



SCREENING OF TEN ADVANCED CHICKPEA LINES FOR BLIGHT AND WILT RESISTANCE

*F. F. JAMIL, I. HAQ, N. SARWAR, S. S. ALAM, J. A. KHAN,
M. HANIF, I. A. KHAN, M. SARWAR and M. A. HAQ.

Nuclear Institute for Agriculture and Biology (NIAB), P. O. Box 128, Jhang Road, Faisalabad, Pakistan

(Received January 21, 2002 and accepted in revised form September 23, 2002)

Ten advanced chickpea lines developed at NIAB were screened for resistance to *Ascochyta* blight and *Fusarium* wilt diseases in different sets of experiments conducted under controlled environment. Inoculation of plants by spore suspension of virulent strains of *Ascochyta rabiei* revealed that one line (97313) was resistant tolerant, two lines (97305, 97392) were tolerant, six lines (97306, 97310, 97311, 97303, 97302, 97393) were tolerant/susceptible and one line (97301) was susceptible. Screening of the same lines against *Fusarium* wilt by water culture method showed that two lines (97301, 97313) were moderately resistant, four lines (97302, 97303, 97306, 97393) were tolerant and the remaining four lines were susceptible. Screening through phytotoxic culture filtrates revealed that two lines (97302, 97313) were less sensitive to culture filtrates of *Ascochyta rabiei* and *Fusarium oxysporum* than the resistant check (CM88). These lines were also analyzed spectrophotometrically for peroxidase enzyme activity. Maximum enzyme activity was detected after 48 hours of inoculation with *A. rabiei* in three lines (97305, 97311, 97313) and resistant check (CM88) while enzyme activity in the remaining lines reached its maximum after 72 hours of inoculation which was comparable to the susceptible check (Pb-1). These studies lead to the conclusion that one line (97313) exhibited resistance against both the diseases and can be used as a source of resistance for further improvement of chickpea germplasm.

Keywords : *Ascochyta rabiei*, *Fusarium oxysporum*, Phytotoxic, culture filtrates, Peroxidase activity.

1. Introduction

Chickpea (*Cicer arietinum* L.) is an important grain legume crop in Pakistan it constitutes about 70% of all pulse crops. It is a good source of protein (20 - 26%) for the masses. Chickpea is not only an important source of human food and animal feed, but, being a leguminous crop, helps in the management of soil fertility particularly in dry-lands. Unfortunately this crop is badly affected by two important and yield limiting diseases i.e. chickpea blight caused by the fungus *Ascochyta rabiei* (pass) Lab. and chickpea wilt caused by *Fusarium oxysporum* f. sp. *Ciceris* (Padwick). Both diseases are highly influenced by environmental conditions, the former being prevalent in cool and humid and the latter in warm and dry environments.

Pathogenic variability has been reported in both the pathogens [1-3]. The pathogens are constantly changing in nature and break the resistance of resistant varieties after sometime. Therefore, characterization and geographic distribution of

virulent races/strains/pathotypes of the pathogens and screening of germplasm, against them is inevitable to identify resistant germplasm.

At NIAB we have established the genetic and pathogenic diversity within *A. rabiei* population of Pakistan and have identified a representative set of virulent *A. rabiei* isolates from different areas to be used for thorough and reliable screening of chickpea germplasm for durable resistance before releasing as varieties. A geographical map of chickpea growing areas of Pakistan has also been constructed to show the prevalence and distribution of these virulent strains to help release resistant varieties for these areas [4].

Phytotoxins are reported to be helpful not only in understanding the mechanism of pathogenesis but also in identifying resistant cells in tissue culture or in screening and breeding for disease resistance. [5-6]. Peroxidases (PO) are reported to have a significant role in plant defense mechanism. They are induced by wounding and infections and presumably involved in the repair of damaged cell

* Corresponding author : niab@fsd.paknet.com.pk

walls [7]. The levels of PO activity and its isozyme patterns have been shown in several plant systems to be altered by stress, chemicals and infections [8]. The present studies were aimed to evaluate the resistant chickpea germplasm against blight and wilt diseases using different techniques.

