# PATHOTYPE VARIATION OF THE REPRESENTATIVE GENOTYPES OF *ASCOCHYTA RABIEI* IN THE BEJA REGION

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## SUMMARY

Seventeen representative genotypes of *Ascochyta rabiei* in the Beja region were analyzed for virulence variation. Virulence characterization was based on rating Ascochyta blight disease on eight differential cultivars using the Riahi *et al.* (1990) scale. Cluster analysis of the virulence revealed five highly virulent phenotypes because at least six of the differential lines were severely infected. Virulence cluster analysis was compared to phylogenetic grouping as determined by Morjane *et al.* (1997) using RFLP assay with microsatellite probes. The correlation coefficient between the virulence and RFLP similarity matrices was low (0.02). This low association indicates that DNA polymorphism is independent of virulence because of virulence instability in Tunisia probably due to the utilization of non resistant cultivars and the occurrence of sexual reproduction between the isolates.

*Key words*: virulence, Ascochyta blight, host parasite interaction.

## INTRODUCTION

Ascochyta blight, caused by *Ascochyta rabiei* is a devastating disease of chickpea (*Cicer arietinum* L.) an economically important crop in the dry area of West Asia and North Africa. The disease occurs in most chickpea growing countries and can result in up to 100% loss of the crop when winter sown crops. In the Mediterranean area, losses are reduced by spring sowing but this not favours high production as the crop growth is limited by the onset of the hot dry summer season. Considerable efforts have been directed at controlling the disease through the development of resistant cultivars (Singh, 1997). However, this resistance is broken by the appearance of new pathotypes (Udupa

and Weigand, 1997a). Therefore understanding the pathogen variability of *A. rabiei* is essential for genetic resistance breeding.

Pathogen variability of *A. rabiei* has been demonstrated by many authors and occurs in several regions as in North Africa, Middle East, India and USA (Kaiser, 1973; Harrabi *et al.,* 1988, Gowen *et al.*, 1989; Mmbaga, 1997). Despite differences in aggressiveness within the isolate, significant genotype x isolate interaction shows that variability of *A. rabiei* populations can be characterized as a specialization of isolates to different cultivars of one host. Such isolates could then be considered as having the same virulence phenotype or pathotype. According to the differential set used, Vir and Grewal (1974) found 10 pathotypes among field isolates from India. Six races (pathotypes) were identified among 50 isolates from Syria (Reddy and Kabbabeh, 1985). Recently using a differential set of three cultivars with different levels of resistance, Weigand and Udupa (1997a) identified three distinct pathotypes with different levels of aggressiveness.

*A. rabiei* identification based on pathogenic characterization suffers from several problems: (*i*) there is a variability in the aggressiveness within the same pathotype (*ii*) the lack of standardization in the assay procedure and rating disease scale leads to inflated estimates of pathotypic diversity and variability (*iii*) the different differential hosts used to characterize *A. rabiei* populations are unable to detect new virulence. Some new variants are virulent to the whole set which presupposes a rapid evolution of virulence. This combined with the difficulties of exploring germplasm resistance for host differential completion makes the introgression of new resistance more difficult.

Molecular techniques such as RFLP and RAPD have been shown to be reliable tools for the characterization *A. rabiei* populations (Weizing *et al.,* 1991; Morjane *et al.*, 1994). Using a microsatellite sequence (GA-TA)$_4$ as a probe Udupa and Weigand (1997b) were able to diagnose *A. rabiei* pathotypes found in Syria. This genetic relationship with virulence supports the view that pathotypes are stable and composed of discernable clonal lineage. Using the combination of mi-

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crosatellite probes $(CA)_8$, $(CAA)_5$, $(CAG)_5$ and $(GATA)_4$, Morjane *et al.* (1997) identified 17 different genotypes from 156 *A. rabiei* isolates collected from the Beja region (North West of Tunisia). Phylogenetic studies separated these genotypes into six clusters at a similarity coefficient of 0.24 (Table 1). The purpose of this work was therefore to investigate the virulence of these genotypes and determine the relationships between pathotype and genotype grouping. A prediction would be that if asexual reproduction occurs in the Beja region an association between genotype and virulence grouping would be found. In this paper the mechanisms of virulence evolution of *A. rabiei* populations in the Beja region are discussed on the basis of the analysis of virulence.

