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INFLUENCE OF INOCULUM LEVELS OF *RHIZOCTONIA SOLANI* AND SUSCEPTIBILITY ON NEW POTATO GERMPLASM

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Abstract

The influence of inoculum levels (10, 15 and 20 of *R. solani* AG-3) of the isolate CL-58 to Rhizoctonia disease was evaluated by using two potato cultivars (Cardinal and Desiree) and three advanced breeding lines (SH-216-A, TPS-9813 and CIP-393594-61). Evaluation of eyes germination, death of emerging sprouts, incidence and severity of stem and stolon canker, black scurf and yield (six disease parameters) revealed that as the inoculum level of *R. solani* AG-3 increased in the soil, there was a significant (P=0.05) increase in all disease parameters measured. Moreover, 20 g inoculum was ascertained as the most effective dose for green house screening.

The susceptibility in 14 potato cultivars and advanced breeding lines was investigated by using isolate CL-58 of *Rhizoctonia solani* anastomosis group 3 (AG-3) under greenhouse conditions. Evaluation 6 disease parameters of each cultivar and advanced breeding ranked Desiree and SH-216-A as susceptible and Cardinal and CIP-393574-61 as resistant (P=0.05).

Introduction

Black scurf caused by *Rhizoctonia solani* Kühn (teleomorph = *Thanatephorus cucumeris* (Frank) Donk) was found prevalent in all potato production agro ecological zones of Pakistan (Rauf, 2002). *R. solani* can affect potato plants from planting to harvest. It colonizes belowground potato plant surfaces in response to root and shoot exudates (Jeger *et al*., 1996; Keijer *et al*., 1996). Among 14 anastomosis (AGs 1-13, AG-BI) groups reported, AG 3 has been found the major cause of *Rhizoctonia* disease of potato in field surveys (Abe & Tsuboki, 1978; Bandy *et al.*, 1984; Chand Logan, 1983; Ceresini *et al.*, 2002). The growth of potato plants is delayed in all phases (Hide *et al.*, 1985; Banville, 1989). The fungus proliferates on emerging eyes, sprouts, roots and stolons to form an extensive network of mycelium. It causes inhibition in eyes germination, killing of underground sprouts, stem canker and stolon canker resulting in poor and uneven stand of weak plants and subsequent yield reduction (Banville, 1989). Infected plants generally produce smaller (< 3 cm diameter) (Banville, 1989) and malformed progeny tubers (Carling *et al*., 1989) and comparatively small number of stem (Scholte, 1989).

Both soil-borne and tuber-borne inocula are important in disease development. Sowing of disease free seed in soil infested with *R. solani* AG-3 can not work out. Severity of black scurf is determined by the inoculum present in the soil (Scholte, 1992). The soil-borne nature of the fungus makes management of the disease challenging and expensive to control by single control measure / tactic. The most economical way of managing this disease is by using resistant varieties. Resistance to fungal diseases such as *Rhizoctonia* canker, have traditionally been scarcely considered in any potato breeding

program. Reliable sources of resistance to *Rhizoctonia* have not been reported, however, differences in disease expression of cultivated varieties and breeding lines have been reported which has encouraged some screening for varietal differences in susceptibility (Frank *et al*., 1976; Kyritsis & Wale, 2002).

Information on black scurf of potato in Pakistan is scarce (Ahmed *et al*., 1995a, & 1995b) because it was not considered an important disease. Rauf (2002) reported that *R. solani* anastomosis group 3 is the primary cause of black scurf in Pakistan, like in most parts of the world (Banville *et al.,* 1996). This disease was found prevalent in all the eight potato production agro ecological zones of Pakistan and was most widespread and prevalent in zone 2, a major potato production area comprising of Sahiwal, Pakpatan, Okara, Sialkot, Jhang and Faisalabad districts of the Punjab province (Rauf, 2002). Approximately 70-80 % of the potatoes produced in Pakistan are grown in this zone. This zone is also an important source of seed potatoes distribution for almost all the potato production areas of Pakistan. Imported and basic seed is also multiplied in this zone (Zanoni, 1991). *R. solani* is carried on infested seed tubers from this zone to all potato production zones of Pakistan thus adding more to existing inoculum load.

