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IDENTIFICATION OF BLIGHT RESISTANT GENOTYPES FROM LOCAL AND EXOTIC CHICKPEA GENETIC RESOURCES

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Abstract

Four hundred and forty eight chickpea genotypes obtained from local and exotic sources were screened against blight at two stages during winter 2002-03. None of the genotypes was highly resistant at any stage, whereas 46 genotypes at seedling in the greenhouse and 94 at pod formation stage in the field were resistant. Thirty genotypes were resistant at both the stages and these are suggested to test under multilocational/agronomic trials for further varietal development. Based on relationship among two stages it is suggested that screening could better be done at seedling stage for preliminary selection and then genotypes with high level of resistance at seedling should be reconfirmed at pod formation stage. Common genotypes, which were resistant at seedling and adult plant stage, are suggested to be utilized in breeding programme to build disease resistance pyramids due to complex nature of *Ascochyta* blight. Disease at seedling and adult plant stage exhibited high association although level of infection was higher at seedling stage. It is suggested to screen huge germplasm lines at seedling stage under greenhouse conditions to save time and labour. Genotypes that give higher level of resistance at seedling stage could be screened at adult plant stage under field conditions.

Introduction

Chickpea is an important grain legume crop sown under rainfed conditions in Pakistan, especially Thal where about 80% of the crop is being cultivated (Khan *et al*., 1991). It is rich and cheap source of vegetable protein for human nutrition (Hulse, 1991). Although many biotic and abiotic stresses affect this crop but blight disease caused by *Ascohcyta rabiei* (Pass.) Lab., has been considered as most devastating (Iqbal *et al*., 2003). Disease epidemics in almost all the chickpea growing countries of the world have been reported (Sattar, 1933; Benlock, 1941; Biggs, 1944; Zalpoor, 1963; Kausar, 1965; Radulescu *et al*., 1971; Kaiser, 1973; Malik & Tufail, 1984). Chickpea breeders in Pakistan have concentrated their efforts to develop blight resistant cultivar that gave rise to promising germplasm (Iqbal, 2002).

Although blight can be controlled by the application of seed dressing and foliar fungicides, use of disease free seeds and destruction of plant diseased debris but under certain conditions these approaches are not feasible (Bashir & Ilyas, 1983; Malik *et al*., 1991; Rauf *et al*., 1996). Therefore importance of resistant cultivars is an established fact recognized by the researchers. Identification and use of resistant sources against pests and diseases is an important 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 & Singh, 1984; Nene & 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

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is yet debatable. Since the host plant resistance provides the cheapest and the most practicable control of blight, therefore, present study was conducted under high load of inoculum with assumptions that advanced chickpea breeding material has high level of resistance at this stage.

Materials and Methods

In order to identify the sources of resistance to blight, 448 chickpea germplasm lines obtained from various sources were evaluated during winter season of 2002-03 (Table 1). These lines were tested under greenhouse and 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±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. The inoculum was prepared from 15 days old culture of *A. rabiei* multiplied on chickpea grains according to the procedure developed by Ilyas & 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 under field conditions

Same set of germplasm was screened under field conditions during simultaneous crop seasons of 2002-03. 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. The inoculum was applied daily in the evening till the 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 were analysed for variance and correlation for each source to compare genotypes and disease at two stages within and between germplasm sources using computer software MS Excel for Windows following the methods by Singh & Chaudhry (1985).

Results

Differences among genotypes originated from various sources were observed for disease rating at both stages with varying degrees of magnitudes (Table 1). More variation was exhibited by disease rating at two stages that was strengthened by variance due to genotypes although genotypes obtained from ICARDA, ICRISAT and BARI, Chakwal were at lower level for significance. The material obtained from AZRI, Bhakhar that constitutes about one fourth of total germplasm gave higher level of significance along with NIAB, Faisalabad (contributing 20% of total) and NARC, Islamabad (contributing 31% of total germplasm) where differences were observed ($p< 0.00$) for both the factors i.e., genotypes and disease rating. Table 1 also presents correlation between disease at two stages for all the sources. Both the stages exhibited significantly positive association although magnitude was low in the material obtained from NIAB.

