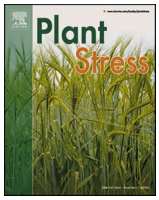
Plant Stress 12 (2024) 100476

Contents lists available at ScienceDirect

Plant Stress

journal homepage: www.sciencedirect.com/journal/plant-stress



Exploration of resistance level against Black Scurf caused by *Rhizoctonia solani* in different cultivars of potato

Muhammad Rafiq [a,*], Amna Shoaib [b], Arshad Javaid [b], Shagufta Perveen [b], Muhammd Umer [c], Muhammad Arif [d], Chunsong Cheng [a,*]

[a] Jiangxi Key Laboratory for Sustainable Utilization of Chinese Materia Medica Resources, Lushan Botanical Garden, Chinese Academy of Science, Jiujiang City, Jiangxi Province, 332900, PR China
[b] Department of Plant Pathology, Faculty of Agricultural Sciences, University of the Punjab, Quaid-i-Azam Campus, Lahore 54590, Pakistan
[c] Forestry College, Research Centre of Forest Ecology, Guizhou University, Guiyang 550025, China
[d] Department of Plant Protection, Faculty of Agriculture, Sakarya University of Applied Sciences, Arifiye Sakarya 54580, Turkey

ARTICLE INFO

Keywords:
Rhizoctonia solani
Potato
Screening
Potato cultivars
Black scurf

ABSTRACT

Black Scurf is one of the destructive fungal diseases of potato crops caused by fungus *Rhizoctonia solani*. Cultural practices and fungicide applications are insufficient in effectively combating the pathogen, while utilizing resistant cultivars has become one of the most economical and effective way to control disease. About ten commercial potato varieties were screened out against the disease by artificially inoculating the plants with *R. solani* and these potato varieties were grouped based on disease incidence and disease severity. Four varieties (Ronoldo, Rubi, Challenge, and Sadaf) were grouped as resistant, three varieties (SH-5, Sante and Astrex) as moderately susceptible and three varieties (Karoda, Mosica and Simply red) as susceptible with significantly highest disease incidence ($P \leq 0.05$). Disease also reduced number and size of tubers, while the disease incidence showed negative ($P < 0.001$) correlation with chlorophyll and protein contents, and activities of catalase (CAT), peroxidase (POX) and polyphenol oxidase (PPO) enzymes. It was concluded that a combination of phenotypic and physiological indices could be used to identify black scurf resistance in potatoes.

Introduction

Potato (*Solanum tuberosum* L.) is the world leading non grain crop, being grown in 150 countries after wheat, rice and maize (Muleta and Aga, 2019). It is one of the most important staple food crop worldwide (Larkin and Griffin, 2021). It is a globally indispensable crop with considerable economic, nutritional, and food security value (Devaux et al., 2021). Among the potato growers, China and India are the largest producers (Anstalt 2021). *Rhizoctonia solani* reported in 1858 by Julius Kuhn (Minier, 2019) is the emerging threat to potato crop in Pakistan and in most of the potato-producing areas of the world (Siddique *et al.*, 2020), forming black, irregular lumpy encrustations on the surface of potato tubers reducing the quality and market value from 30 to 50 % (Kulkarni and Chavhan, 2017). It is a seed- and soil-borne pathogen, which survives through sclerotia and mycelia in infected seeds or soil in tropical environments. In soil, infected plant debris is the major carrier that may arise from potato or weed hosts (Das and Pattanayak, 2022). The pathogen is not only a problem for the potatoes (Solanaceae) but causing diseases in other plant families including Poaceae, Amaranthaceae, Fabaceae, Brassicaceae, Rubiaceae, Araceae, Malvaceae, Moraceae and Linaceae (Mayo *et al.*, 2015; Verwaaijen *et al.*, 2017; Ajayi-Oyetunde and Bradley, 2018). The pathogen attack on above ground results in upward leaf rolling, shoot stunting, purple pigmentation in the upper leaves while it destroys the root system in below-ground plant parts (Malik et al., 2014: Tsror, 2010). All these symptoms may appear on infected potato plants either separately or in combination (Zheng *et al.*, 2014; Muzhinji *et al.*, 2018).