2. Materials and Methods

2.1 Screening of chickpea germplasm against *Ascochyta blight* by using spore suspension

Seeds of ten test lines/cultivars were obtained from chickpea breeders of NIAB. These seeds alongwith seeds of two standard varieties; Aug 424 (susceptible) and CM88 (resistant) were sown in plastic pots kept in a growth room (temp. 20 ± 2 °C; rel. humidity, 70 - 80%; light, 24000 - 26000 Lux fluorescent + Incandescent).

Four highly virulent isolates, one each from Chakwal, Kaghan, NARC and Attock were selected from a culture stock of about 200 isolates collected from the major chickpea growing areas of the country over a number of years and preserved under liquid nitrogen. Cultures of these virulent isolates were multiplied on autoclaved chickpea seeds. Ten-day-old cultures were used for preparation of spore suspension. Two-week-old seedlings grown in pots were sprayed with spore suspension (10^6 /ml) of *A. rabiei* isolates. Disease data was recorded on a 9 point rating scale [9] twelve days after inoculation.

2.2 Screening of chickpea lines against *Fusarium wilt* by water culture method

Mass inoculum of highly virulent isolate FOC 9917 obtained from NIAB collection was prepared by growing the fungus on Czapek-dox liquid medium. A concentration of 6.5×10^5 spores per ml was used. Seeds of chickpea lines were surface sterilized with sodium hypochlorite and germinated in plastic pots containing sandyloam soil. Ten days old seedlings were transplanted in 100 ml conical flasks containing 50 ml inoculum and held tightly with cotton plugs. Data were recorded after 10 days by using 1 - 5 scale as described by Haware and Nene, 1982 [10] with slight modification.

1.	0% Mortality	Highly resistant.
2.	10% or less mortality	Moderately resistant.
3.	11 - 20% mortality	Tolerant.
4.	21 - 50% mortality	Susceptible.
5.	51% or more mortality	Highly susceptible.

2.3 Screening of chickpea lines against culture filtrates of *A. rabiei* and *Fusarium oxysporum f. sp. Ciceris* (FOC)

Culture filtrate of FOC was produced as described by Bajwa *et al.*, 2000 [11] and that of *Ascochyta rabiei* as reported by Alam *et al.*, (1989) [12]. Five ml of culture filtrate was taken in small vials (cap. 7.5 ml) in three replicates for each variety/line. Two weeks old cuttings of the lines/varieties were dipped in these vials and then placed in incubator at 25°C. CM88 and AUG 424 were used as resistant and susceptible checks respectively. The symptoms were observed daily. Data were recorded when susceptible check completely died.

2.4 Enzyme assay

These chickpea lines alongwith Pb 1 (susceptible) and CM88 (resistant) were also analyzed spectrophotometrically for peroxidase enzyme. Plant tissues from control and inoculated plants were collected at 0,24,48 and 72 hours after inoculation with *A. rabiei*. Extraction and activity of peroxidase were determined as described [13].

3. Results and Discussion

3.1 Screening of chickpea germplasm against *ascochyta blight* by using spore suspension

Of the advance lines, one line (97313) was found resistant/tolerant, two lines (97305 and 97392) were tolerant, six lines (97302, 97303, 97306, 97310, 97311, 97393) were tolerant/susceptible and one line (97301) was found susceptible (Table 1). One line (97313) detected as resistant/tolerant may be recommended for cultivation as a blight resistant material. Most of the tested varieties/lines were found as susceptible or tolerant/susceptible and no one showed absolute resistance indicating the scarcity of resistance in existing chickpea germplasm. It suggests the need for evolution of chickpea varieties having durable resistance to *Ascochyta blight*. Blight resistant varieties have been released from time to time, which become susceptible after some time probably due to appearance of virulent strains/pathotypes of the pathogen [2]. Variability in pathogenicity of *A. rabiei* has been reported from many countries. [14, 15].

