Research Journal of Agricultural Science, 57 (3), 2025; ISSN: 2668-926X

http://doi.org/10.59463/RJAS.2025.3.41

COMPARATIVE EVALUATION OF AN OPTIMIZED PROTOCOL FOR TESTING RESISTANCE TO DOWNY MILDEW IN SUNFLOWER RACE 730

Denis PITARIU[1] , Emilian ONISAN[1*] , Marinel Nicolae HORABLAGA [1,2],Petru RAIN[2]
[1] University of Life Sciences "King Mihai I" from Timisoara, Faculty of Agriculture, Romania, Calea Aradului, 119, Timisoara, Romania
[2]Agricultural Research and Development Station Lovrin,*Strada Principală nr. 200, Loc. Lovrin, jud. Timiș.*
**Corresponding author:emilian.onisan@usvt.ro*

Abstract. *Sunflower downy mildew, caused by Plasmopara halstedii, is one of the most aggressive diseases affecting this crop, causing significant losses ranging between 20% and 80%, depending on the pathogen race, the crop development stage, and the hybrid's susceptibility. Curently, sunflower breeding programs focused on obtaining resistance genes, which has induced pressure for the development of increasingly aggressive races. While initially we spoke of races such as race 100 or 300, currently we can discuss race 730, 734, and even in some cases, race 774 or 777. Race 730, used in our study, is among the most widespread races in Europe, exhibiting a high capacity for adaptation and for overcoming the resistance conferred by the genes Pl1, Pl2, Pl3, Pl4, Pl9, Pl10, Pl11, and Pl12, which are not sufficient to provide the genotype with resistance to this race.In our study, genotypes derived from interspecific crosses of wild sunflower species were selected. Following the research, protocols were established to determine the optimal timing for embryo inoculation, with the goal of improving the efficiency of the methods used for detecting resistance or susceptibility. The results obtained indicated a higher success rate of inoculation when the embryo radicle was between 0.5 and 2 mm, with the inoculation rate gradually decreasing as the embryo developed. The study also allowed for the creation of new sunflower genotypes resistant to race 730, with the prospect that these lines may be registered in international gene banks in the future.*

Keywords: *Plasmopara halstedii ,race 730 ,phenotypic screening ,optimized protocol ,plant breeding*

INTRODUCTION

Sunflower (*Helianthus annuus* L.) is a crop of global importance, ranking fourth among the main oilseeds and sources of vegetable oil in the world. In 1975, global production was approximately 10 million tons, coming from 9.6 million hectares [2]. By 2018, this had grown to 52 million tons across 27 million hectares, with production increasing twice as fast as the cultivated area, thanks to improved yields obtained through hybrid varieties and better agronomic practices [16,17]. The main producing countries have changed over time, with Ukraine, Russia, and Argentina now accounting for over half of global production.

Plasmopara halstedii, the pathogen that causes sunflower downy mildew, is one of the most destructive pathogens affecting sunflower crops worldwide [6]. Biodiversity studies suggest the pathogen originated in the wild sunflower center of diversity in South America [12]**.** Its effects are manifold, influencing plant health, crop yield, and the sustainability of sunflower production. Early infections can cause the death of seedlings before establishment, significantly reducing the plant population in a field. Infected plants often exhibit reduced growth, resulting in stunting and hampered development. Yellowing or whitening of the leaves, especially along the veins, is common [9]. Furthermore, white sporulation can sometimes be observed on the underside of the leaves and cotyledons. Severe infections can lead to the production of sterile or unviable seeds, directly impacting production therefore, molecular protocols have been developed to detect the pathogen in seeds [13]. The pathogen can spread through the plant's vascular system, causing widespread symptoms and, sometimes, plant death[8]. *P. halstedii* evolves rapidly, generating more aggressive races that can overcome existing resistance genes in commercial cultivars

http://doi.org/10.59463/RJAS.2025.3.41

[7,10,11], which complicates disease management [4]. The pathogen has acquired resistance to frequently used fungicides, such as metalaxyl, limiting chemical control options[1].

