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INHERITANCE OF RESISTANCE TO *FUSARIUM* WILTS IN CHICKPEA

Khalid Mahmood*, Muhammad Saleem and Muhammad Ahsan

Department of Plant Breeding and Genetics, University of Agriculture, Faisalabad, Pakistan
*Corresponding author's e.mail: khalidbully@yahoo.com

The present study was undertaken to investigate the inheritance of wilt resistance of chickpea genotypes under field conditions. Inheritance of resistance to *Fusarium oxysporum* was studied in a set of crosses among six resistant CM-98, Aug-786, Bittal-98, Balksar-2000, Wanhar-2000, Punjab-2000 and one disease susceptible parent viz., AUG-424. F_1, F_2 and F_3 generations indicated that resistance was conferred by a single recessive allele at the same locus in the six resistant parents. The resistance was successfully transferred from resistant parents to susceptible parent by hybridization and pedigree and bulk selection.
Keywords: *Cicer arietinum* L., *Fusarium oxysporum*, wilt resistance, legume

INTRODUCTION

Chickpea (*Cicer arietnium* L.), a cool season food legume grown in tropical, sub-tropical and temperate regions of the world is the leading pulse legume in Pakistan. The major chickpea growing countries are India, Pakistan and Turkey in Asia, Ethiopia in Africa, California and Washington states in the USA, Mexico and Australia. In Pakistan, the crop is grown under three farming systems: the rainfed system, contributing 88% of the total chickpea area, where it is grown as sole crop or mixed with others; the rice based system constituting 11% of the total chickpea area, where the crop is grown on residual moisture after rice and irrigated system constituting 1% of the total area. The area under the crop is about 1028.9 thousand ha as winter crop with a production of 479.5 thousand tones (Anonymous, 2008-09). The major chickpea production belt is the 'Thal' comprising the districts of Bhakhar, Mianwali, Leyyah, Khushab and parts of Jhang as well as Attock, Rawalpindi, Jehlum and Chakwal districts of Potohar. Since most of the chickpea crop is grown under the rainfed system, fluctuations in rainfall can severely limit productivity.

The two largest chickpea growing countries i.e., India and Pakistan, which account for nearly 85% of the chickpea area in the world have low average yield levels of 598 kg/ha and 466 kg/ha, respectively. The low yield of chickpea in countries with extensive area under the crop is the factor responsible for overall low production of the crop in the world. This is primarily due to paucity of productive cultivars, non-availability of good quality seed and resistance against various biotic and abiotic stresses. Of the various fungal diseases, chickpea wilt caused by *Fusarium oxysporum* sp. *ciceris* causing the plant to wilt, is a serious problem especially in the rainfed area. Therefore, introduction and evolution of chickpea genotypes performing better in both rainfed and irrigated conditions is key to enhance productivity. An estimated annual loss of 12 million rupees was reported in Pakistan (Sattar *et al.,* 1953). Attempts were made to estimate loss in yield on a per plant basis. Earlier wilting caused more loss than late wilting. Seeds harvested from wilted plants were lighter and duller than those from healthy plants (Haware and Nene, 1980). At least 4 races of this fungus have been reported (Haware and Nene, 1982), however, no information on existence of races in Pakistan is available. 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.

Differences in time of wilting of chickpea (*Cicer arietinum* L.) in response to Race 1 of *Fusarium oxysporum* f.sp. *ciceris*, are confirmed (Kumar and Haware 1982). C-104 wilts later than JG-62 and the difference in time of wilting appears to be inherited as a single gene with early wilting partially dominant to late wilting (Upadhyaya *et al*. 1983). Considered in relation to earlier studies, the observations indicate that at least two genes are involved in the inheritance of resistance in chickpea to Race 1 and offer an explanation for previous difficulties in interpreting the inheritance of resistance (Pathak *et al.*1975). Resistance to race 2 of *Fusarium* wilt is controlled by two genes, the first of which must be present in the homozygous recessive form, and the other in the dominant form, whether homozygous or heterozygous for complete resistance. Early wilting results if the other gene is homozygous recessive. Late wilting occurs if both loci are dominant. The existence of difference among chickpea cultivars

in the time taken to express the initial symptoms of *Fusarium* wilt was observed (Gumber *et al*., 1995).

