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RESPONSE OF ADVANCED LINES OF CHICKPEA AGAINST CHICKPEA BLIGHT DISEASE

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Abstract

In order to have resistance material 277 advanced lines of chickpea received from various research organizations were screened during the rabi season 2005-06 for the source of resistance against chickpea blight disease by artificial inoculation of the germplasm with pycniosspore suspension of the pathogen. The screening revealed 02, 38, 39, 49 and 149 lines to be highly resistant (immune), resistant, moderately resistant, susceptible and highly susceptible. Out of 126 lines received from Pulses Research Institute, (PRI) Faisalabad, none of the lines responded highly resistant or resistant while 12 lines such as 06025, 06026, 06027, 06031, 06035, 06040, 06041, 06056, Vinhar, Bitter-98, Pb-2000 and Paidar-91 responded to be moderately resistant. Out of 83 lines received from Nuclear Institute of Agriculture and Biology, (NIAB) Faisalabad, 7 lines *viz.*, 06223, 06224, 06270, 06271, 06272, 06277 and 06278 displayed resistant response while other 7 lines such as 06214, 06217, 06218, 06220, 06225, 06237, and 06279 exhibited moderately resistant response. Out of 36 advanced lines of National Agricultural Research Centre, (NARC) Islamabad, 13 lines (CMC-70T, CMC-55D, NCS-0510, CMC-59S, NCS-0516, NCS-0602, NCS-0612, NCS-0613, NCS-0614, NCS-0616, NCS-0621, NCS-0623 and NCS-0526) responded to be resistant while 14 lines such as NCS-0518, NCS-0513, NCS-0512, NCS-0610, NCS-0603,CMC-186M, NCS-0617, NCS-0619, NCS-0620, NCS-0625, NCS-0527, NCS-0529, NCS-0531, and Pb-2000 responded to be moderately resistant. Out of 32 advanced lines received from International Crop Research Institute for the Semi Area Tropics, (ICRISAT) India, 2 lines such as EC-516792, ICCV-98815 were found to be highly resistant (asymptomatic). Eighteen lines (EC-516709, EC-516729, EC-516771, EC-516793, EC-516878, EC-516895, EC-516934, EC-516957, EC-516967, EC-516974, EC-517003, EC-517011, EC-517039, EC-517073, ICC-6304, ICC-6945, ICCV-04537 and ICCV-98818) exhibited moderately resistant response. Thus the germplasm of NARC, and that of ICRISAT, India, consisted of greater number of resistant lines as compared to that of NIAB, Faisalabad and PRI, Faisalabad.

Introduction

Chickpea (*Cicer arietinum* L.), a post-monsoon rabi crop, is cultivated on an area of 1073 thousand hectares of barani as well as irrigated area of Pakistan with an annual production 842 tones per hectare, an average yield being 784 kg/ha (Anon., 2007). Thus production of this crop is very low as compared to the production in other chickpea growing countries, due to several biotic and abiotic factors affecting the crop. Among the biotic factors responsible for reducing the yield of chickpea in Pakistan is the blight disease caused by *Ascochyta rabiei* (Pass.) Labr. In Pakistan, the disease may result in 50-70% crop losses (Malik & Bashir, 1984) and under conditions conducive for the development of disease; losses may run to complete failure of the crop (Nene, 1984). The disease expresses itself as circular spots on leaves and pods and as elongated lesions on petioles and stems. The spots on leaves may coalesce and the entire leaf may become

scorched. Seeds within the pods may also develop lesions and become shriveled (Nene, 1982; Akeem, 1999). The pathogen survives and the disease perpetuates through infected seeds and crop refuses (Kaiser *et al*., 1973; Maiden, 1987). The pathogen has been reported to be highly variable and consists of several patho-types and races (Luthra *et al*., 1939; Bedi & Aujla, 1969; Vir & Grewal, 1974; Reddy & Kabbabeh, 1985; Singh, 1990; Sarwar *et al*., 2000). The disease can be managed by the removal and destruction of dead plant debris, crop rotation, deep sowing of seed (Sattar, 1933), inter-cropping of chickpea with cereals (Luthra & Bedi, 1935), by fungicidal seed treatment (Tripathi *et al*., 1987) and foliar application of fungicides (Kaiser *et al*., 1973; Singh & Singh, 1990) but the use of host resistance is the most effective and economical way of management of *Ascochyta* blight of chickpea (Reddy & Nene, 1987). However, the presence of physiological races is one of the problems in exploiting host resistance (Singh & Pal, 1993). This study was undertaken with the objective to identify the sources of resistance against *Ascochyta* blight disease by screening of chickpea germplasms originated from Pulses Research Institute, Faisalabad, NIAB, Faisalabad, NARC, Islamabad and ICRISAT, India.

