HELIA, **21**, Nr. 28, p.p. 69-74, (1998) UDC 632.4:633.854.78

Plasmopara halstedii IN SUNFLOWER (*Helianthus annuus* L.): A NEW METHOD FOR TESTING RESISTANCE

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Received: September 18, 1997
Accepted: April 20, 1998

SUMMARY

In sunflowers, the incorporation of resistance to the fungus *Plasmopara halstedii* generally involves the use of methodologies that are not very accessible due to their high cost. The use of simple techniques would permit easier access to cultivars resistant to this parasite. This paper describes an approach combining two techniques for an early screening test for downy mildew resistance in sunflower. The whole-seedling immersion technique was applied to immature germinated seeds of different sunflower cultivars in a suspension of *Plasmopara halstedii* zoosporangia. This methodology did not produce any negative or modifying effect on resistance to the mildew, it was capable of producing similar disease reactions when the genotypes under evaluation were naturally infected by this pathogen in other experiments. It is concluded that this new and innovating technique allows resistant sunflower genotypes to be identified at very low cost in breeding programs.

Key words: **Breeding, downy mildew, immature seed germination, *Plasmopara halstedii*, resistance test, sunflower.**

INTRODUCTION

Downy mildew is one of the most important diseases affecting the sunflower crop (*Helianthus annuus* L.). This disease appears when *Plasmopara halstedii* (Farl.) Berlesse & de Toni, an obligate parasite, infects susceptible genotypes. Mildewed sunflower seedlings can die before or after emergence, thus decreasing the stand of plants. Infected adult plants can show different degrees of dwarfing and produce little if any seeds (Sackston, 1992; Mouzeyar *et al.*, 1994).

In some countries there is a chemical method capable of controlling disease effects. However, the appearance of *Plasmopara halstedii* isolates resistant to met-

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alaxyl (Albourie, 1996) raises the question of whether this chemical control would be effective in the future. Use of genetically resistant cultivars is preferred because they constitute a less costly, more reliable control and they will not pollute the environment.

Sunflower resistance to *Plasmopara halstedii* is controlled by major dominant genes called "*Pl* " (Vear and Leclercq, 1971). In susceptible cultivars, the incorporation of resistance is obtained by crossing genotypes that have a *Pl* gene followed by a backcross and selection program. This method normally takes between six to seven crop cycles, which corresponds to an equal number of years when only one generation a year is grown..

Table 1: Number of immature and scarified seeds cultivated and germinated in Petri dishes of different sunflower cultivars and response to inoculations with *Plasmopara halstedii*

	Number of seeds			Disease reaction	
Cultivar	cultivated	germinated	%(1)	S(2)	R(2)
B1	1610	982	61	982	0
FIBA	1520	532	35	532	0
U1	58	50	86	50	0
U8	56	51	91	51	0
U46	60	53	88	53	0
HA335	49	42	86	0	42
HA338	41	40	98	0	40
RHA34054	49	91	0	49	

(1) [number of germinated seedlings / number of cultivated seeds]
(2) expressed as number of germinated and inoculated seedlings : S=susceptibility, R=resistance

In order to reduce the time necessary for the incorporation of resistance to this pathogen, *in vitro* techniques have been used to produce several generations per year (Pereyra *et al.*, 1991; Francke, 1995). However, the use of *in vitro* techniques requires special equipment (i.e. laminar flow chamber, test tubes, culture medium, etc.) and trained personnel. Therefore, the use of methodologies that can both simplify the work of improving resistance to this pathogen and reduce costs, should be considered as an useful alternative.

This work shows the use of the immature seed germination technique in the evaluation of *Plasmopara halstedii* resistance in sunflower.

MATERIALS AND METHODS

The parasite: *Plasmopara halstedii* was isolated from mildewed plants which were collected from the crops in South-East Buenos Aires province of Argentina. Fresh inoculum was obtained by multiplying the fungus on the inbred line HA 89, which is susceptible to all the known races (Gulya *et al.*, 1991).