Two hundred and forty nine chickpea mutants developed through gamma irradiation along with their respective parents and susceptible check Aug-424 for resistance to *Fusarium* wilt was screened in natural wilt sick plot during 2003-2004 seasons. All the 4 parent genotypes showed highly susceptible reaction to *Fusarium* wilt. Out of a total of 249 morphological mutants of 4 genotypes, 75 mutants exhibited highly resistant reaction (less than 10 %) followed by 31 mutants resistant (11 to 20%), 34 mutants moderately resistant / tolerant (21 to 30%), 35 mutants susceptible (31 to 50%) and 75 mutants were highly susceptible (50 to100%), (Shah *et al*., 2009).

Three hundred and twenty one genotypes of chickpea from different sources 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 (Ahmad *et al.,* 2010).

We hope this information will be useful to chickpea researchers all over the world. Also, principles that have been used to develop these techniques should be useful in working out techniques for other diseases elsewhere. The use of resistant cultivars to control wilt is the best and the cheapest method.

The objective of this study is to find the nature of inheritance of *Fusarium oxysporum*, sp. *Ciceri* resistance under field conditions for the development new wilt resistant genotypes.

MATERIALS AND METHODS

To study inheritance of resistance to chickpea wilt, six resistant parents viz., CM-98, Aug-786, Bittal-98, Balksar-2000, Wanhar-2000, Punjab-2000 and one susceptible (Aug-424) were crossed during February 2006-07. To ascertain disease classes and genetic difference, Aug-424 was also crossed with each other. But the cross was not successful because of severe fungal effect of the pathogen. Six successful crosses were manipulated and F_1, F_2 progenies were raised and evaluated for disease reaction under wilt sick bed during crop seasons 2006-2009. To ensure uniform incidence of the disease under field conditions, artificial sickbed was prepared and maintained by Department of Plant Breeding and Genetics in their field area by the following method.

1. Selected plot of adequate size that it is isolated from other chickpea fields to avoid spread of the fungus inoculum from this plot to others. The plot should have been cropped in the previous year with chickpea traces of wilt incidence.
2. Planted a highly susceptible cultivar (Aug-424) in this plot. Ensure a good plant population and carry out normal agronomic operations.
3. By the end of the season, at least 20 to25% of the plants should show wilt symptoms. After harvesting and threshing, scatter the debris uniformly all over the plot.
4. Repeat steps 2 and 3 in the next season.
5. Initiated screening in the same plot. Plant a susceptible cultivar after every two test rows in the whole field. These rows will serve as checks, and will help in monitoring and maintaining the wilt sickness of the plot. The susceptible check rows should show more than 90% wilt. Different level intensity of wilt can be seen in field (Fig.1).

Figure 1. Three Pictures of Wilt field of Experiment at University Experimental area. Different level of intensity of Wilt can be seen in pictures.

Rating Scale

A 6-point scale is used for easy scoring. Interpretation of the scale is as follows: (1, 0%) highly resistant; (2-3, 1-20%) moderately resistant; (4, 21-50%) tolerant; (5,

51-80% susceptible) moderately susceptible; (6, 81% or more) highly susceptible (Table 1).

The F_3 progenies were obtained from random F_2 plants grown under wilt free conditions. To find the nature of inheritance of *Fusarium oxysporum*, sp. *Ciceris* resistance using chi-square test.

RESULTS AND DISCUSSION

The crosses of all the six resistant genotypes with AUG-424 were found to be moderately susceptible in F_1 progenies by the formation of necrotic local lesions on primary leaves and later on no distortion or stunting of plants was observed. The F_2 progenies segregated in a ratio of 3 susceptible and 1 resistant (Table 2), and the F_3 progenies, 1 susceptible : 2 segregating : 1 resistant (Table 3). The data revealed segregation of a single gene with the recessive allele conferring resistance to race 1 of the wilt fungus. The heterozygous populations of all cross combinations in F_3 generation (Fig.1) of are a close fit of actual to the expected distribution for a 1:2:1 ratio indicating resistance to be controlled by a single gene pair (Kumar and Haware, 1982). There was an excess of resistant progenies in the F_3 generation from Wanhar-2000 × AUG-424 and Punjab-2000 × AUG-2000 that could have resulted from a failure of occurrence. Some crosses did not give good fit to the expected ratios for simple inheritance. The existence of polygenic complex and the inheritance of resistance to other races of the pathogen were not yet studied. Meanwhile the resistance of CM-98, Aug-786, Bittal-98, Balksar-2000, Wanhar-2000, Punjab-2000 and other genotypes resistant to race 1 could be transferred to other genotypes by hybridization and followed by pedigree and bulk selection. These results were confirmed by Upadhyaya *et al*. (1983), Kumar (1998), Pande *et al*. (2007), Shah *et al*. (2009) and Ahmad *et al.* (2010) those observed mono genic inheritance for wilt inheritance. Haware and Nene (1982) reported four races of *F. oxysporum*, only a few reports are available on race 2 of *Fusarium* wilt. Under field condition the resistance to race 2 was controlled by a single r ecessive gene (Pathak *et al.,* 1975 and Gumber *et*