The objective of the present study was to ascertain the influence of inoculum levels and to identify an inoculum level to screen new potato germplasm. This inoculum level was subsequently employed to scrutinize potato germplasm for their level of susceptibility towards black scurf.

Materials and Methods

Eleven advanced breeding lines (TPS-9801, TPS-9813, TPS-9802-3, TPS-9805, TPS-13, TPS-9803, CIP-393574-61, CIP-394028-37, SH-53, SH-103, SH-216-A,) and three commercial cultivars (Cardinal, Diamant and Desiree) of potato were obtained from Potato Program, National Agricultural Research Centre (NARC), Islamabad, Pakistan and Punjab Seed Corporation (PSC), Sahiwal. An aggressive isolate of *Rhizoctonia solani* anastomosis group 3 (AG-3, isolate CL-58) was selected while studying the virulence of 18 isolates on 10 potato cvs. / lines from Pakistan (Rauf, 2002).

Two experiments were conducted, to find the optimal inoculum level for screening and the actual screening of potato germplasm against optimal inoculum level of *R. solani* AG 3, CL-58.

Influence of soil-borne inoculum levels of *Rhizoctonia solani* on black scurf: Effect of three levels of inoculum 10, 15 and 20 g per pot were evaluated in 2 potato cultivars (Cardinal, and Desiree) and 3 lines (TPS-9813, CIP-393574-61 and SH-216-A). Each cultivar and advanced breeding line was subjected to following four treatments:

- Sterilized potting mixture (SPM) + sowing of sprouted potato tubers (Control).
- SPM + 10 g inoculum + sowing of sprouted tubers.
- SPM + 15 g inoculum + sowing of sprouted tubers.
- SPM + 20 g inoculum + sowing of sprouted tubers.

Data analysis: Analysis of effect of inoculum levels on eyes germination inhibition (EGI), sprouts killing (SK), stem canker (SKI), stolon canker (black scurf on progeny tuber) and yield reduction were performed by using ANOVA and mean comparison were

done by using the least significant difference (LSD, *P*=0.05) with 2 factor factorial (4 levels of inoculum x 5 potato cultivars/ advanced breeding lines). There were three replicates for each treatment including control arranged in a completely randomized design. For control, tubers were planted in non-inoculated potting mixture. The experiments were conducted in the greenhouse and laboratory of the Department of Plant Pathology UAAR, Pakistan in October, 2003 and repeated in March, 2004 in greenhouse of the Crop disease research Centre (CDRC) Murree.

Screening of potato germplasm against soil-borne inoculum of *R. solani* AG-3 isolate CL-58: Eleven advanced breeding lines (TPS-9801, TPS-9813, TPS-9802-3, TPS-9805, TPS-13, TPS-9803, CIP-393574-61, CIP-394028-37, SH-53, SH-103, SH-216-A) and 3 commercial cultivars (Cardinal, Diamant and Desiree) were screened against *R. solani* AG-3 by using the following two treatments.

- SPM + sowing of sprouted potato tuber (Control).
- SPM +20 g mass inoculum + sowing of sprouted tubers.

Data analysis: Each treatment was replicated 3 times. Treatments were arranged in a completely randomized design. Data regarding 6 disease parameters were subjected to ANOVA and mean comparison were done by using the least significant difference (LSD, *p*=0.05) in MSTATC computer program.

Maintenance and multiplication of inoculum: *Rhizoctonia solani* AG-3 isolate CL-58 was multiplied on potato dextrose agar (PDA) medium (potato starch: 20 g, dextrose: 20 g, agar: 20 g per liter of distilled H_2O. PDA was sterilized in a gas operated autoclave at 121^oC temperature or 15 pounds pressure per square inch (PSI) for 20 min.

Mass inoculum of isolate CL-58 was grown in 250 ml flasks containing 100 g of barley and wheat grains, 2:1 plus 120 ml double distilled / deionized H_2O and autoclaved for 1 hr at 121^oC temperature or 15 PSI for three consecutive days (Balali *et al.*, 1995). Each flask was inoculated with five plugs of 5-mm diameter of the fungus taken from the margins of a 1-wk-old culture of isolate CL-58, grown on PDA medium. Flasks were incubated at 25^oC for 18 days.

