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Identification of resistant sources in chickpea against *Fusarium* wilt under greenhouse condition

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ABSTRACT: Wilt caused by the fungus *Fusarium oxysporum* f. sp. *ciceri* is devastating disease of chickpea in Iran. To identify genetic sources of resistant against wilt under greenhouse conditions, 18 genotypes/cultivar were obtained from the Agricultural Jihad Research Center. Disease observations were recorded at seedling stage and reproductive stage. A considerable variation between genotypes and cultivars was observed in both stages. Disease incidence ranged from 0% to 46.6% at seedling stage and it varied from 0% to 100% at reproductive stage. At seedling stage 2 genotypes (FLIP03 -110C, X98TH75K1-83) were highly resistant. 3 cultivar (Hashem, Azad and Bivanij) and 7 genotypes (SAR79J87K1-85, SAR79J38K8-85, SAR79J61K1-86, SAR79J18K1-86, SAR79J15K3-86, SAR 79J15K3-86, SAR79J78K5-85) were resistant. 2 genotypes (SAR79J78K3-86 and FLIP98-55C) were moderately resistant and 4 cultivar (ILC482, Arman, Gerite and blackchickpea) were susceptible, whereas at reproductive stage 2 genotypes (FLIP03 -110C, X98TH75K1-83) were resistant, Azad cultivar was moderately resistant, Hashem cultivar and 2 genotypes (SAR79J61K1-86, SAR79J18K1-86) were susceptible, 5 cultivar (ILC482, Arman, Gerite, blackbean and Bivanij) and 7 genotype (SAR79J61K1-86, SAR79J38K8-85, SAR79J15K3-86, FLIP98-55C, SAR79J78K5-85, SAR79J78K3-86, SAR79J710K2-85) were highly susceptible. Two genotypes showed steady resistance at both stages. These genotypes may be exploited for the development of resistant cultivars against wilt.

***Keywords*:** Wilt, *Fusarium oxysporum* f. sp. *ciceri*, chickpea, resistance

INTRODUCTION

Chickpea (*Cicer arieatinum* L.) is one of the most important crops growing in the Lorestan of Iran**.** It is an important source of human food and animal feed that also helps in the management of soil fertility particularly in dry lands(Ansar Ahmad, 2010). But the yield and quality of chickpea are influenced by *Fusarium* wilt disease caused by the *Fusarium oxysporum* f.sp. *ciceris* (Padwick) Sato & Matuo (Dueby, 2007). *Fusarium* wilt is one of the most important and destructive vascular disease of chickpea (Dileep kumar, 1999). Yield losses of chickpea due to *Fusarium* wilt are estimated at 10% in India and Spain, 40% in Tunisia and 17% in Iran (Bouslama, 1980; Jamali, 2004). There are eight races of *F. oxysporum* f. sp*. ciceri* (0, 1A, 1B/C, 2, 3, 4, 5 and 6) which are identified by reaction on a set of differential chickpea cultivars (Jimenez-Gasco and Jimenez-Diaz, 2003; Haware and Nene, 1982) and consists of two pathotypes (yellowing and wilting) (Jimenez-Gasco and Jimenez-Diaz, 2002) The most efficient method for the management of disease is using resistant cultivars (Karimi, 2012), 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, 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, 2007).

MATERIALS AND METHODS

To perform this experiment, 18 cultivar/genotypes were obtained from the Agricultural Jihad Research Center of Lorestan province, and associated with susceptible check (ILC482) completely randomized designs block with four replications were tested under greenhouse condition. To prepare inoculum *F. oxysporum* f. sp *ciceri* during growing seasons of 2012-2013 from different fields were sampled and samples of infected transferred to the laboratory. Samples after washing and disinfesting were cultured in Nash & Snyder selective medium and PDA (Potato-Dextrose-Agar). The fungal species were purified by single spore then identification of isolates carried out by using CLA and PDA media and isolates were identified using keys (Nelson, 1983) and (Leslie, 2006).