Table 1. Description of the rating scale for scoring

Rating	Wilt	Field Observation
1	No mortality	No lesions visible
2	10% or less mortality	Few scattered lesions; usually seen after careful examination
3	11-20% mortality	Lesions and defoliation on some plants; little damaging
4	21-50% mortality	Lesions very common and damaging; 25% plants killed
5	51-80% mortality	All plants with extensive lesions causing defoliation and drying of branches; 50% plants killed
6	81% or more mortality	Lesions extensive on all plants; defoliation and drying of branches; more than 75% plants killed

Table 2. Number of resistant and susceptible plants or progenies in F_1 and F_2 generations of the 6 crosses infested with *Fusarium oxysporum* L.

Cross combination	Number of plants observed								
	Gene-ration	Total	Resis-tant	Segre-gating	Suscep-tible	Expected ratio	*d.f*	X^2	P-value
CM-98 × AUG-424	F_1	14	14						
	F_2	367	85	201	81	3:1	2	3.419	0.70-0.50
Bittal-98 × AUG-424	F_1	17	17						
	F_2	396	81	208	107	3:1	2	4.424	0.50-0.30
AUG-786 × AUG-424	F_1	11		11					
	F_2	81	27	39	15	3:1	2	3.725	0.70-0.50
Punjab-2000 × AUG-424	F_1	13	13						
	F^2	508	124	265	119	3:1	2	1.051	0.70-0.50
Wanhar-2000 × AUG-424	F_1	8	8						
	F_2	757	193	388	176	3:1	2	1.243	0.50-0.30
Balksar-2000 × AUG-424	F_1	9	9						
	F_2	145	29	83	33	3:1	2	4.736	0.50-0.30

Table 3. Number of resistant and susceptible plants or progenies in F_3 generations of the 6 crosses infested with *Fusarium oxysporum* L.

Cross combination	Number of plants observed								
	Generation	Total	Resis-tant	Segre-gating	Suscep-tible	Expected ratio	*d.f*	X^2	P-value
CM-98 × AUG-424	F_3	609	155	315	139	1:02:01	2	1.57	0.50-0.30
Bittal-98 × AUG-424	F_3	428	125	201	102	1:02:01	2	4.05	0.30-0.20
AUG-786 × AUG-424	F_3	342	99	147	96	1:02:01	2	6.84	0.10-0.05
Punjab-2000 × AUG-424	F_3	350	108	167	75	1:02:01	2	7.01	0.10-0.05
Wanhar-2000 × AUG-424	F_3	989	271	501	217	1:02:01	2	6.08	0.10-0.05
Balksar-2000 × AUG-424	F_3	343	104	156	83	1:02:01	2	5.36	0.10-0.05

al.,1995). Similar study for screening was conducted by Chaudhry *et al.* (2006) Who reported high level of resistance to *Fusarium* wilt in chickpea germplasm originating from different sources from Pakistan.

CONCLUSION

Diseases are the major constraints in the cultivation of chickpea. During the last 15 years, considerable information became available on chickpea diseases. Progress has been made in identifying sources of resistance to wilt disease of chickpea through screening of germplasm and inheritance of the disease resistance (Chaudhry *et al.* 2006). Existence of mono-genic and di-genic inheritance pedigree and bulk selection methods can be used to increase resistance against wilt disease in chickpea genotypes. Crosses Wanhar-2000 **×** AUG-424 and Punjab-2000 **×** AUG-2000 that could not showed true to fit ratio, needed further evaluation. However concentrated efforts are needed to utilize the information which is available for the improvement of chickpea crop in the Pakistan. Disease surveys are needed to understand the distribution, severity and crop losses caused by the diseases in chickpea. Epidemiological studies of the important diseases will help to formulate effective integrated disease management.

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