

Effect of foliar application of *Ascochyta rabiei* on growth and vesicular arbuscular mycorrhizal status of eight chickpea varieties

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Abstract

Eight different (resistant and susceptible) chickpea varieties were studied for various growth parameters including vesicular arbuscular mycorrhizal status. This study was carried out before and after the spray of the pathogen, *Ascochyta rabiei* and the results were evaluated.

Resistant varieties showed significantly high values for growth parameters studied. Vesicular arbuscular mycorrhizal infections especially arbuscules were recorded high for resistant varieties. In contrast susceptible varieties showed a noticeably low values for all growth parameters. However as far as their mycorrhizal status is concerned, significantly high values for vesicular infections were observed.

Introduction

Chickpea is the most important pulse of Pakistan, which is cultivated on about one million hectare per annum with a yield of 550kg/ha. The growth and yield of the crop has always been affected by *Ascochyta rabiei* (Pass) Laber (Nene, 1982). Low ambient temperatures and high relative humidity being conducive conditions for the blight to become an epidemic over night. The pathogen attack all aerial parts of plant giving rise to dark necrotic spots on leaves, stem and pods. As the infection progresses the pycnidia are formed in concentric rings (Hafiz, 1951). When stem is infected girdling commonly occurs causing breakage at this point. Plant parts above the lesion die rapidly (Agris, 2000).

According to Krishna *et al.*, (1984) the genetically resistant plant species can better tolerate the impact of disease due to their inherent susceptibility to VA mycorrhizal infection. There are numerous examples of cultivars within plant species that differ in the extent of colonization by mycorrhizal fungi, and their responsiveness to them (Parke and Kaeppler, 2000). Mycorrhizae are highly evolved, mutualistic associations between soil fungi and plant roots. The partners in this association are members of the fungus kingdom (Basidiomycetes, Ascomycetes and Zygomycetes) and most vascular plants (Harley & Smith, 1983; Kendrick, 1992; Brundrett, 1991). In the mycorrhizal literature, the term *symbiosis* is often used to describe these highly interdependent mutualistic relationships where the host plant

receives mineral nutrients while the fungus obtains photosynthetically derived carbon compounds (Harley & Smith, 1983; Smith & Read, 1997). Mycorrhizal associations involve 3-way interactions between host plants, mutualistic fungi and soil factors. Rhizosphere microbial changes occur when mycorrhizae are formed. When these associations are formed the fungi live both within root tissue and external to those tissues. They could have direct interactions with other soil organism or they could influence those organisms indirectly by changing host plant physiology especially root physiology and in turn pattern of exudation into the '*mycorrhizosphere*'. Numerous attempts have been made to summarize the literature on mycorrhiza-disease interactions and to draw conclusions if possible (Azcon-Aguilar & Barea, 1996; Bagyaraj, 1984; Caron, 1989; Dehne, 1982; Hooker *et al.*, 1994; Ingham, 1988; Jalali & Jalali, 1991; Linderman, 1988; Linderman & Paulitz, 1990; Schenck, 1983 & 1989; Schenck & Kellam, 1978; Schoenbeck, 1979, St-Arnaud *et al.*, 1995; Zak, 1964). However, conclusions generally have not been possible, largely because the data are few and the experimental systems rarely, if ever, have been comparable (Schenck, 1983 & 1989). Furthermore, the reactions of plants, with or without mycorrhizas, to various types of pathogens (whether fungi, bacteria, virus, or nematode) may be very different, making comparisons virtually impossible. Nonetheless, various authors have discussed the reports of interactions and the mechanisms that seem to be involved. Most

mechanisms seem to fall into the general categories of: 1) enhanced nutrition, 2) competition for host photosynthates and infection sites, 3) morphological changes in roots and root tissues, 4) changes in chemical constituents of plant tissues, 5) reduction of abiotic stresses, and 6) microbial changes in the mycorrhizosphere (Linderman & Paulitz, 1990; St-Arnaud *et al.*, 1995; Zak, 1964). All or any combination of these mechanisms could be involved in disease interactions with the arbuscular mycorrhizal (AM) fungi in any specific situation. The extent to which they modify the disease reaction is influenced by many factors. Thus, the relative importance of different mechanisms or combinations thereof on plant tolerance to diseases as affected by different factors can be addressed.

A preliminary study has been conducted to compare the genetically different varieties of chickpea as regards their potential to form VA mycorrhiza and susceptibility towards *Ascochyta* blight.