The sclerotia are resistant to available fungicides and can survive in harsh environmental conditions making it the tough pathogen to manage (Rahul *et al.*, 2016). The chemical fungicides are not preferred in sustainable agriculture due to their negative impact on health of terrestrial, aquatic and other soil organisms (Kumar *et al.*, 2018). The crop rotation due to limited potato growing area is not recommended as potato is playing key role in food security of Pakistan (Majeed and Muhammad, 2018). The use of resistant germplasms is the most appropriate approach to control the black scurf disease of potato

* Corresponding authors.
E-mail addresses: rafiq@lsbg.cn (M. Rafiq), chengcs@lsbg.cn (C. Cheng).

https://doi.org/10.1016/j.stress.2024.100476
Received 10 August 2023; Received in revised form 11 March 2024; Accepted 6 May 2024
Available online 7 May 2024

(Rahman and Rauf, 2022). The response of each cultivar is different towards the *R. solani* but no potato cultivar has been listed as resistant to the pathogen (Jeger *et al.*, 1996: Zrenner *et al.*, 2021). Therefore, the current study was conducted to explore the resistance source in potato against the black scurf disease.

Materials and methods

Isolation and identification of Rhizoctonia solani

R. solani was isolated from the infected potato tubers collected from the local market, Lahore, Pakistan. The samples were cut into small pieces, surface-sterilized with 1 % solution of sodium hypochlorite, and placed on 2 % malt extract agar (MEA) medium. After one week of incubation at 25 °C, mono-hyphal cultures were performed and re-inoculated on fresh MEA plates to get the pure culture of the fungus.. The fungus mycelia was observed macroscopically (WY830S, compound microscope attached with camera) for morphological identification including colony morphology (color and size) and microscopically (moniloid cells, right-angle branching, constriction as well as formation of septa near the point of hyphal branching) by a compound microscope with a digital camera using a lacto phenol cotton blue stained slide mounted with a small portion of the mycelial mass. For ITS-based identification, the DNA was extracted from 7-day-old culture (Abuhena *et al.* 2022), amplification of the DNA was carried out in PCR mixture. The Taq DNA polymerase was used in 20 µl PCR reaction mixtures. PCR conditions included 35 cycles with denaturation, annealing, and extension steps. (Al-Fadhal *et al.* 2019). For confirmation of the pathogen, the obtained sequence was subjected to BLAST-N (http://www.ncbi.nlm.nih.gov/BLAST/). The homology of the pathogen was checked, and it was confirmed that the pathogen is *Rhizoctonia solani* from the database. The pathogen was multiplied on pearl millet seeds for later use in the screening experiment.

Procurement of potato varieties

Ten commercial potato varieties (Mosica, Karoda, Simply red, Challenge, Sante, Ronoldo, Astrex, SH-5, Rubi and Sadaf) were collected from Punjab Seed Corporation, Lahore, Pakistan. The Punjab seed corporation provide the seeds to from their cold store to the researchers that's why dormancy of the selected potato varieties was broken by keeping them under the shade for one week to allow them to start their normal physionlocal activities and tubers of uniform size were selected for sowing to provide homogeneous environment to all varieties being screened out.

Pot experiment

Soil was sterilized with 2 % formalin solution to remove any sort of pathogen available in soil as we wanted to make sure that the soil must not contain any pathogen before, filled (7 kg pot^{-1}) in pots (30 cm × 25 cm length and width) and fungal inoculum (20 g) was added in each pot while only boiled pearl millet seeds (20 g) were added in pots of negative control treatment (Khan *et al.*, 2016). The seed tubers were sown in pots, irrigated and kept for a week for establishment of the fungal inoculum. The experiment was designed according to Completely Randomized method with five replicates of each treatment.

