JACOBS PUBLISHERS

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# Jacobs Journal of Agriculture

Research Article

## Analysis of Genetic Components of Resistance to *Ascochyta rabiei* in Chickpea

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*Received:* 05-19-2017

*Accepted:* 06-02-2017

*Published:* 06-05-2017



### Abstract

Blight caused by *Ascochyta rabiei* is one of the most devastating diseases of chickpea that causes yield losses over 80% in some years and may induce total failure to the crop under epidemic conditions. In this study, we tried to understand the role of epidemiological factors of chickpea blight such as incubation and latent periods under greenhouse conditions. A principal component analysis (PCA), hierarchical and multiple regression analyses were performed. The overall analyses of the results revealed that incubation and latent period explain very well the intensity of the disease in the tested lines. The length and the growth of the lesions are also components to consider, as they are significantly correlated to the note of the intensity of the disease. In contrast, sporulation and sporulation capacity are weakly correlated with the severity of the disease.

**Keywords:** Chickpea; Blight; Epidemic; Incubation Period; Latent Period

### Introduction

Works realized on blight of chickpea have permitted to highlight the importance of the environmental factors that act directly on the development of the disease such as humidity and temperature in one hand [1-3], and in another hand those who affect the expression of the disease as the inoculum concentration and physiological state of the plant [4,5]. These results were used to develop techniques and screening evaluation of plant material against the disease. However, they did not clarify the relationships between the host and the pathogen. These relationships should be defined, to explain sometimes the contradictory experimental results and implement control strategies and genetic improvement of plant resistance.

Resistance usually affects the multiplication of the pathogen rather than its dissemination [6]. The development of the epidemic is determined by the amount of primary inoculum, and its degree of multiplication, described by the apparent infection rate. Resistance could reduce the primary inoculum than infection rate or both at once. If primary inoculum is reduced, the epidemic is delayed, whereas if infection rate is small, the epidemic is limited [7]. Indeed many varieties differ in their ability to delay the development of the disease with different combinations of resistance components that lead to the expression of slow disease or slow "slow rusting" [8,9] and "slow blasting" in rice [11]. The intensity of the disease is the cumulative result of several factors of infection which are: the latent period, the sporulation and the infectious period [6,12]. The latent period is one of the com-

ponents of plant disease resistance that can reduce the rate at which disease epidemics develop [13].

This study aims to evaluate the importance of each component of the resistance in the development of the disease, using multiple lines with different levels of susceptibility to *A. rabiei*. A comprehensive approach to improve the partial resistance of chickpea to *A. rabiei*, could then be seen by selecting one or more of these components. A quantitative analysis of resistance components was carried out on 20 lines of chickpea, against race 4 of *A. rabiei*. Six parameters were measured or calculated as AUDPC over a period from the $4^{th}$ to the $28^{th}$day after inoculation; incubation and latent periods, lesion length, lesion growth per day, the production of spores (or sporulation ) and the capacity of production of spores (or capacity of sporulation).

## Material and Methods

The experiment was conducted in a greenhouse. The seeds of twenty lines of chickpea were sown in pots of 22cm of diameter, 18 cm height, filled with sterilized soil, with 4 seeds per pot. A seed of susceptible cultivar ILC 263 was planted in the center of each pot as a check. The greenhouse temperature was set at 22°C during the day and 18°C overnight. The plants were divided according the randomized complete block design.

To produce inoculum of race 4, a two-pieces of stem infected and stored at -20°C were incubated in the media "chickpea agar" in a Petri dish to prepare an initial suspension of spores. 250ml Erlenmeyer flasks containing 50ml of liquid medium were inoculated. Incubation was carried out at 22°C under continuous light and without agitation during seven days. The cultures were harvested, and added with sterile water, then crushed with a mixer type Blender. The spore suspensions obtained after filtration were adjusted to $5x10^5$ spores per ml, after counting using a hemocytometer. Two milliliters of Tween 80 per liter of suspension were then added to promote spore dispersal. The inoculation was performed with a spore suspension on young plants aged of 10 days with 3 to 4 leaves by spraying 1.5ml per plant. The plants were then left one to two hours at room temperature to dry and sprayed lightly with distilled water using a hand sprayer. The plants were placed on a pallet, in a sealed plastic cage inside which has been deposited a film of water of about 1 mm thick. After three days of incubation, the lid of the cage was removed and the plants were sprayed three times per day with distilled water during one week. Blight symptoms were recorded four days after inoculation and at regular intervals every two days until the end of the experiment (21 days after inoculation).