Materials and Methods

Two hundred and seventy seven advanced lines of chickpea received from PRI, Faisalabad (126), NIAB, Faisalabad (83), NARC, Islamabad (36), and ICRISAT, India (32) were planted and screened in field research area of the Department of Plant Pathology, University of Agriculture, Faisalabad for the source of resistance against *Ascochyta* blight disease by artificial inoculating of the germplasm mentioned above Table 1. The screening was carried out during the rabi season of 2005-06. Each of the test lines was sown in single row sub-plot of 4 meter long with row to row distance of 30cm and plant to plant distance of 15cm. One row of highly susceptible cultivar, Pb-1, was planted after every two test lines as a spreader-cum-indicator row. A susceptible cultivar KC-4991 was also included in the ICRISAT material of the nursery as their local check. When most of test lines were at mid pod stage, the nursery was inoculated with a spore suspension of *A. rabiei* @ approximately 200,000 spores per ml of the H_2O.

Isolation of *A. rabiei* and preparation of inoculum: Previous year's chickpea pods, severely suffering from characteristic blight symptoms were collected from chickpea field and refrigerated at 5-8°C until used for the isolation of *A. rabiei*. The isolation was carried out by the procedure followed by Ilyas & Iqbal (1986). Infected pods, by holding them in a forceps, were surface sterilized in the flame of spirit lamp in such a way that only charring of outer pod layer (and surface sterilization) could occur but the inner pod layer remained intact. The charred and surface sterilized pods were then pressed open and infected seeds were taken out of these pods aseptically with help of another flame sterilized forceps. The naturally *A. rabiei* infected seeds, thus obtained, were plated on autoclaved chickpea seed meal agar (CSMA) medium in Petri plates and were incubated at 20 ± 2°C for 15 days. The colonies of *A. rabiei* coming out of blighted seeds were isolated and purified by spore streak method (Pathak, 1986). The purified culture was maintained at 5°C until used. The composition of CSMA medium was: Chickpea seed meal 20g, glucose 20g, agar agar 20g and sterilized water to make volume one liter.

Table 1. List of cultivars/ lines from different sources for screening against *Ascochyta rabiei* disease.

Sources	Varieties/test lines
Pluses Research Institute, Ayub Agricultural Research Institute, Faisalabad (PRI)	06025, 06026, 06027, 06031, 06035, 06040, 06041, 06056, Vinhar, Bittle-98, Pb-2000, Paidar-91, Bitter-98, Balkasar, Pb-91, C-44, 05141, 06005, 06006, 06020, 06022, 06023, 06042, 06053, 06054, 06055, 06057, 06058, 06059, 06060, 02009, 02023, 02044, 02075, 02093, 90261, 98004, 98154, 05142 to 05182, 06001 to 06004, 06007 to 06019, 06021,06024, 06028, 06029, 06030, 06032, 06036, 06037, 06038, 06043 to 06052, 02004, 02006, 02052, 02060, 02080, 93127, CM-98, Pb-1 (Local Check)
Nuclear Institute of Agriculture and Biology, Faisalabad (NIAB)	06223, 06224, 06270, 06271, 06272, 06277, 06278, 06214, 06217, 06218, 06220, 06225, 06237, 06279, 06203, 06205, 06219, 06221, 06233, 06239, 06252, 06275, 06278, 06260, 06264, 06265, 06266, 06268, 062174, 06282, 06201, 06202, 06204, 06206 to 06213, 06215, 06216, 06222, 06226 to 06232, 06234, 06236, 06238, 06240 to 06251, 06253 to 06256, 06259, 06261 to 06263, 06267, 06269, 06273, 06275, 06276, 06280, 06281, Pb-1 (Local Check)
National Agricultural Research Center, Islamabad (NARC)	CMC-70T, CMC-55D, NCS-0510, CMC-59S, NCS-0516, NCS-0602, NCS-0612, NCS-0613, NCS-0614, NCS-0616, NCS-0621, NCS-0623, NCS-0626, NCS-0518, NCS-0513, NCS-0512, NCS-0610, NCS-0603,CMC-186M, NCS-0617, NCS-0619, NCS-0620, NCS-0625, NCS-0527, NCS-0529, NCS-0531 and Pb-2000, NCS-0515, NCS-950259, NCS-0611, NCS-0615, NCS-0618, NCS-0534, NCS-0533, NCS-0622, CM-2000, Pb-1 (local Check)
International Crop Research Institute for the Semi Area Tropics (ICRISAT), India	EC-516792, ICCV-98815, EC-516709, EC-516729, EC-516771, EC-516793, EC-516878, EC-516895, EC-516934, EC-516957, EC-516967, EC-516974, EC-517003, EC-517011, EC-517039, EC-517073, ICC-6304, ICC-6945, ICCV-04537, ICCV-98818, EC-516796, EC-516916, EC-517025, EC-517030, ICC-4033, and ICCV-04537, ICC-12968, ICC-14344, ICC-15996, ICC-4991, ICC-4991 (Check), Pb-1 (local Check)