Disease assessment

After 90 days of sowing, as shown in the Table 1, the disease incidence and severity were recorded on potato tubers according to the rating scale described by Malik et al. (2014).

Total chlorophyll and carotenoid contents

The ethanolic leaf extract (0.1 g) was used to estimate the total chlorophyll content of leaves by taking the absorbance in spectrophotometer (Epoch 2 BioTek) for estimation of chlorophyll a (645 nm), chlorophyll *b* (663 nm) and carotenoid (470 nm) in the extract (Lichtenthaler and Buschmann, 2001).

Table 1
Disease severity scale for the assessment of Black Scurf of potato.

Disease severity grades	Percentage of disease
0	No disease symptoms
1	≤ 1 % tuber surface affected
2	1 −10 % tuber surface affected
3	11 −20 % tuber surface affected
4	21 - 50 % tuber surface affected
5	> 50 % tuber surface affected

Total protein content and antioxidant enzymes

About 0.5 g of plant material was crushed 10 mL of phosphate buffer (0.1 M, pH 7.5), centrifuged at 3000 rpm, separating the supernatant by following the method of Lowry (Pomory, 2008). The same extract was used to access the activities of other antioxidants viz., catalase (CAT), peroxidase (POX) and polyphenoloxidase (PPO). For the CAT activity, the reaction mixture containing [0.1 mL enzyme extract + 2.9 mL of buffer having 20 mM H_2O_2 solution in 0.05 M sodium phosphate buffer (pH 7.0)] was checked for the reduction in absorbance at 240 nm (Weydert and Cullen, 2010). For POX assay, the reaction mixture containing 2 mL phosphate buffer (0.1 M; pH 6.8) and 1 mL of pyrogallol, filled with 1 mL of 0.5 M H_2O_2, terminating the reaction by adding 2.5 N H_2SO_4. Finally, the absorbance of the reaction mixture was taken at 420 nm against a blank (Sinsabaugh, 2010). The PPO activity was assayed by measueing absorbance of the reaction mixture [enzyme extract: 0.5 mL, sodium phosphate buffer: 2 mL, and catechol 0.2 mL) at 495 nm (Cheema and Sommerhalter, 2015)

Yield assessment

Potato tubers harvested after 90 days to record number and weight of tubers pot^{-1} and tuber weight.

Statistical analysis

MS Excel program was used to calculate the Standard errors of means. Data was subjected to analysis of variance, after that treatment's means were separated by Tukey's HSD software Statistix 8.1.

Results and discussion

Morphology and molecular identification of Rhizoctonia solani

The colony of the right-angled hyphal branching along with the constriction as well as formation of septa at the point of branching was observed (Fig. 1). The amplicon of 618 bp revealed 99 % homology with 20 different strains of *R. solani* deposited in genebank including MK 026954.1; KX 674530.1; KX 674529.1; KF 907725.1 and KF 907721.1.

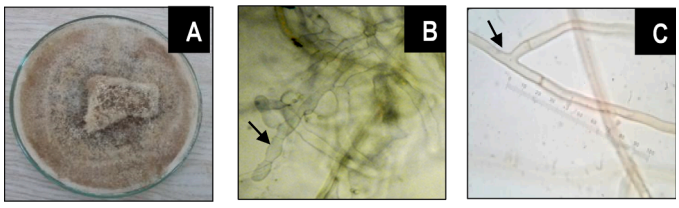


Fig. 1. (A-C): Macroscopic and microscopic characters of *Rhizoctonia solani*. **A:** Culture plate; **B:** Hyphae and moniloid cells (40X); **C:** Right angle branching, a slight constriction at the base of the branch and cross walls (septa) near the branch (40X).