Preparation of the pots: The potting mixture (clay, sand, and farmyard manure, 1:1:1) was sterilized with 37% commercial formalin. One part of formalin was diluted with nine parts of water. The potting mixture was placed over a cemented path in layers. The mixture was then moistened with formalin solution and covered with a polyethylene sheet for 48 hours. The mixture was exposed to air for 4-5 days until the formalin vaporized or volatilized.

Clay pots (8"x11") filled with sterilized potting mixture, were inoculated with weighed inoculum of isolate CL-58, mixed to the depth of 5-cm, four days prior to sowing of one whole sprouted tuber (35-50 mm diameter.) with 3-4 eyes / pot. Each tuber was placed in a hole, to the depth of 4-5 cm and covered with potting mixture and watered weekly as required. Pots were kept in greenhouse at 25^oC under natural light.

Parameters studied: The following parameters were assessed on each plant; inhibition in eyes germination (EGI), sprouts killed (SK), stem canker index (SCI), stolon canker

index (STCI), black scurf disease index (BSDI) and reduction in yield (compared to non inoculated control).

Two sets of experiment were made. Data regarding percent eyes germination and sprouts killed was recorded 30 days after sowing, by harvesting first set of experiment whereas the data regarding rest of the parameters was observed after 90 days of sowing by harvesting second set of experiment. The experiment was repeated once.

The total number of germinated eyes in each replication of a treatment was recorded at the time of sowing. Percent eye germination and sprouts killed were calculated as follows:

$$\text{Eyes germination EG (\%)} = \frac{\text{Number of eyes germinated in each treatment}}{\text{Total number of eyes}} \text{ X } 100$$

$$\text{EGI (\%)} = 100 - \text{EG \%}$$

$$\text{Sprouts killed (\%)} = \frac{\text{Number of sprouts killed in each treatment}}{\text{Total number of sprouts}} \text{ X } 100$$

Stem canker incidence and severity was expressed collectively as stem canker index (SCI). Severity was assessed on 0-5 visual disease rating scale as described by Frank *et al*., (1976) using the following formula:

$$\text{SCI (\%)} = \frac{\text{Number of stems in each rating} \times \text{rating}}{\text{Total number of stems}} \text{ X } 100$$

where, 0 = no lesion; 1 = single lesion less than 25 mm; 2 = single lesion 25-50 mm (or composite of small lesions totaling less than 50 mm); 3 = single lesion >50 mm (or composite of small lesion totaling > 50 mm but not girdling the stem; 4 = lesion(s) less than 25 mm which is girdling the stem; and 5 = lesion(s) more than 25 mm which is girdling the stem.

Stolon canker index (STCI) was assessed with the same procedure as described under stem canker index.

Black scurf disease incidence and severity was expressed as BSDI and calculated using the following formula:

$$\text{BSDI (\%)} = \frac{0(n_1) + 1(n_2) + 2(n_3) + 3(n_4) + 4(n_5) + 5(n_6)}{\text{N (Total number of tubers)}} \text{ X } \frac{100}{5}$$

where, n_1 = number of tubers in 0 rating; n2 = number of tubers in 1 rating; n_3 =number of tubers in 2 rating; n_4 = number of tubers in 3 rating; n_5 = number of tubers in 4 rating; n_6 = number of tubers in 5 rating.

Black scurf severity was assessed on a visual disease rating scale 0-5 based on percent tuber surface showing disease symptom as described by Ahmad *et al*., (1995).

where 0 = no symptoms on potato tubers; 1 = less than 1 % tuber area affected; 2 = 1-10 % tuber area affected; 3 =11-20 % tuber area affected; 4 =21-51 % tuber area affected; 5 = 51 % or more tuber area affected.

