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SOURCES OF MULTIPLE RESISTANCES IN CHICKPEA GERMPLASM AGAINST *ASCOCHYTA BLIGHT*, FUSARIUM WILT AND STUNT VIRUS DISEASES

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ABSTRACT

Ascochyta blight, Fusarium wilt and stunt virus disease, being the three major diseases of chickpea in Pakistan, cause appreciable yield losses and now and then upset the production statistics of the crop in the country. The screening of 63 chickpea lines in Ascochyta blight, Fusarium wilt and stunt virus disease nurseries revealed the expression of multiple resistance. A test line 14177 was found to be moderately resistant to Ascochyta blight but immune to wilt and stunt virus disease. One line 14133 was resistant to blight but immune to wilt infection. Four chickpea lines 662-1, 816, 914, 985 were moderately resistant to wilt and stunt virus disease; one line 936 was moderately resistant to wilt but immune to stunt virus infection. Three chickpea lines 113-1, 1435, C-44 were moderately resistant to both stunt virus and Ascochyta blight disease; one line 984, was immune to stunt virus but moderately resistant to Ascochyta blight disease while two lines 270, 14144 were resistant to stunt virus but moderately resistant to Ascochyta blight disease.

Key words: *Cicer arietinum* L., *Ascochyta rabiei*, *Fusairum oxysporium* f. sp. *ciceri*, stunt virus disease.

INTRODUCTION

Chickpea (*Cicer arietinum* L.) is cultivated as a post monsoon winter crop in barani areas of Pakistan and it ranks second to India in term of area occupied by it. In Pakistan, chickpea is cultivated on an area of 1028.9 thousands hectares with an annual production of 479.5 thousands tones, an average yield being 466 kg/ha (Anon., 2006). This is an extremely low yield as compared to potential yield of commercial chickpea cultivars sown in the country. Besides, other factors, diseases play a major role in reducing chickpea yield in Pakistan. Although the crop is attached by a number of diseases, but Ascochyta blight, Fusarium wilt and stunt virus disease, caused by pea leaf roll virus, are the three most important diseases (Nene and Reddy, 1987). Chickpea blight caused by *Ascochyta rabiei* (Pass.) Lab. is a devastating disease in areas where and when cool, cloudy humid weather (15-25 ^{0}C and > 150 mm rainfall) persists during the crop season (Pande et al., 2005). The disease causes serious grain yield losses (Malik and Bashir, 1984; Kaiser and Hannan, 1988; Guar and Singh, 1996) and may result in complete failure of the crop (Malik and Bashir, 1984). The disease perpetuates through infected seed and crop debris (Naid and Nirala, 1979; Maiden, 1987). Chickpea wilt caused by *Fusarium oxysporum* f. sp. *ciceri* (Padwick) is both seed and soil borne and the pathogen can survive in the soil, even in the absence of host, for six year (Iqbal *et al* ., 2005). The disease appears when high temperature, (> 25 ^{0}C) and moisture stress prevails (Satter *et al.*, 1953; Hawere *et al.*, 1996). In Pakistan the disease may cause 10-50 percent crop losses (Khan *et al*., 2002). The chickpea stunt virus disease caused by pea leaf roll virus in transmitted by *Aphis crassivora* (Nene and Reddy, 1976) and is characterized by marginal browning and thickening of leaflets and stems. The disease has been reported to cause 50 percent losses in USA (Kaiser and Danesh, 1971). This disease is responsible for reduction in pod number, number of seed per plant, 100 seed weight and seed yield of the plants (Ilyas *et al.*, 1990). Since chemical control measures for chickpea disease are too expensive and biological control measures are too difficult to be applied by a farmer, the most ideal and cheapest method of controlling these diseases is the use of resistant varieties. This paper reports the screening of sources of multiple resistance in a chickpea germplasm against Ascochyta blight, Fusarium wilt and stunt virus disease.

MATERIALS AND METHODS

Sixty four chickpea lines were screened for the sources of resistant in each of the chickpea Ascochyta blight nursery, chickpea wilt nursery and chickpea stunt virus disease nursery at the Department of Plant Pathology, University of Agriculture, Faisalabad, Pakistan.

I- Chickpea Ascochyta Blight Nursery

Each of the 63 test lines was planted in a single row subplot of 3 meter length with plant to plant distance 15 cm and row to row 30 cm. A highly susceptible check cultivar C-727 was planted in a single row after every two test lines of the germplasm. At mid pod stage, the test lines were sprayed with the spore suspension of *A. rabiei* (1 x 10^{-5} spores / ml) until the full appearance of disease on check cultivar C 727. The inoculum was prepared by the mass

culturing technique described by Ilyas and Khan (1986). The development of the disease was aided by the continuous spray of fresh water every day. The data for disease severity were recorded to assess the level of resistance or susceptibility of each test line, using 0-9 grades disease rating scale (Reddy and Singh , 1984) where 1 is no visible disease symptoms on any plant and 9 is profuse lesions on all plants, stem girdling on more than 50 percent of the plants and many plants skilled.