Mass culturing of *A. rabiei*: The mass preparation of inoculum of *A. rabiei* was carried out by the method of Ilyas & Khan (1986). The materials used for mass preparation of inoculum were 30 x 24cm size polypropylene bags; 2.5cm plastic of the same diameter, cotton plugs and chickpea seeds. The chickpea seeds were soaked in tap water for about 6 hours and then were boiled for about 30 minutes. The boiled seeds were spread on paper towels to absorb free moisture and were surface dried. The soaked and boiled seeds were then put into polypropylene bags @ 500 g bag^{-1}. The open end of each bag was passed through 2.5cm plastic ring. A cotton plug was inserted into the mouth of bag passing through the ring. The bags with seed inside were autoclaved at 20 psi for 30 minutes twice with an interval of 24 hours with an idea to render them free from the bacterial endospres, if any. The seeds were then inoculated with *A. rabiei* by inserting three to four 6 mm agar plugs cut from a 15 days old culture of *A. rabiei* using sterile cork borer. Under aseptic

condition in laminar flow chamber, 50 mg of streptomycin was also added to each bag in order to inhibit bacterial contamination. Upon cooling the bags were inoculated with *A. rabiei* culture grown in 90 mm Petri dishes. After plugging the mouth of bags with cotton, these were incubated at 20 ± 2°C for 10 days for further development of pycnidial culture of *A. rabiei*.

Inoculation of International chickpea blight nursery: At the time of artificial inoculation of test lines of the nursery plants pycnidial inoculum on chickpea seeds from 5-7 bags were thoroughly meshed in 20 liter of sterile water to prepare spore suspension of *A. rabiei* (200,000 spores/ml). The spore suspension was screened through a muslin cloth to remove seed debris and was sprayed on the test lines. The inoculation spray was applied every day in the evening till the development of blight symptoms on the susceptible chickpea lines. The development of blight was further aided by daily spray of water. The data on disease severity of test lines were recorded, when check lines were completely blighted and majority of their plants were dead, by using 1-9 grades disease rating scale described by Nene (1984) where 1 (Asymptomatic immune), 2-3 (Resistant), 4-5 (Moderately Resistant), 6-7 (Susceptible) and 8-9 (Highly susceptible) stand for various degrees of response.