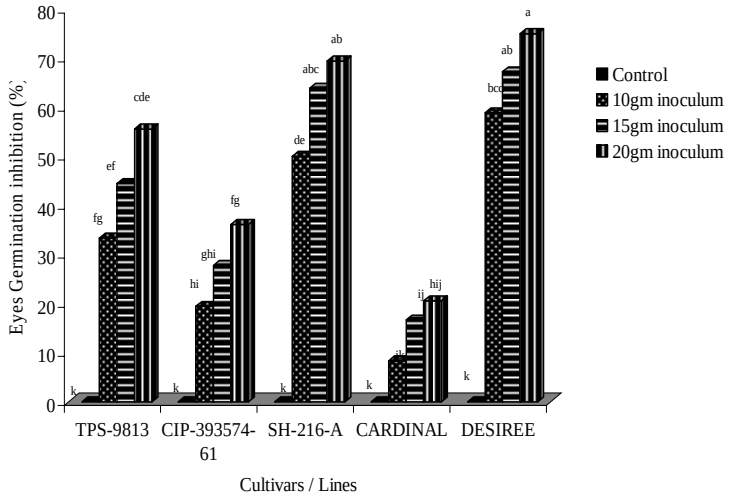

Fig. 1. Effect of graded soil-borne inoculum of *Rhizoctonia solani* AG 3 isolate CL-58 on eyes germination inhibition.
*Means followed by same letters are not significantly different according to LSD (p=0.05) LSD= 11.24

Total yield reduction in each pot was recorded as:

$$\text{Yield reduction (\%)} = \frac{\text{100-weight of potato tubers in each pot}}{\text{Mean weight of potato tubers in control}} \text{ X } 100$$

Results and Discussion

Influence of soil-borne inoculum levels of *Rhizoctonia solani* on black scurf: The percentage of eye germination in all potato cultivars and advanced breeding lines decreased significantly as the concentration of inoculum increased from 5-20 g (Fig. 1). The greatest reduction in eye germination was observed at 20 g inoculum level in all cultivars and advanced breeding lines tested. Inhibition of eye germination in Desiree and SH-216-A were not different from each other (p=0.05) but were significantly lower than other cultivars and lines evaluated.

There was statistically significant increase in the percentage of dead sprouts in all cultivars and advanced breeding lines as the amount of inoculum increased (Fig. 2). At 10 g inoculum level sprouts killing percentage was similar in Desiree and SH-216-A and not different than TPS-1813 at 20 g inoculum level.

The maximum stem canker index was observed in case of Desiree and SH-216-A at 20 g inoculum (Fig. 3). The highest stem canker index in all potato cultivars and advanced breeding lines tested was observed at the highest inoculum level (20 g).

Stolon canker index significantly increased with increase in soil-borne inoculum levels except in cv. Cardinal where no significant difference was observed in stolon canker index when compared with control at 10 and 15 g inoculum levels (Fig. 4). The highest stolon canker index was observed at 20 g inoculum level in all cvs. / line. There was a significant increase in the development of black scurf on progeny tubers with an increase in inoculum level (Fig. 5). Induction of sclerotia on Cardinal and line CIP-393574-61 were statistically similar.

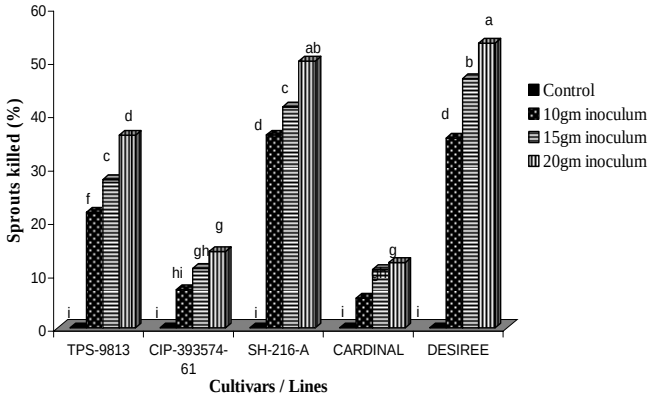

Fig. 2. Effect of graded soil-borne inoculum of *Rhizoctonia solane* AG 3 isolate CL-58 on sprouts killing.
*Means followed by same letters are not significantly different according to LSD (p=0.05) LSD= 5.04