**Table 1.** Classification of the 17 representatives genotypes of *A. rabiei* in the Beja region at similarity of 0.24 as determined by Morjane *et al.* (1997).

| Cluster | Genotypes |
|---|---|
| I | Tar1, Tar2, Tar3, Tar4 |
| II | Tar5 |
| III | Tar6, Tar7 |
| IV | Tar8 |
| V | Tar9 |
| VI | Tar10, Tar11, Tar12, Tar13, Tar14, Tar15, Tar16, Tar17 |

**MATERIALS AND METHODS**

**Pathogenicity test.** The differential set is composed of eight cultivars with different sources of resistance (Singh and Reddy, 1990); ILC72, ILC195, ILC482, ILC1929, ILC3279, ICC13416, FLIP8346C and FLIP 8479C. Seeds of the various cultivars were sown at random in 60x48 cm plastic trays with 12 seeds per cultivar. Three replications were used. The trays were placed in a growth chamber and maintained at 22°C with 16 hours of light. Twelve days after sowing, chickpea seedlings were sprayed until run off with a fresh spore suspension prepared from a single spore culture as described by Weising *et al.* (1991). At this stage the plants were 10 cm in height. Seventeen isolates corresponding to the representative genotypes of the Beja region were used (Table 2). The inoculum was standardized at $10^6$ spores ml$^{-1}$. The inoculated plants were immediately placed in a dew chamber and incubated for 48 hours and then returned to the growth room. Three weeks after inoculation the plants were evaluated for

disease reaction based on the Riahi *et al.* (1990) scale which consists of measuring the linear infection index (LII). The latter corresponds to the ratio between the total length of the lesion (TLL) and the total length of the plant (TPL).

**Table 2.** Genotypes determined by Morjane *et al.* (1997) with the reference number and origin.

| Genotypes | Isolate number | Origin |
|---|---|---|
| Tar1 | 1133 | ABCD |
| Tar2 | 1268 | A |
| Tar3 | 1270 | A |
| Tar4 | 1273 | A |
| Tar5 | 1313a | A |
| Tar6 | 1313b | A |
| Tar7 | 1314 | A |
| Tar8 | 1322 | A |
| Tar9 | 1325 | A |
| Tar10 | 1323 | AD |
| Tar11 | 122 | E |
| Tar12 | 1131 | ABCD |
| Tar13 | 125 | E |
| Tar14 | 128 | E |
| Tar15 | 153 | D |
| Tar16 | 151 | D |
| Tar17 | 1329 | A |

A: Hamman Sayala, B: Oued Zargua, C: Beja/bousalem, D: Beja, E: Oued Beja.

**Statistical analysis.** Using virulence phenotypes we computed estimates of the Euclidian distances between all pairs of the $n = 17$ isolates. This distance is calculated by:

$$d_{ij} = \sqrt{\Sigma (x_{ik} - x_{jk})}$$

where $x_{ik}$ and $x_{jk}$ ($k = 1..m$) represent the variable values for isolates $i$ and $j$ and $m$ is the number of variables, here the number of cultivars used as a differential set ($m = 8$). $d_{ij}$ served as elements of the $n$x$n$ proximity matrix, D. The isolates were classified by custer analysis using the average linkage method (UPGMA; unweighted pair group method using arithmetic averages). Cluster analysis was performed by the statistic software version 4.5 (1993) with D serving as input to the analysis. A dendrogram was obtained depicting the relationships between isolates.

To measure the correspondance between this classi-

fication (based on virulence phenotypes) and that of Morjane *et al.* (1997) based on molecular markers, we calculated the correlation coefficient between both dendrograms. This was achieved by computing the average dissimilarity between pairs of isolates as determined from the dendrograms. To estimate the distance between two isolates, from the dendrogram, we used the placement of the nearest node joining these two isolates in the classification. We thus generated two distance matrices based on the two classifications. These should be similar if the dendrogram based on virulence accurately depicts the relationships between isolates as estimated by the molecular data. The concordance was measured by the linear correlation coefficient ($r$) and the Spearman rank correlation coefficient ($r_s$).

## RESULTS

Cluster analysis of the virulence data obtained after analysis of the reaction of the 17 genotypes on a differential set composed of 8 varieties, revealed 6 clusters at a similarity coefficient of 0.5 (Fig. 1). The virulence characteristics of each cluster are summarized in Table 3. Isolates are considered virulent when the mean disease rated from 4.5 to > 10 on the Riahi *et al.* (1990) scale. Cluster A containing the most predominant genotypes (Tar1 and Tar12; Morjane *et al.*, 1997) is particularly virulent to all varieties of the differential set. The 10 genotypes (Tar2, 3, 5, 7, 8, 9, 14, 15, 16 and 17) of cluster B are pathogenic to all varieties except ILC72. Clusters C (Tar4), D (Tar13) and F (Tar10) composed each of one genotype are differentiated by their avirulence to one variety respectively ILC3279, ICC1316, and FLIP 8346C. Cluster E (Tar6) is avirulent on both ILC482 and ILC72.