P. *halstedii* spores can persist in the soil for many years, serving as a reservoir for future infections and complicating crop rotation strategies. *P. halstedii* poses a severe threat to sunflower production, causing plant death, stunting, yield losses, and the rapid breakdown of resistance strategies, making the adoption of integrated and innovative approaches essential for sustainable crop. The pathogen begins its life-cycle by the germination of oospores that have survived in the soil, infecting the sunflower roots. Due to the persistent and evolving nature of *P. halstedii*, effective management requires an integrated approach that combines genetic, chemical, cultural, and biological methods.

Breeding sunflowers for resistence to *P.halstedii* is the most durable control method. Over 40 dominant resistance genes (Pl) have been identified and introduced into commercial varieties, but the pathogen frequently evolves new races that can overcome single-gene resistance [6]. Combining multiple resistance genes and introducing quantitative (horizontal) resistance can enhance durability. Coating seeds with fungicides, particularly oxathiapiprolin, offers powerful protection against various *P. halstedii* isolates and is more effective than older fungicides, such as mefenoxam [1]. Alternating with non-host crops for at least four years reduces the viability of oospores in the soil and the risk of disease.

Resistance to *P. halstedii* is classically classified into two broad physiological systems. Type 1 resistance acts extremely early, before the pathogen can establish feeding structures (haustoria). The plant essentially prevents the oomycete from gaining entry[5]. Type 2 resistance allows the pathogen to enter, and sometimes even form haustoria, but blocks its systemic spread before it can reach and colonize the meristem. Type 2 resistance is often associated with quantitative or partial resistance, though some Pl genes also produce Type 2 responses depending on the sunflower genotype × pathogen race combination[5]. Over 20 major resistance genes (Pl) have been identified and mapped (e.g., Pl1, Pl2, Pl5, Pl6, Pl8, Pl13, Pl15, Pl22, Pl36, Pl37, Pl38). These genes confer resistance to specific pathotypes of *P. halstedii*, but new races of the pathogen can overcome the resistance offered by a single gene, making gene stacking (pyramiding) and continuous breeding essential[4].

In our study, we aim to establish the optimal protocol for sporulation induction (i), HA89 susceptible lines were used to determine the best inoculation stage of germinated seeds and the most suitable temperature for spore development. Furthermore, our study evaluates downy mildew resistance in interspecific populations (ii) of HA89 x *Helianthus tuberosus* and HA89 x *Helianthus praecox* (from USDA) . These populations were developed using biotechnological strategies such as immature embryo rescue to facilitate gene transfer [15, 18, 20] ,screened to identify the presence and type of resistance genes.

MATERIAL AND METHODS

Germplasm

The genetic material used in this study was obtained from the USDA Gene Bank and consisted of interspecific hybrids derived from crosses between the cultivated line HA89 and wild *Helianthus* species. The analyzed genotypes were developed from decedents of several origians of *Helianthus praecox* and *Helianthus tuberosus* (Table 1).

Table 1 .

Genotypes origins used for the experiment.

Genotype code	Pedigree
E0201	HA89 x *Helianthus praecox* (PI 468848)
E0202	HA89 x *Helianthus praecox* (PI 468860)
E0203	HA89 x *Helianthus praecox* (PI 494604)
E0204	HA89 x *Helianthus praecox* (PI 494600)
E0205	HA89 x *Helianthus praecox* (PI 494601)

http://doi.org/10.59463/RJAS.2025.3.41

E0206	HA89 x *Helianthus praecox* (PI 494602)
E0207	HA89 x *Helianthus praecox* (PI 494603)
E0208	HA89 x *Helianthus tuberosus* (PI 650095)
E0209	HA89 x *Helianthus tuberosus* (PI 650099)
E0210	HA89 x *Helianthus tuberosus* (PI 650107)

Experimental design for protocol optimization

In order to determine the best protocol procedure , HA89 –public susceptible lines were used to determine the best period to inoculate the germinated seeds and the best temperature to induce sporulation. Futthernore, to achieve this objective we used 100 seeds of sunflower.

Two types of germinated seeds were used for the experiment to determine the best protocol for inoculation. For the first type, the seeds were germinated for a period of 4-5 days ,until the radicle reached a size of 0,5-2,0 mm. For the second type the seeds were germinated for a period of 7-10 days ,until the radicle reached a size of 4-6 mm.

The pathogen used was *P. halstedii* Race 730 ordered from France, Geves company.