The scale 0 to 9 was used to assess the disease severity [5,14]. The observations were taken concerning:

- The incubation period: the number of days between the date of inoculation and the appearance of the first lesion on the plant.

- The latent period: number of days between inoculation and the first appearance of pycnidia on the lesions. Pycnidia were regularly observed under a binocular microscope, until the appearance of cirrus.

- Lesion length: one to two lesions on the main stem of each plant were marked and their size was measured every two days.

- Spore production: for each plant at the end of the experiment all lesions on stems were measured, sampled and cut into small fragments. These were then placed in moistened Whatman paper Petri dishes, and then incubated for 24 hours. These pieces of stems were then agitated in beakers containing 3ml of water for four hours. The spore concentration was evaluated under microscope, using a malassez cell counter. The same samples were re-incubated for a second time, and the experiment was continued under the same conditions as above. The total production of spores is equal to the sum of the number of spores obtained in two harvests.

From the data lengths of the lesions and the total production of spores, the other two parameters were calculated:

- The growth of lesions per day: ratio of the length of the lesion.

- The number of days during which the growth was observed.

For statistical methods, a principal component analysis (PCA), the hierarchical classification and correlation study were performed.

## Results

The first results are summarized in Table 1. It represents the grouping of lines depending on the severity of the disease and the different components of resistance. Two major groups of lines, significantly different, are distinguished. The first group (g1) can be divided into two sub-groups:

- A short incubation period (S)
- A medium incubation period (M)

The second group (g2) was composed by genotypes with a long period of incubation (L).

The statistical analysis has identified three groups of cultivars for latent period, significantly different:

-A short latent period (S)
- A medium latent period (M)
- A long latent period (L)

Lesion length, measured on the stems of chickpea ranged from

1 to 20 mm. Three groups of lines can be distinguished:

- A large lesions (L)
- A medium lesions (M)
- A small lesions (S)

Both extremes groups are significantly different.
The average growth of the lesions was 1.09mm/day, with a maximum of 2mm and a minimum of 0.22mm. Three groups of lines are characterized:

- Rapid growth (Ra) greater than 1.7mm/day
-Medium growth (M) between 0.86 and 1.54mm/day
- Slow growth (Sl) not exceeding 0.56mm/day

Spore production at 28 days was zero for ILC 182 and reached a maximum of $3.3x10^6$ spores for ILC 482. The tested lines are not significantly different.

The sporulation capacity ranged from 0 to $7.3x10^4$ spores per mm lesion for ILC 182 and ILC 482, respectively. There is no significant difference between the lines.

The average of intensity of the disease measured at 15 and 21 days after inoculation, were respectively 5.13 and 5.75. Data from the two readings are giving identical ranks. They are grouped as follows:

The first group (g1) is divided into three sub-groups:

- Very susceptible (VS) with a score above 8
- Susceptible (S) with a score ranging from 6.7 to 8
- Moderately susceptible (MS) with a score of 5 to 6. The second group (g2) with lines considered resistant (R) with rating less than 4.

## Multiple Regression Analysis

The correlation matrix of the components of resistance indicates a strong negative correlation between latency and incubation period with the disease severity assessed at 15 days after inoculation (r=-0.94) and a positive correlation with a growth and a length of lesions with disease severity (Table 2). In addition, the notes of the disease intensity, rated at 21 days after inoculation, are highly correlated with the incubation and latent period. Lesion and growth length are negatively correlated with incubation and latent period (Table 2). In contrast, sporulation and sporulation capacity are weakly correlated with all others components, especially the intensity of the disease assessed at 21 days (Table 2).

## Explanatory Components of Resistance

The overall analysis of the results revealed that incubation and latent periods explain very well the intensity of the disease for the tested lines ($R^2$=0.91). In particular, the incubation period explains alone 87% of the variability in the model, with a probability highly significant. However, we note slight differences between these two components for the group of moderately susceptible lines.

Individual analysis of the results allows a more detailed study. It shows that, for each line, specific components explain well the intensity of the disease, 15 or 21 days after inoculation. Components and characteristics of each line which are best related to the intensity of the disease are listed in Table 3.

The intensity of the disease, observed at 15 days, is best explained, in large majority of lines, by sporulation, lesion growth and the incubation period. In contrast, at 21 days, lesion length, and sporulation, to a lesser extent, the growths of the lesions are the components that characterize best the disease severity.