Table 1. Analysis of variance and correlation between two stages of *Ascochyta* blight in local and exotic chickpea germplasm.

Source of germplasm	Number of genotypes	Genotypes	Stages	Error	Correlation between 2 stages
Arid Zone Research Institute (AZRI), Bhakkar	109	21.21 (P<0.00)	5.12 (P<0.00)	1.49	0.55** (df=109-2)
Nuclear Institute for Agriculture & Biology (NIAB), Faisalabad	86	7.72 (P<0.00)	18.67 (P<0.00)	1.71	0.64** (df=86-2)
International Centre for Agricultural Research in Dry Areas ICARDA), Syria	41	2.20 (P<0.02)	32.98 (P<0.00)	1.13	0.32* (df=41-2)
International Centre for Research in Semi-arid Tropics (ICRISAT), India	16	3.17 (P<0.02)	6.13 (P<0.03)	1.06	0.52** (df=16-2)
Gram Research Station (GRS) Karak	46	6.67 (P<0.00)	1.84 (P<0.04)	0.39	0.89** (df=46-2)
Barani Agricultural Research Institute (BARI), Chakwal	13	3.04 (P<0.02)	13.88 (P<0.00)	0.88	0.56** (df=13-2))
National Agricultural Research Centre (NARC), Islamabad	137	8.83 (P<0.00)	57.03 (P<0.00)	0.79	0.84** (df=137-2)

* and ** are significant at 5 and 1 percent level of probability, respectively.

Indifferences in the results relating correlation might be attributed through radiation effect in the material originated from NIAB or evolution of new genes for disease resistance at two different stages.

Figure 1 presents the frequency for germplasm in each disease rating for all the sources at both stages, seedling and pod formation. It is quite evident that disease resistant lines were aggregated at seedling stages in all the sources except in the germplasm obtained from NARC and ICARDA where number of resistant lines was higher at pod formation stage. In the material developed by NARC, it could be due to conducive environment and continuous breeding for disease resistant cultivars, whereas ICARDA material consisted kabli chickpea that is more tolerant to blight as a group. On the other extreme, susceptible lines were more in all the sources at pod formation stage except in the material obtained from BARI, Chakwal and NARC where the number of susceptible lines was reduced. Differences in disease rating at two stages indicated the presence of different genes for resistance at two stages.

The germplasm screening during the season revealed that none of the genotype was highly resistant at any stage, whereas 46 genotypes at seedling in the greenhouse and 94 at pod formation stage in the field were resistant (Table 2). Thirty genotypes were resistant at both the stages. Out of these, one (92A048) was from AZRI, nine (NB 02169, NB 02173, NB 02175, NB 02178, NB 02179, NB 02180, NB 02181, NB 02183, NB 02184) were from NIAB, seven (ILC-7374, FLIP97-132C, FLIP98-176C, FLIP98-226C, FLIP99-54C, FLIP00-50C, FLIP00-55C) were from ICARDA, one (KR-4) from Karak and twelve (FLIP98-198C, FLIP98-80C, FLIP97-195C, X98TH10, SEL96TH11507, NCS-9905, NC9903, NC9904, Dasht, Parbat, Balkasar, NIFA-88) from NARC. The genotypes listed in Table 2 were resistant at seedling and adult plant stage and are suggested to test under multilocational/agronomic trials for varietal development.

High relationship among two stages in the material obtained from all the sources indicated that screening could be conducted at any of these stages, but to minimize labour and resources, screening could better be done at seedling stage because one third of resistant genotypes were consistent at both the stages. Screening of germplasm for blight resistance at seedling stage under greenhouse conditions is easier as compared to field conditions where it is very difficult and costly to maintain moisture level that is conducive for disease development. The genotypes with considerable level of resistance at seedling could be reconfirmed at pod formation stage for further utilization in breeding programme. In the present study it was observed that four resistant checks were at par with other 26 germplasm lines that indicated the efforts made by the breeders working on chickpea for developing resistant sources during past two decade.