3.2 Screening of chickpea lines against *Fusarium wilt* by water culture method

Out of 10 lines, only two lines (97301 and 97313) were found moderately resistant, and four

Table 1. Screening of some chickpea lines for resistance to *Ascochyta* blight by using four highly virulent isolates of *A. rabiei* collected from different areas.

A. rabiei Isolates collected from

Chickpea cultivar /line	Chakwal	Kaghan	NARC	Attock	Average	Reaction
Aug 424	9.0	9.0	9.0	9.0	9.0	S
CM 88	2.8	5.3	4.5	5.7	4.57	R/T
97301	8.2	7.8	6.5	8.8	7.82	S
97302	7.97	7.0	4.7	7.2	6.72	T/S
97303	6.4	7.4	5.8	7.1	6.67	T/S
97305	4.0	5.9	4.6	5.6	5.02	T
97306	5.7	6.7	5.6	6.2	6.05	T/S
97310	5.5	6.8	6.2	6.4	6.22	T/S
97311	5.8	7.5	5.2	7.25	6.44	T/S
97313	3.3	5.5	4.1	5.5	4.5	R/T
97392	4.0	6.8	6.2	5.4	5.6	T
97393	7.0	7.3	7.3	7.0	7.15	T/S

Scale: R (resistant) = 1 - 3; R/T (resistant/tolerant) = 3.1-4.5.

T (Tolerant) = 4.6 - 6; T/S (Tolerant/Susceptible) 6.1-7.5

S (Susceptible) = 7.6 - 9.0

Table 2. Screening of chickpea lines against FOC 9917 by water culture method.

Lines	Disease %age	Rating scale	Degree of resistance
97301	8	2	Moderately resistant
97302	16	3	Tolerant
97303	17	3	Tolerant
97305	25	4	Susceptible
97306	20	3	Tolerant
97310	30	4	Susceptible
97311	26	4	Susceptible
97313	10	2	Moderately resistant
97392	23	4	Susceptible
97393	14	3	Tolerant
Aug 424	77	5	Highly susceptible
CM 88	17	3	Tolerant

- | | | |
|----|-----------------------|-----------------------|
| 1. | 0% Mortality | Highly resistant. |
| 2. | 10% or less mortality | Moderately resistant. |
| 3. | 11 - 20% mortality | Tolerant. |
| 4. | 21 - 50% mortality | Susceptible. |
| 5. | 51% or more mortality | Highly susceptible. |

lines, i.e. 97302, 97303, 97306 and 97393 were tolerant and the rest were found susceptible. None of the lines was found highly resistant showing scarcity of the resistant sources against FOC [16]. Moreover, the performance of the two moderately resistant lines, 97301 & 97313, was comparatively better than CM-88 (resistant check). These two lines were also found less sensitive against culture filtrates of the same isolate showing good correlation between the two methods of screening.

3.3. Screening of chickpea lines against culture filtrates of *A. rabiei* and FOC

Disease symptoms started appearing within 3 days in both the culture filtrates. The susceptible check completely died after 4 days. Out of 10 lines

screened against culture filtrate of *A. rabiei* the lines 97302 and 97313 were less sensitive as compared to the resistant check (CM 88) and the rest of the lines were highly sensitive (Table 3). When screened against FOC culture filtrates two lines, (97301 and 97313) were found less sensitive as compared to the resistant check, CM 88, (Table 4). Bajwa *et al.*, (2000) [12] screened the chickpea germplasm under field conditions and against the culture filtrates of FOC, and found a positive correlation between the two methods of screening for disease resistance.

3.4 Enzyme assay

Peroxidase activity reached its maximum after 48 hours in lines, 97305, 97311, 97313 and in

Table 3. Screening of chickpea lines against culture filtrate of *Ascochyta rabiei*.