Materials and Methods

Chickpea plants were sampled from the fields of the department of plant pathology, university of Agriculture Faisalabad, where an area of about 15 x 52 feet was allocated for sowing of thirteen varieties. Seeds of each variety were sown in specified rows while a row of test plants (highly susceptible) was planted in the end.

Out of these thirteen varieties, eight were selected for regular sampling. These varieties were 184W, NE1256, AUG 970, ILC 1256, CM 72, ICC 6304, ILC 2548, C 679 respectively. Five harvests were taken. Two harvests were made before and three after the spray of the pathogen. From the early beginning (when seedlings were 4-6 inches high) and till the appearance of blight symptoms on that plant, pathogen suspension was sprayed ten times with an interval of two days. Each time the inoculum of *Ascochyta rabiei* growing on boiled chickpea seeds were washed in water (1Kg/ 20 litres of Water) and sprayed on the plants with the help of a sprayer. Plants were dug out carefully without damaging the fine root system and nodules at the time of harvest. These samples were brought back to the Biocontrol Research Lab., Department of Botany, University of the Punjab, for further study.

The growth parameters noted were root/shoot lengths, fresh dry weights of root and shoot, number of nodules and vesicular arbuscular infections.

List of different chickpea varieties used for analysis

Sr. No.	Variety code Number
1.	184 W*
2.	NE1256*
3.	AUG 970 ⁺
4.	ILC 1256 ⁺
5.	CM 72 ⁺
6.	ICC 6304 ⁺
7.	ILC2548*
8.	C 679 ⁺

*= Susceptible variety

⁺= Resistant variety

For VA mycorrhizal assessment, root system were carefully washed under tap water and preserved in F.A.A. (Formaline, acetic acid alcohol in 5:5:90). Clearing of roots was done in 10% KOH in autoclave at 15lb/inch² pressure. Dark colored roots were bleached in 30% H₂O₂ and neutralised in 0.1N HCl. Staining was done in trypan blue (0.05% in lactic acid, phenol and glycerine in 1:1:1 ratio) following the method of Phillips and Hayman (1970) with some modifications (Iqbal and Nasim, 1991). Stained root pieces were mounted in lactophenol and observed under the microscope. The parameters recorded for the assessment of VAM infections were percentage of general mycelial infections, percentage of arbuscular infections, percentage of vesicular infections, extent of infections, number of arbuscules and vesicles per 100 cm of root length. Presence or absence of different fungal structures in root pieces was recorded under the heading of percentage frequency of occurrence. The number of vesicles and arbuscules was recorded by randomly focusing the root piece at 10X and counting the number all along the length of the root piece. However the extent of mycelium was recorded with the help of a pre-calibrated ocular micrometer. Data was statistically analyzed by applying Duncan's New Multiple Range Test, (Steel and Torrie, 1980). Microphotography was done with the help of a Minolta X700 with a microscope adapter tube.

Results

Shoot and Root length

At the time of first harvest maximum values (16.5 cm) for shoot length were recorded for varieties 4 and 7. While variety number 3 had lowest values (12.4cm) for the shoot length (Fig. 1).

At the time of second harvest the pattern of maximum and minimum values changed. Almost overlapping values (26.95 and 26.53cm) of shoot length were recorded for two varieties like Nos. 7

and 8 respectively, while variety # 2 has minimum value (18cm) for shoot length.

At the later stages i.e. at third, fourth and fifth harvests variety number 8 had significantly high values. At third harvest variety No. 6 had overlapping values as regards shoot length (Fig.1).

Root length was maximum (6.25cm) for variety number 7 while lowest value was recorded

for variety number 3. At second and third harvests the highest values were recorded for 7th variety while at fourth and fifth harvest the maxima shifted to variety No. 4. The plants of variety No. 4 had their root growth up to a significant length i.e. 17cm at the time of last harvest. The varieties kept on varying as regard having minimum values for root growth (Fig.1).

