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IDENTIFICATION OF RESISTANT SOURCES FOR MULTIPLE DISEASE RESISTANCE IN CHICKPEA

Sheikh Muhammad Iqbal, Abdul Ghafoor, Ahmad Bakhsh, Iftikhar Ahmad and Altaf Sher
National Agricultural Research Centre, Islamabad, Pakistan
Email: **iqbalsh65@hotmail.com**

ABSTRACT

Wilt caused by *Fusarium oxysporum* f.sp. *ciceri* is a devastating disease of chickpea in Pakistan. To identify genetic sources of resistance against wilt disease under artificial disease condition, one hundred and forty five genotypes obtained from various sources were evaluated. Disease observations were recorded at seedling and reproductive stages. A considerable variation among the genotypes observed at both the stages. Disease incidence ranged from 0% to 57.2% at reproductive stage and it varied from 0% to 100% at seedling stage. At seedling stage, 14 genotypes (90395, C-235, C-44, CM2000, ILC 182, FLIP97-129C, FLIP97-172C, FLIP98-107C, FLIP98-227C, FLIP98-230C, FLIP98-231C, FLIP98-38C, FLIP98-54C, ILC7374, KC-89) were resistance, 65 tolerant and 66 were susceptible. On the contrary, at reproductive stage, no genotype was resistant, 12 (90395, C-235, C-44, E101XPB91, FLIP98-107C, FLIP98-226C, FLIP98-227C, FLIP98-230C, FLIP98-231C, FLIP98-38C, FLIP98-54C, ILC7374) were tolerant and 133 susceptible. Six genotypes (FLIP98-44C, ILC7795, FLIP97-217C, FLIP98-56C, FLIP99-48C, FLIP97-195C) were tolerant to blight under green house conditions, whereas other six genotypes (FLIP98-230C, FLIP98-226C, FLIP97-219C, FLIP97-229C, FLIP97-132C, FLIP98-231C) were resistant at adult plant stage under field conditions. Five genotypes were identified with genes for tolerance against both the diseases which could be tested under wide range of environments and be utilized for developing high yielding cultivars with dual tolerance through building pyramid resistance.

Key words: Wilt, *Fusarium oxysporum* f.sp *ciceri,* chickpea, germplasm, resistance.

INTRODUCTION

Chickpea is one of the most important food legumes being cultivated in almost all over the world including temperate and sub-tropical regions. The crop faces various problems throughout the growing areas, some related to specific regions and some under wider range of climatic conditions. Among biotic stresses, chickpea blight and wilt are the most serious and are non-linear in comparison. Both of these diseases are serious and often reported in chickpea growing areas of India. Iran, Pakistan, Nepal, Burma, Spain, Tunisia, Bangladesh, Ethiopia, Malawi, Mexico, Peru, Syria and the USA (Nene *et al*., 1984). The yield losses due wilt may vary from 10-90% (Jimenez-Diaz *et al.*, 1989). According to an estimate the annual loss of US $ 1 million may be caused by this disease in Pakistan (Sattar, 1933). Wilt has reduced the share of chickpea from 50% in 1950s to 10% in 1990s on irrigated lands in Pakistan (Haqqani *et al*., 2000). An annual yield loss of 12-15% in chickpea, caused by wilts and root rot, in Spain was estimated by Trapero-Casas and Jimenez-Diaz (1985). The production of chickpea in California declined largely because of chickpea wilt (Buddenhagen *et al*., 1988). At ICRISAT, it was found that early wilting causes more loss than late wilting and the seeds harvested from late wilted plants were less heavy and duller than that from healthy plants (Haware and Nene, 1980). At least 7 races of this fungus have been reported (Haware and Nene, 1982; Philips, 1988; Jimenez-Diaz *et al*., 1989). However, no information on existence of races in Pakistan is available, though variation in isolates of the fungus, collected from different sites has been reported by Iftikhar *et al* (2002). Chemical control of wilt is not much effective and economical because the pathogen is soil as well as seed-borne in nature and difficult to eradicate. Fungal chlamydospores can survive in soil up to 6 years even in the absence of the host plants (Haware *et al*., 1996). The use of resistant cultivars to control wilt is the best and the cheapest method. A massive screening program for wilt resistance was carried out at ICRISAT, India and more than 50 germplasm accessions were identified as resistant.