Results and Discussion

The screening of two hundred and seventy-seven advance lines of chickpea germplasms received from various research institutes revealed 02, 38, 39, 49 and 149 lines to be highly resistant, resistant, moderately resistant, susceptible and highly susceptible (Table 3). Out of 126 lines received from PRI, Faisalabad, none of the lines responded immune or resistant while 12 lines such as 06025, 06026, 06027, 06031, 06035, 06040, 06041, 06056, Vinhar, Bitter-98, Pb-2000, and Paidar-91 responded to be moderately resistant. The remaining lines responded susceptible to highly susceptible (Table 2), out of 83 lines received from NIAB, Faisalabad, 7 lines such as 06223, 06224, 06270, 06271, 06272, 06277 and 06278 displayed resistant response while another set of 7 lines *viz.*, 06214, 06217, 06218, 06220, 06225, 06237, and 06279 exhibited moderately resistant response. Among the remaining lines of NIAB, 16 and 53 lines responded to be susceptible and highly susceptible respectively. Out of 36 advanced lines of NARC, Islamabad 13 lines i.e., CMC-70T, CMC-55D, NCS-0510, CMC-595, NCS-0516, NCS-0602, NCS-0612, NCS-0613, NCS-0614, NCS-0616, NCS-0621, NCS-0623, and NCS-0626 responded to be resistant while 14 lines such as NCS-0512, NCS-0513, NCS-0518, NCS-0603, NCS-0610,CMC-186, NCS-0617, NCS-0619, NCS-0620, NCS-0625, NCS-0527, NCS-0529, NCS-0532 and Pb-2000 responded to be moderately resistant. Among the remaining lines of NARC, Islamabad, 07 and 02 were susceptible and highly susceptible (Table 2). Out of 32 advanced lines received from ICRISAT, India 2 lines such as EC-516792 and ICCV-98815, were found to be asymptomatic i.e., highly resistant. Eighteen lines such as EC-516709, EC-516729, EC-516771, EC-516793, EC-516878, EC-516895, EC-516934, EC-516957, EC-516967, EC-516974, EC-517003, EC-517011, EC-517039, EC-517073, ICC-6304, ICC-6945, ICCV-04537 and ICCV-98818 exhibited resistant response. The 6 moderately resistant lines were EC-516796, EC-516916, EC-517025, EC-517030, ICC-4033, and ICCV-04537. None of these 32 lines was susceptible but 6 advanced lines displayed highly susceptible response. The germplasm of NARC, Islamabad and that of ICRISAT, India consisted of greater number of resistant lines as compared to that of NIAB, Faisalabad and PRI, Faisalabad.

Table 2. Response of cultivars/ lines from different sources after artificial inoculation with *A. rabiei*.

Disease rating	Response	Varieties/test lines
1	Highly resistant (Asymptomatic)	EC-516792, ICCV-98815
2-3	Resistant	06223, 06224, 06270, 06271, 06272, 06277, 06278, CMC-70T, CMC-55D, NCS-0510, CMC-59S, NCS-0516, NCS-0602, NCS-0612, NCS-0613, NCS-0614, NCS-0616, NCS-0621, NCS-0623, NCS-0626, EC-516709, EC-516729, EC-516771, EC-516793, EC-516878, EC-516895, EC-516934, EC-516957, EC-516967, EC-516974, EC-517003, EC-517011, EC-517039, EC-517073, ICC-6304, ICC-6945, ICCV-04537, ICCV-98818
4-5	Moderately Resistant or Moderately Susceptible	06025, 06026, 06027, 06031, 06035, 06040, 06041, 06056, Vinhar, Bitter-98, Pb-2000, Paidar-91, 06214, 06217, 06218, 06220, 06225, 06237, 06279, NCS-0518, NCS-0513, NCS-0512, NCS-0610, NCS-0603,CMC-186M, NCS-0617, NCS-0619, NCS-0620, NCS-0625, NCS-0527, NCS-0529, NCS-0531 and Pb-2000, EC-516796, EC-516916, EC-517025, EC-517030, ICC-4033, and ICCV-04537
6-7	Susceptible	Bittle-98, Balkasar, Pb-91, C-44, 05141, 06005, 06006, 06020, 06022, 06023, 06042, 06053, 06054, 06055, 06057, 06058, 06059, 06060, 02009, 02023, 02044, 02075, 02093, 90261, 98004, 98154, 06203, 06205, 06219, 06221, 06233, 06239, 06252, 06275, 06278, 06260, 06264, 06265, 06266, 06268, 062174, 06282, NCS-0515, NCS-950259, NCS-0611, NCS-0615, NCS-0618, NCS-0534, NCS-0533
8-9	Highly Susceptible	05142 to 05182, 06001 to 06004, 06007 to 06019, 06021,06024, 06028, 06029, 06030, 06032, 06036, 06037, 06038, 06043 to 06052, 02004, 02006, 02052, 02060, 02080, 93127, CM-98, Pb-1 (Local Check), 06201, 06202, 06204, 06206 to 06213, 06215, 06216, 06222, 06226 to 06232, 06234, 06236, 06238, 06240 to 06251, 06253 to 06256, 06259, 06261 to 06263, 06267, 06269, 06273, 06275, 06276, 06280, 06281, Pb-1 (Local Check), NCS-0622, CM-2000, Pb-1 (local Check), ICC-12968, ICC-14344, ICC-15996, ICC-4991, ICC-4991 (Check), Pb-1 (local Check)

Table 3. Category wise numbers of cultivars/ lines from different sources after artificial inoculation with *A. rabiei*.