Disease and tuber growth

Ten potato varieties were grouped on the basis of disease incidence and disease severity. Four varieties (Ronoldo, Rubi, Challenge and Sadaf) exhibited significantly lower disease incidence (>15 %) and equated to a severity rating of 2, therefore were grouped as moderately resistant. SH-5, Sante and Astrex were classified under moderately susceptible group presented significantly greater disease incidence of 61, 87 and 71 %, respectively and 40–50 % area of tubers affected due to the infection caused by *R. solani*. Karoda, Mosica and Simply red performed as susceptible exhibited the significantly highest disease incidence (96–98 %) and were equated to a severity rating of 5 on account of affected tubers area (80–100 %) (Table 2).

Disease also affected tuber's number and size in moderately susceptible and susceptible groups. Mean tuber number and weight in moderately susceptible plants were significantly decreased by 22.68–31.25 % ($P < 0.05$) and 28.05–35.50 % ($P < 0.001$), respectively as compared to their respective non-inoculated treatments. Likewise, number and weight of tubers were more significantly decreased by 22.22–33.33 % ($P < 0.05$ or 0.01) and 38.11–44.39 % ($P < 0.0001$) in susceptible group, respectively (Table 3).

Physio-chemical attributes

The physiological and biochemical attributes of three groups of potato varieties are summarized in Table 4 and 5. In non-inoculated healthy potato leaves, the median values of total chlorophyll content, carotenoid, total protein content as well as activities of CAT, POX and PPO were ranged between 6.45–10.39 mg g^{-1}, 4.40–6.60 mg g^{-1}, 0.83–1.85 mg g^{-1}, 4.71–8.02 U g^{-1} FW, 1.46–3.33 U g^{-1} FW and 1.59–2.57 U g^{-1} FW, respectively. Two-sample paired *t*-test revealed that the investigated parameters in inoculated plants were differed significantly ($P < 0.05$ or $P < 0.01$ or $P < 0.001$) among the group in comparison to their respective controls.

In resistant group, the photosynthetic pigment, total protein content and POX activity were insignificantly affected when inoculated with *R. solani*, however CAT and PPO activities increased very significantly ($P < 0.01$) by 23–34 % and 25–34 %, respectively with respect to corresponding healthy plants grown in un-inoculated soil. Infection caused by *R. solani* in moderately susceptible group resulted in significant reduction of 23–31 % ($P < 0.05$ or 0.01), 43–48 % ($P < 0.05$ or 0.001), 26–34 % ($P < 0.01$ or 0.001) and 34–39 % ($P < 0.001$ or 0.0001) in total chlorophyll content, total protein content, CAT and POX activity, respectively as compared with their respective control. Likewise, genotypes in susceptible group showed reaction to pathogen infection by drastically affecting physiological attributes. Accordingly, total chlorophyll content and POX activity decreased significantly by 25–32 % and 34–45 % at $P < 0.01$, respectively, while total protein content and activities of CAT and PPO decreased significantly by 53–58 %, 32–42 % and 30–35 % at $P < 0.001$, or $P < 0.0001$ respectively.

Table 2
Disease incidence (%) and rating scale for assessment of black Scurf disease on different potato varieties (90 DAS).

#	Potato variety	Black scurf incidence (%)	Black scurf severity (%)	Rating	Degree of susceptibility/ Resistance
1	**Rubi**	4.70±1.45**f**	**2**	**2**	**Moderately resistant (R)**
2	**Sadaf**	4.00±1.01**f**	**2**		
3	**Ronoldo**	11.00 ±2.34**ef**	**5**		
4	**Challenge**	14.00±1.53**e**	**3**		
5	**SH-5**	62.00±2.73**c**	**40**	**4**	**Moderately susceptible (MS)**
6	**Sante**	87.00±3.52**b**	**50**		
7	**Astrex**	71.00±4.18**d**	**50**		
8	**Karoda**	98.00±2.34**a**	**100**	**5**	**Susceptible (S)**
9	**Mosica**	97.00±3.85**a**	**80**		
10	**Simply red**	97.00±3.34**a**	**78**		

Letters show significant difference ($P \leq 0.05$) as determined by LSD test. ± shows standard errors of means of five replicates.
0–5 rating scale where 0 = No symptom; 1 = less than 1 %; 2 = 1–10 %; 3 = 11–20 %; 4 = 21–50 %; and 5 = 51 % or more tuber area affected.