The second serious disease, blight caused by *Ascohcyta rabiei* (Pass.) Lab. has been considered the most devastating one till recent past (Iqbal *et al*., 2003, Iqbal *et al*., 2004). Disease epidemics in almost all the chickpea growing countries of the world have been reported (Radulescu *et al*., 1971; Kaiser, 1973; Malik and Tufail, 1984). Even after one hundred years of research on blight, many questions are unsolved that indicated the complexity of this disease. Although blight can be controlled by the application of foliar and seed dressing fungicides, use of disease free seeds and destruction of plant diseased debris, but under certain conditions, these approaches are not feasible (Bashir and Ilyas, 1983; Malik *et al*., 1991; Rauf *et al*., 1996). Importance of resistant cultivars is an established fact recognized by the researchers

therefore, identification and use of resistant sources against pests and diseases is an integral component of genetic improvement programme. Previously a number of chickpea resistant lines/cultivars have been identified against *Ascochyta* blight at national and international levels (Haq *et al*., 1981; Hawtin and Singh, 1984; Nene and Reddy, 1987). With the co-existence of host-pathogen complex, genetic breakdown of resistant genes is likely to work, especially in chickpea blight where genetic mechanism is yet debatable.

A little information is available in germplasm evaluated for dual resistance against these two important diseases due to their occurrence under contrasting environments. The present study was therefore, undertaken with the hypothesis to identify genotypes of chickpea for resistance against both of these fungal diseases from diverse genetic resources.

MATERIALS AND METHODS

One hundred and forty five chickpea genotypes obtained from National Agricultural Research Centre (NARC), Islamabad, Nuclear Institute of Agriculture and Biology (NIAB), Faisalabad and International Centre for Agricultural Research in Dry Areas (ICARDA), Syria were screened for their reaction against wilt and blight under field and green house conditions. These two sets of experiments were undertaken under two different environmental conditions.

Screening against wilt under green house conditions

Inoculum of the fungus was prepared by soaking sorghum grains in tap water overnight and then as surface dried by spreading on paper towels in laboratory under a ceiling fan. Surface dried seeds were put into conical flasks @ 250g flask^{-1} 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 seven days old actively growing culture of *F. oxysporum* f.sp. *ciceri* by adding a 6 mm agar plugs using sterile cork borer. After plugging, these flasks were incubated at 25^{o}C for 7 days. At the time of inoculation, each of the flasks containing inoculum was mixed in 2 kg of soil, which was put in the earthen pots (20 x 15 cm) to develop artificial wilt sick field. Each of the test lines was sown in two replications. Data on the number of wilted seedlings in each pot for each test line were recorded 40 days after sowing and percent disease incidence was calculated for each test line by keeping number of wilted plants as numerator and total number of plants as devisor. The level of resistance and susceptibility of each test line was determined by using 1-9 rating scale given by Iqbal *et al*. (1993) 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).

Screening against wilt under field conditions

These genotypes were also screened in a wilt sick plot at NIAB, Faisalabad. A mixture of various isolates of wilt fungus was used to develop wilt sick plot. Experiment was planted on 14th October 2003 in an augmented design and a highly wilt susceptible genotype (AUG 424) was repeated after every two test entries in two replications. Each genotype was planted in a 4 m single row plot, whereas inter and intra-row distance were maintained at 30 cm and 10 cm, respectively. Disease data were recorded at reproductive (near physiological maturity) stage. Data on wilted plants of test entries were recorded when 100% killing of the susceptible check had occurred. The percentage of wilt incidence of each entry was calculated and the level of resistance and susceptibility of each test entry was determined by using 1-9 disease rating scale (Iqbal *et al*., 1993).

Screening against blight under green house conditions

Same set of genotypes were screened against blight under greenhouse as well as field conditions. Seeds of test lines were surface sterilized with Clorox solution (0.1% available chlorine) for 2 minutes and sown in disposable pots (7.5 x 15 cm) filled with sterilized soil and sand mixture (2:1). Each pot contained five seedlings and a susceptible check (C 727) was kept as control for comparison. Pots were kept under greenhouse at 20$\pm$2 ^{0}C in natural light for 15 days before inoculation. Pots were watered from the top prior to inoculation. Two week old seedlings were inoculated by spraying aqueous spore suspension having a concentration of 5 x 10^{5} spores ml^{-1}. The inoculum was prepared from 15 days old culture of *A. rabiei* multiplied on chickpea grains according to the procedure developed by Ilyas and Khan (1986). The inoculated seedlings were incubated in humid chamber for 72 hours in the greenhouse. Disease observations were taken when susceptible check was completely killed and recorded on 1-9 disease rating scale (Singh *et al*., 1981).