Preparation fungal suspension

To producing fungus suspension *Fusarium* isolates cultured on plate has been PDA and placed within incubator at 25°C temperature. From 5 days culture, a block of 5 mm diameter was removed and the vials of 100 ml containing 50 ml of medium PDB (200 g potato, 20g dextrose, 1,000 mm distilled water that were cultured for a period of 3 days on a shaker speed of 120 rpm were exposed to fungal growth. Then contents of each flask are smooth, after which the spore concentration was determined using hematositometer slide .In Pathogenicity of spore suspension was used to 1×10^6.

Crop plants to inoculation

The produced seeds for 5 min by sodium hypochlorite 0.5% sterile and three times for 5 min are washed with distilled water. Then transferred to sand containing pots and after 15 days, chickpea seedlings out of sand and with spore suspension 1×10^6 for 1-2 min are inoculated by using root-dipping method. In root-dipping method after creating a small wound in root and near the crown by sterile scalpel, seedling root are kept inside spore suspension for 1-2 minutes. For seedlings inoculated with fungal isolates are used from plants 8-10 cm in length. One day before inoculation pots were watered to operate was done easily inoculation. Immediately inoculated seedlings in pots containing sterilized soil are planted (1: animal manure, 1 sand: 1 soil) two days before they watered, and after planting watered. The check seedlings roots for 1.5 min dipped in distilled water and then planted in the pots, planting pots kept in the greenhouse at 20-25ċ. Record was performed 2 stages: at seedling stage and reproductive stage. Data on the number of wilted seedlings in each pot for each test genotype and cultivar were recorded 50 days after sowing and percent disease incidence was calculated for each test genotype and cultivar by using the formula

$$\text{Wilt incidence} = \frac{\text{No of wilted plants}}{\text{Total no. of the plants}} \times 100$$

The level of resistance and susceptibility of infected plants were evaluated by using common ICARDA method, Highly resistant: Less that %2 plant are infected.(HR), Resistant: 2-10% plants infected.(R), Moderately resistant: 11-20% plant infected.(MR), Susceptible: 21-50% plant infected.(S), Highly Susceptible: up to 50% plant are infected. (HS)

RESULTS AND DISCUSSION

Results

The disease incidence of 18 chickpea genotypes and cultivar was recorded at seedling and reproductive stage (Table 1). According to disease incidence these chickpea genotypes and cultivar were grouped in five categories (Fig. 1)

According to our results 2 genotypes were found highly resistant, 7 genotype and 3 cultivar resistant, 2 genotypes moderately resistant and 4 cultivar susceptible at seedling stage. whereas, 2 genotypes observed resistant, 1 cultivar moderately resistant, 1 cultivar and 2 genotypes susceptible and 5 cultivars and 7 genotypes highly susceptible at reproductive stage. In this experiment in 2 stage of recorded more genotypes/cultivars and check cultivar were infected plants percentage various and check cultivar had the most percentage infected plants.

The disease incidence at physiological maturity stage increased invariably in all the genotypes and cultivar as compared to that at seedling stage (Table 1). A considerable variation between genotypes and cultivars was observed in both stages. Disease incidence ranged from 0% to 46.6% at seedling stage and it varied from 0% to 100% at reproductive stage. With the progress of time and the creation of new physiological stages of chickpea, the percentage of infected plants has increased**.** Increasing of percentage of infected plants, at reproductive comparison with seedlings stage were due to increasing temperature.

Development of disease were slow in resistant genotypes and fast in susceptible genotypes. As the resistant cultivars and genotypes at reproductive stage also became susceptible thus field screening at reproductive stage seems to be more reliable. *Fusarium* than temperature sensitive and with increasing ambient temperature its activity increases, so that the temperature is above 25°C the most activity and consequently the most percentage of infected plants at high temperatures, which happens to coincides with the chickpea flowering.