Lines / varieties	R1	R2	R3	R4	C1	C2
97301	+++		+++	++	-	-
97302	++	+	+	+	-	-
97303	++	++	+++	+++	-	-
97305	+++	-	+++	+++	-	-
97306	+++	+++	+++	++	-	-
97310	+++	++	+++		-	-
97311	++	++			-	-
97313	+	+	++	+	-	-
97392	++	++	++	++	-	-
97393	+++	+++			-	-
AUG 424	+++	+++	+++	++	-	-
CM88	++	++	++	++	-	-

- = No reaction, healthy. + = Mild Yellowing.
 ++ = Leaves burning 50%. +++ = complete burning/killing.

Table 4. Screening of chickpea germplasm against culture filtrates of FOC.

Lines/ varieties	R1	R2	R3	Cont
97301	++	+	+	-
97302	+	++	+	-
97303	+++	++	+++	-
97305	+++	+++	+++	-
97306	++	+++	+++	-
97310	+++	++	+++	-
97311	+++	+++	++	-
97313	+	++	++	-
97392	+++	+++	+++	-
97393	+++	+++	+++	-
CM88	++	++	+++	-
Aug 424	+++	+++	+++	-

- = Healthy (no reaction). + = Slight yellowing/burning.
 ++ = Drooping/complete burning of leaves.
 +++ = Wilting.

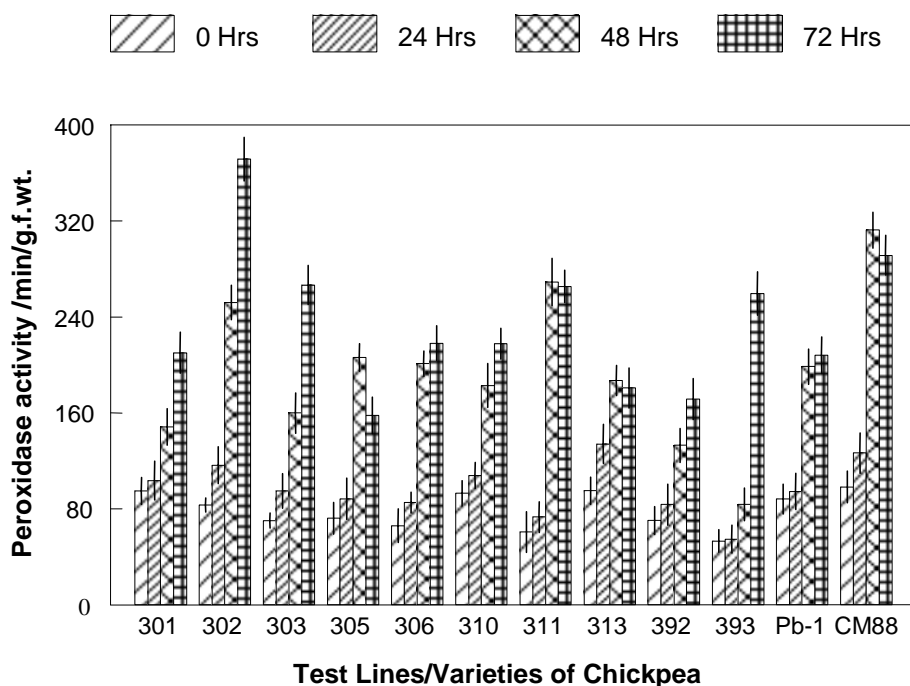


Fig 1: Peroxidase activity in various test lines / varieties of chickpea at different time intervals after inoculation with *Ascochyta rabiei*.

resistant check, CM88, (Fig.1) while in other lines the enzyme activity reached its maximum after 72 hour, which was comparable to susceptible check (Pb-1). PO is known to be involved in oxidative polymerization of hydroxycinnamyl alcohols to yield lignin [8] and cross-linking isodityrosin bridges in cell walls. These compounds act as barriers against pathogen invasion and hence constitute part of host resistance mechanism [17]. PO also produces free radicals and hydrogen peroxide, which are toxic to several pathogens [18]. It suggests that high activity of PO in resistant varieties of chickpea constitutes part of its resistance mechanism against *A. rabiei* by enhancing phenol and lignin accumulation.