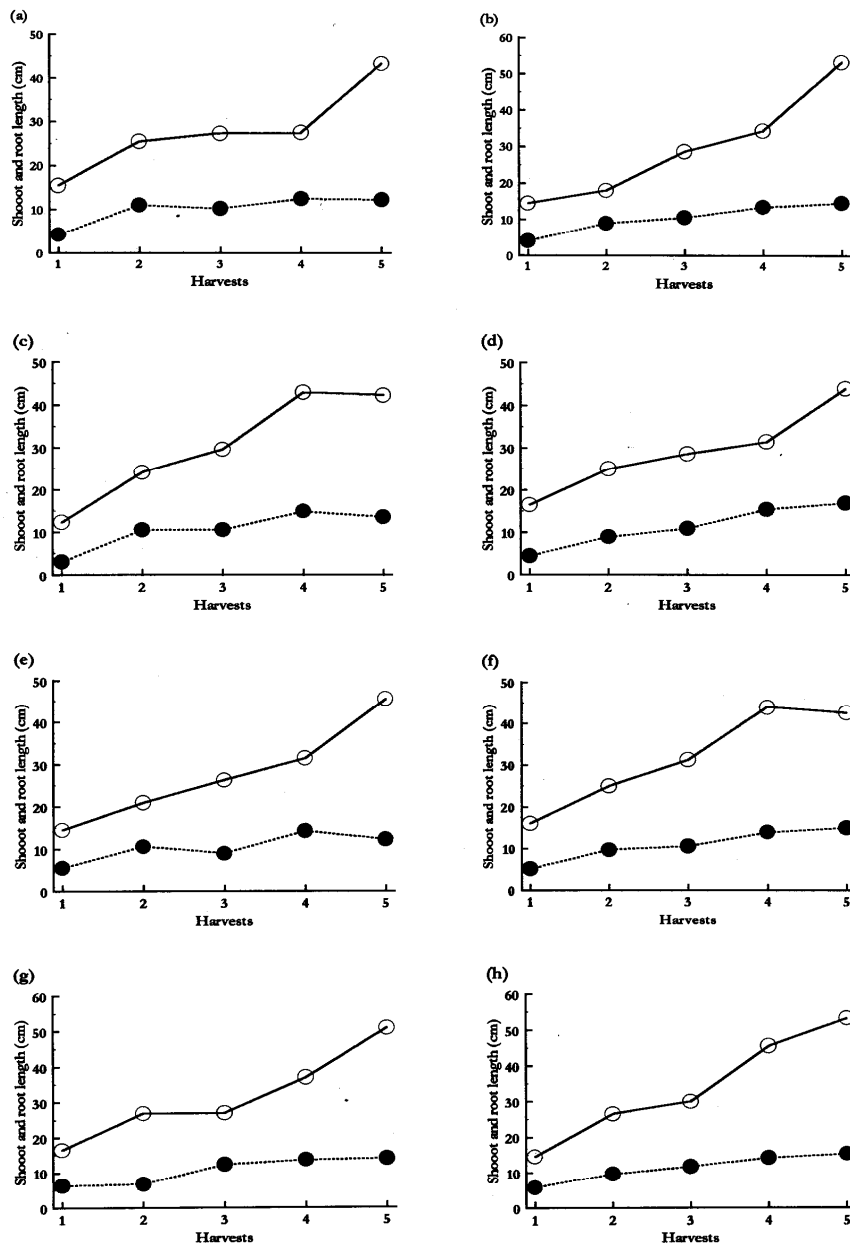


Fig. 1: Shoot length (○) and root length (●) of various chickpea varieties before (harvests 1 & 2) and after (harvests 3-5) the spray of the pathogen. (a)184W*, (b)NE 1256*, (c)AUG 970+, (d)ILC 1256+, (e)CM 72+, (f)ICC 6304+, (g)ILC 2548* and (h)C 679+.

(★ Stands for susceptible varieties and † for resistant varieties).

Number of nodules

The plants of resistant chickpea varieties produce maximum number of nodules.

At first harvest variety number 5 had significantly high number (10.00) of nodules at $P=0.05$ analyzed by applying Duncan Multiple Range Test (Fig.2). The varieties No.2 and 4 had overlapping number of nodules born by their root

system. The values were 5.6 and 5.0 respectively. At subsequent harvests the maxima fluctuated within varieties 8, 7, 5, 6. At the time of second and fifth harvests variety No.8 had significantly high values while at third and fourth harvests variety No.7 was having the top values. At fourth harvest varieties 5 and 6 also shared the maxima for the number of nodules with variety No.7.