II- Chickpea wilt nursery

Each of the 63 chickpea germplasm lines was sown in a single row subplot of 4 meter length with row to row distance 30 cm and plant to plant distance 15 cm in a wilt sick plot at the experimental area of the Department of Plant Breeding and Genetics, University of Agriculture, Faisalabad. The cultivar AUG-480 included in wilt nursery served as a susceptible check. The nursery was raised with general agronomic practices. The data on the number of wilted plants as well as the total number of plants in each test line were recorded and incidence of wilt for each test line was determined by using following formula

$$\text{Disease Incidence} = \frac{\text{No of wilted plants in the test line}}{\text{Total No. of Plants of the test line}} \times 100$$

The level of resistance and / or susceptibility in each test line was determined by using 1-9 grades rating scale of Iqbal *et al.,* (1993), where 0 = Immune (None of the plant wilted) 1= highly resistant (1-5 % plant wilted), 3 = Resistant (6-10 % plant wilted), 5 = Moderately resistant(11-20 % plants wilted), 7 = Susceptible (21-50 % plants wilted) and 9 = Highly susceptible (more than 50 % wilted plants).

III- Chickpea Stunt Virus Disease Nursery

Here also each of the 63 test lines was sown in a single row subplot of 4 meter length with row to row distance 30 cm and plant to plant distance of 15 cm. A highly susceptible cultivar ICC 918 was planted as a check after every two test lines. The entries of the nursery were subjected to natural invasion and build up of *Aphis crassivora* aphids, the vector of PLRV and consequently to infection of chickpea plants of each test line. Chickpea plants suffering from stunt virus disease were recognized by the characteristic symptoms of inter-node shortening, marginal browning and thickening of leaflets, proliferation of branches and leaflets with reddish ting (Nene and Reddy, 1976). After pod filling stage but before plant maturity, plants suffering from stunt virus infection and total number of plants for each test line were counted. The incidence of chickpea stunt virus infection for each test line was estimated by using the following formula.

$$\text{Incidence} = \frac{\text{Total No. of diseased plants in the test line}}{\text{Total No. of plants of the test line}} \times 100$$

The level of resistance / susceptibility of each test line was determined by using 0-9 grades self design rating scale where 0 = Immune (No infection at all), 1 = Highly resistant (1-5 % plants diseased), 3 = Resistant (6-10 % plants diseased), 5 = Moderately resistant (11-20 % plant diseased), 7 = Susceptible (21-30 percent plant diseased) and 9= Highly susceptible (more than 30 percent plant diseased).

RESULTS AND DISCUSSION

I- Chickpea Ascochyta Blight Nursery

None of the 63 chickpea test lines was formed to be immune or highly resistant to Ascochtya blight infection (Table -1). However , eleven lines such as 201, 218, 283, 970, 1045, 1065, 1145, 14130, 14133, 14154 and 14162 were formed to be resistant while thirteen lines such as 102, 113-1, 240, 270, 832, 913, 981, 984, 1435, 14144, 14177 CM-870 and C-44 were moderately resistant to blight disease. Out of the remaining lines, 19 and 20 lines were susceptible and highly susceptible respectively.

II- Chickpea Fusarium Wilt Nursery

Out of the 63 test lines, three lines i.e., 1315, 14133 and 14177 were immune i.e 100 percent free from Fusarium wilt infection (Table -2) and none was found to be highly resistant or resistant. However, six lines such as 46, 662-1, 816, 914, 936 and 985 were moderately resistant to wilt infection. Of the remaining test lines 15 and 39 lines were susceptible to highly susceptible to Fusarium wilt infection respectively.

III- Chickpea Stunt Virus Disease Nursery

Out of the 63 test lines eight lines such as 15-1, 48-1, 936, 984, 1114, 1115, 1431 and 14177 were found to be 100 percent free form stunt virus infection i.e., immune (Table 3). None of the test lines was found highly resistant. However, seven lines such as 46, 270, 832, 908,942,1434-1, 14144 were found to be resistant while nineteen lines such as 1-3, 6-1, 113-1, 128, 283, 588, 660-1, 662-1, 816, 854, 896, 907, 910, 914, 927, 985, 1435, CM-72 and C-44 were moderately resistant to stunt virus disease. The remaining test lines were susceptible to highly susceptible.

A comparative perusal of the test lines resistant to blight, wilt or stunt virus disease for a search of multiple resistance source to all the three or to any two diseases revealed that only one test line i.e., 14177 displayed multiple resistance to the three diseases. It was moderately resistance to blight but immune to wilt and stunt virus infection. Two test lines i.e 14133 and 14177 were resistant and moderately resistant to blight respectively but immune to wilt infection. Four test lines such as 662-1, 816, 914, 985 were moderately resistant to wilt and stunt virus infection. Three test lines such as 113-1, 1435, C-44 were moderately resistant to both stunt virus and Ascochyta blight disease; one test line such as 984, was immune to stunt virus but moderately resistant to Ascochyta blight disease while two lines i.e 270 and 14144 were resistant to stunt virus but moderately resistant to Ascochyta blight disease.