The linear and the Spearman rank correlation coefficients between the classification based on virulence and on genetic polymorphism of the 17 genotypes described by Morjane *et al.* (1997) were non significant ($r = 0.019$, $P = 0.82$ and $r_s = -0.040$, $P = 0.63$), indicating a weak relationship between virulence and polymorphism and suggesting that DNA polymorphism is independent of virulence. Genotypes of pathotype B (Tar2, 3, 5, 7, 8, 9, 14, 15, 16 and 17) are highly polymorphic and are not included in the same lineage of the phylogenetic tree (Morjane *et al.,* 1997).
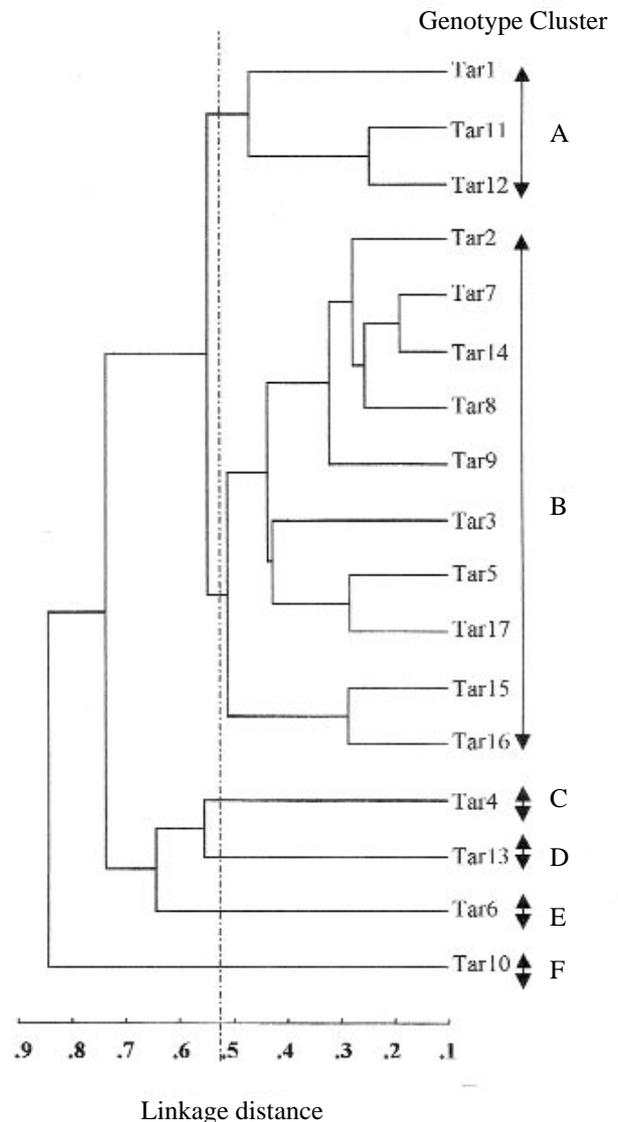


**Fig. 1.** Dendrogram of 17 representative genotypes of *A. rabiei* in the Beja region based on mean disease rated with the Riahi *et al.* (1990) scale on 8 chickpea differential cultivars listed in Table 2 using the unweighted pair group arithmetic mean (UPGMA) program.

## DISCUSSION

Using a differential set of eight cultivars, pathotype variability was observed in the *A. rabiei* population of the Beja region. These pathotypes differed only in the addition or subtraction of one or two virulence phenotypes revealing the occurrence of virulent pathotypes in the Beja region. Predominant genotypes (Tar1, and Tar12) as observed by Morjane *et al.* (1997) were highly

**Table 3.** Virulence grouping of the 17 representative genotypes in the Beja region and their virulence patterns on eight differential cultivars.

| Virulence cluster | Genotypes | Virulence on differential cultivars* |
|---|---|---|
| A | Tar1, Tar11, Tar12 | 1, 2, 3, 4, 5, 6, 7, 8 |
| B | Tar2, Tar3, Tar5, Tar7, Tar8, Tar9, Tar14, Tar15, Tar16, Tar17 | 1, 2, 3, 4, 5, 7, 8 |
| C | Tar4 | 1, 3, 4, 5, 6, 7, 8 |
| D | Tar13 | 1, 2, 4, 5, 6, 7, 8 |
| E | Tar6 | 1, 2, 3, 5, 7, 8 |
| F | Tar10 | 1, 2, 3, 4, 5, 6, 8 |

1: ILC1929; 2: ILC3279; 3: ICC1316; 4: ILC482; 5: ILC195; 6: ILC72; 7: FLIP8346C; 8: FLIP8479C.