Correlation matrix of physiological and biochemical attributes in potato varieties in response to Rhizoctonia solani

The correlation coefficients for overall change in potato plant's disease, physiological and biochemical attributes inoculated with *R. solani* in comparison to non-inoculated plants for all 10 potato genotypes is presented in Table 6. Results revealed that disease incidence was negatively and strongly ($P < 0.001$ or 0.0001) associated with total chlorophyll content, total protein content and with activities of antioxidant enzymes (CAT, POX and PPO). However, rest of physiological and biochemical variables were positively and significantly correlated with each other. Accordingly, a highly significant correlation ($P < 0.001$) of total protein content was recorded with CAT, POX and PPO as well as with total chlorophyll content ($P < 0.01$). Total chlorophyll content of inoculated plants exhibited significant ($P < 0.01$) correlation with POX and PPO, while non-significant correlation with CAT. Moreover, reaction of all ten genotypes against pathogen inoculation resulted in highly significant ($P < 0.001$) correlation between three enzymes (Table 6).

Discussion

Black scruf disease affecting stem and stolons is responsible for 30–50 % yield losses have been reported the oldest and widespread diseases of potatoes around the world as well as in all potato producing agro–ecological zones of Pakistan (Carling *et al.*, 1989: Rauf *et al.*, 2015). The management of the disease through traditional cultural and chemical methods make it difficult due to persistent sclerotia and soil–borne nature of the pathogen (Abdel-Kader *et al.*, 2010).

Screening of ten potato varieties against *R. solani* was carried out in artificially infested pot condition. On the basis of disease severity scale (Ahmed et al., 1995), four varieties (Ronoldo, Rubi, Challenge and Sadaf) were grouped as moderately resistant, three (SH-5, Sante and Astrex) as moderately susceptible and remaining three (Karoda, Mosica and Simply red) as susceptible. Disease also reduced tuber's number and size in moderately susceptible and susceptible groups. Malik et al. (2014) and Rauf *et al.* (2015) screened out the potato cultivars by using same disease rating scale of Ahmed et al. (1995) and grouped different cultivars into resistant, susceptible and moderately susceptible. They studied different yield and growth parameters and on the basis of their finding they also reported that no resistant cultivar is available, and the pathogen more reduced the quality than the yield. Likewise, Mohsan *et al.* (2016) used the same disease rating scale to categorize 18 potato varieties/cultivars against black scurf disease. They reported three varieties/lines (FD 74–21, FD 73–73 and SL 15–10) as resistant, one (FD 61–3) as moderately resistant, six as moderately susceptible and eight as susceptible against black scurf disease. Sante, Simply red, Astrex and Karoda cultivars are widely cultivated in Punjab but now have become susceptible to *R. solani*.

The physiological and biochemical attributes (the photosynthetic pigment, total protein content and POX activity) in moderately resistant group were affected insignificantly. However, CAT and PPO activities increased significantly by 23–34 % that would be the result of activation plant defense mechanism to acquire local or systemic, inducible or constitutive resistance against pathogen (Awan *et al.*, 2018). However, in moderately susceptible and susceptible groups the pathogen caused significant reduction of 23–48 % and 25–58 %, respectively in the stress markers (photosynthetic pigment, total protein content, CAT, POX and PPO), which indicated the inability of plant to identify pathogen and to activate its defense mechanism to combat the pathogen attack on time

Table 3
Effect of *Rhizoctonia solani* on tuber attributes of different potato varieties (90 DAS).

