

IDENTIFICATION OF RESISTANT SOURCES IN CHICKPEA AGAINST *FUSARIUM* WILT

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Abstract

Wilt caused by *Fusarium oxysporum* Schlechtend. Fr. f. sp. *ciceris* is a devastating disease of chickpea in Pakistan. In the present study 321 genotypes from different sources were evaluated under controlled condition to identify genetic sources of resistance against this disease at seedling and reproductive stage. Disease reaction at two stages revealed considerable variation among the genotypes. At seedling stage disease incidence varied from 0 to 29.3% whereas at reproductive stage ranged from 0 to 57%. At seedling stage 173 genotypes were resistant, 54 were tolerant and 94 were susceptible, whereas at reproductive stage, 102 genotypes were resistant, 36 were tolerant and 183 were susceptible. Eighty two genotypes showed steady resistance at both stages. These genotypes may be exploited for the development of resistant cultivars against wilt.

Introduction

Chickpea (*Cicer arietinum* L.) is the most important pulse crop of Pakistan. It is an important source of human food and animal feed that also helps in the management of soil fertility particularly in dry lands (Singh & Saxena, 1996). It can be a promising alternative crop for rotation with barley, peas and wheat in dry land areas (Auld & Lee, 1981). The productivity of chickpea in Pakistan is below world average and has been uncertain, erratic and low with about 10% of the world's production (Auckland & Vander-Maesan 1980). Wilt disease is one of the factors responsible for its low yield.

Fusarium wilt caused by *Fusarium oxysporum* Schlechtend Fr. f. sp. *ciceris* (Padwick) Matuo & K. Sato, is the most important soil-borne disease of chickpea throughout the world and particularly in the Indian Subcontinent, the Mediterranean Basin, and California (Haware 1990; Jalali & Chand 1992; Nene & Reddy, 1987). Attacks of the *Fusarium* wilt pathogen can destroy the crop completely (Halila & Strange, 1996) or cause significant annual yield losses. Annual chickpea yield losses due to *Fusarium* wilt were estimated at 10% in India (Singh & Dahiya, 1973; Trapero-Casas & Jiménez-Díaz, 1985) and 40% in Tunisia (Bouslama, 1980). Early wilting causes more loss than late wilting, but seeds from late-wilted plants are lighter, rough and dull than those from healthy plants (Haware & Nene, 1980). The cheapest, economical and the most ideal way of managing chickpea wilt, is the use of resistant cultivars. Chemical control of wilt is not feasible and economical because of the soil as well as seed-borne nature of the pathogen. Fungal chlamydospores can survive in soil up to 6 years in the absence of the host plants (Haware *et al.*, 1996). The most practical and cost-efficient method for management of *Fusarium* wilt of chickpea is the use of resistant cultivars (Nene & Haware 1980; Nene & Reddy 1987; Bakhsh *et al.*, 2007). Present study was undertaken to evaluate the newly developed genotypes of chickpea for resistance against local isolates of wilt fungus in order to identify new genetic sources of resistance.

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Material and Method

Three hundred and twenty one genotypes obtained from various National and International institutes (AZRI, NIAB, ICARDA, BARI and NARC) were screened for their level of resistance/susceptibility against *Fusarium* wilt under greenhouse and field conditions at National Agricultural Research Centre, Islamabad (Table 1). Inoculum of the fungus was prepared on sorghum grains. The sorghum grains were soaked in tap water overnight and then surface dried by spreading on paper towels in laboratory under a ceiling fan. Surface dried seeds were put into conical flasks @ 250g/flask and the flasks were closed by inserting cotton plugs. These flasks were autoclaved at 15 psi for 20 minutes. The sterilized flasks after cooling were inoculated with 7 days old actively growing culture of *F. oxysporum* f. sp. *ciceri* by adding 4 mm agar plugs with sterile cork borer. These flasks were incubated at 25°C for 7 days. At the time of inoculation each flask containing inoculum was mixed in 2 kg of sterilized soil, which was put in the sterilized disposable pots (20 x 15 cm) for plantation of chickpea genotypes. Each of the test lines was sown in two replications. For susceptible check AUG 424 was repeatedly planted after every two test entries. Data on the number of wilted seedlings in each pot for each test line were recorded 35 days after sowing.