\* Ascochyta blight rated on the Riahi *et al.,* scale (1990). Isolates causing mean disease ratings from 1 to 4.49 and 5 to >10 were considered avirulent and virulent respectively.

virulent because they severely attack the whole differential set. The predominance of the most aggressive genotypes was also described in Syria (Udupa and Weigand, 1997a). Evolution of the pathogen population to highly virulent phenotypes is essentially due to host selection and rapid adaptability of the isolates to overcome resistance. The use of different sources of resistance in chickpea and pyramiding resistance in the Middle East and Asia have increased the virulence of pathogen populations and a rapid breakdown of new sources of resistance is frequently observed (Mmbaga, 1997). Utilization of the susceptible cultivar Amdoun by Tunisian farmers does not explain the predominance of highly virulent pathotypes. Moreover the recent introduction (1990) of the resistant cultivars Kasseb (FLIP8446) and Chetoui (ILC3279) in the Beja region which then became immediate susceptible to the representative genotypes of the Beja region shows the poor influence of host selection against the high adaptability of virulence of pathogen populations in Tunisia. Highly pathogenic genotypes presumably evolved through host selection in Turkey and Syria where resistant chickpea cultivars are deployed and then migrated through infected seeds. The predominance of genotypes Tar1 and Tar12 both representing 60% of the pathogen population in the region (Morjane *et al.,* 1997) strengthen the hypothesis of seed contamination. Recycling of contaminated chickpea seeds, a cultural practice used by Tunisian farmers, would increase the predominance and spread of Tar1 and Tar12 genotypes through asexual reproduction during seed germination and plant development. The occurrence of a predominant genotype (genotype H) over a large area (Syria and Lebanon) with a high frequency also suggests a probable migration of this genotype through infected seeds (Udupa and Weigand, 1997b).

Despite using controlled conditions and the same cultivars to characterize *A. rabiei* populations, some inconsistencies have been observed according to the results of Udupa and Weigand (1997a). These Authors showed with respect to the high resistance level of ILC3279, that resistance of ILC 482 to the isolates necessarily implies the resistance of ILC3279. In contrast to their results, in our study, ILC3279 and ILC482 showed susceptibility and resistance respectively to Tar6 isolates. This discrepancy could not be totally explained by the utilization of a different protocol to test pathogenic variability of *A. rabiei* but suggests that the differential set used are inappropriate to test the pathogenicity. Usually differential series are chosen on the basis of the differential reaction of different cultivars to several isolates collected from various regions. According to that differential reaction, specific resistant genes are attributed to each line of the differential set. However these genes have not yet been characterized in chickpea. Since pathotype specific and non specific resistance coexist in chickpea (Chiha *et al.,* 1997), the absence of identification and characterization of the source and the nature (specific or non specific) of resistance of each line will lead to the establishment of a differential set inappropriate for pathotype characterization. Moreover the presence of genotypes which show virulence to the whole differential set presupposes that the series would be completed by the introduction of new sources of resistance.

An attempt to correlate the representative genotypes of *A. rabiei* in the Beja region with virulence phenotype was carried out in order to clarify the pathotype evolution through DNA patterns. Correlation was very low showing no relationship between genetic polymorphism and virulence. This observation might be explained by frequent parasexual recombination allowing the selection of virulence phenotypes to occur independently of molecular polymorphism. High genotype diversity within the *A. rabiei* population in Beja observed by Morjane *et al.* (1994, 1997) and the existence of both

mating types in Tunisia, essential for sexual reproduction in infested chickpea debris (Kaiser *et al.,* 1997), are consistent with the expectation for sexually reproducing organisms. Furthermore, the absence of host selection in Beja and the occurrence of several pathotypes may lead to pathotype instability of the observed genotypes and consequently non virulence/polymorphism relationships. However in Syria pathotype association with the most predominant genotypes was related to the selection and stability of highly virulent genotypes through the deployment of selective resistance pressure (Udupa and Weigand, 1997b).

In conclusion host selection in the Beja region has probably contributed little to the selection of virulent pathotypes. Highly virulent pathotypes were probably selected through the utilization of new resistant chickpea varieties in Middle East countries where the seeds are produced. The introduction of contaminated seeds into the Beja region led to the spread of highly virulent pathotypes without influence of host selection. Therefore a strict certification of chickpea seeds should be applied at the frontier to avoid rapid breakdown of newly introduced sources of resistance in Tunisia. The assumption that sexual reproduction may contribute to genotype variability is possible since 17 different genotypes were identified from 156 collected isolates in the Beja region (Morjane *et al.,* 1997). However this genetic variability does not necessarily imply variability of the virulence phenotype. In fact, recombination may occur in neutral DNA sequences which change DNA patterns without affecting the virulence which essentially evolves through host selection. Since any of the genotypes could be associated to a virulence phenotype, the identification of DNA markers for virulence would be made possible by mating virulent and avirulent isolates to establish a progeny population that segregate for virulence. Artificially crosses between low and high virulent *A. rabiei* isolates have been successfully performed (Gestlinger *et al.,* 1997). The molecular polymorphism of the progeny was analyzed and segregation analysis of the polymorphic markers with the virulence will permit the identification of DNA markers for virulence.

## ACKNOWLEDGMENTS

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