level	Potato variety	No. of tubers (pot^{-1})			Weight of tubers (g pot^{-1})		
		NI	I	% D	NI	I	% D
R	**Rubi**	8.50±0.75	7.40ns ±0.81	12.30	225±4.98	217ns ±7.75	3.40
	Sadaf	8.60±0.87	8.20ns ±1.12	4.40	231±9.42	218ns ±7.37	5.50
	Ronoldo	6.60±1.78	6.40ns ±0.75	3.00	243±9.04	215ns ±12.32	11.4-
	Challenge	8.40±0.68	8.20ns ±0.38	2.60	216±10.01	211ns ±10.69	2.50
MS	**SH-5**	9.60±1.29	6.60*±0.71	31.30	238±9.79	171*** ±9.64	28.00
	Sante	9.40±0.51	6.80*±0.49	27.50	256±9.58	165***±13.21	35.50
	Asrex	9.60±0.68	7.40*±0.75	22.80	238±12.49	175***±7.02	26.50
S	**Karoda**	8.00±0.55	5.40**±0.67	33.30	257±15.67	143***±7.43	44.40
	Mosica	8.80±0.38	6.80**±0.58	22.70	234±10.97	139***±9.85	40.608
	Simply red	7.20±1.53	5.60*±0.25	22.20	254±14.29	157***±7.44	38.00

NI: Non-inoculated; I: Inoculated;% D: Decrease in inoculated treatment with respect to its non-inoculated control. R: resistance; MS: moderately susceptible and S: susceptible.
ns, *, **, *** non-significant or significant at $P \leq 0.05$, 0.01 and 0.001 using independent two sample *t*-test for comparison of inoculated vs. non-inoculated control plants within each genotype.

Table 4
Effect of *Rhizoctonia solani* on physiological attributes of different potato varieties with respect to its non-inoculated control (40 DAS).

Potato variety	Total chlorophyll content (mg g^{-1})			Carotenoids (mg g^{-1})			Total protein content (mg g^{-1})		
	NI	I	% I/D	NI	I	% I/D	NI	I	% I/D
Rubi	6.50	6.00ns	**5.40**	5.00	4.90ns	**2.30**	1.00	1.40ns	**−33.00**
Sadaf	9.80	8.90ns	**9.50**	4.60	4.50ns	**3.20**	1.10	1.40ns	**−25.00**
Ronoldo	7.00	6.60ns	**6.00**	4.10	3.90ns	**4.40**	1.20	1.50ns	**−22.00**
Challenge	6.60	6.00ns	**9.40**	5.30	4.90ns	**9.00**	0.80	1.10ns	**−37.00**
Sante	10.00	7.30**	**26.90**	5.40	4.70ns	**11.60**	1.30	0.70**	**48.00**
Astrex	7.60	5.30*	**30.90**	5.70	4.90ns	**15.00**	1.00	0.60*	**47.70**
SH-5	7.90	6.00*	**22.80**	5.30	4.50ns	**13.70**	1.90	1.00**	**45.00**
Mosica	6.60	4.90**	**25.20**	6.40	5.20ns	**18.90**	0.90	0.40***	**57.70**
Simply red	8.00	5.70**	**27.90**	5.60	4.80ns	**15.60**	0.80	0.40***	**53.00**
Karoda	8.00	5.40**	**31.70**	5.80	4.80ns	**16.90**	0.90	0.40***	**55.00**

Table 5
Effect of *Rhizoctonia solani* on biochemical attributes of different potato varieties with respect to its non-inoculated control (40 DAS).