Table 1. Disease rating of chickpea genotypes and cultivars against *Fusarium* wilt at seedling and reproductive stage

NO	Variety	Infected plants percentage at seedling stage	Reaction	Infected plants percentage at reproductive stage	Reaction
1	Arman	31.2%	Susceptible	98%	highly susceptible
2	Azad	7%	Resistant	20%	moderately resistant
3	Hashem	10%	Resistant	48%	Susceptible
4	Gerite	46.6%	Susceptible	93.3%	highly susceptible
5	Black chickpea	35%	Susceptible	95%	highly susceptible
6	bivanij	10%	Susceptible	91.6%	highly susceptible
7	Check ILC482	25%	Resistant	100%	highly susceptible
8	SAR79J78K3-86	20%	moderately resistant	76.4%	highly susceptible
9	SAR79J78K5-85	9%	Resistant	81.2%	highly susceptible
10	FLIP 98-55C	20%	moderately resistant	98%	highly susceptible
11	SAR79J15K3-86	8%	Resistant	86%	highly susceptible
12	SAR79J710K2-85	10%	Resistant	73.3%	highly susceptible
13	SAR79J87K1-85	6.25%	Resistant	48%	Susceptible
14	SAR79J38K8-85	9%	Resistant	70%	highly susceptible
15	SAR79J61K1-86	10%	Resistant	81.2%	highly susceptible
16	FLIP03 -110C	0	highly resistant	9%	Resistant
17	X98TH75K1-83	2%	highly resistant	8%	Resistant
18	SAR79J18K1-86	8%	resistant	50%	Susceptible

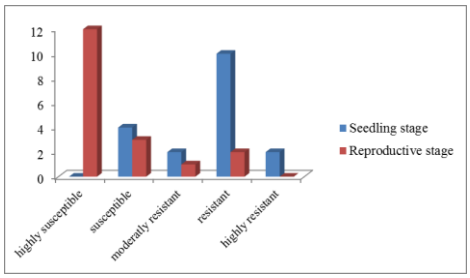

Figure 1. Classification of chickpea genotypes and cultivars with respect to their wilt response at seedling and reproductive stage.

Discussion

According to our results 2 genotypes were found highly resistant, 7 genotypes and 3 cultivars resistant, 2 genotypes moderately resistant and 4 cultivars susceptible at seedling stage. whereas, 2 genotypes observed resistant, 1 cultivar moderately resistant, 1 cultivar and 2 genotypes susceptible, 5 cultivars and 7 genotypes highly susceptible at reproductive stage. Similar studies were made by Zote, (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. Similarly, Govil and Rana (1984) evaluated 239 cultivars representing a range of variability among Indian and Iranian germplasm in wilt sick plot for years. None was found to be immune but the maximum resistance was shown by Indian cultivars such as P-597, P-621, P-3649, P-4128 and P-4245. 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. Our study revealed that at seedling stage majority of the genotypes and cultivar were resistant whereas at reproductive stage majority of the genotypes and cultivar appeared to be highly susceptible. Similarly, various workers have reported variation in wilt resistance at two stages (Nene, 1981; Haware 1996). Tullu (1996) reported variation in chickpea genotype that was consistently and uniformly resistant. These findings are quite in conformity with our results.

Iftikhar, (1997) screened 31 chickpea germplasm lines received from ICARDA and found that all of them were highly resistant to wilt disease. Whereas, Bajwa*,* (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*,* (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*,* (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. Whereas, Naser Ahmad, (2010) evaluated 321 test lines against *Fusarium* wilt under greenhouse and field conditions and found 173 resistant, 54 tolerant and 94 susceptible at seedling stage. Whereas, 102 genotypes were observed resistant, 36 tolerant and 183 susceptible at reproductive stage. Iqbal, (2010) screened 145 chickpea genetic sources of resistance against wilt disease under artificial disease condition and found, 14 genotypes were resistant, 65 tolerant and 66 were susceptible at seedling stage, on the contrary, at reproductive stage, no genotype was resistant, 12 were tolerant and 133 susceptible. Nazir & khan (2012) screened 137 chickpea germplasm lines/cultivars for resistance to against wilt disease in a wilt sick plot none of the test lines were found immune and resistant. The most efficient method for the management of disease is using resistant cultivars (Karimi, 2012). The resistant genotypes and cultivars 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 and cultivars 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 susceptible cultivars at seedling stage may be categorized as early wilting cultivars 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. These genotypes can be used in hybridization program for the development of chickpea resistance cultivars for commercial cultivation in the country.

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