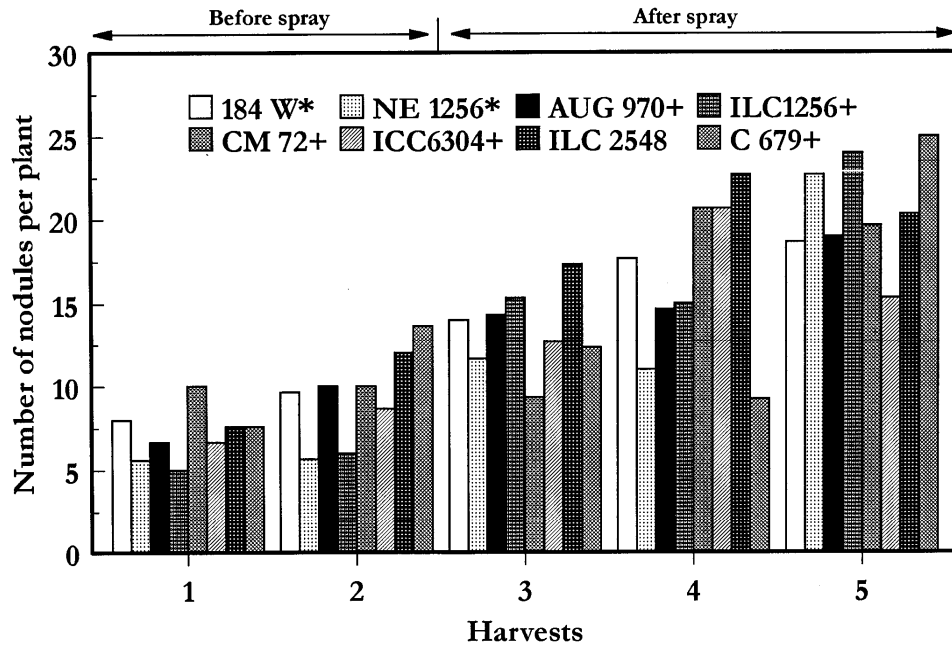


Fig. 2: Number of nodules per plant in eight chickpea varieties before and after the spray of the pathogen.

(* = susceptible and + = resistant varieties)

Fresh and Dry Weight of Shoot

For fresh weight of shoot maximum fresh weight for the aerial portion of the plant was recorded in the case of varieties 8, 7, 6 and 1. At first and second harvests variety No.8 had maximum readings (6.33 and 21.00 g, respectively), while at third, fourth and fifth harvests the varieties which topped the results were 7, 6 and 1 scoring significantly high values as 14.00, 26.40 and 34.60 g, respectively. The results were proved significant by DMR test at $P=0.05$ (Fig.3). For crown oven dry weight the varieties Nos. 3, 8, 6 and 7 had appreciable readings before and after the spray of the pathogen means throughout the growth phase. At the time of first harvest the plants dry weight value was reported maximum for variety No.1 (Fig.3). However, at subsequent growth stages variety No.8 had

appreciably high values as compared to susceptible variety as proved by DMR test at $P=0.05$ level. However, the insignificantly differing values as compared to variety 8 were recorded for varieties 7, 6 and 7 at third, fourth and fifth harvests respectively. The susceptible chickpea varieties had significantly low values for the oven dry weight of crown (Fig.3).

Fresh and Oven Dry Weight of Root

At first and second harvest stages variety No.8 had maximum reading scoring 1.7 and 3.9 g weight of fresh plant roots (Fig.4). At later stages of crop growth the maximum values shuffled between varieties No. 2, 3 and 4.

The values for oven dried weight also followed the same pattern (Fig.4). At the first two harvests variety No.8 had significantly high values while at subsequent growth stages some other

varieties like 2nd, 3rd, 6th and 7th had overlapping values with variety No.8. The highest value ranged

between 0.23 g to 0.63 g from 1st till last harvest (Fig.4).