The disease data were recorded at two stages of plant growth i.e., at seedling stage and at reproductive stage (near physiological maturity). The data on wilted plants of test entries at seedling stage were recorded when killing of the susceptible check had occurred. The second stage data on wilted plants were recorded at the initiation of physiological maturity. The wilt incidence of each test entry was calculated by the following formula:

$$\text{Wilt incidence} = \frac{\text{Number of wilted plants}}{\text{Total number of plants}} \times 100$$

The level of resistance and susceptibility of each test line was determined by using 1-9 rating scale given by Iqbal *et al.*, (2005) where 1=highly resistant (0-10% plants wilted), 3=resistant (11-20% plants mortality), 5=moderately resistance (21-30% mortality), 7=susceptible (31-50% mortality) and 9= highly susceptible (more than 50% mortality).

A mixture of various isolates of wilt fungus was used to develop wilt sick plot. Experiment was planted in an augmented design having two replication. Each genotype was planted in a 4 m plot. Row to row and plant to plant distances were maintained at 30 cm and 10 cm, respectively. A highly wilt susceptible genotype, AUG 424, was repeatedly planted after every two test entries. At reproductive stage data on wilted plants of test entries were recorded at 100% killing of the susceptible check.

Results

The disease incidence of 321 chickpea genotypes was recorded at seedling and reproductive stage (Table 1). A significant results of t-test of present studies showed a distinct variation among the chickpea lines against wilt resistance at $P = 0.05$ (Table 2). According to disease incidence these chickpea lines were grouped in three categories (Fig. 1). It was observed that 173 lines were resistant, 54 were tolerant while 94 were susceptible to the wilt disease at seedling stage in the greenhouse. Whereas, in

Table 2. T-test for reaction of chickpea lines against *Fusarium* wilt at seedling and reproductive stage.

SOV	t	df	Mean	Mean difference	Std. error mean	Std. deviation	95% Confidence interval of the difference		Sig.
							Lower	Upper	
Seedling	14.959	320	17.7349	17.73489	1.18559	21.24157	15.4024	20.0674	0.00
Flowering	20.320	320	29.6445	29.64455	1.45890	26.13833	26.7743	32.5148	0.00

the field sick-bed, 102 genotypes were resistant, 36 were tolerant and 183 were susceptible at reproductive stage (Table 3). The disease incidence at physiological maturity stage increased invariably in all the genotypes as compared to that at seedling stage (Fig. 2). On an average basis 53.9% disease resistance was recorded at early stage and 31.78% at reproductive stage, whereas 29.3% disease incidence was observed at seedling stage and 57% at reproductive stage. The disease incidence of tolerant genotypes remains 16.8% to 11.36% (Table 3) ranging disease rating from 0% (at seedling stage) to 20% (at reproductive stage).

There was a wide variation between genotypes for their disease reaction at two stages i.e., at seedling stage and reproductive stage. Development of disease is slow in resistant lines and fast in susceptible lines. As the resistant lines at reproductive stage also became susceptible thus field screening at reproductive stage seems to be more reliable.

Discussion

Due to the prevalence of drought conditions in the country *Fusarium* wilt has gained importance in Pakistan. Our results indicate that the incidence and the severity of the disease was less in the greenhouse and higher in the field. One of the reasons might be that the crop often has the chances of disease escape as the wilt disease is temperature dependent and the level of inoculum may vary at different places. The resistance source of *Fusarium* wilt in chickpea germplasm is not uncommon and a number of other workers have also reported the occurrence against high level of resistance of *Fusarium* wilt (Ahmad & Sharma 1990; Reddy *et al.*, 1990; Iqbal *et al.*, 1993; Ahmad *et al.*, 1990; Iftikhar *et al.*, 1997; Yu & Su, 1997).

According to our results 173 lines were found resistant, 54 tolerant and 94 susceptible at seedling stage. Whereas, 102 genotypes were observed resistant, 36 tolerant and 183 susceptible at reproductive stage. Similar studies were made by Zote *et al.*, (1983) who studied sources of resistance to chickpea wilt and reported that none of the 42 lines of *Cicer arietinum* tested in a wilt sick plot infested with *F. oxysporum* f. sp. *ciceri* were highly resistant, 4 developed less than 10% and 6 others less than 29% disease. While, Patel *et al.*, (1985) studied the reaction of chickpea lines to *Fusarium* wilt and screened 34 supposedly resistant germplasm lines from ICRISAT and 3 promising varieties as potted plants for germination and for wilting at 40 and 80 days after sowing in soil infested with the Arnej isolate of *F. oxysporum* f. sp *ciceri*. Similarly, Zote *et al.*, (1986) further studied that only 5 chickpea lines out of 15 tested for three successive years showed less than 10% wilt incidence. Khalid (1993) evaluated 122 test lines against *Fusarium* wilt under field conditions and found 37 of them to be resistant while all the remaining test lines exhibited moderate resistance to highly susceptible reaction. Whereas, Kapoor *et al.*, (1991) evaluated 39 varieties for resistance to *F. oxysporum* f. sp. *ciceri*.