Among the resistant lines, four components seem particularly important to explain the intensity of the disease in 21 days:

- Lesion length for ILC 72, ILC 7795 and ILC 182
- The incubation period for ILC 200
- The growth of lesions for ILC 7374
- The growth and sporulation of lesions for ICC 12004

However, for some lines, there are no components that can explain the intensity of the disease in two reading dates. Indeed, none of the components studied reaches an acceptable level to be included in the model. This is the case of lines like ILC 605, ILC 201, ILC 482, P-1252-1, ILC 195, and ILC 8068 and in particular, the resistant line ILC 3279.

## Classification of Lines

A principal component analysis (PCA) and hierarchical analysis were performed with the data (AUDPC) from this study. In principal component analysis, the main plane describes an average of 93.4% of the total variability analysis (Fig. 1). The first principal component also involves all variables with a more reduced sporulation and sporulation ability. However, in the composition of the second component, we have mainly 43.6% for sporulation and 33.2% for sporulation capacity which represents a total of 76.8% in the construction of the second factor.

The projection lines on the two main axes highlights one group of relatively homogeneous lines representing seven resistant lines (Fig. 1). Most other lines are distributed in the graph to the left of the axis 2. These lines, as described above, have resistance components highly variable (Table 1).

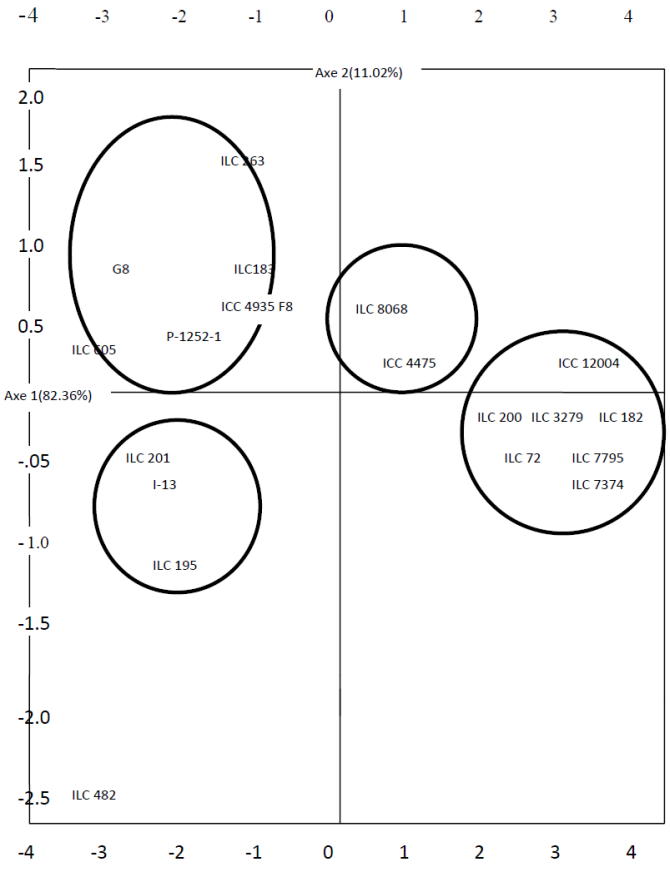


**Figure 1.** Principal component analysis of 20 lines of chickpea under axes 1 and 2.

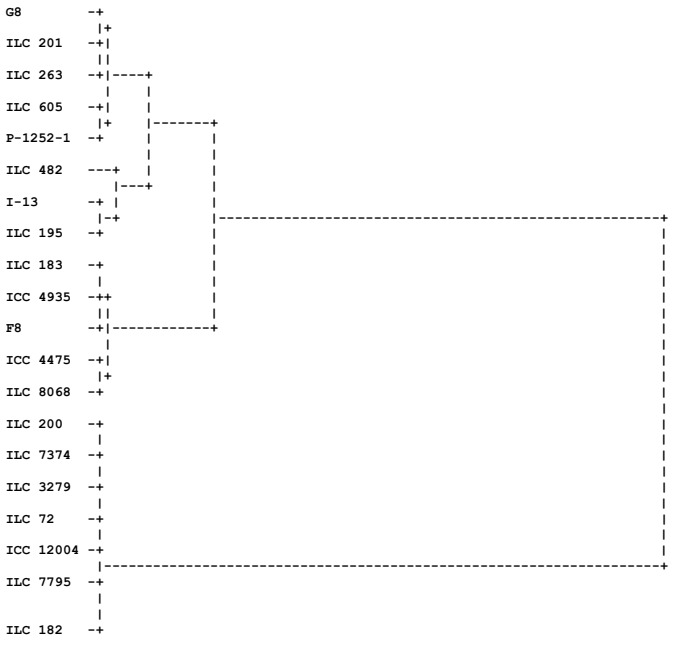


**Figure 2.** Hierarchical classification of chickpea lines against Ascochyta blight.

The hierarchical classification (Fig. 2) shows the same group of line resistant's than the PCA. This classification clearly distinguishes three groups of lines: the resistant with the group of ILC 200, the moderately susceptible with the group of ILC 8068 and the susceptible with the group of ILC 201. The separation between these three groups is for resistant lines with a rating of less than 3.5 and the moderately susceptible lines with a rate from 3.5 to 5.4 and for the susceptible lines with a rate higher than 5.4. This result confirms the result that we have had previously concerning the 9 points scale that would be the note of 4 which separate resistant from the susceptible lines.