Discussion

About 7% of germplasm was resistant at both the stages against chickpea blight that had been considered an important disease throughout the world in chickpea growing countries (Iqbal, 2002). The increased number of resistant genotypes at seedling stage (46 genotypes) and adult stage (94 genotypes) indicated the efforts made by chickpea breeders in the country for developing resistant cultivars as most of the material included in this experiment was advanced lines provided by the researchers. Although none of the genotypes was highly resistant at any of these stages that still blaze a scope for higher level of sustainable resistance (Iqbal, 2002). Screening techniques along with conducive environmental conditions at NARC for screening chickpea germplasm against blight can

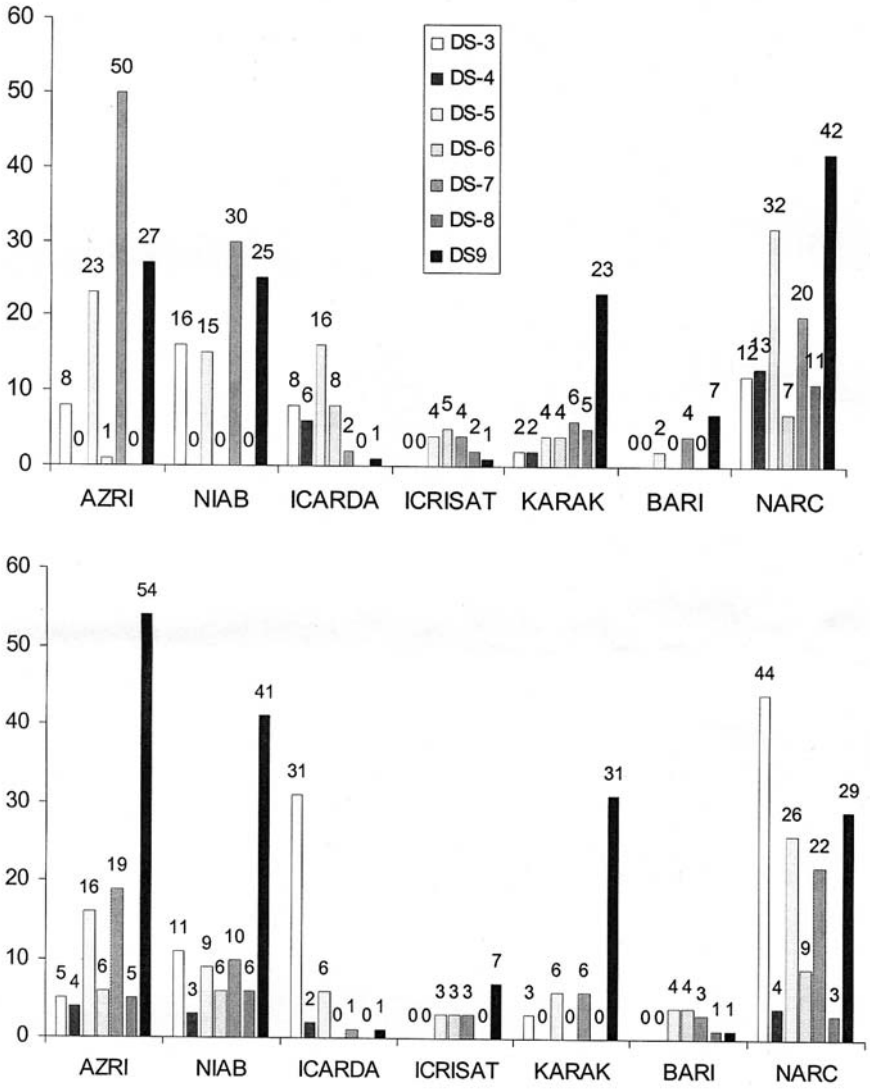

Fig. 1. Frequency of genotypes obtained from local and exotic sources for disease ratings at seedling (upper) and pod formation stage (lower).

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 have been utilized in breeding programmes somewhere (Singh *et al*., 1984; Reddy & Singh, 1990; Crino *et al.,* 1985; Bashir & Haware, 1986; Ilyas *et al*., 1991, Hussain *et al*., 2002). Similarly in the present study, 26 lines were observed resistant although some of the lines previously reported as resistant did not prove their resistance at NARC under high level of inoculum.