Screening against blight under field conditions

Same set of germplasm was screened under field conditions during simultaneous crop seasons of 2003-04. One row of 4 m length was planted for each genotype in two replications. Susceptible check (C 727) was planted after every two rows of the germplasm for disease spread and comparison. When the entries were in early flowering stage, they were spray-inoculated with spore suspension of *A. rabiei* @ 5 x 10^{5} spores ml^{-1} and the inoculum was applied daily in the evening till appearance of blight. Continuous spray of water supported to maintain RH for

development of disease. The data for blight at vegetative stage was recorded according to Singh *et al*., 1981. Data for both sets of experiments for both the diseases were analyzed for variance and correlation to compare genotypes and disease at two stages using computer software MS Excel for Windows following the methods by Singh and Chaudhry, (1985).

RESULTS AND DISCUSSION

One hundred and forty five chickpea genotypes were screened against wilt and blight at two stages revealed significant resistance for disease stages and genotypes for both the diseases (Table 1). The high proportion of variable for disease stages suggested the improvement of screening techniques especially under field conditions. It was observed that 14 lines (90395, C-235, C-44, CM2000, FLIP97-129C, FLIP97-172C, FLIP98-107C, FLIP98-227C, FLIP98-230C, FLIP98-231C, FLIP98-38C, FLIP98-54C, ILC7374, KC-89) were resistant to wilt. In case of screening in the filed in sick-bed, no line was found resistant while 12 lines (90395, C-235, C-44, E101XPB91, FLIP98-107C, FLIP98-226C, FLIP98-227C, FLIP98-230C, FLIP98-231C, FLIP98-38C, FLIP98-54C, ILC 7374) were moderately resistant. It was observed that out of 14 resistant lines under greenhouse condition, ten lines were moderately resistant under field conditions. The economical and the ideal way of managing chickpea wilt, is the use of resistant cultivars, which are not common in the existing chickpea germplasm and similarly in our present investigation, no genotype was resistant to wilt under sick bed conditions.

Zote *et al*., (1983) studied the 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, four developed less than 10% and six 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 moderately resistant to highly susceptible reaction. Iftikhar *et al*., (1997) screened 31 chickpea germplasm lines received from ICARDA, and found that all of them were highly resistant to wilt disease. The discrepancies in the results, especially on screening experiments are mainly attributed towards the material and environmental conditions where the experiments are conducted. It is suggested that resistant material identified at one or other site should be screened over the locations under conducive environments by the experts to minimize experimental errors. Screening of chickpea germplasm for the sources against *Fusarium oxysporum* f.sp. *ciceri* revealed that the incidence and the severity of the disease were high under sick beds, whereas under natural conditions the crop often has the chances of disease escape. Wilt disease is temperature dependent and the level of inoculum may vary at different spots within one field, therefore it is necessary to screen the material under uniform disease intensity. Our results indicated that the source of resistance to *Fusarium* wilt in chickpea germplasm is not much common although a number of workers have also reported the occurrence against high level of resistance of *Fusarium* wilt (Pathak *et al*., 1982, Zote *et al*., 1983, Ahmad and Sharma 1990, Kaushal and Singh, 1990, Reddy *et al*., 1990. Iqbal *et al*., 1993, Ahmad *et al*., 1990, Iftikhar *et al*., 1997, Yu and Su, 1997). Against blight six genotypes (FLIP98-44C, ILC7795, FLIP97-217C, FLIP98-56C, FLIP99-48C, FLIP97-195C) were tolerant under green house conditions at seedling stage, whereas other six genotypes (FLIP98-230C, FLIP98-226C, FLIP97-219C, FLIP97-229C, FLIP97-132C, FLIP98-231C) were resistant at adult plant stage. It is important to note that although inoculation with same quality and intensity was applied at both these two screening sites but tolerance was recorded on different sets of genotypes that might be due different genes involved in blight resistance in the material screened. Among germplasm six genotypes in each case under green house and field conditions were resistant to chickpea blight that had been considered an important disease throughout the world in chickpea growing countries (Iqbal, 2002).