These sources of resistance could further be evaluated for their yield potential and other desirable agronomic traits if these are there. The resistant lines with desirable agronomic traits and appreciable yield potential could either be released directly as commercial cultivars or these may be used as source resistant parents to transfer their resistance into commercial cultivars lacking resistance, through conventional breeding procedures. Any how prior to such transfer of their resistance to the commercial cultivars, a thorough study on the genetic basis of their resistance (i.e., vertical or horizontal) must be determined against the prevalent virulence of *Ascochyta rabiei*, *Fusarium oxysporum* and stunt virus. Moreover, studies on the nature and diversity of resistant genes are essential for exploiting a particular resistance source in resistance breeding programs.

Earlier genetic studies revealed that resistance of chickpea against *Ascochyta blight* disease is due to either a single dominant gene or recessive gene (Singh and Reddy, 1983) Allelic studies by Tewari and Pande (1986) have revealed the presence of three independently segregating dominant genes for resistance in chickpea in chickpea cultivar 1215-1, EC 26446 and PG 82-1 and a recessive gene in BRG 8. Inter allelic interaction, additive gene effects and dominance influencing resistance has also been reported (Dey and Singh, 1993) Recent studies on the inheritance of Ascochyta blight resistance indicate that several quantitative trait loci (QTL) also control resistance (Tekeoglu *et al.,* 2000; Collard *et al.,* 2003; Flandez *et al.,* 2003). Thus Ascochyta blight resistance is a complex endeavor suggesting that there is a range of different resistance sources with different genes of resistance. Sindhu *et al,* (1983) reported that resistance of three resistant chickpea lines (1231, 32/35-8/7 and 35/35-32/2) is conditioned by a single recessive gene (rfo) while susceptibility is conditioned by its dominant allele (Rfo). The genetic basis of resistance of chickpea against stunt virus disease, and the nature and diversity of genes controlling resistance is yet not known. However, pyramiding of different resistance genes, operative against Ascochyta blight, Fusarium wilt and stunt virus disease, into commercial cultivars may facilitate building up the level of resistance and increasing durability of resistance in commercial cultivars.

Table 1. Chickpea lines exhibiting various levels of resistance / susceptibility to *Ascochyta* blight.

0 Immune or Completely Resistant	1 Highly Resistant	3 Resistant	5 Moderately Resistant	7 Susceptibility	9 Highly Susceptibility
None	None	201, 218, 283, 970, 1045, 1065, 1145, 14130, 14133, 14154, 14162	102, 113-1, 240, 270, 832, 913, 981, 984, 1435, 14144, 14177 CM-870 and C-44	1-3, 15-1, 366, 660-1, 662, 896, 907, 908, 927, 942, 957, 985, 1071, 1114, 1115, 1134, 1434-1, 1437, CM. 72	6-1, 46, 48-1, 128, 190, 293, 588, 816, 854, 905, 910, 914, 936, 999, 1064, 1119, 1315, 1431, 6306, C. 727

Table 2. Chickpea germplasm lines exhibiting various levels of resistance / susceptibility to the incidence of *Fusarium* wilt disease.

Disease incidence and response of germplasm lines					
0 Immune or Completely resistant	1-5 Highly resistant	6-10 Resistant	11-20 Moderately Resistant	21-50 Susceptibility	51-100 Highly Susceptibility
1315, 14133, 14177	None	None	46-662-1, 816, 914, 936, 985	128, 218, 366, 660-1, 907, 908, 984, 999, 1145, 1437, 14130, 14154, C. 44, C 72, CM 870	1-3, 6-1, 15-1, 48-1, 102, 113-1, 190, 201, 240, 270, 283, 293, 588, 832, 854, 896, 905, 910, 913, 927, 942, 957, 970, 981, 1045, 1064, 1065, 1071, 1114, 1114, 1119, 1134, 1431, 1434-1, 1435, 6306, 14144, 14162, and AUG-480

Table 3. Chickpea germplasm lines exhibiting various levels of resistance / susceptibility to the incidence of stunt virus disease.

Disease incidence and response of germplasm lines					
Zero or Completely resistant	1-5 Highly resistant	6-10 Resistant	11-20 Moderately Resistant	21-30 Susceptibility	31-100 Highly Susceptibility
15-1, 48-1, 936, 984, 1114, 1115, 1431, 14177	None	46, 270, 832, 908,942, 1434-1, 14144	1-3, 6-1, 113-1, 128, 283, 588, 660-1, 662-1, 816, 854, 896, 907, 910, 914, 927, 985, 1435, CM-72 and C-44	102, 190, 201, 218, 240, 293, 366, 905, 913, 970, 981, 999, 1045,1064, 1065, 1071, 1134,1145, 1315, 6306, 14130, 14133, 14154, 14162, CM-870	957, 1119, 1437, 1CC. 918 (Susc Check)

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