The host: Different cultivars were used: 1) a synthetic variety (B1), of local origin, formed by the following inbred lines: HA 89, HA 124, HA 234, HA 290, HA 301, HA 302 and HA 303 from the USA, and CM 400 from Canada; 2) an open pollinated population (FIBA), originated from IMPIRA-INTA an old Argentinian variety; 3) three inbred lines, U 8, U 28 and U 46 selected in Balcarce from materials of American and Canadian origin, and 4) three inbred lines selected in USA: HA 335, HA 338 and RHA 340.

The synthetic B1, the population FIBA and the lines U 8, U 28 and U 46 are susceptible to the local race/s of *Plasmopara halstedii* (Gorostegui, 1971; Kesteloot, unpublished results; UIB, 1993). The American lines HA 335, HA 338 and RHA 340 are resistant to the seven known races of *Plasmopara halstedii* (Gulya *et al.*, 1991).

Immature seed germination technique: The immature seed germination technique used is described by Torresán *et al.* (1996). Fifteen days after anthesis, several seeds were taken from various open pollinated capitula of B1 and FIBA, and from different self-pollinated capitula of the six inbred lines. These seeds were scarified by cutting off one third of the blunt end and by eliminating part of the pericarp, seminal membrane and embryo. The scarified seeds were soaked in 100 mg/g of gibberelic acid for one hour and then washed with distilled water. After this, the scarified seeds were placed in unsterilized petri dishes on a filter paper and a fine layer of cotton, both of which were moistened with distilled water. Then the seeds were incubated in the laboratory at room temperature under low intensity light.

Inoculation technique and disease assessment: The whole-seedling immersion technique (Miller and Gulya, 1987) was utilized. All scarified seeds with a radicle of 10 to 15 mm in length were soaked for 3 to 4 h in an inoculum suspension containing approximately 2×10^4 zoosporangia/ml of distilled water. The inoculated seedlings were grown in trays containing sterilized soil. These trays were placed in a greenhouse for 15 days with a temperature of 20 to 25°C. When the first true leaves in seedlings were 2 cm long, the trays containing them were covered with a transparent plastic for 16 to 18 h in order to increase the relative humidity and to accelerate the appearance of symptoms.

The host response was evaluated following Mouzeyar *et al.* (1993). A compatible reaction (susceptibility) was scored when the cotyledons and the first pair of seedling leaves showed the presence of fungal sporulation. An incompatible reaction (resistance) was scored when no symptoms were seen on both cotyledons and first pair of seedling leaves.

RESULTS AND DISCUSSION

The number of immature and scarified seeds germinated as well as the percentage of germination are presented in the left hand part of Table 1. The highest values in germination were obtained with the inbred lines (average= 90%); the lowest ones

were those of the FIBA (35%). The synthetic B1 had intermediate values (61%). Similar results were obtained with these genotypes by Torresán (1992). The variability of the percentage of germination of immature sunflower seeds found in this experiment is similar to at obtained by Torresán *et al.* (1996). The number of seedlings obtained for the different genotypes in both experiments is adequate to implement this technique in breeding programs concerned with the evaluation or improvement of the resistance to *Plasmopara halstedii*.

The responses to the inoculations produced with *Plasmopara halstedii* through the whole-seedling technique of the cultivars evaluated are listed in the right hand half of Table 1. All the inoculated seedlings of the materials selected in Balcarce (B1, FIBA, U8, U28 and U46) showed some fungal sporulation on cotyledons and the first pair of true leaves; these cultivars were classified as susceptible. These disease reactions are similar to those obtained from the same cultivars naturally infected by *Plasmopara halstedii* in previous work (Gorostegui, Op.c.; Kesteloot, Op.c.; UIB, Op.c.).

The American lines HA335, HA338 and RHA340 were considered resistant due to the complete lack of sporulation on the first true leaves. Therefore, all these results confirm the lack of resistance genes to the local race of *Plasmopara halstedii* in B1, FIBA, U8, U28 and U46, and the resistance of the American lines when they are tested using this methodology.