Table 1: Mean squares for wilt and blight ratings and stages in chickpea genotypes

Source of Variation	df	Wilt	Blight
Rating	144	3.84 (P<0.00)	4.24 (P<0.00)
Stages	1	422.41 (P<0.00)	7.30 (P<0.01)
Error	144	1.11	1.10
Total	289		

Table 2:- Resistant genotypes selected from germplasm obtained from local and exotic sources screened at seedling and pod formation stages

	Rating	Green house conditions	Field conditions
Wilt	1	C-235, 90395	Nil
	3	FLIP98-230C, FLIP98-231C, FLIP98-227C, FLIP98-54C, ILC7374, ILC182, FLIP98-38C, FLIP98-107C, FLIP97-129C, KC-89, C-44, CM2000, FLIP97-172C	Nil
Blight	3	FLIP98-44C, ILC7795, FLIP97-217C, FLIP98-56C, FLIP99-48C, FLIP97-195C	FLIP98-230C, FLIP98-226C, FLIP97-219C, FLIP97-229C, FLIP97-132C, FLIP98-231C

Table 3:- Identified sources of resistance for wilt and blight in chickpea

Variety	Source	Wilt under green house	Wilt under field	Blight under green house	Blight under field
FLIP98-227C	ICARDA	3	5	5	5
FLIP98-230C	ICARDA	3	5	5	3
FLIP98-54C	ICARDA	3	5	5	5
ILC7374	ICARDA	3	5	5	5
FLIP98-226C	ICARDA	5	5	5	3

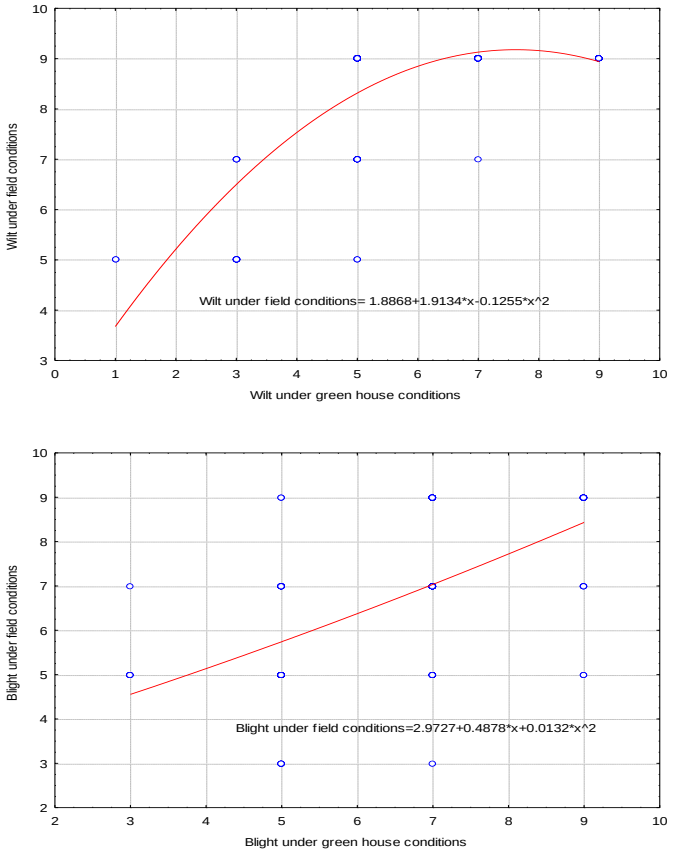

Fig. 1:- Relationship between screening under green house and field conditions for wilt (upper) and blight (lower)

Although none of the genotypes was highly resistant at any of these stages that still demands development of higher level of sustainable resistance (Iqbal & Ghafoor, 2005). Five genotypes were identified with genes for tolerance against both the diseases which could be utilized for developing high yielding cultivars with dual tolerance (Table 3). In Pakistan chickpea is grown under rainfed conditions where availability of moisture is uncertain that cause sever wilt under water scarcity and blight during excessive rains. Therefore, these genotypes are suggested to test under chickpea growing areas with close monitoring for disease reaction and yield potential. Linear relationship between two stages of screening for chickpea blight as presented in the Figure 1 indicated relative usefulness of screening at either site, whereas screening for wilt under natural conditions might be a misleading information, therefore screening against wilt is suggested only under sick bed conditions. Screening techniques along with conducive environmental conditions at NARC for screening chickpea germplasm against blight can be extended to national and international researchers because the material identified at this location is likely to withstand high levels of inoculum. Most of the chickpea lines reported as resistant by earlier researchers like, Singh *et al*., 1984; Reddy and Singh, 1990; Crino *et al.*, 1985: Bashir and Haware (1986) and Ilyas *et al*. (1991) have been utilized in breeding programmes somewhere or else. Genotypes with indifference reaction at two stages are needed to be investigated for mode of resistance at particular stage as not to loose genes for yield potential. Infection might be due to different genes involved for resistance mechanism at various plant stages or may be because of variation in mode of infection at various stages (Reddy and Singh, 1993). Anyhow this situation is yet to be resolved by conducting more experiments on mode of inheritance and infection of *Ascochyta* blight.

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