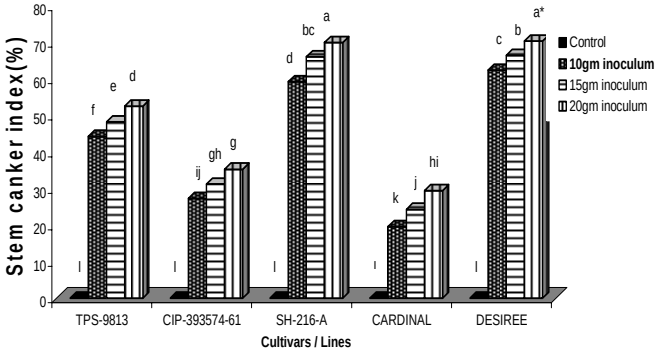

Fig. 3. Effect of graded soil-borne inoculum of *Rhizoctonia solani* AG 3 isolate CL-58 on stem canker index.
*Means followed by same letters are not significantly different according to LSD (p=0.05) LSD= 3.30

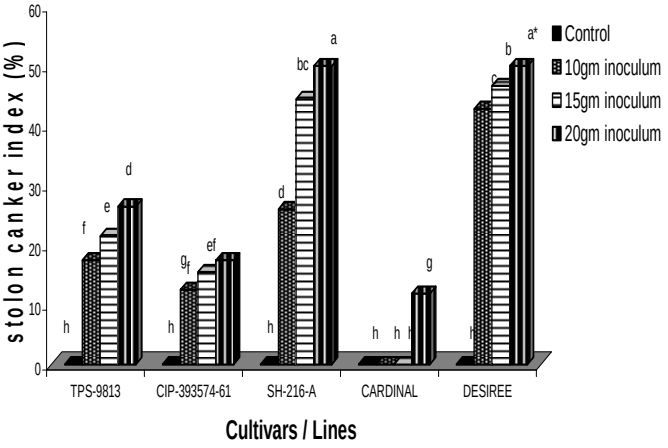

Fig. 4. Effect of graded soil-borne inoculum of *Rhizoctonia solani* AG 3 isolate CL-58 on stolon canker index.
*Means followed by same letters are not significantly different according to LSD test (p=0.05) LSD= 2.81

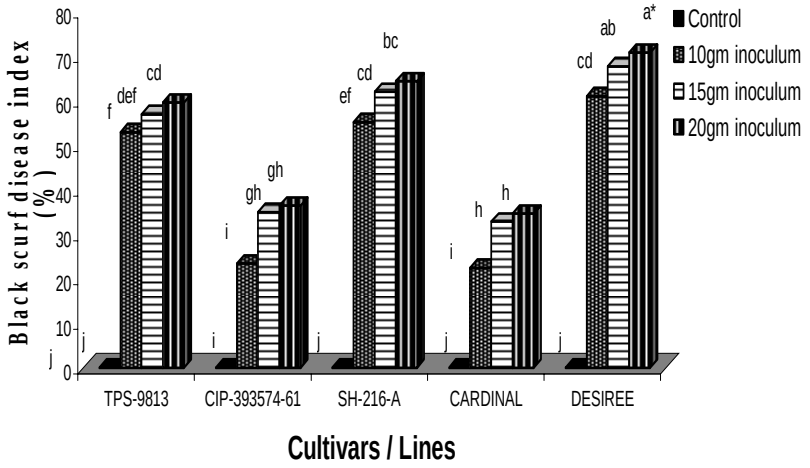

Fig. 5. Effect of graded soil-borne inoculum of *Rhizoctonia solani* AG 3 isolate CL-58 on black scurf disease index.
*Means followed by same letters are not significantly different according to LSD (p=0.05)

