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Short communication

Resistance to *Penicillium hirsutum* Dierckx in garlic accessions

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Abstract

Among the factors affecting the quality and yield of garlic production, blue mold caused by – *Penicillium* spp. – is responsible for economical losses in many countries. Allicin, present in garlic bulbs, has been suggested as having antifungal activity against some *Penicillium* species. This study was conducted to evaluate the response of garlic accessions against *Penicillium hirsutum* infection and to compare this response with bulb allicin content. Twelve garlic accessions were inoculated with *P. hirsutum*, and assayed in greenhouse and growth chamber experiments. Plant growth parameters and the fungal production of conidia were evaluated. Significant differences were found among the accessions. Accessions Castaño and Morado were most resistant whereas AR-I-125 and Fuego were always severely affected by the disease. A low correlation was found ($r = 0.17$) between allicin content and tolerance, indicating that allicin is not the main factor involved in the resistance against *P. hirsutum*.

Garlic (*Allium sativum*) is an important and widely distributed crop used as a spice and for medicinal purposes (Agarwal, 1996). *Penicillium* decay is one of the factors responsible for economical losses in garlics grown in Argentina and other countries (Smalley, 1954; Avila et al., 1977; Bruna, 1985). This pathogen is responsible for the blue mold disease in garlic and it infects field-grown plants and stored bulbs. *Penicillium hirsutum* was the causal agent of garlic blue mold in Chile (Bruna, 1985), Italy (D'ercole, 1972), Argentina (Avila et al., 1977) and USA (Smalley and Hansen, 1962). Smalley and Hansen (1962) observed that crude garlic extracts can inhibit the growth of some *Penicillium* isolates *in vitro* and attributed this phenomenon to a differential sensitivity to allicin, the predominant thiosulfinate found in garlic. In their study, only a crude extract was used and the possibility remains that substances other than allicin may be involved in the inhibition of fungal growth. To date, no garlic resistance against this pathogen has been reported. Smalley and Hansen (1962) tested eight *Allium sativum* clones, among a number of other *Allium* species, as possible hosts for *P. hirsutum*. They found that all the garlic clones were highly and equally susceptible. The identification and utilization of resistant or tolerant garlic accessions can help the management of blue mold of garlic. In the present study, the variability for resistance against this pathogen was investigated in twelve garlic accessions and compared with their bulb-allicin content.

Eleven *Allium sativum sativum* (Morado, Norteño, Licán, Nieve, Unión, Perla, AR-I-158, AR-I-125, Sureño, Gostoso, Fuego) accessions and one *Allium sativum ophioscordoron* (Castaño) accession

from the Argentine garlic collection (INTA La Consulta, Mendoza) were used in this study. All the experiments were initiated when garlic cloves had broken dormancy and the Dormancy Ratio (D.R. = length of sprouting leaf/length of storage leaf × 100) reached 75% (Burba et al., 1993). For each accession, cloves with similar weight (deviation from the mean weight (<0.5 g) were used and a monosporic isolate of *P. hirsutum* was used as an inoculum source.

One hundred and twenty cloves from each garlic accession were peeled and surface sterilized. Half of them were inoculated by making two small wounds on each clove with a sterile needle, which was dipped in a conidial suspension of *P. hirsutum* of known concentration (5.68×10^6 conidia ml^{-1}). The other half (60 cloves) was inoculated with sterile water and evaluated as non-inoculated controls. The inoculated and control cloves were planted in plastic pots containing sterile substrate, distributing twelve cloves/pot, resulting in five replications per treatment. Pots were distributed in the greenhouse according to a randomized complete block design. Plants were grown for 50 days, harvested and the following variables were measured: total dry matter (partitioned by leaves, pseudo-stalks, and roots), percentage of survival, percentage of asymptomatic plants, total leaf area, stalk diameter and plant height. For data analysis, variables were expressed as ratios of the non-inoculated (control) treatments. The data were analyzed by analysis of variance ANOVA and for each variable means comparisons among garlic accessions were done by the Tukey test ($\alpha = 0.05$ and 0.01).