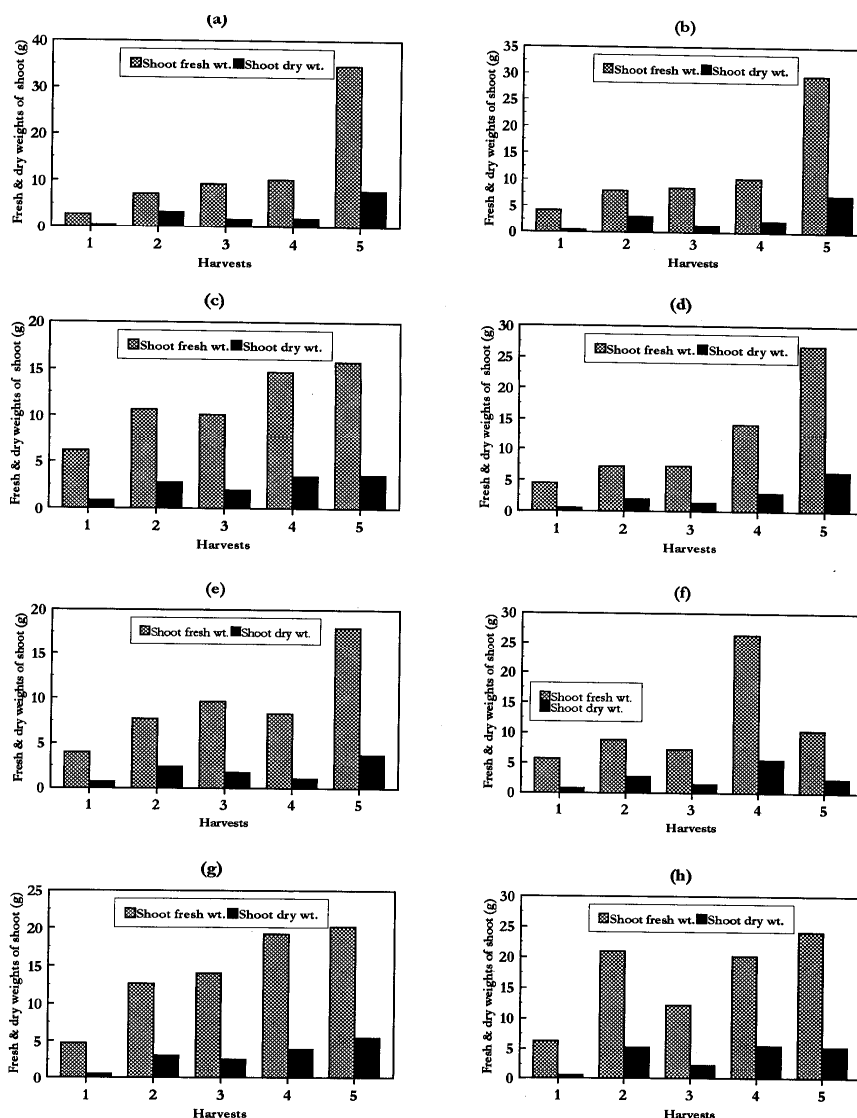


Fig. 3: Fresh and dry weight of shoot of various chickpea varieties before (harvests 1 & 2) and after (harvests 3-5) the spray of the pathogen. (a) 184W*, (b) NE 1256*, (c) AUG 970+, (d) ILC 1256+, (e) CM 72+, (f) ICC 6304+, (g) ILC 2548* and (h) C 679+. (* Stands for susceptible varieties and + for resistant varieties).

Number of Arbuscules and Vesicles

The formation of arbuscules in chickpea varieties increased till 3rd and 4th harvest time but tended to stabilize or decreased thereafter (Fig.5). The varieties like No. 6, 1, 7, 2, 3, 4 and 8 had maximum number of arbuscules at various stages of plant growth. At first, second and third harvests, varieties 6th, 1st and 7th had significantly high values for number of arbuscules as proved by DMR test at $P=0.05$. At fourth harvest variety 2nd, 3rd, 4th and 8th had top most values, which were overlapping with each other. At the last harvest, 1st

and 2nd variety had an appreciable number of arbuscules in the root cortex.

Vesicle formation showed a step rise from 1st till 3rd, 4th and 5th harvests depending upon different chickpea varieties. In 1st, 2nd and 5th varieties the formation of vesicles increased steadily till the final stage, while in the remaining chickpea varieties the number dropped after 3rd and 4th harvest (Fig.5). The maximum number of varieties kept varying between varieties 7th, 4th, 1st, 6th and 2nd.