## Discussion

Among the six components of the resistance studied, incubation and latent periods explain best the intensity of the disease, for the 20 lines studied. These two components are highly correlated between them and the disease severity obtained at 15 and 21 days after inoculation (Table 4). These results were also found by Benali et al. [15] for *Mycosphaerella pinodes* on pea. The incubation and latent periods are long for the 7 resistant lines (above 10 and 20 days, respectively), while it is much shorter on the susceptible lines (between 4 and 9 days for the incubation period and between 7 and 13 days for the latency period) (Table 1). These two components which correspond to the phases of installation and maturation of the fungus seem to be very important to evaluate the resistance of lines in *A. rabiei*. Several studies have also reported similar observations [16-18].

**Table 1.** Classification of lines in homogeneous group's according to the intensity of disease and the different components of resistance.

| Line | Disease severity (1) | | Incubation p. (2) | | Latent p. (3) | | | Lesion length (4) | | | Lesion growth (5) | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | g1 | g2 | g1 | g2 | g1 | g2 | g3 | g1 | g2 | g3 | g1 | g2 | g3 |
| G8 | VS | | S | | S | | | L | | | Ra | | |
| ILC 605 | VS | | S | | S | | | L | | | Ra | | |
| ILC 201 | VS | | S | | S | | | | M | | | M | |
| ILC 263 | VS | | S | | S | | | | M | | | M | |
| ILC 482 | S | | S | | S | | | L | | | Ra | | |
| P-1252-1 | S | | S | | S | | | | M | | | M | |
| ILC 183 | S | | M | | S | | | L | | | | M | |
| I-13 | S | | M | | S | | | L | | | | M | |
| ILC 195 | S | | M | | S | | | L | | | | M | |
| ICC4935 | S | | M | | S | | | L | | | | M | |
| F8 | MS | | M | | S | | | | M | | | M | |
| ILC 8068 | MS | | M | | | M | | L | | | | M | |
| ICC4475 | MS | | M | | | M | | | M | | | M | |
| ILC 200 | | R | | L | | | L | | | S | | | Sl |
| ICC 12004 | | R | | L | | | L | | | S | | | Sl |
| ILC 3279 | | R | | L | | | L | | | S | | | Sl |
| ILC 72 | | R | | L | | | L | | | S | | | Sl |
| ILC 7795 | | R | | L | | | L | | | S | | | Sl |
| ILC 7374 | | R | | L | | | L | | | S | | | Sl |
| ILC 182 | | R | | L | | | L | | | S | | | Sl |

(1) Disease severity; VS: very susceptible; S: susceptible; MS: moderately susceptible; R: resistant
(2) Incubation period; S: short; M: medium; L: long
(3) Latent period; S: short; M: medium; L: long
(4) Lesion growth; L: large; M: medium; S: short
(5) Lesion growth; Ra: rapid; M: medium; Sl: slow

**Table 2.** Correlation matrix of component of resistance.

| Components of resistance | Disease severity 1 | Incubation P. | Latent P. | Lesion L. | Lesion G. | Sporulation | Sporulation cap. |
|---|---|---|---|---|---|---|---|
| Incubation P. | -0.94 | | | | | | |
| Latent P. | -0.94 | 0.91 | | | | | |
| Lesions L. | 0.73 | -0.72 | -0.73 | | | | |
| Lesion G. | 0.80 | -0.77 | -0.81 | 0.69 | | | |
| Sporulation | 0.34 | -0.33 | -0.41 | 0.24 | 0.23 | | |
| Sporulation cap. | 0.34 | -0.32 | -0.38 | 0.25 | 0.26 | 0.71 | |
| Disease severity 2 | 0.97 | -0.93 | -0.91 | 0.73 | 0.78 | 0.36 | 0.35 |

