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DETECTION OF RESISTANT SOURCES FOR COLLAR ROT DISEASE IN CHICKPEA GERMPLASM

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Abstract

Ninety-eight chickpea germplasm accessions received from International Centre for Agricultural Research in the Dry Areas (ICARDA), Syria and NARC, Pakistan were evaluated under greenhouse conditions where temperature, ranged from 8-24°C and humidity was maintained above 80% by sprinkling fresh water at National Agricultural Research Centre, Islamabad, during the year 2006 to identify sources of genetic resistance against collar rot disease incited by the fungus *Sclerotium rolfsii*. The fungus was isolated from diseased chickpea plants present in experimental fields, purified and maintained on PDA at 4°C for further screening process. Mass culture of the pathogen was prepared on wheat grains and used according to the procedure described by Sugha *et al*., (1991). Out of 98 germplasm accessions only 5 genotypes viz., FLIP 97-132C, FLIP 97-85C, FLIP 98-53C, ILC -5263 and NCS 9905 exhibited highly resistant response to disease while 9 genotypes viz., FLIP 96-153C, FLIP 97-129C, FLIP 97-172C, FLIP 97-185C, FLIP 98-227C, FLIP 98-107C, FLIP 98-230C, ILC-182 and NCS 9903 displayed resistant reaction. Twenty five genotypes displayed moderately resistant to tolerant response while the remaining were susceptible to highly susceptible to this disease. These resistant sources can further be exploited in breeding program for the development of disease resistant commercial cultivars.

Introduction

Chickpea (*Cicer arietinum* L.) is an important source of protein enriched human food and animal feed, particularly for the low-income population of Southeast Asia (Suzuki & Konno, 1982). Being a sub-tropical and drought resistant crop, it grows most successfully in cooler and dry climates. Amid different factors causative towards its low production, natural constraints, chiefly diseases are the most significant. Due to these ailments, there is an extremely low yield in Pakistan as compared to potential yield of commercial chickpea cultivars (Ilyas *et al.*, 2007). Collar rot caused by *Sclerotium rolfsii* Sacc., is one of the several fungal diseases affecting this crop and is reported almost all over the world whereever chickpea is grown (Nene *et al*., 1984).

Collar rot is a fast spreading and destructive disease of chickpea. It causes significant losses in yield where ever the crop is grown under environmental conditions favorable for its development. This soil-borne pathogen causes rot of collar region on a wide range of plant species belonging to families Compositae and Leguminosae whereas members of Graminae are less susceptible to this disease (Mahen *et al*., 1995). The most common hosts are legumes, crucifers and cucurbits. Seedling mortality from 54.7 to 95.0% in chickpea due to infection of *S. rolfsii* has been reported by Mathur & Sinha (1968, 1970) and Kotastthane *et al*., (1976). As the genetic resistance is regarded, the only practicable and cost-effective control for such a devastating soil-borne pathogen is selection of cultivars. Therefore, the present study was conducted to screen the chickpea germplasm against *S. rolfsii* for the identification of resistant sources in available germplasm accessions.

Table 1. Frequency distribution of accessions in various disease reaction groups.

Disease rating	Disease reaction	No. of accessions	% of total
1	Resistant (HR)	5	5.1
3	resistant (R)	9	9.2
5	Tolerant (T)	25	25.5
7	Moderately Susceptible (MS)	32	32.6
9	Highly susceptible (HS)	27	27.5

Materials and Methods

The pathogen, *Sclerotium rolfsii*, was recovered from the sections of infected chickpea plants found in experimental fields of National Agricultural Research Centre (NARC), Islamabad and was cultured on potato dextrose agar medium (PDA). The fungus developed was purified and maintained on PDA at 4ºC for further screening process. Mass culture of the pathogen was prepared on wheat grains according to the procedure described by Sugha *et al*., (1991).