All the garlic accessions showed statistical differences between inoculated (I) and control (C) treatments for at least two of the variables measured, with the exception of Castaño where no differences were noted between I and C for all the variables analyzed (except for production of conidia/clove which was not considered in inoculated–control comparisons) (Table 1). The growth components most severely affected were total dry matter, root dry matter and stalk diameter. The total dry matter provides an idea of how the disease affects the overall growth of plants, regardless of the effect on any specific organ. For this variable, ANOVA was highly significant ($P \leq 0.01$) and four statistically different groups were identified (Table 1). Castaño

was the most resistant accession while Fuego and AR-I-125 were the most susceptible (Figures 1a and b). At the end of the greenhouse experiment the pathogen was re-isolated from symptomatic plants and identified, confirming that *P. hirsutum* (IMI 386756) was responsible for the observed effects. Overall, significant differences ($P < 0.01$) among accessions were noted for all ten variables evaluated and means comparisons clustered the garlic accessions into two to five different groups (Table 1). Castaño was the most resistant accession for eight of the ten variables. Morado was usually ranked after Castaño and, for seven of the ten variables these two accessions were not statistically different, indicating that Morado was also resistant. On the other hand, AR-I-125 was the most susceptible for all the variables considered. Fuego was also susceptible and similar in disease response to AR-I-125 for six of the variables.

The fungal growth and sporulation on garlic cloves were tested in the growth chamber experiment. Cloves from each accession were sterilized and inoculated by performing one small wound and adding 3 μl of the conidial suspension. Negative controls were inoculated with sterile water. Cloves were placed in plastic containers, covered with transparent plastic film and incubated in a growth chamber for 12 days. Temperature inside the growth chamber was 22 °C for optimal growth of the fungus (Smalley and Hansen, 1962) and air humidity inside the containers was close to 100%. At the end of the experiment conidia from each lesion were collected and quantified using a hemocytometer. ANOVA was highly significant ($P < 0.001$) and means comparison separated five different groups (Table 1). As for the variables analyzed in the greenhouse experiment, AR-I-125 was the most susceptible. Union showed a similar, not statistically different, susceptible behaviour. The highest level of resistance was represented by a group of accessions and included Castaño and Morado. Figures 1c–h shows how cloves from Morado and AR-I-125 were differentially affected by the fungal infection.

Morado showed production of the least amount of conidia/clove. Interestingly, this accession developed an intense red pigment in the tissues surrounding the fungal colony (Figure 1c). All the other garlic accessions did not produce a red pigment or the pigment was faintly pink-coloured.

Table 1. Comparison of plant parameters, conidial production and bulb allicin twelve garlic accessions

Variables		Garlic accessions											
		Castaño	Morado	AR-I-158	Norteño	Unión	Nieve	Licán	Perla	Gostoso	Sureño	Fuego	AR-I-125
Total dry matter/pot	I/C[a]	0.986	0.818**	0.777**	0.778***	0.743***	0.740**	0.737**	0.728**	0.686***	0.679**	0.544**	0.406***
	SD[b]	A	B	B	B	B	B	B	B	B	BC	CD	D
Leaves dry matter/pot	I/C	0.907	0.898	0.824*	0.851	0.779	0.776*	0.813	0.775**	0.726**	0.701**	0.577**	0.438**
	SD	A	AB	ABC	ABC	BC	BC	ABC	BC	CD	CD	DE	E
Roots dry matter/pot	I/C	0.971	0.761*	0.680**	0.669**	0.701**	0.690**	0.635**	0.679**	0.617**	0.677**	0.438***	0.405***
	SD	A	B	B	B	B	B	B	B	BC	B	CD	D
Pseudo-stalks dry matter/pot	I/C	1.041	0.818***	0.821*	0.857	0.761	0.782	0.812	0.753*	0.730**	0.659**	0.673**	0.382***
	SD	A	BC	BC	B	BC	BC	BC	BC	BC	C	C	D
Stalk diameter	I/C	0.974	0.933	0.858**	0.896	0.853*	0.827**	0.480**	0.818**	0.806**	0.756**	0.592*	0.482**
	SD	A	AB	ABC	ABC	ABCD	BCD	BCD	BCD	CD	D	E	E
Plant height	I/C	0.922	0.968	0.789	0.873	0.801	0.778	0.767	0.757	0.684*	0.705	0.729	0.401
	SD	A	A	AB	AB	AB	AB	AB	AB	B	B	AB	C
Survival (%)	I/C	1.000	0.966	0.966	0.983	0.983	0.966	0.983	0.966	0.983	0.949	0.800**	0.749**
	SD	A	A	A	A	A	A	A	A	A	A	AB	B
Asymptomatic plants/pot (%)	I/C	0.966	0.900	0.883	0.833	0.833	0.855	0.855	0.855	0.816	0.750**	0.416**	0.330**
	SD	A	AB	AB	AB	AB	AB	AB	AB	AB	B	C	C
Total leaf area/pot (cm²)	I/C	0.974	0.948	0.850	0.882	0.843	0.811	0.830	0.847	0.827	0.763	0.608**	0.378**
	SD	A	A	AB	AB	AB	AB	AB	AB	AB	BC	C	C
Production of conidia/clove (×10⁶)	I	320.3	237.21	365.86	273.4	589.2	310	321	335.68	336.55	523.53	576	723
	SD	BCDE	E	BCDE	DE	AB	CDE	BCDE	BCDE	BCDE	BCD	BC	A
Mean bulb allicin content (mg g⁻¹ Dw[c])	SD	13.111	8.165	11.298	14.761	16.705	15.927	10.283	10.879	12.546	17.4	8.3983	11.285
		BC	E	CD	B	A	AB	D	D	C	A	E	D