After 14 days of growth – the sporulation was induced in several temperatures to determine the best temperature of sporulation:a temperature 16℃ ,with a 12/12 photo-period ,a temperature of 18℃ with a 12/12 photo-period and an temperature of 20℃ with a 12/12 photo-period

Inoculation with *P. halstedii* took place when the sunflower seeds were at the germination stage . Inoculation was performed at this stage so that the infection was as obvious as possible.

The inoculation of the seeds was done in the fridge at the temperature of: 18℃ for 6 h in full bath of spores of DM collected from the infected plants.

Experimental design for germplasm testing

For the first objective, we used line HA89 crossed with two different wild species. All descendants were developed until F2 population in greenhouse. The F2 seeds of each descendant was tested in order to determine the types of resistance and genes implicated. According to the chi-square(χ^2) test the ratio of the genes in F2 was determine. Genotypes with dominant genes where advanced furthere, in order to mantain the genes.

Statistical analyses

For the first objective, concerning the optimization of the working protocol, the percentage sporulation success rates were monitored. Based on the collected data, interpretations and optimizations of the protocol were performed. For the evaluation of genotypes from wild species, segregation in the F2 generation was studied. The chi-square(χ^2) test was applied in order to evaluate the conformity of the experimental data with the theoretical Mendelian ratios (3:1, 1:1, 1:3, and 15:1), to determine the types of genes involved in resistance.

RESULTS AND DISCUSSIONS

According to the obtained results, interpretations were possible in terms of the inoculation time of the seeds but also of the temperatures. The temperatures used were 16℃,18℃,20℃. The inoculation for type A seeds was when the radicle length was 0.5-2mm. And for type B seeds ,the radicle was between 4-6mm long.

The results of type A germination (0.5-2.0mm) according to table 2 were clearly superior to the inoculation times of type B seeds.

Table 2.

Results regarding efficiency in inoculation for seed type A.

Seed Type	**Sporulation Temperature (°C)**	**No. of Tested Seeds**	**% Successful Infection**	**Observations**
Type A	16	100	45%	Moderate sporulation;

http://doi.org/10.59463/RJAS.2025.3.41

Type A	18	100	92%	Best sporulation
Type A	20	100	63%	Reduced spore viability

Note: Type A represent seeds germinated of the period of 4-5 days with a radicle of 0.5-2 mm length

The percentages of successful infestations in type A vary from 45% to 92% depending on the sporulation induction temperatures. While in type B it varies from 30% to 58%.

The best results were obtained when the sporulation temperature was 18℃, the radicle length was 0.5-2mm and the germination period was 4-5 days. These factors resulted in an infection rate of 92%.

Table 3.

Results regarding efficiency in inoculation for seed type B.

Seed Type	Sporulation Temperature (°C)	No. of Tested Seeds	% Successful Infection	Observations
Type B	16	100	30%	Low infection rate;
Type B	18	100	58%	Moderate infection;
Type B	20	100	41%	Reduced sporulation

Note: Type B represent seeds germinated of the period of 7-10 days with a radicle of 4-6 mm length

In objective II from the F2 generation of the created interspecific population , we observed the following types of genes as a result of the chi-square test , performed on the analyzed population, regarding the ratio of susceptible and resistent plants to plasmopara.

The results show that EO202 and EO209 lines posses dominant genes , making them the most interesting and promising for further breeding improvement. The segregation analysis showed unique genetic patterns among the populations. Lines E0202 and E0209 were distinctly aligned with a 3:1 ratio, providing strong evidence for the existence of a single dominant resistance gene.

Conversely, lines E0201, E0203, and E0207 conform to a 1:1 distribution, indicating regulation by one heterozygous gene or a likely backcross situation. In the meantime, resistance in lines E0208 and E0210 seems to be recessive, as suggested by their conformity to a 1:3 ratio.

Ultimately, the leftover lines (E0204–E0206) did not fit these straightforward models; their behavior indicates a more intricate inheritance, potentially involving epistatic interactions or polygenic effects .

Table 4.