Sick bed of the disease was prepared by mixing the wheat grains impregnated with *S. rolfsii* in the soil prior to sowing of chickpea seeds (Sugha *et al*., 1991) in iron trays of 24x15x4 inches size. Ninety eight chickpea germplasm accessions were obtained from International Centre for Agricultural Research in the Dry Areas (ICARDA), Syria and NARC, Pakistan. Prior to sowing, seeds of each accession were surface sterilized with 0.1% clorox solution, before sowing.

Five seeds of each accession were sown in the sick bed in the greenhouse keeping 2 inches row to row and 1.5 inches plant to plant distance. Data on seedling mortality were recorded 15 days after sowing. The percentage of mortality for each germplasm line was calculated and the level of resistance/susceptibility was grouped according to disease rating scale of Iqbal *et al*., (2005) where: 0 = No mortality, 1= less than 1% mortality, 3 = 1-10% mortality, 5 = 11-20% mortality, 7 = 21-50% mortality and 9 = 51% or more mortality.

Results and Discussion

In the present study, 98 chickpea germplasm accessions were screened against collar rot pathogen under green-house conditions. Only 5 genotypes, FLIP 97-132C, FLIP 97-85C, FLIP 98-53C, ILC-5263 and NCS 9905 were found highly resistant to this disease, whereas, 9 genotypes FLIP 96-153C, FLIP 97-129C, FLIP 97-172C, FLIP 97-185C, FLIP 98-227C, FLIP 98-107C, FLIP 98-230C, ILC 182 and NCS 9903 were identified as resistant (Table 1). Twenty five genotypes were found moderately resistant/tolerant while 32 were moderately susceptible and 27 were highly susceptible to the disease.

The frequency of highly resistant lines is generally very low as only 5 genotypes were highly resistant and 9 were resistant. This shows a high level of aggressiveness of the pathogen or relatively narrow diversification of genetic material under study. Hussain *et al*., (2005), screened 57 cultivars and found only one genotype highly resistant. Sugha *et al*., (1991) evaluated 210 chickpea lines/cultivars from different sources. None of these were resistant or even moderately resistant.

Table 2. Level of resistance/susceptibility of chickpea germplasm accessions against collar rot.

Disease reaction	Germplasm lines
Highly resistant	FLIP 97-132C, FLIP97-85C, FLIP98-53C, ILC- 5263, NCS 9905
Resistant	FLIP96-153C, FLIP 97-129C, FLIP97-172, FLIP97-185C, FLIP 98-227C,FLIP 98-107C, ILC-182, NCS9903, FLIP 98-230C
Tolerant	FLIP00-69C, FLIP85-29, FLIP97111C, FLIP97174C, FLIP97179C, FLIP97-219C, FLIP97-254C, FLIP98-128C, FLIP98-133C, FLIP98-176C, FLIP98-226C, FLIP98-22C, FLIP98-231C, FLIP98-33C, FLIP98-37C, FLIP98-44C, FLIP98-56C, FLIP99-26C, FLIP99-45C, ICCV97117, ICCV97126, ILC1929, NCS2001, NCS950210, NCS9904.
Susceptible	FLIP00-46C, FLIP00-55C, FLIP0063C, FLIP00-66, FLIP00-67, FLIP00-71C, FLIP00-72C, FLIP90-131C, FLIP92-113C, FLIP92-28C, FLIP96-154C, FLIP97-116C, FLIP97-120C, FLIP97-121C, FLIP97-131C, FLIP97-195C, FLIP97-220C, FLIP97-221C, FLIP97-267C, FLIP97-280C, FLIP98-15C, FLIP98-23C FLIP98-91C, FLIP99-46C, FLIP99-73C, ILC7374, ILC7795, NCS9906, NCS9911, Parbat, PB-91XICC13508, PCH15.
Highly susceptible	FLIP00-50C, FLIP00-65C, FLIP00-70C, FLIP00-73C, FLIP97-110C, FLIP97-217C, FLIP97-229C, FLIP97-258C, FLIP97-261C, FLIP98-130C, FLIP98-174C, FLIP98-19C, FLIP98-229C, FLIP98-38C, FLIP98-54C, FLIP99-34C, FLIP99-47C, FLIP99-48C, ICCV97119, ICCV97121, NCS950204, NCS950209, NCS950219, NCS950235, NCS950259, NCS9914, NCS9917.