[a]I/C – ratio inoculated/control. Mean I/C values were calculated summing up the I/C values from the 5 replications and dividing the result by 5.
[b]SD – significant differences found among garlic genotypes based on mean comparisons of their I/C values (Tukey test).
[c]Dw – dry weight.
*Significant differences between I and C for each garlic genotype at $P \leq 0.05$. **Significant differences between I and C for each garlic genotype at $P \leq 0.01$.
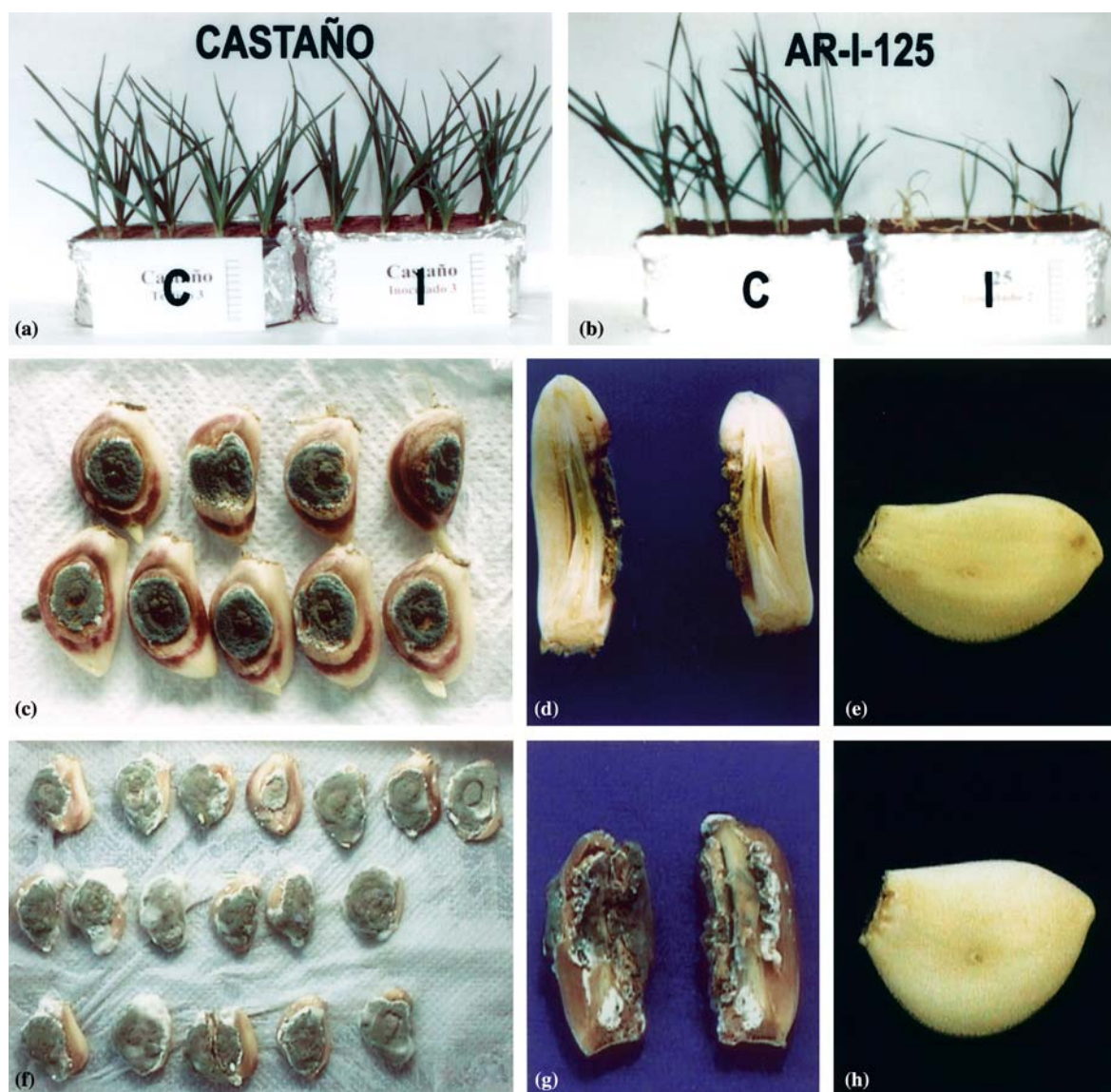
Figure 1. Effect of *Penicillium hirsutum* inoculation on garlic growth in greenhouse (a, b) and growth chamber (c–h) experiments after 50 and 12 days, respectively. (a, b) Effect on plant growth, biomass production and plants survival in inoculated (I) and control (C) plants from accessions Castaño (a) and AR-I-125 (b). (c, f) Production of conidia in inoculated cloves of Morado (c) and AR-I-125 (f). (d, g) Longitudinal sections of inoculated cloves of Morado (d) and AR-I-125 (g). (e, h) Negative controls (not inoculated) of Morado (e) and AR-I-125 (h).