	Pluses Research Institute, (PRI)	Nuclear Institute of Agriculture and Biology, Faisalabad (NIAB)	National Agricultural Research Center, Islamabad (NARC)	International Crop Research Institute for the Semi Area Tropics, (ICRISAT), India
1	-	-	-	02
2-3	-	7	13	18
4-5	12	7	14	06
6-7	26	16	07	-
8-9	88	53	02	06

The international chickpea blight nursery of ICRISAT, India and the blight nursery of NARC, Islamabad, includes mostly promising and resistant lines. The NARC, Islamabad, after local screening, supply these resistant lines to various research organizations within Pakistan while ICRISAT after local screening, supply their germplasm to various research organizations in different countries for their further evaluation against various races, patho-types, virulences etc., existing in the countries supplied with the germplasm. This is the reason why most of the chickpea lines of the nurseries of ICRISAT, India and NARC, Islamabad were found to be resistant to *Ascochyta* blight in Pakistan. These sources of resistance identified from chickpea blight nursery, can be exploited in breeding programs for the development of disease resistant commercial cultivars, if these are found to possess other desirable agronomic characters and passing through the proper channel of approval and obtaining the status of an approved variety. These can be released directly as commercial cultivars. The results presented by Nasir *et al*., (2000) are similar to present studies when they screened 14 chickpea cultivars, 29 imported chickpea lines and 38 local breeding lines to 4 Australian isolates of *A. rabiei* and found that all of the Australian chickpea cultivars tested were susceptible to *A. rabiei*, however, 7 imported lines and three local breeding lines were found to be resistant to *A. rabiei*. Work on the wild relatives (Shah *et al*., 2005) of *Cicer* genus were also carried out for the search of resistance source in different part of the world gave promising results. Ilyas *et al*., (2007) screened 173 germplasm lines/varieties of chickpea received from various research organizations against chickpea blight disease by artificially inoculating the germplasm under a plastic tunnel. Three lines viz., 03039, 03041 and 03053 of PRI, Faisalabad exhibited highly resistant response while 5 lines from NIAB, Faisalabad 03115, 03131, 03133, 03143 and 03159 were found to be highly resistant to *A. rabiei* infection. Three lines from Arid Zone Research Institute, Bukhar viz., 93A-086, 93A-111 and 93A-3354 exhibited highly resistant response. Screening of 356 chickpea germplasm accessions of different origins revealed that none of the genotypes was found highly resistant. However, 7 genotypes (FLIP94-90C, FLIP95-68C, FLIP95-47C, FLIP97-132C, FLIP97- 227C, FLIP98-224C and FLIP98-231C) were resistant and 75 were moderately resistant (Iqbal *et al*., 2002). Intensive resistance screening work is being regularly carried out at International Center for Agricultural Research in the Dry Areas, (ICARDA) Syria. Screening of germplasm accessions for resistance to 6 races of *A. rabiei* identified in Syria has resulted in identification of many lines possessing resistance to one or more races but not to all races (Singh & Reddy, 1990). Kabuli type germplasm accessions ILC-200, 3856, 5928 had resistance to 5 races. ILC-72, 2001, 2506, 2956, 3279 and Kabuli type breeding line flip 83-48C had resistance to 4 races. The desi type germplasm accessions ICC-3996 was resistant to 3 races. Pal & Singh (1990) had reported that ILC-3864, 3870 and 4421 were resistant to blight. In

general 'Kabuli' type chickpea is more resistant than 'desi' type. Almost all the lines of chickpea showing multiple race resistance belong to Kabuli type. This could be due to the fact that the region (Asia Minor) which is supposed to be original habitat of chickpea almost exclusively cultivated Kabuli type (Singh & Reddy, 1990). In a study with 5 races of *A. rabiei* Singh & Pal (1993) found that out of 81 chickpea genotypes screened, none was resistant to all the races. Two genotypes GG-715 and ICC-76 were resistant to 3 races, while 3 genotypes, H 86-8, H 86-100 and HK 86-120 were resistant to 2 races.

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