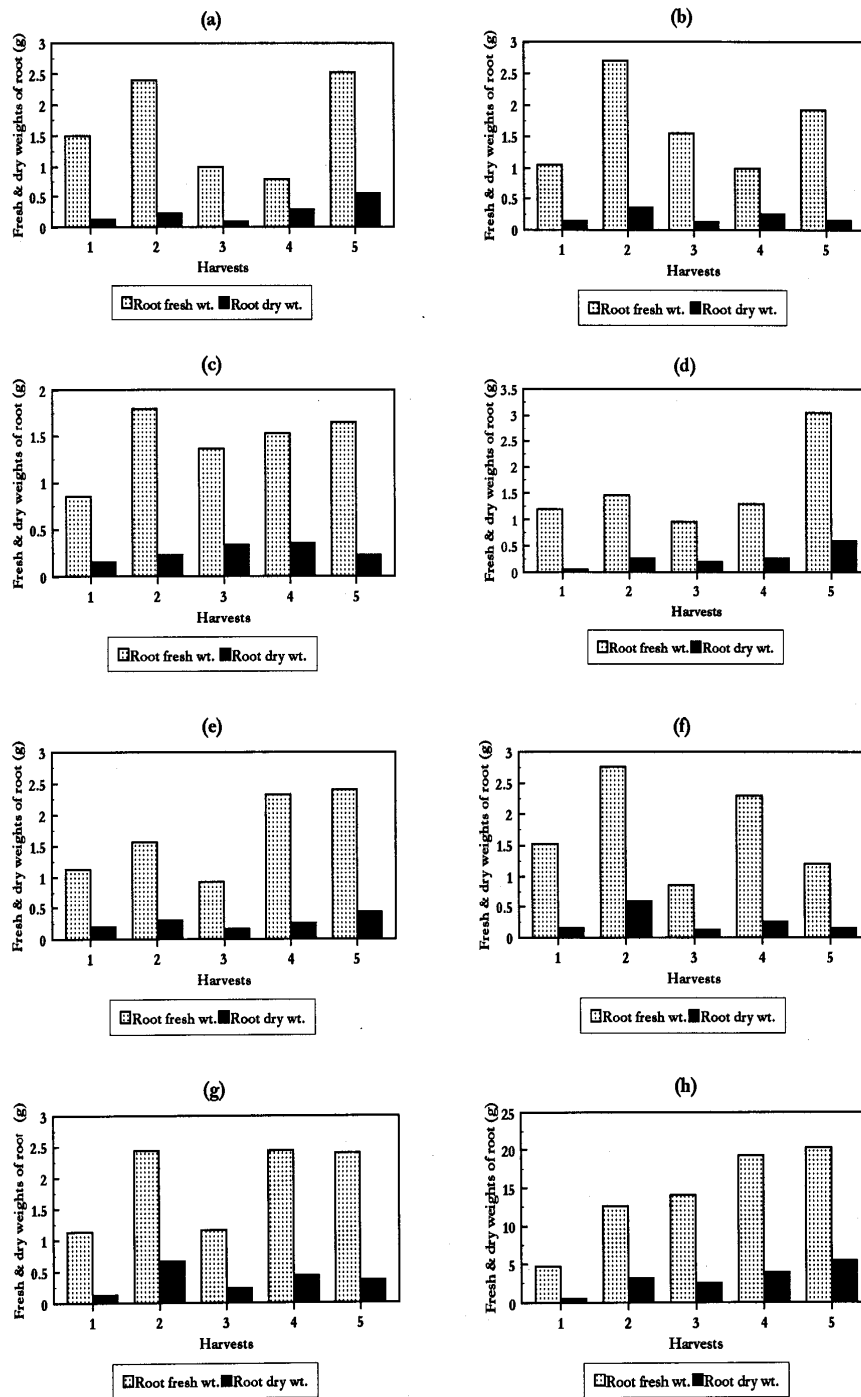


Fig 4: Fresh and dry weight of root of various chickpea varieties before (harvests 1 & 2) and after (harvests 3-5) the spray of the pathogen. (a)184W*, (b)NE 1256*, (c)AUG 970+, (d)ILC 1256+, (e)CM 72+, (f)ICC 6304+, (g)ILC 2548* and (h)C 679+. (* Stands for susceptible varieties and + for resistant varieties).