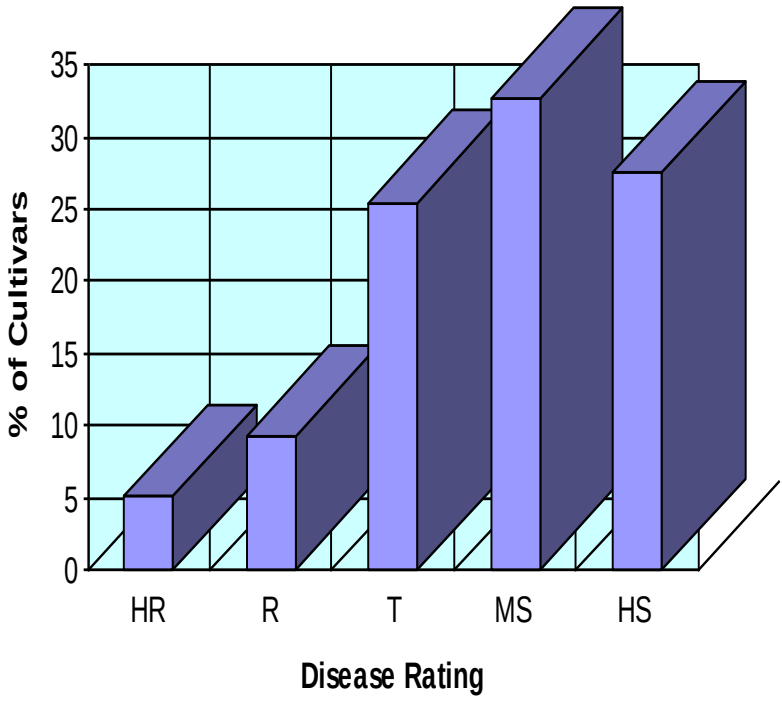

Fig. 1. Disease reaction of ninety-eight chickpea germplasm against collar rot caused by *Sclerotium rolfsii*.

The results suggest that method of disease screening described here could be used as a rapid method to screen resistant cultivars against *Sclerotium rolfsii.* Although, this method gave some grouping, but it also has some disadvantage of favouring the growth of contaminant species that develop on PDA. These resistant sources can further be exploited in breeding program for the development of disease resistant commercial cultivar by determining their genetics.

In legumes, few methods have been developed for screening against soil borne diseases. Examples were the green-house (Nwakpa & Ikotum, 1988) and field screening (Feryand Dukes, 1986) for resistance of legume cultivars against *Sclerotium rolfsii.* In the green-house method, plant must be wounded before inoculation with sclerotia. For other crops and pathogens, other screening methods have been developed (Jimenez & Lockwood, 1980; McBlain, 1991; Olah & Schmitthenner, 1984), including the mycelium-inoculum-layer (Walker & Schmitthenner, 1984), hypocotyls-injection method (Hass & Buzzell, 1976) used for screening of soybean (*Glycin max* (L.) Merr. Cultivars against *Phytophthora soja*. These methods because of damage to the plant stem, are said not to be appropriate for thin stem plants such as soybean (Pazdernik *et al*., 1997) and cowpea. A recent screening method was developed called agar plug-inoculation method, which could be used on thin stemmed seedling (Pazdernik *et al*., 1997).

The approach developed in the current study is quick, as it could be completed within 12 days in the green house. This is the first report showing a rapid green house method to screen chickpea germplasm against collar rot. This is very important, since, it is cheaper, requires no special skills, avoid classical strategies that follow long term screening trials both in the green house and field as used in previous trials on cowpea resistance to *S. rolfsii* (Feryand Dukes, 1986; Nwakpa & Ikotum, 1988). Moreover, it was observed during the present study that collar rot at seedling stage causes a high level of infection, therefore, a large number of germplasm lines can be screened at seedling stage under green-house conditions saving much time and labour.

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