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PHOMOPSIS RESISTANCE ON LEAVES AND STEMS OF *Helianthus petiolaris*

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SUMMARY

Wild species of sunflower (*Helianthus annuus*) play an important role in the genetic improvement of this oleaginous crop since they have different characters of economic importance to be transferred into the cultivated form using interespecific hybridization. The purpose of our present research program is to preserve and characterize the genetic resources of both naturalized and exotic wild sunflowers having an agronomic interest. One of the first steps was to describe different accessions of annual wild species for biotic stress in order to detect potential resistance sources. *H. petiolaris* is one of the wild sunflower species that can be found in Argentina and we had selected before a certain number of these species on the basis of their *Sclerotinia sclerotiorum* responses on leaves and stems. This genetic material was then inoculated on leaves and stems but with *Phomopsis helianthi*, a fungus which produce stem canker in sunflower. This work shows the variability of brown necrotic responses after Phomopsis inoculations on *H. petiolaris* leaves and stems. Some half-sib families were detected to have good disease tolerance to Phomopsis as well as Sclerotinia and they will be backcrossed in order to increase the diversity for this disease resistance in the cultivated germplasm.

Key words: **sunflower, wild species, diseases, Phomopsis resistance, Sclerotinia resistance**

INTRODUCTION

Breeders make use of wild species of the *Helianthus* genus to find sources of disease resistance to be employed in cultivated sunflowers (*H. annuus*) (Fick and Miller, 1997). *H. petiolaris* is a wild species which is widely distributed and naturalized in Argentina, particularly in the central-west of the sunflower growing region (Cantamutto and Poverene, 2002). We have not found literature sources characterizing this wild species nor evaluating its potential use in sunflower breeding pro-

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grams. Therefore, a description of this abundant genetic resource in our country was considered of interest, particularly for disease resistances.

The stem canker in sunflower is a fungal disease caused by *Phomopsis helianthi* (Gulya *et al*., 1997). Although sunflower crops with serious attacks of this pathogen have not been detected in Argentina yet, the disease produced severe damages in sunflower crops in Uruguay (INIA, 2006) and it is always present in the sunflower crops grown in the Mesopotamia region of our country (Fálico *et al*., 2003). Thus, the main objective of this work was to evaluate the responses of some *H. petiolaris* populations, naturalized in Argentina, to *P. helianthi* inoculation. The relationship between both *P. helianthi* and *S. sclerotiorum* responses is also shown.

MATERIALS AND METHODS

During 2005, 105 half-sib families of 6 *H. petiolaris* accessions were evaluated for their response to *S. sclerotiorum* inoculation of leaves and stems (Cáceres *et al*., 2005). Then we selected three groups of 5 families each, according to their relative performance (*i.e*., high, intermediate and low) in Sclerotinia resistance tests. In 2006, 25-day seedlings were transplanted in the UIB's experimental field, in a randomized complete block design with two replicates. The sunflower hybrid DK 3881, of well-known reaction to Phomopsis inoculation (Castaño *et al*., 1997; Verschoor *et al*., 1998), was grown in adjacent plots.

Ph1, a *P. helianthi* isolate obtained from diseased stems of cultivated sunflower in INTA Paraná, Entre Ríos, was used. Only 29 families were inoculated, using the protocols described by Castaño *et al*. (1993). Agar disks of 7 mm in diameter contained the young mycelium of the pathogen. Two leaves per plant in 50% plants/plot were inoculated 70 days after transplanting. One week later, two stems per plant were inoculated in the remaining plants. The resistance check was inoculated on the same dates as the *H. petiolaris* plants. Sprinkler irrigation of around 5 mm/day was applied until Phomopsis symptoms were scored. Brown necrotic lesions on leaf veins and stems were measured 12 days after inoculation. The inoculated leaves and stems were removed and destroyed right after the score.

Only data higher than 7mm of Phomopsis lesions were considered for statistical analyses. Mean values per plant and plot was calculated. Analysis of variance and F-test were made to detect genotype effects (Reza-Hoshmand, 1998). The least significant difference test (LSD) was used to differentiate the half-sib families by Phomopsis response and three groups with different levels of resistance were formed. Two of them (*e.g*., groups G1 and G2) contained half-sib families with values of brown necrotic lesions statistically similar to either maximum or minimum values, respectively. The half-sib families placed in the third group, G3, had intermediate values of brown necrotic lesions.

RESULTS AND DISCUSSION

The 29 half-sib families of *H. petiolaris* showed an average of brown necrotic lesions on leaves and stems of 20 and 24 mm, respectively (Table 1). The hybrid DK 3881 had the mean values of 16 mm on leaves and 47 mm on stems. Estimated coefficients of variability were similar to those calculated after Phomopsis inoculations on cultivated sunflowers (Castaño *et al*., 1997; Verschoor *et al*., 1998; García *et al*., 2000).

Table 1: Means of brown necrotic lesions on leaves and stems after Phomopsis inoculation of 29 half-sib families of *Helianthus petiolaris* naturalized in Argentina

Half-sib family no.	Phomopsis lesions	
	Leaves, (mm)	Stems, (mm)
3	23*	27
9	20	30
11	19	22&
13	22*	27
15	24*	15&
16	20	19&
22	22*	18&
24	21*	28
28	20	28
30	20	23&
32	22*	21&
34	19	16&
36	21*	26
42	20	32*
45	19	29
48	25*	32*
49	22*	21&
50	24*	11&
54	20	23&
57	17&	19&
64	20	16&
68	14&	27
69	20	45*
71	17&	22&
75	17&	19&
79	21*	22&
87	19	27
99	22*	33*
104	16&	-
Mean	20	24
CV (%)	12	29
$LSD_{.05}$	4	14

Note: Relative resistance level: *low, intermediate, &high.