It is possible that this substance could have a role, *in vivo*, in restricting the growth and spread of the fungus. The pigment was not observed in the negative controls (Figures 1c and e) indicating that this is a response to the fungal infection, rather than a general response to any non-specific stress, such us the mechanical wounds produced by inoculation. Also, a cork-like structure appeared in cloves from most of the accessions and always surrounded fungal colonies, whereas negative controls lacked this tissue. The amount and appearance of it varied, depending on the garlic accession. It is speculated that this tissue could play a defense role, acting as a physical barrier against the spread of the fungus.

The differential responses of Morado and Fuego were confirmed by monitoring the conidial production of sprouted and non-sprouted inoculated

cloves. For each accession, 36 sprouted and 36 not-sprouted cloves were peeled, sterilized, inoculated and incubated as described for the growth chamber experiment. Conidia from twelve randomly selected cloves were quantified 5, 10 and 15 days after inoculation. In agreement with their behaviour in greenhouse and growth chamber experiments, Fuego was significantly more susceptible than Morado regardless of the physiological status of the cloves (data not shown). Sprouted cloves were slightly more susceptible (not significantly different) than not-sprouted ones, for both cultivars.

Bulb allicin contents for the twelve garlic accessions were determined by standard HPLC prodedures (Lawson et al., 1991; Lawson and Hughes, 1992). For each garlic accession four independent extractions were performed and quantified. Bulb allicin contents varied across accessions and mean values ranged from 8.16 to 17.39 mg g^{-1} dry weight. Five statistically different groups were observed (Table 1). Sureño, Union and Nieve had the highest allicin concentration, while Fuego and Morado had the lowest. Morado, which restricted the fungal growth and conidial production the most, showed the lowest allicin content whereas accession Union, which allowed an intensive fungal growth and conidial production, had one of the highest allicin concentrations. Allicin content and total dry matter (total dry matter values were considered to be representative of the tolerance behaviour), had a low correlation coefficient value, $r = 0.17$. This indicates that allicin does not play a major role in the resistance against *P. hirsutum*. Our results are in close agreement with those reported by Durbin and Uchytil (1971). By testing the effect of allicin on the growth of 11 *Penicillium* isolates *in vitro*, and their virulence when infecting garlic cloves, these authors found that among the isolates capable of decaying garlic, there was no correlation between the degree of fungal growth inhibition by allicin and the virulence on garlic cloves. Furthermore, they observed that one penicillia was not inhibited by allicin *in vitro* and yet could not decay garlic.

In conclusion, the variables analyzed in greenhouse and growth chamber experiments showed that the most susceptible accessions were AR-I-125 and Fuego, while Castaño and Morado were the most resistant. The low correlation observed between bulb allicin content and disease resistance indicates that this compound is not the main factor involved in the resistance against *P. hirsutum* and suggests that other defense mechanisms must be involved.

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