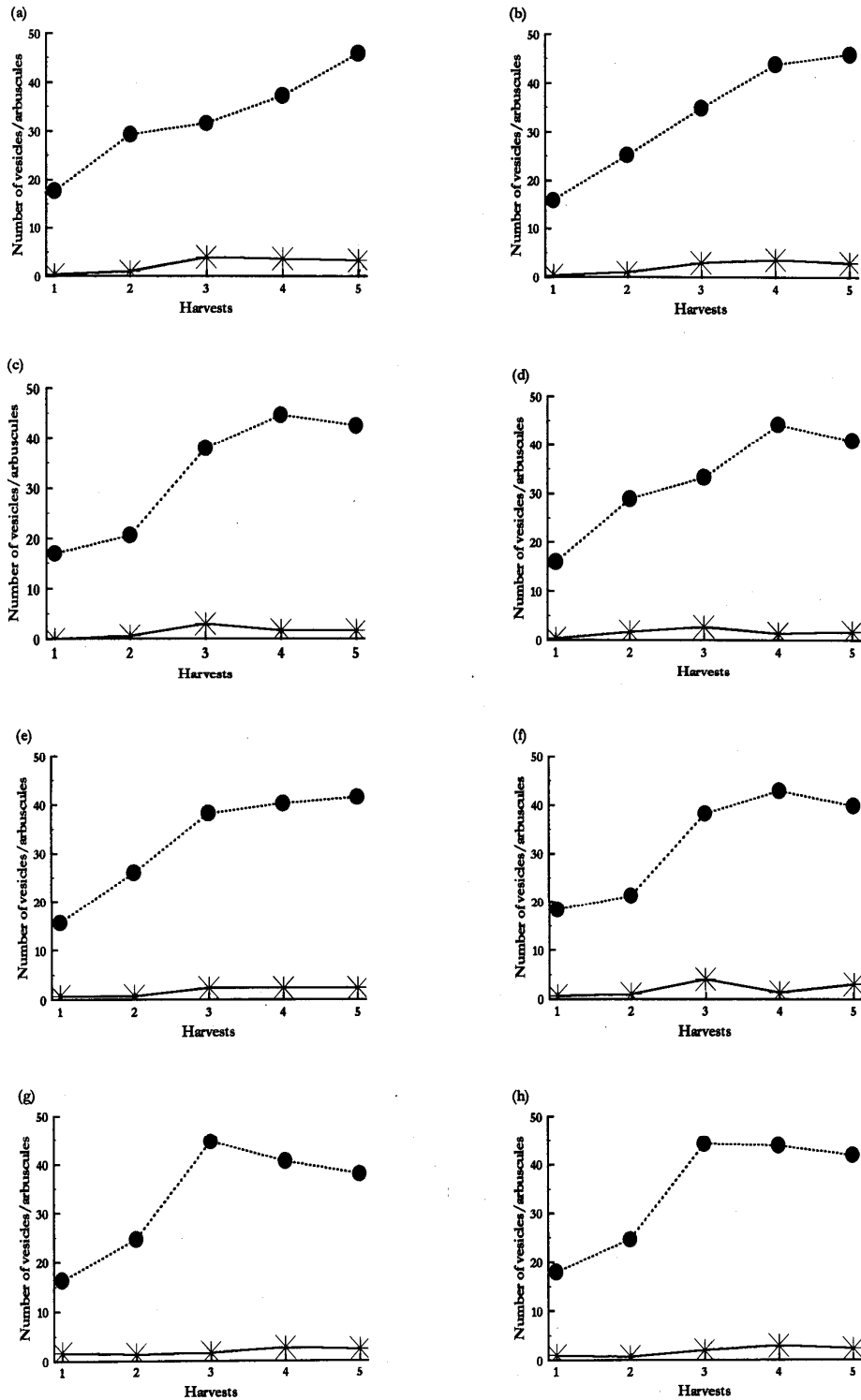


Fig. 5: Number of arbuscules (✱) and vesicles (●) per mm² of root cortex of various chickpea varieties before (harvests 1 & 2) and after (harvests 3-5) the spray of the pathogen. (a) 184W*, (b) NE 1256*, (c) AUG 970+, (d) ILC 1256+, (e) CM 72+, (f) ICC 6304+, (g) ILC 2548* and (h) C 679+.

(* Stands for susceptible varieties and + for resistant varieties).

Table 1: Extent of VAM mycelium (mm/cm of root) in the root corticle cells of eight chickpea varieties at five harvests. Two harvests were taken before and three harvests were taken after the spray of the pathogen.

Harvests ▼	Chickpea varieties							
	1 184 W*	2 NE 1256*	3 AUG 970+	4 ILC 1256+	5 CM 72+	6 ICC 6304+	7 ILC 2548*	8 C 679
1	4.00 ±0.50	2.63 ±0.32	2.16 ±0.76	2.50 ±1.05	1.80 ±0.29	2.50 ±0.50	3.00 ±1.00	2.83 ±0.29
2	5.00 ±0.5	4.33 ±0.57	4.66 ±0.57	5.00 ±1.00	4.33 ±1.15	4.83 ±0.76	5.00 ±1.00	5.00 ±1.0
3	3.50 ±0.50	3.83 ±0.29	3.60 ±0.26	4.16 ±0.29	3.83 ±0.76	3.83 ±0.76	4.00 ±0.50	3.83 ±1.04
4	4.50 ±0.50	5.10 ±0.29	3.80 ±0.76	3.33 ±0.57	4.50 ±0.50	4.10 ±0.29	4.00 ±1.00	3.83 ±0.76
5	4.33 ±0.57	4.80 ±0.29	3.80 ±0.76	3.80 ±0.76	4.80 ±0.76	3.80 ±0.29	3.50 ±0.50	4.30 ±0.57

(Mean values in each cell are accompanied by standard deviation. * stands for susceptible and + for resistant varieties)

Extent of VAM Mycelium

The trend for the extent of VAM mycelium varied throughout the plant growth. The varieties which had maximum values at different harvests were variety No. 1, 4, 7, 8, 4, 2 and 5 (Table 1).