The spores of *Plasmopara halstedii* stored at 4°C for 5 to 7 days maintain their infectious capacity (Gulya *et al.*, 1991). Therefore, the technique presented here could be used in situations where there is a lack of synchronization between the time of sporangia production and that of the inoculation.

With the introduction of a *Pl* gene in susceptible material and after the first backcross, it is necessary to perform a test on the offspring in order to identify the resistant seedlings in the population. The method described can differentiate susceptible from non-susceptible plants. If we keep in mind that in vertical resistance, such as in the sunflower-*Plasmopara halstedii* interaction, the observed resistance at the seedling level is a positive test for resistance in adult plants (Day, 1974), only the seedlings that do not show sporulation should be transplanted in order to continue backcrossing.

The seedlings obtained through immature seed germination are vigorous and strong; these same seedlings can be transplanted directly in the field or in a greenhouse where crossings are performed. This technique can be performed by people with little training because there is no *in vitro* step. Due to the relatively small number of procedures to be performed with this technique, a large number of plants can be evaluated for resistance.

With this technique it is possible to obtain resistant adult plants producing a satisfactory number of seeds. The life cycle of the plant can be reduced by 25 to 30 days when compared with the conventional techniques.

The use of the immature seed germination and whole-seedling techniques have allowed us to reproduce the expected reactions of susceptibility and resistance to *Plasmopara halstedii* in sunflower seedlings. Thus, the method described here provides similar results as when the sunflower plants are naturally infected by this pathogen.

ACKNOWLEDGEMENT

The authors thank Dafne Rodríguez for her assistance in translating the manuscript into English.

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Plasmopara halstedii EN GIRASOL: UN NUEVO MÉTODO PARA EVALUAR LA RESISTENCIA.

RESUMEN

En el girasol, la incorporación de resistencia al hongo *Plasmopara halstedii* involucra generalmente el uso de metodologías no muy accesibles debido a su costo excesivo. Es por ello que la utilización de técnicas nuevas capaces de sortear esta barrea permitiría que el proceso de incorporación de genes de resistencia a este parásito sea menos oneroso. Este artículo describe una estrategia, que combina dos técnicas diferentes aplicadas sobre plántulas de girasol, que puede ser utilizadas en una selección precoz por la resistencia al mildiú. La técnica de inmersión de la semilla entera, en una suspensión acuosa conteniendo los zoosporangios del hongo, fue aplicada a semillas inmaduras germinadas de diferentes variedades de girasol. Estas metodologías no produjeron efectos negativos sobre la resistencia al mildiú, dado que con su ayuda se ha podido reproducir, en las plántulas evaluadas, reacciones similares a aquéllas producidas sobre plantas, del mismo genotipo, pero infectadas naturalmente a campo.

Plasmopara halstedii EN TOURNESOL: UNE NOUVELLE MÉTHODE POUR TESTER LA RÉSISTANCE.

RÉSUMÉ

L'incorporation de la résistance à *Plasmopara halstedii* en tournesol implique l'utilisation de quelques méthodologies dont leurs protocoles ne sont pas très faciles à mettre en pratique à cause de son coût élevé. C'est ainsi que l'emploi de techniques nouvelles capables de contourner cette barrière ferait que le procès d'incorporation des gènes de la résistance à ce parasite soit moins cher. Cet article a pour but décrire l'utilisation de deux techniques en combinaison qui peuvent aider à trier des génotypes, dans le stade de plantule, résistants et dans générations précoces. La technique qui prenne en compte l'imbibition de la graine entière, dans une suspension aqueuse contenant les zoosporanges du pathogène, a été appliquée aux semences immatures germées des différents variétés de tournesol. Chez le tournesol, ces méthodologies n'ont pas produit un effet nuisant la résistance au mildiou puisque elles ont aidé à reproduire de réponses, sur les plantules évaluées, similaires à celles observées sur des plantes, du même génotype, mais attaquées naturellement. Ces techniques nouvelles, et à la fois innovatrices, permettent donc d'identifier des génotypes résistants à *Plasmopara halstedii* à moindre coûts dans les programmes de sélection.