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

Source	Stages	Number	Genotype
AZRI	Seedlings	8	92A048, NCS095, NCS98K4E, NCS98K4, 9LA256, 21104, 93A046, 96A3249
	Pod formation	5	92A048, 93A082, 96A4504, NCS98AK17, 93A122
NIAB	Seedlings	16	NB 02169, NB 02173, NB 02175, NB 02178, NB 02179, NB 02180, NB 02181, NB 02183, NB 02184, NB 02127, NB 02172, NB 02177, NB 02171, NB 02176, NB 02118, NB 02129
	Pod formation	11	NB 02169, NB 02173, NB 02174, NB 02175, NB 02178, NB 02179, NB 02180, NB 02181, NB 02183, NB 02184, NB 02186
ICARDA	Seedlings	8	ILC-182, ILC-7374, FLIP97-132C, FLIP98-176C, FLIP98-226C, FLIP99-54C, FLIP00-50C, FLIP00-55C
	Pod formation	31	ILC-7374, FLIP97-132C, FLIP98-176C, FLIP98-226C, FLIP99-54C, FLIP00-50C, FLIP00-55C, PCH-15, FLIP98-229C, FLIP97-110C, FLIP97-116C, FLIP97-121C, FLIP97-131C, FLIP97-174C, FLIP97-185C, FLIP97-195C, FLIP97-229C, FLIP98-22C, FLIP98-38C, FLIP98-56C, FLIP98-230C, FLIP98-231C, FLIP98-19C, FLIP99-33C, FLIP97-85C, FLIP97-219C, FLIP98-37C, FLIP98-53C, FLIP98-128C, FLIP98-130C, FLIP98-107C
ICRISAT	Seedlings	0	
	Pod formation	0	
KARAK	Seedlings	2	KR-4, KR-17
	Pod formation	3	KR-4. KR-2. KR-8
BARI	Seedlings	0	
	Pod formation	0	
NARC	Seedlings	12	FLIP98-198C, FLIP98-80C, FLIP97-195C, X98TH10, SEL96TH11507, NCS-9905, NC9903, NC9904, Dasht, Parbat, Balkasar, NIFA-88
	Pod formation	44	FLIP97-135C, FLIP98-222C, FLIP97-217C, FLIP97-121C, FLIP97-168C, FLIP97-111C, FLIP98-174C, FLIP98-198C, FLIP98-80C, FLIP98-20C, FLIP97-195C, FLIP98-181C, FLIP97-179C, X98TH91, SEL96TH11488, CMNK-287-3-K, X98TH37, X98TH10, SEL96TH11507, FLIP82-150C, NCS-9903, NCS-9911, NCS-9905, NCS-9904, 92280, 96051, BC 6-5, DC-1, (C44xE100YM)xNIFA88, NC950220, NC950219, NCS950235, NC9903, NC9914, NC9904, NC9911, NC950258, NC950264, NC950235, NCS98K4, Dasht, Parbat, Balkasar, NIFA-88

Indifferences in the results relating correlation might be attributed through mutations or evolution of new genes for disease resistance at two different stages. Genetic mechanism of this complex disease is yet to be explored for enhancing improvement in yield potential and disease resistance level. Number of resistant genotypes at pod formation stage was just double to that of at seedling stage that indicated the importance of screening methodology during early stage of the crop. Thirty genotypes including 4 varieties were observed resistant at both the stages, hence these could be exploited for yield potential. The 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 & Singh, 1993). Anyhow this situation is yet to be resolved by conducting more experiments on mode of inheritance and infection of *Ascochyta* blight.

Although we used aggressive inoculum in screening experiments but for more surety, the resistant lines identified in the present study need to be retested for confirmation. Similarly ICARDA has identified resistant sources to *Ascochyta* blight (Reddy & Singh, 1984; Singh *et al*., 1984). Some of ICARDA lines i.e., ILC-72 and ILC-3279 have resistance in several countries, but none of these are resistant in India and Pakistan. Therefore resistant genotypes originating from ICARDA along with presently identified are needed to be retested using more aggressive pathotypes. It is established that the fungus *A. rabiei* is highly variable and the pathotypes present in Pakistan and India are more aggressive than those prevalent in the Mediterranean region (Singh *et al*., 1984).

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