Discussion

Numerous reports indicate that where arbuscular mycorrhizas reduce disease, the reduction could be duplicated by correcting the nutrient deficiency that is induced by reducing phosphorus (P) to plant growth -limiting levels, a common practice in AM research. Only rarely, were the experimental plants with or without arbuscular mycorrhizas of comparable size, and even if they were, their physiology would likely be quite different. The explanation for the reduced disease with mycorrhizas or enhanced P nutrition is that plants are more vigorous and therefore better able to resist or tolerate root disease. However, the enhanced plant vigor provides better substrate for obligate pathogens and pathogens causing foliage diseases to infect and multiply (Meyer & Dehne, 1986). Some studies indicate that P-induced changes in root exudation could affect (reduce) spore germination by the pathogen (Graham & Menge, 1982).

In the present survey work an attempt has been made to correlate the occurrence of VA mycorrhizal association with the incidence of *Ascochyta* blight which is an air borne pathogen and makes foliar entry. This study being preliminary of the kind indicates few findings. The biomass of chick pea plants of resistant varieties remained highest as various parameters were recorded. This may be attributed to the genetic ability of the cultivars to extract nutrition from the soil as suggested by. As regards other parameters like number of arbuscules is concerned, it reduced in the susceptible varieties during the early stages

of plant growth. However during later stages an appreciable number was recorded. These results deviate than those of Hetrick *et al.* (1996). These authors have reported that the establishment and development of VAM infections was early and rapid in the resistant cultivars. Possible explanation for this deviation may be that in the case of diseased plants, there is a decline in the rate of photosynthesis and hence the production of photosynthates is reduced as a result.

There are numerous examples of cultivars with in plant species that differ in the extent of colonization by mycorrhizal fungi and their responsiveness to them. These include *Arachis hypogaea* (Kasava Rao *et al.*, 1990), *Hordeum vulgare* (Boan *et al.*, 1993), *Medicago sativa* (Lackie *et al.*, 1988), *Oryza sativa* (Dhillion 1992), *Pennisetum americanum* (Krishna *et al.*, 1985), *Triticum aestivum* (Hetrick *et al.*, 1996), *Vigna unguiculata* (Mercy *et al.*, 1990) and *Zea mays* (Toth *et al.*, 1990). The present study thus extends the list of host plants, the cultivars of which were screened for the ability to form VAM and response towards *Ascochyta* blight.

These studies exemplify the range of variations present among genotypes with in species of plants and with in species of mycorrhizal fungi for the ability to form a mutually beneficial interaction. These genetic interactions coupled with environmental factors such as soil fertility, light intensity variation in the density of mycorrhizal populations in soil and microbial competition with in the rhizosphere have made it challenging to harness the mycorrhizal interaction in agronomic applications (Park and Kaeppler, 2000).

Chickpea plants assimilate higher amounts of phosphate. The deficiency of phosphorus in the soil not only affects plant growth but also the nodule formation and symbiotic nitrogen. VA mycorrhizal fungi known to involve in symbiosis

with nodule forming bacteria particularly in phosphorus deficient soils. In the present study a negative correlation has been observed between VAM colonization of the roots and susceptibility of the host plant towards *Ascochyta* blight. The results show an overall increase in biomass, nodule formation and VAM colonization with a reduced disease severity. Investigation of Schoenbeck and Schizer (1972) and Daft and Okusawya (1973) indicated that VAM plants showed resistance to various infections and disease severity was reduced by mycorrhizal plants. It has been found that the rusted wheat plants has lesser VAM infections than their healthy counterparts (Nasim, Unpublished data). However, a positive correlation has been found between disease susceptibility and colonization in a study conducted on maize by Toth *et al.*, (1990).

The genetic basis of the plant mycorrhizal fungal interaction has not been fully characterized. Genetic control of the interactions between VAM fungi and host plant exist both with in host as well as symbiont species (Duc *et al.*, 1989). The genetic relationship between AM symbiosis phenotypes (e.g. percent of root colonized) and organismic traits such as yield, biomass accumulation and disease resistance is of particular interest from a plant improvement perspective.

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