Results regarding segregation ratio on resistant and susceptible on P.halstedii of sunflower genotypes of *Helianthus praecox.*

Code	Mendelian ratios				The ratio closest to the data ($\chi^2 < 3.84$)
	3:1	**1:1**	**1:3**	**15:1**	
E0201	17.28	1.96	54.61	230.50	**1:1**
E0202	2.61	12.96	98.61	113.16	**3:1**
E0203	17.28	1.96	54.61	230.50	**1:1**
E0204	5.33	9.00	85.33	141.07	—
E0205	5.33	49.00	192.00	13.07	—
E0206	6.45	51.84	198.45	10.25	—
E0207	33.33	0.00	33.33	326.67	**1:1**

Genotyopes E0201, E0203, and E0207 of *H.praecox* show χ^2 values below 3.84 for the 1:1 ratio, indicating that resistance in these populations is likely controlled by a single heterozygous gene or originates from a segregating backcross-type structure. This suggests incomplete fixation of the resistance allele and a simple inheritance model.

http://doi.org/10.59463/RJAS.2025.3.41

Furthermore genotype E0202 of *H.praecox* fits the 3:1 ratio, which is characteristic of one dominant resistance gene segregating in the F_2 population. This means that only one allele is required to confer resistance, making E0202 a promising source of dominant resistance.

Additionally , genotypes E0204, E0205, and E0206 *H.praecox* do not fit any simple Mendelian ratio. Their χ^2 values are higher than 3.84 for all models, suggesting complex inheritance.

Table 5.

Results regarding segregation ration on resistant and susceptible on P.halstedii of sunflower genotypes of *Helianthus tuberosus.*

Code	Mendelian ratios				The ratio closest to the data (χ^2 < 3.84)
	3:1	1:1	1:3	15:1	
E0208	81.12	7.84	6.45	569.18	**1:3**
E0209	0.85	33.64	155.52	37.13	**3:1**
E0210	149.81	31.36	0.48	878.60	**1:3**

Genotype E0208 of *H. tuberosus* , fits a 1:3 ratio, which indicates the presence of a recessive resistance gene. This means that plants must be homozygous recessive to express resistance. Such genes are rarer but important for pyramiding strategies.

On the other hand , genotype E0209 of *H. tuberosus* shows a strong fit for the 3:1 ratio, meaning resistance is controlled by a single dominant gene, similar to the situation observed in the H. praecox line E0202. This population is highly valuable for breeding because dominant resistance is easier to introgress.

Moreover , genotype E0210 *H. tuberosus* fits the 1:3 ratio, like E0208, again pointing to recessive genetic control. These recessive genes can provide durable or race-specific resistance depending on their mode of action.

CONCLUSIONS

Our results determine a more effective and dependable method for assessing sunflower resistance to *Plasmopara halstedii* race 730. According to our results when seeds were inoculated during the early germination phase (0.5–2.0 mm radicle length) at 18°C a percent of inoculation success of more them 90% success was registered.

Furthermore, genotypes resulted from interspecific populations identified multiple sources of resistance. Lines E0202 resulted from sunflower species *Helianthus praecox* and E0209 resulted from sunflower species *Helianthus tuberosus* ,exhibited a distinct 3:1 segregation ratio, suggesting dominant resistance genes and rendering them extremely useful for future breeding efforts. On the other hand other sources showed 1:1 or 1:3 ratios, indicating distinct genetic mechanisms of resistance different form the dominant one. The genetic sources found in this research can act as valuable genetic resources in breeding initiatives, either for direct incorporation into new lines and hybrids or as sources of resistance

BIBLIOGRAPHY

AHMED, S., TOURVIEILLE DE LABROUHE, D., DELMOTTE, F., (2012). Emerging virulence in Plasmopara halstedii, the sunflower downy mildew. Evolutionary Applications, 5(6), pp. 567-582.

BAN, R., KOVACS, A., KOROSI, K., PERCZEL, M., TUROCZI, G., (2021). New and High Virulent Pathotypes of Sunflower Downy Mildew (Plasmopara halstedii) in Seven Countries in Europe. Journal of Fungi, 7(7), 549.

BUZNA, C., HORABLAGA, M. N., JURJESCU, A. L., SALA, F. (2024). Comparative study of seeds agronomic attributes and of yield in some sunflower genotypes. *Scientific Papers. Series "Management, Economic Engineering in Agriculture and Rural Development"*, 24(4), pp. 131–140

COHEN, Y., RUBIN, A.E., (2020). Oxathiapiprolin-based fungicides for the control of *Plasmopara halstedii* in sunflower. *Crop Protection*, 136, 105237.