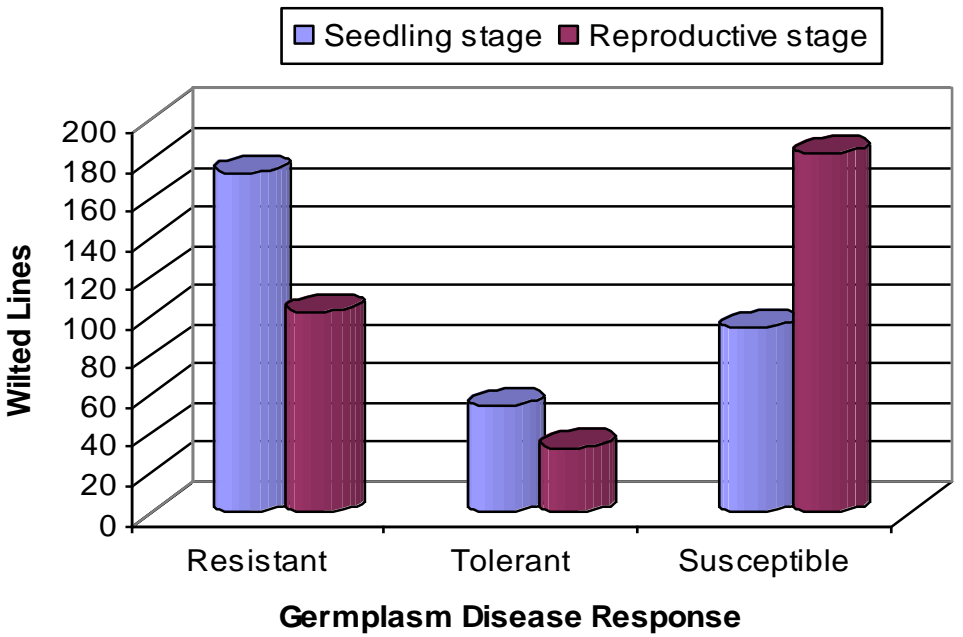


Fig. 1. Classification of chickpea genotypes with respect to their wilt response at seedling and reproductive stage.

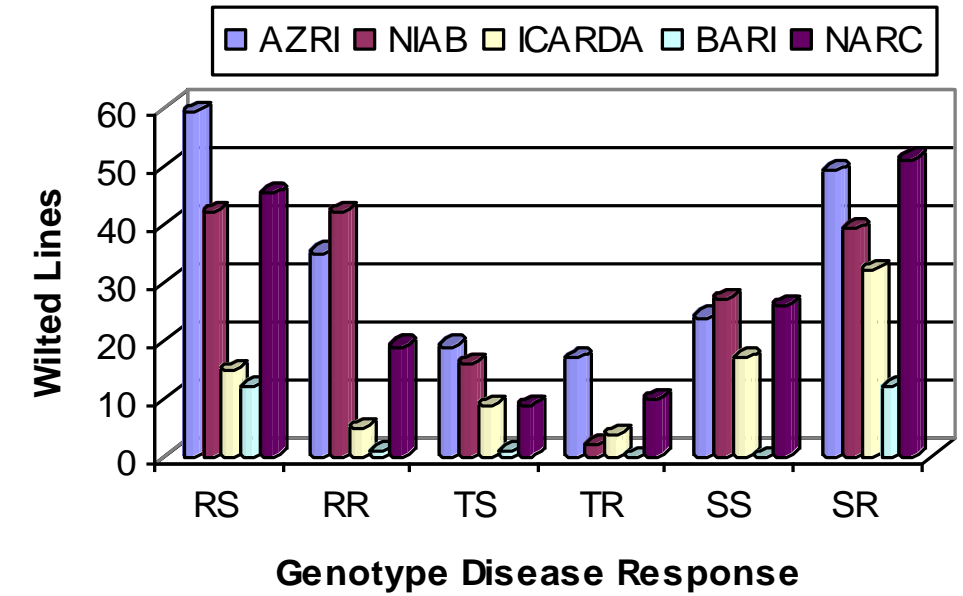


Fig. 2. Genotypes wilt response at seedling and reproductive stage. (RS= Resistance at seedling stage, RR= Resistance at reproductive stage, TS= Tolerance at seedling stage, TR= Tolerance at reproductive stage, SS= Susceptible at seedling stage, SR= Susceptible at reproductive stage)

