a humid chamber and mycelial disks of 0.5 cm diameter were placed at the edge of the leaf main vein. The humid chamber consists of a plastic box (70 cm x 40 cm) with water-soaked foam material covered with two layers of Whatmann filter paper No 1. The chambers were covered with transparent plastic film and placed in a humid growth chamber. The detached leaf tests were conducted under controlled environmental conditions at 20°C, 12 h light/day and 90% humidity.

Data recording

The following observations and measurement were recorded.

Leaf lesion:

The length of the necrotic tissue along the leaf main vein. The maximum expansions were measured in cm four days postinfectionally.

***Stem lesions*:**

The lesion length was recorded four days after infection in cm.

***Wilting scoring*:**

Percent of plants showing severe wilting symptoms after mid-stem infection.

***Sclerotial index*:**

Occurrence of sclerotia in the infected tissue (leaf surface, stem pith) measured as % of plants showing sclerotia.

Statistical analysis

Analysis of variance for field randomized block designs and randomized detached leaf tests were performed with data from each genotype and year using plot means calculated from measurement for each trait. According to Degener *et al.* (1998), plants showing no successful infection were excluded from the calculations. Correlations among traits were determined by parametric and non-parametric statistical procedures (Pearson's and Spearman's correlation coefficients, respectively) using mean values of each genotype across the years.

RESULTS AND DISCUSSION

The mean level of successful infection over all experiments exceeded 80%, ranging from 70% for mid-stem infection to 100% for detached leaf test.

Analysis of variance revealed highly significant ($p<0.01$) differences among genotypes for all fungal resistance traits. Despite genotype - year interactions the order of ranking from tolerant to susceptible species for all recorded traits and years remains nearly unaffected. However, there exist remarkable differences between the recorded resistance levels.

The overall results of highly tolerant and susceptible genotypes are depicted in Table 4. The genotypes *H. tuberosus*, *H. giganteus*, *H. grosseserratus* and *H. atrorubens* could be classified as highly susceptible to all traits. Two accessions of *H. maximiliani* (AC 7 and ACM) and *H. salicifolius* showed considerable levels of resistance to mid-stem infection, whereas *H. divaricatus* and *H. maximiliani* AC 7 exhibited resistance to leaf infection. Therefore, *H. maximiliani* AC 7 harbors a considerable level of resistance to both mid-stem infection and leaf infection. All other perennial species examined in this study were classified in a group having intermediary resistance level to *Sclerotinia* mid-stem and leaf infection.

Relationships between mid-stem infection, detached leaf test and field leaf test showed highly significant ($p < 0.01$) positive to strong positive correlation as shown in Table 3. Detached leaf test and leaf test conducted under field conditions were closely associated. Associations between mid-stem lesion length on one side and percent wilted plants and occurrence of sclerotia in the stem tissue on the other also showed highly significant positive correlations ($r = 0.90^{**}$ for percent wilted plants, $r = 0.88^{**}$ for sclerotial index).

Table 3: Phenotypic correlations among perennial *Helianthus* species for three traits of resistance to *Sclerotinia* artificial infection

Trait	Field leaf test	Mid-stem infection
Detached leaf test	0,866**	0,649**
Mid-stem infection	0,622**	

The typical spreading of the fungal mycelia from infected leaves over the petiole to the stem as observed in the natural process of mid-stem infection in cultivated sunflower could only be observed in the highly susceptible genotypes *H. tuberosus*, *H. giganteus*, *H. grosseserratus* and *H. atrorubens*. Furthermore, only the above-mentioned species showed sclerotia both in the affected stem pith and on the surface of the inoculated leaf and additionally the occurrence of severe wilting due to the completely macerated stem tissue.

Tolerant plants usually showed dry, superficial and black lesions with well-defined boundaries around the necrotic tissue, whereas susceptible plants exhibited soft and watery, pale brown lesion without clear boundaries. As reported previously (Cerboncini *et al.*, 2000), resistance of perennial species to mid-stem infection is closely related to the morphological and induced characteristics of the affected tissue. The occurrence of melanin-like dark incrustations in the highly tolerant species could be a practical indicator for the involvement of induced mechanisms e.g. induced phenolics and related enzymatic processes in the resistance reaction.

Sclerotinia sclerotiorum is distributed worldwide and attacks more than 400 genera of the plant kingdom. The disease complex is very complicated and no single source of resistance has been detected up till now (Seiler and Rieseberg, 1997). Furthermore, it does not seem reasonable to expect to evaluate a single gene for resistance to a polyphagous fungus with such a wide host range. Resistance of *Helianthus annuus* to *Sclerotinia* is considered as polygenic trait (Robert *et al.*, 1987). The infected plant parts (leaf, mid-stem, capitulum and root) may differ considerably among themselves in the level of resistance. Therefore, each form of attack can be considered as different disease. In order to obtain varieties with superior overall resistance, it is necessary to simultaneously breed each plant part for resistance.