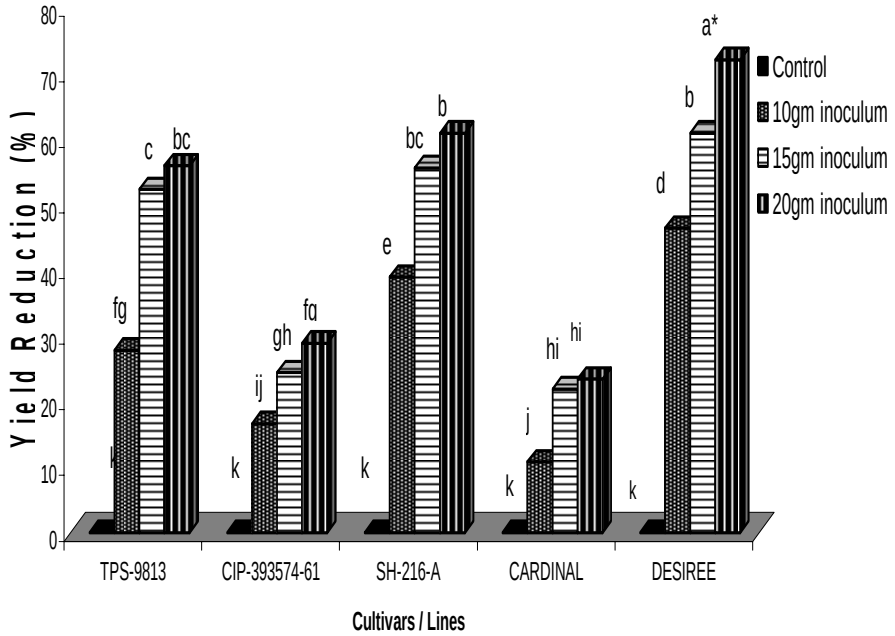

Fig. 6. Effect of graded soil-borne inoculum of *Rhizoctonia solani* AG 3 isolate CL-58 on yield reduction.
*Means followed by same letters are not significantly different according to LSD (p=0.05) LSD= 5.86

Statistically significant increase in yield was observed in all cultivars / lines with increase in the density of soil borne inoculum (Fig. 6). Desiree had significantly greatest loss in total yield at 20 g inoculum level followed by YR at 15 g inoculum level. The maximum YR in all potato genotypes tested was, however, observed at 20 g inoculum level.

Table 1. Screening of potato germplasm against *Rhizoctonia solani* AG 3 isolate CL-58 against six disease producing symptoms.

Cultivars / lines	Disease Parameters (%)					
	Eyes germination inhibition	Sprouts killed	Stem canker index	Stolon canker index	BSDI*	Yield reduction
TPS-9801	66.67 $_{abc}$**	44.84 $_{b}$**	64.40 $_{b}$**	38.47 $_{b}$**	64.40 $_{b}$**	68.03 $_{a}$**
TPS-9813	57.41 $_{cde}$	36.10 $_{de}$	52.60 $_{de}$	26.50 $_{f}$	59.60 $_{bc}$	55.91 $_{cd}$
TPS-9802-3	50.00 $_{ef}$	33.33 $_{e}$	53.70 $_{de}$	25.40 $_{f}$	62.40 $_{bc}$	51.33 $_{de}$
TPS-9805	63.89 $_{bcd}$	33.33 $_{e}$	51.30 $_{ef}$	26.90 $_{ef}$	61.00 $_{bc}$	58.34 $_{bc}$
TPS-13	60.19 $_{bcde}$	38.77 $_{cd}$	55.10 $_{cd}$	29.70 $_{de}$	53.60 $_{d}$	52.22 $_{de}$
TPS-9803	64.82 $_{abcd}$	27.77 $_{f}$	48.50 $_{fg}$	26.50 $_{f}$	59.60 $_{bc}$	41.41 $_{f}$
CIP-393574-61	39.82 $_{fg}$	14.28 $_{h}$	35.33 $_{h}$	19.50 $_{g}$	36.40 $_{e}$	28.84 $_{g}$
CIP-394028-37	63.71 $_{bcd}$	28.75 $_{f}$	50.20 $_{ef}$	25.40 $_{f}$	58.40 $_{cd}$	47.81 $_{e}$
SH-53	65.56 $_{abc}$	43.3 $_{b}$	62.00 $_{b}$	33.31 $_{c}$	61.00 $_{bc}$	57.23 $_{bc}$
SH-103	54.44 $_{de}$	21.66 $_{g}$	45.60 $_{g}$	25.40 $_{f}$	53.60 $_{d}$	39.45 $_{f}$
SH-216-A	70.37 $_{ab}$	50.00 $_{a}$	70.00 $_{a}$	50.00 $_{a}$	64.40 $_{b}$	60.90 $_{b}$
Cardinal	29.63 $_{g}$	12.22 $_{h}$	29.40 $_{i}$	11.88 $_{h}$	39.60 $_{e}$	31.38 $_{g}$
Diamant	64.63 $_{abcd}$	41.66 $_{bc}$	57.50 $_{c}$	30.33 $_{d}$	62.00 $_{bc}$	54.43 $_{cd}$
Desiree	75.00 $_{a}$	53.33 $_{a}$	70.42 $_{a}$	50.00 $_{a}$	70.80 $_{a}$	72.09 $_{a}$
LSD (5%)	**10.67**	**4.08**	**3.71**	**2.96**	**5.96**	**4.89**

*Black scurf, *Black scurf disease index.