FAOSTAT, (2020). *Crops and livestock products*. Food and Agriculture Organization of the United Nations. Statistical Database.

GASCUEL, Q., MARTINEZ, Y., BONIFACE, M.C., VEAR, F., PICHON, M., GODIARD, L., (2015). The biodiversity of Plasmopara halstedii in South America suggests a potential origin of the pathogen in the wild sunflower center of diversity. BMC Evolutionary Biology, 15, 188.

GULYA, T.J., TOURVIEILLE DE LABROUHE, D., MASIREVIC, S., PENAUD, A., RASHID, K., VIRANYI, F., (1998). Proposal for standardized nomenclature and identification of races of *Plasmopara halstedii* (sunflower downy mildew). *Helia*, 21(29), pp. 11-30.

HORABLAGA, M. N., BUZNA, C., SALA, F. (2024). Productivity of some sunflower hybrids – comparative analysis. *Scientific Papers. Series "Management, Economic Engineering in Agriculture and Rural Development"*, 24(1), pp. 475–485.

IOOS, R., ANDRIEUX, A., MARQUER, B., FREY, P., (2007). A simplified protocol for the molecular detection of Plasmopara halstedii in sunflower seeds. Seed Science and Technology, 35(1), pp. 177-181.

JULLIEN, I., TOURVIEILLE DE LABROUHE, D., (2006). A new seedling immersion test to detect sunflower resistance to Plasmopara halstedii. Helia, 29(45), pp. 13-20.

MARTÍN-SANZ, A., RUEDA, S., GARCÍA-CARNEROS, A.B., MOLINERO-RUIZ, L., (2020). First Report of a New Highly Virulent Pathotype of Sunflower Downy Mildew (*Plasmopara halstedii*) Overcoming the Pl8 Resistance Gene in Europe. *Plant Disease*, 104(2), 598.

ONIȘAN, E., PETOLESCU, C., TENCH-CONSTANTINESCU, A., PETRESCU, I., HORABLAGA, M. N., SIMINA, A., GORINOIU, G., CAMEN, D., POPESCU, S., MATE, C., ȘARAC, I. (2025). Application of Biotechnology for Sunflower Inbred Line Development: Immature Embryo Rescue. *Applied Sciences*, 15(3), 1576

ONIȘAN, E., SARAC, I., PETRESCU, I., DORIN, C. (2023). New breeding methods in sunflower hybrid research and development. *Scientific Papers. Series A. Agronomy*, 66(1)

ONIȘAN, E., SĂRAC, I., PETRESCU, I., POPESCU, S., PETOLESCU, C. (2024). Transgenic sunflower genotypes: production, evaluation, and improvement strategies. *Journal of Horticulture, Forestry and Biotechnology*, 28(1), pp. 141-147

RADWAN, O., BOUZIDI, M.F., VEAR, F., PHILIPPON, J., TOURVIEILLE DE LABROUHE, D., NICOLAS, P., MOUZEYAR, S., (2003). Identification of non-TIR-NBS-LRR markers linked to the Pl5/Pl8 locus for resistance to downy mildew in sunflower. *Theoretical and Applied Genetics*, 106, pp. 1438–1446.

SEILER, G.J., GULYA, T.J., (2016). Sunflower Diseases and Pests. In: *Sunflower: Chemistry, Production, Processing, and Utilization*, AOCS Press, pp. 329-373.

TOURVIEILLE DE LABROUHE, D., WALSER, P., SERRE, F., ROCHE, S., VEAR, F., (2012). Race 730 of *Plasmopara halstedii*, a new threat to sunflower in France. *Phytopathologia Mediterranea*, 51(3), pp. 455-463.

TROJANOVÁ, Z., SEDLÁŘOVÁ, M., LEBEDA, A., (2017). Microscopy of *Plasmopara halstedii* infection in sunflower: from zoospore docking to systemic spread. *Plant Pathology*, 66, pp. 1442–1455

VIRÁNYI, F., GULYA, T.J., TOURVIEILLE DE LABROUHE, D., (2015). Downy Mildew of Sunflower. In: *Sunflower Diseases*. APS Press, St. Paul, MN, USA.