Our study revealed that at seedling stage majority of the genotypes were resistant whereas at reproductive stage majority of the genotypes appeared to be susceptible. Similarly, various workers have reported variation in wilt resistance at two stages (Nene *et al.*, 1981; Haware 1996). A high level of variability has been reported between isolates of same race collected from different areas and between isolates of different races (Sivaramakrishnan *et al.*, 2002). Similarly the isolates from different areas of Pakistan varied highly with respect to their virulence (Iftikhar *et al.*, 2002). Tullu (1996) reported variation in chickpea genotype that was consistently and uniformly resistant. These findings are quite in conformity with our results.

Cultivation of resistant cultivars is the most effective and economical way of controlling the disease (Jimenez-Diaz *et al.*, 1993). The current study was conducted to identify resistant cultivars against the prevalent isolates of wilt existing in Pakistan. Iftikhar *et al.*, (1997) screened 31 chickpea germplasm lines received from ICARDA and found that all of them were highly resistant to wilt disease. Whereas, Bajwa *et al.*, (2000) found that out of 32 genotypes only one line was resistant, 4 lines were tolerant, and 27 were susceptible to highly susceptible against *Fusarium* wilt. Iqbal *et al.*, (2005) also report the sources of resistance against *Fusarium* wilt in chickpea germplasm originating from national and international research institutes. They identified 14 chickpea lines to be resistant to wilt at seedling stage but no line found to be resistant at reproductive stage. Chaudhry *et al.*, (2006) among 414 varieties/ germplasm accessions evaluated for *Fusarium* wilt and found 35 test lines resistant, 208 intermediate, 77 susceptible and 94 highly susceptible. Next year Chaudhry *et al.*, (2007) screened 196 chickpea germplasm lines/cultivars for resistance to wilt disease in a wilt sick plot. None of the test line was found immune or highly resistant. Infantino *et al.*, (2006) presented advances in conventional and innovative screening methods for disease resistance.

Current study was carried out in a wilt sick plot prepared with mixture of isolates representing different chickpea growing areas, the genotypes identified as resistant in this study will maintain their response across the locations. Most of the genotypes that showed resistant response at seedling stage appeared to be susceptible at physiological maturity stage. This phenomenon could be accounted due to the prevalence of disease for a short period at seedling stage and for a long period at the reproductive stage. Since high temperature plays an important role for the development of disease and the high temperature prevailed for a short period at seedling stage due to onset of the winter in December and it prevailed for a long time at reproductive stage due to the onset of summer at the time of flower initiation. Therefore, disease prevailed for a longer time at reproductive stage of observation. Consequently, most of the genotypes that were resistant at seedling stage became susceptible at reproductive stage. This means that such genotypes required long wilting time. Therefore, the genotypes used in the present study may be divided into two categories, early wilting genotypes and late wilting genotypes. The resistant genotypes at seedling stage may be planted in areas where disease occurs at seedling stage only. Delay in sowing can also help to escape disease from such areas. On the other hand the genotypes that showed resistance or tolerance at both the stages are most suitable for exploitation in breeding programs or for direct sowing in wilt prone areas. The resistant genotypes expressed resistance against a mixture of isolates, they may possess multiple genes for resistance against this disease. The susceptible genotypes at seedling stage may be categorized as early wilting genotypes and at reproductive stage may be classified as late wilting genotypes. There was a common relationship between disease severities at two stages. This indicated that different genotypes could be utilized according to prevalence of disease at various growth stages. This study provides us valuable information about the resistance sources, which exist in the world collection of

chickpea germplasm against a virulent isolate of *F. oxysporum* f. sp. *ciceri* in Pakistan. These lines can be used in hybridization programme for the development of chickpea resistance cultivars for commercial cultivation in the country.

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