**Means within the column followed by the same letters are not significantly different according to LSD test (*p=0.05*).

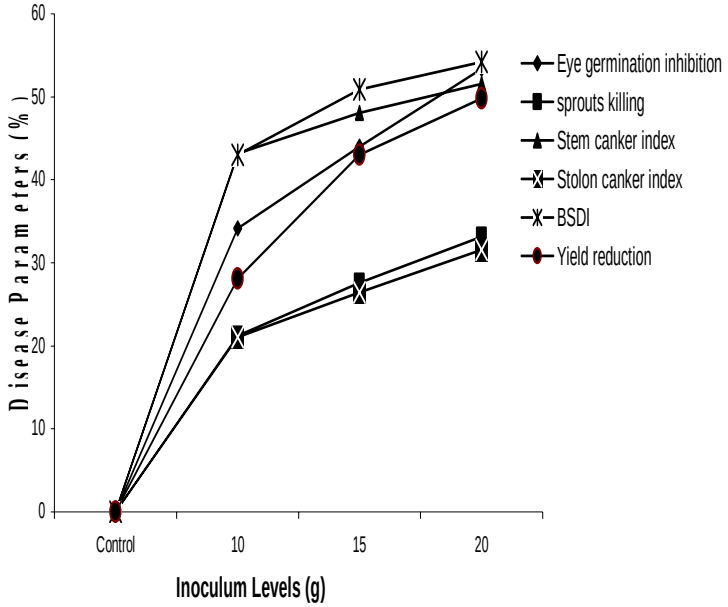

Fig. 7. Effect of graded soil-borne inoculum of *Rhizoctonia solani* AG 3 isolate CL-58 on six disease parameters.

As the inoculum level increased in the soil there was a significant increase in inhibition in eyes germination, sprouts killing, incidence and severity of stem canker, stolon canker, black scurf and decrease in total yield reduction among cultivars and advanced breeding lines tested. The maximum disease was observed at the highest inoculum level i.e., 20 g. Standard curve between soil-borne inoculum levels and disease parameters development was established (Fig. 7). Results were similar to the findings of

Kyritsis & Wale (2002) with respect to black scurf and stem canker. In this study, four additional parameters were also evaluated, which have not been investigated before (possibly true for eye germination, but not for the number of dead sprouts and incidence and severity of stem and stolon canker). Simons & Gilligan (1997) and James & McKenzie (1972) suggested that density of seed-borne inoculum had a much smaller effect on the development of black scurf on the tubers and cankers on stems and stolons. The amount of black scurf produced on tubers, stem and stolons were found higher in some plots, sown with the lower density of tuber-borne inoculum. This was almost certainly due to the contributing effect of varying quantities of soil-borne inoculum (Gilligan *et al.,* 1996). When the soil samples were collected from these plots, the density of *R. solani* surviving in the soil was relatively high i.e., *R. solani* was present in 336 out of 480 soil samples collected using a pellet soil-sampler method (Simons & Gilligan, 1997). Therefore, the soil-borne inoculum contributes more to the black scurf than tuber-borne inoculum. The importance of soil-borne inoculum as a cause of black scurf is also acknowledged by Van Emden (1965). Weinhold *et al.,* (1982) also demonstrated that inhibition in eyes germination, killing of underground sprouts and severity of stem canker is directly linked to reduction in yield of progeny tubers. The highest disease in all genotypes was observed at 20 g inoculum level which was ascertained as the optimal inoculum level for infection and screening new potato germplasm.

Screenings of potato germplasm against soil-borne inoculum of *Rhizoctonia solani* AG 3 isolate CL-58: For management of black scurf disease of potato, based on host plant resistance, 14 cultivars and advanced breeding lines were screened against soil-borne inoculum of *R. solani* AG-3 isolate CL-58. Results revealed that soil-borne inoculum of *R. solani* isolate CL-58 significantly inhibited eyes germination, increased the killing of emerging sprouts, stem canker index, stolon canker index, black scurf on progeny tubers and reduced yield in all potatoes germplasm tested, as compared to control (non inoculated soil). There was a significant difference between inoculated and non-inoculated soil (Table 1). Eyes germination inhibition varied among the cvs. / lines tested. The highest inhibition of eye germination was exhibited by Desiree (-75%), which was significantly greater than Cardinal (29.6%) and CIP-393574-61 (39.8%).