**Table 3.** Resistance components explaining best the disease severity for each line.

| Line | Disease severity at 21 days | Component explaining best the disease severity at 15 days | Component explaining best the disease severity at 21 days |
|---|---|---|---|
| G8 | 8.75 | Incubation and sporulation period | Incubation and sporulation period |
| ILC 263 | 8.60 | Sporulation capacity | - |
| ILC 183 | 7.40 | Lesion growth and sporulation | Lesion length |
| I-13 | 7.07 | Latent and sporulation period | Latent and sporulation period |
| ICC 4935 | 6.70 | Lesion growth | - |
| F8 | 6.00 | Lesion growth | Lesions growth and sporulation capacity |
| ICC 4475 | 5.63 | Incubation period | - |
| ILC 200 | 3.55 | Incubation period and sporulation | Incubation period |
| ICC 12004 | 3.47 | Lesion length and sporulation | Lesion growth and sporulation |
| ILC 72 | 3.05 | Lesion growth | Lesion length |
| ILC 7795 | 2.52 | - | Lesion length |
| ILC 7374 | 2.35 | Sporulation | Lesion growth |
| ILC 182 | 2.33 | - | Lesion length |

In our study, incubation and latent periods are correlated but are not fully associated. The incubation period alone explains 87% of the variability of the response of lines to the pathogen in the model, but not enough to explain the behavior of intermediate resistance lines. Thus, ICC 4475 and ILC 8068 have been separated from the group of susceptible lines, for the duration of the latent period, using the method step-by-step. These two lines are also highlighted in the graphic representation of CPA (Fig. 1). This component of the resistance could therefore provide additional information to that given by the incubation period, to better understand the behavior of certain lines to *A. rabiei*. The importance of the latent period was also highlighted to explain the resistance of potato cultivars to *Phytophthora infestans* [6]. In contrast, the latent period is not an essential component of partial resistance in the model rice (*Oryza sativa*) - *Magnaporthe grisea* (anamorph: *Pyricularia oryzae*) [19,20].

Length and particularly the growth of the lesions are also components to consider, as they are significantly correlated to the note of the intensity of the disease. Resistant lines have small lesions (less than 5 mm) and slow growth (less than 0.6mm/day). Meanwhile, susceptible lines show lesions of 7 mm or more, with a growth of more than 0.8mm/day. Lesion length is considered by several authors as an important criterion for estimating the intensity of the disease to *Ascochyta* blight of chickpea. This is the case of the rating scale of 9 points of Reddy et al. [14] and the measurement of the linear index of infection of the method of Riahi et al. [21].

In contrast, sporulation and sporulation capacity are weakly correlated with the rating of the severity of the disease, as well as other components of the resistance. These two variables are relatively independent of one another: they represent 43.6 and 33.2 of the variability of the factor 2 in the principal component analysis, while the other variables are mainly related to the factor 1 (Table 3). This result is surprising, because there is generally a relationship between sporulation and lesion length for necrotrophic parasites. Indeed, in saprophytic fungi, the sporulation surface grows proportionally with the extension of necrosis induced by these parasites [22]. The results found in this study could be related to the method of estimating the sporulation between lines and between plants of the same genotype. Indeed, infectious periods differ in the time between lines and sporulation tissues of some lines that had not reached their maximum production of spores for the period of 28 days. The mean spore production per line and per plant showed that, line that produce fewer spores are the most resistant, in contrast lines that produce more spores are not the most susceptible (Table 2). Gowen et al. [23] also found that sporulation was not correlated with the pathogenicity of strains. However, this result is to modulate because when sporulation was measured, at the end of the experiment, the lesions of lines with the very short latent period, had already produced and may be most of their spores have been released. In our study, although the incubation and latent periods are the two most important components to explain the intensity of the disease on all lines, individual analysis helped to identify several characteristic components of each line. These components can be classified into three groups:

- The length and growth of lesions that correspond to the growth of the pathogen (axis 1),

- The incubation period which corresponds to the infectious period of the plant by the fungus (axis 1),

- Production of spores which corresponds to the reproductive phase (axis 2).

For resistant lines, the length and the growth of lesions appear to be the most important parameters to explain the resistance to *A. rabiei*. They correspond to one or more mechanisms of plant defense which limit the parasite growth at each site of stem infection. In a similar study on wheat Septoria (*Septoria nodorum*), Jeger [24] found that four factors are involved and

combined independently in cultivar resistance, these factors represent the percentage of necrosis in sporulation, growth and the establishment of the fungus. For necrotrophic fungus, it appears that the establishment and growth of the fungus are important components of resistance. Partial disease resistance is characterized by a reduced rate of epidemic development in a host population including lower infection frequency and a longer latent period [25,26].

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