Analyses of variance detected significant ($\alpha<0.05$) differential responses of the half-sib families to both Phomopsis resistance tests, on leaves and stems.

The LSD value for the leaf test was 4mm. Twelve genotypes were placed in group G1 and they present the lowest level of resistance in our trial. Five half-sib families (*e.g.* 57, 71, 75, 104, 68) showed the best performance (G2) and similar lesion values. The intermediate group, G3, included 12 genotypes.

The LSD value was 14 mm for the stem test. Four half-sib families were in G1. Fifteen genotypes were grouped in G2 (30, 54, 79, 11, 71, 32, 49, 16, 57, 75, 22, 64, 34, 15 and 50). Nine genotypes were placed in G3. In this resistance test, all *H. petiolaris* half-sib families showed lower necrotic lesion values than the cultivated DK 3881.

Spearman correlation coefficient of ranks ($r_s=0.02$) was not significant. This result suggests that a general increase of lesion length on leaves was not associated with either an increase or a decrease of lesions on stems. In spite of that, three half-sib families (57, 71 and 75) were placed in group G2 after the two Phomopsis inoculations. These genotypes could have common resistance genes controlling brown necrosis lesions on both, leaves and stems.

Of those three half-sib families with high performance to the Phomopsis infections, two (57 and 75) have been included in this experiment on account of their good performance in Sclerotinia inoculations during 2005. This suggests the possibility to detect genotypes of *H. petiolaris* with adequate level of resistance to both Phomopsis and Sclerotinia infections.

Further experiments are needed to evaluate the repeatability of the results. In spite of this, our sunflower breeding group will multiply next summer some *H. petiolaris* families by sib crosses. Afterwards, they will be crossed with inbred lines of cultivated sunflower in order to valuate the transmissibility of Phomopsis resistance to the interspecific offspring.

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RESISTENCIA HACIA *Phomopsisu* EN HOJAS Y TALLO DE LA ESPECIE *Helianthus petiolaris*

RESUMEN

Las especies silvestres de girasol (*Helianthus annuus*) tienen un papel significante en la mejora genética de este cultivo oleaginoso – ellas poseen diferentes características de importancia económica que pueden ser transferidas en la forma cultivada mediante la hibridación interespecies. La finalidad de esta investigación es de conservar y caracterizar los recursos genéticos, de las especies de girasol silvestre, naturalizadas, tanto como exóticas, que son del interés económico. Uno de los primeros pasos fue descripción de las especies silvestres anuales en la colección, en el sentido de reacción al estrés biótico, y con el fin de encontrar unas fuentes de resistencia eventuales. *H. petiolaris* es una de las especies silvestres de girasol que puede encontrarse en La Argentina, y nosotros hemos elegido un cierto número de esas especies, sobre la base de la reacción de sus hojas y tallos hacia *Sclerotinia sclerotiorum*. Las hojas y los tallos de ese material genético fueron luego infectados artificialmente con *Phomopsis helianthi*, un hongo que causa el cáncer del tallo en girasol. Este trabajo demuestra que existía la variabilidad en la reacción necrótica hacia *Phomopsis* en las hojas y tallos de *H. petiolaris*. Algunas familias que están en semiparentesco, demostraron buena tolerancia hacia *Phomopsis* tanto como hacia *Sclerotinia* y ellas serán reversiblemente cruzadas, para aumentar la diversidad de esta resistencia en el germoplasma cultivado.

RÉSISTANCE AU *Phomopsis* DANS LES FEUILLES ET LES TIGES DE L'ESPÈCE *Helianthus petiolaris*

RÉSUMÉ

Les espèces sauvages de tournesol (*Helianthus annuus*) jouent un rôle important dans l'amélioration génétique de cette culture oléagineuse car ils possèdent différentes caractéristiques d'importance économique qui peuvent être transmises à la forme cultivée par hybridation interspécifique. Le but de notre actuel programme de recherche est de conserver et de définir les ressources génétiques autant des tournesols naturalisés que des espèces sauvages exotiques ayant un intérêt pour l'agronomie. L'une des premières étapes a été de décrire les espèces sauvages annuelles de la collection du point de vue de la réaction au stress biotique dans le but de détecter des sources potentielles de résistance. *H. petiolaris* est l'une des espèces sauvages de tournesol que l'on peut trouver en Argentine et nous en avons choisi un certain nombre pour la réaction de leurs feuilles et de leurs tiges envers le *Sclerotinia sclerotiorum*. Les feuilles et les tiges de ce matériel génétique ont ensuite été infectées artificiellement de *Phomopsis helianthi*, champignon qui provoque le chancre de la tige chez le tournesol. Cet article montre la variabilité des réactions nécrotiques brunes après des inoculations de *Phomopsis* sur les feuilles et les tiges de *H. petiolaris*. Certaines familles de demifratrie ont montré une bonne tolérance envers le *Phomopsis* ainsi qu'envers le *Sclerotinia* et elles seront rétrocroisées pour que la diversit de la résistance à cette maladie soit augmentée dans le plasma germinatif cultivé.