Potato variety	Catalase activity (U g^{-1} fresh weight)			Peroxidase activity (U g^{-1} fresh weight)			Polyphenol oxidase activity (U g^{-1} fresh weight)		
	NI	I	% I/D	NI	I	% I/D	NI	I	% I/D
Rubi	5.40	6.60*	**−23.20**	3.50	4.20ns	**−21.80**	1.80	2.40*	**−33.20**
Sadaf	4.50	5.70**	**−27.80**	3.20	4.10ns	**−27.60**	2.00	2.60*	**−25.20**
Ronoldo	4.70	6.00**	**−29.90**	3.30	3.90ns	**−16.20**	2.20	2.90*	**−34.00**
Challenge	4.50	6.00**	**−34.10**	2.20	2.60ns	**−18.10**	1.70	2.30*	**−32.80**
Sante	4.10	3.00***	**25.80**	2.90	1.70***	**41.90**	2.30	1.80ns	**−22.20**
Astrex	5.40	4.00***	**25.80**	3.30	1.70***	**49.90**	2.40	2.00ns	**−19.80**
SH-5	6.40	4.20**	**34.00**	2.10	1.10***	**44.60**	3.00	2.30ns	**−10.60**
Mosica	5.40	3.60***	**34.10**	1.50	1.00**	**35.50**	2.60	1.80***	**29.60**
Simply red	6.60	4.00***	**38.20**	1.50	0.90**	**37.70**	2.90	1.90***	**33.30**
Karoda	5.40	3.30***	**39.30**	1.80	1.00**	**44.50**	2.60	1.80**	**35.60**

ns, *, **, *** non-significant or significant at $P \leq 0.05$, 0.01 and 0.001 using independent two sample *t*-test for comparison of inoculated vs. non-inoculated control plants within each genotype.
NI: Non-inoculated; I: Inoculated;% I/D: increase/decrease in inoculated treatment.

Table 6
Correlation matrix (Pearson's two tailed) of disease incidence, physiological and biochemical attributes in potato varieties in response to *Rhizoctonia solani*.

Attributes	DI	POX	PPO	TCC	TPP
CAT	−0.85***	0.85***	0.85***	0.50ns	0.85***
DI		−0.92****	−0.83***	−0.74**	−0.94***
POX			0.81***	0.76**	0.88***
PPO				0.72**	0.96***
TCC					0.74**

DI: disease incidence; TPP: total protein content; CAT: catalase; POX: peroxidase; PPO: polyphenol oxidase; TCC: total chlorophyll content ns, **, *** non-significant or significant at $P \leq 0.01$ and 0.001.

(Awan *et al.*, 2018). The correlation coefficients also revealed that the disease incidence was negatively and strongly associated with stress markers that further confirmed the pathogen establishment exclusively in host cells and at the same time overburdening of plant defense machinery.

Conclusion

Black Scurf, a damaging fungal disease in potatoes caused by *Rhizoctonia solani*, is best managed with resistant cultivars. Among ten commercial potato varieties, four were identified as resistant, three as moderately susceptible, and three as highly susceptible to the disease. The disease also reduced tuber quantity and size, while negatively correlating with chlorophyll, protein content, and the activities of

certain enzymes. Combining phenotypic and physiological indicators is effective in identifying resistant potato varieties. This study suggests that cultivars with resistance should be cultivated in the future for better, sustainable growth for food security, as potato is the staple food of many countries.

CRediT authorship contribution statement

Muhammad Rafiq: Writing – original draft, Investigation. **Amna Shoaib:** Investigation, Supervision. **Arshad Javaid:** Supervision. **Shagufta Perveen:** Methodology. **Muhammd Umer:** Data curation, Software. **Muhammad Arif:** Writing – review & editing. **Chunsong Cheng:** Investigation, Funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Acknowledgment

The authors gratefully acknowledge financial support from Jiangxi Province Double Thousand Talent-Leader of Natural Science Project (JXSQ2023101038), Jiangxi Province Urgently Overseas Talent Project (2022BCJ25027), and The Key Research Projects in Jiangxi Province (20223BBH8007 & 20232BBG70014); This work was also funded by the Science and Technology Innovation Team Project in Key Areas of Jiujiang City Base and Talent Plan (S2022TDJS029), Jiangxi Key Lab Laboratory for Sustainable Utilization of Chinese Materia Medica Resources (2024SSY07083).

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.stress.2024.100476.

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