Several wild species has been identified as potential sources of resistance to *Sclerotinia* ssp. with different results, depending on the evaluated plant part (Thompson *et al.*, 1978; Škorić, 1987; Serieys 1987). In our investigation, we eval-

Table 4: Mean, ranges, sclerotial index and average wilting for traits of resistance to *Sclerotinia* in selected tolerant and susceptible perennial *Helianthus* species

Trait	Year	Tolerant resp. susceptible genotypes [cm]			Sclerotial index mean %	Average wilting %
		Genotypes	Mean	Range		
Detached leaf test	1999	*H. divaricatus*	4.55	4.2 – 4.8 a**	0	
		H. maximiliani AC 7	4.40	4.0 – 4.9 a**	0	
		H. nuttallii	9.18	8.4 – 9.6 b**	74	
		H. tuberosus	8.53	8.1 – 9.4 b**	86	
		H. giganteus	8.60	8.0 – 9.2 b**	83	
	1998	*H. divaricatus*	1.83	1.4 – 2.3 a**	0	
		H. maximiliani AC 7	1.80	1.2 – 2.4 a**	0	
		H. nuttallii	6.80	5.9 – 7.8 b**	50	
		H. tuberosus	6.90	5.8 – 8.1 b**	75	
		H. giganteus	7.40	6.3 – 8.4 b**	45	
Field leaf test	2001	*H. divaricatus*	2.38	1.9 – 2.9 a**	0	
		H. maximiliani AC 7	2.50	1.8 – 3.1 a**	0	
		H. atrorubens	9.05	7.4 – 10.4 b**	56	
		H. tuberosus	7.45	6.4 – 9.4 b**	45	
	1999	*H. divaricatus*	1.83	1.4 – 2.3 a**	0	
		H. maximiliani AC 7	1.80	1.2 – 2.4 a**	0	
		H. nuttallii	6.80	5.9 – 7.8 b**	50	
		H. tuberosus	6.90	5.8 – 8.1 b**	75	
		H. giganteus	7.40	6.3 – 8.4 b**	45	
Field stem test	2000	*H. salicifolius*	1.05	0.6 – 1.6 a**	0	0
		H. maximiliani AC M	1.29	0.8 – 1.7 a**	0	0
		H. maximiliani AC 7	1.20	0.9 – 1.5 a**	0	0
		H. tuberosus	7.90	6.6 – 8.9 b**	90	75
		H. giganteus	7.30	6.0 – 9.1 b**	100	100
		H. grosseserratus	8.24	7.1 – 9.1 b**	90	75
	1999	*H. salicifolius*	0.30	0.2 – 0.4 a**	0	0
		H. maximiliani AC M	0.89	0.5 – 1.2 a**	0	0
		H. maximiliani AC 7	1.00	0.7 – 1.2 a**	0	0
		H. tuberosus	6.60	4.8 – 8.6 b**	75	50
		H. giganteus	6.83	6.1 – 8.0 b**	90	75
		H. grosseserratus	6.08	5.1 – 7.3 b**	90	50
	1998	*H. salicifolius*	0.80	0.3 – 1.6 a**	0	0
		H. maximiliani AC M	1.03	0.4 – 1.7 a**	0	0
		H. maximiliani AC 7	1.05	0.5 – 1.7 a**	0	0
		H. tuberosus	9.10	6.9 – 11.4 b**	100	100
		H. giganteus	9.23	6.3 – 11.7 b**	90	100
		H. grosseserratus	8.10	5.9 – 10.4 b**	75	90

uated one population accession of *H. maximiliani* comprising a considerable level of resistance to *Sclerotinia* mid-stem and leaf infection. Such genotypes with superior levels of resistance to the different forms of fungal attack are of most desirable quality with respect to introgression of resistance traits related to *Sclerotinia* disease into the *Helianthus annuus* genome.

Since all resistant genotypes evaluated in this trial were sexually incompatible with the *Helianthus annuus* genome (Table 1), newly developed biotechnological methods (Wingender *et al.*, 1996; Henn *et al.*, 1998; Binsfeld *et al.*, 2000) may prove to be useful in overcoming the severe injuries caused by *Sclerotinia sclerotiorum* in cultivated sunflowers.

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RESISTENCIAS CONTRA *Sclerotinia sclerotiorum* (Lib.) de Bary EN LA POPULACIÓN NATURAL DE GIRASOL (*Helianthus*)

RESUMEN

Sclerotinia sclerotiorum (Lib.) de Bary llego a ser una de las enfermedades de girasol más importantes. El hongo puede dañar todos los órganos del girasol (*Helianthus annuus*), en particular el capitulo, las hojas y los tallos. En el presente investigatiòn experimental de especies silvestres de *Helianthus* perennes se investigó a traves de infección artificial sobre la resistencia contra la infección de la *Sclerotinia*. Las variedades investigadas mostraron grandes differencias significativas en su reacción de resistencia contra infecciones de tallo y hoja. Se pudo evaluar tres especies con resistencia elevada contra infecciones de tallo y dos de hoja. Como existen resistencias considerables contra el hongo *Sclerotinia* en populaciones perennes del género *Helianthus* y todos los genotipos resistentes no son sexualmente compatible con los populatciones de *H. annuus*, alcanzan una importancia especial de empleados nuevos metodos biotecnologicos de crianza para la transferencia de las resistencias existentes.

RÉSISTANCES CONTRE *Sclerotinia sclerotiorum* (Lib.) de Bary EN LA POPULATION NATUREL DU TURNESOL (*Helianthus*)

RÉSUMÉ

Sclerotinia sclerotiorum (Lib.) de Bary est l´une des plus importantes maladies du tournesol (*Helianthus annuus*). Le pathogène peut attaquer tous les organes du tournesol, en particulier le capitule, les feuilles et les tiges. On a déterminé, par infections artificielles, les niveaux de résistance de l´espèce sauvage contre l´infection de la feuille et la tige. L´analyse statistique a montré qu´il avait considérable différences signifiante entre l´espèce concernant leur degré de tolérance. Nous avons évalué trois espèces avec des résistances supérieures contre l´infection de la feuille et deux qui montre considérables niveaux de tolérance de l´infection a la tige. Parce que toutes des génotypes évaluées ne sont pas compatibles avec le génome annuel du tournesol, le développement des nouveaux méthodes biotechnologique atteints une importance particulière pour améliorée les degrés de la résistance contre l´attaque de *Sclerotinia*.