A significantly highest percentage of sprout death was observed in Desiree (53.33%) and line SH-216-A (50 %) when compared with the other treatments (Table 1). Cardinal had the lowest percentage of sprout killing with 12.2 % and line CIP-393574-61 with 14.28 % sprouts killing were statistically ranked as the least affected, as statistically no difference was observed in number of sprouts killed between these two followed by SH-103 in which 21.66% sprouts were killed.

The most severe stem canker was observed in Desiree with 70.4% and line SH-216-A, 70% stem canker index, whereas Cardinal exhibited significantly less stem canker (29.40 %) followed by line CIP-393574 – 61, when compared with the rest of cultivars and lines (Table 1).

The highest incidence and severity of stolon canker was observed in Desiree and SH-216-A (50 %) followed by the line TPS-9801 with 38.47 % stolon canker index (Table 1), whereas, the lowest severity of stolon canker index was observed in cv. Cardinal (11.88%) followed by the line CIP-393574–61 with 19.50% stolon canker index. Significant amount of black scurf developed on progeny tubers of all the cvs. / lines, in response to soil-borne inoculum of *R. solani* isolate CL-58 as compared to control (Table 1). The highest severity of black scurf disease was exhibited by Desiree (70.8 %) followed by line SH-216-A and

line TPS-9801 (64.4%), whereas, Cardinal and CIP-393574-61 exhibited statistically the lowest BSDI i.e., 39.60% and 36.40% The highest yield reduction was observed in Desiree (72.1%) and the lowest yield reduction was observed in line CIP-393574-61 (28.8%) and Cardinal (31.4%). Potato cultivars and lines, differed significantly ($P<0.05$) in their susceptibility, to the soil-borne inoculum of *R. solani*, but none of the cultivars and lines exhibited complete resistance to the soil-borne inoculum of *R. solani* as compared to control. Statistically, significant differences ($P< 0.05$) were observed in percent eyes germination, percent sprouts killed, severity of stem canker, stolon canker and black scurf diseases and yield (Table 1). Cardinal and line CIP-393574-61 exhibited, resistant behavior excluding stolon and stem canker index, where significant differences were observed between these two. Whereas, Desiree and line SH-216-A were statistically the most susceptible cultivars, except two parameters viz. black scurf disease index and yield reduction, in which significant difference was found between these two varieties. Desiree and line SH-216-A were among the most susceptible and Cardinal and line CIP-393574-61 among the least affected (resistant) potato germplasm. Other cultivars and lines exhibited intermediate response to *Rhizoctonia* disease. Our finding were consistent with the results of Kyritsis & Wale (2002) who asserted that cultivars tested differed significantly in their susceptibility towards soil-borne inoculum of *R. solani* but none of the cultivar tested showed complete resistance to the disease. However, their data was based on only two parameters i.e., stem canker and black scurf disease index.

Cultivar Desiree was approved in 1969, since then it is being extensively grown in all the potato production zones of Pakistan, especially in the Punjab province. It is not only susceptible to black scurf; it is also highly susceptible to common scab (*Streptomyces scabies*), *Verticillium* wilt (*Verticillium albo-atrum*) and early frost (Zanoni, 1991). Therefore, cultivation of Desiree may be discouraged to avoid build up of *R. solani* inoculum in these areas. Cardinal and line CIP-393574-61 were found to exhibit a high level of partial resistance to *Rhizoctonia* disease when compared to other breeding lines and cultivars. Cardinal was released in 1975 and since then it is being cultivated in all potato growing areas of Pakistan. It is a high yielding red skinned variety which is also resistant to powdery scab, *Verticillium* wilt, *Fusarium* dry rot and black scurf (Zanoni, 1991). Therefore, it could be grown as a resistant cultivar in areas where it is a problem whereas CIP-393574-61 may be considered as resistant line while breeding for resistance.

Acknowledgment

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