4. Conclusions

It is concluded from these studies that one line 97313 which exhibited resistance against both the diseases can be used as a source of resistance for further improvement of chickpea germplasm.

References

[1] A. Porta-Puglia, Proceedings of the consultative meeting on breeding for disease resistance in Kabuli chickpea. ICARDA,

Aleppo, Syria. Singh K.B., Sexena, M.C. eds., (1992) 135.

- [2] F. F. Jamil, M. Sarwar, I. Haq and N. Bashir, Pakistan Journal of Botany **27** (1995) 193.
- [3] R.M. Jimenez-Diaz, A. Trapero-Casas and J. Cabrera de La Colina, Vascular wilt diseases of plants, NATO AS/Series, Berlin, Germany, Springer-Verlag. Tjamos, E. C. and Beckman, C. Eds., **H28** (1989).
- [4] F. F. Jamil, N. Sarwar, M. Sarwar, J. A. Khan, J. Geistlinger and G. Kahl, Proc. 2nd Natl. Conf. of Plant Pathol., (Sept. 27 - 29, 1999, Univ. of Agric., Faisalabad), S. M. Khan, R. A. Chohan and A.M. Khan Eds., (2000) 214.
- [5] H. S. Song, S. M. Lim. and J. M. Widholaw, Phytopathology, **84** (1994) 948.
- [6] M. Maiero, G. A. Bean and T. J. Ng., Phytopathology, **81** (1991) 1030.
- [7] H. Burecka, and A. Miller, Plant Physiol., **53** (1974) 569.
- [8] R. F. Oliveira, F.S. Pascholatic and B. Leite, Fitopatologia Brasileira, **22** No.2 (1997) 195.

- [9] K. Weising, D. Kaemmer, J. T. Epplen, F. Weigand, M. Saxena and G. Kahl, *Curr. Genet.*, **19** (1991) 483.
- [10] M. P. Haware and Y. L. Nene, *Plant Disease*, **66** (1982) 809.
- [11] S. S. Alam, J. N. Biltan, A. M. Z. Slawin, D. J. Williams, R. N. Sheppard and R. N. Strange, *Phytochemistry*, **28** (1989) 2627.
- [12] K. M. Bajwa, I. A. Khan, S. S. Alam, I. Ahmed, and M. A. Gill, *Pak. J. Phytopathol.*, **12** No.1 (2000) 62.
- [13] N. Sarwar, M. Sarwar, F.F. Jamil and R. Perveen, *Proc. 2nd Natl. Conf. Plant Pathol.* (Sept. 27 - 29, 1999, Univ. of Agric., Faisalabad), S. M. Khan, R.A. Chohan and A. M. Khan Eds., (2000) 228.
- [14] S. R. Gowen, M. Orton, B. Thurley and A. White, *Tropical Pest Management*, **35** (1989) 180.
- [15] Y. L. Nene, *Ascochyta blight and winter sowing of chickpea* (Eds.): M. C. Saxena and K. B. Singh. Martinus Nijhoff/Dr. W. Junk. Hague, Netherland. (1984) 17.
- [16] A. Porta-Puglia and A. In Fantino. *Pak. J. Phytopathol.*, **9**, No.10 (1997) 26.
- [17] W. N. Okey, E. J. Duncan, G. Sirju-charran and T. N. Sreenivasan, *J. Phytopathol. (Berlin)* **145**, No.7 (1997) 295.
- [18] M. Peng and J.A. Kuc', *Phytopathol.*, **82** (1992) 696.