BMC Plant Biology

RESEARCH Open Access

Response to oxalic acid: an important supplement screening against stem rot resistance in groundnut (*Arachis hypogaea* L.)

H. V. Veerendrakumar[1,2], Bangaru Kiranmayee[1], R. P. Vasanthi[2], A. R. Nirmal Kumar[2], Manish K. Pandey[1*] and Hari Kishan Sudini[1*]

Abstract

Background Stem rot, caused by the soil-borne pathogen *Sclerotium rolfsii*, pose a serious challenge in the groundnut (*Arachis hypogaea* L) cultivation. Although this disease is widespread globally but had most adverse impact in groundnut growing regions of United States, India, and Australia. The pathogen primarily targets the crown region of the plant, resulting in systemic collapse and potentially leading to yield losses up to 80%. Effective genetic control measures are essential to mitigate the impact of this disease on groundnut production. Realizing the time and resource-consuming complex field-based phenotyping, the availability of easy and repeatable phenotyping methods may fasten the process of donor and gene discovery efforts.

Results Multi-season phenotyping was performed for stem rot on 184 minicore germplasm accessions, including checks, under two conditions: sick field screening and response to oxalic acid assay. This study demonstrated medium to high heritability (52–63% broad-sense heritability) and significant environmental influence (36%). The response to the oxalic acid assay showed a high proportion of similarity (approximately 80%) with the percent mortality observed in the sick field indicating an easy way of performing precise phenotyping. Notably, seven genotypes—ICG163, ICG721, ICG10479, ICG875, ICG11457, ICG111, and ICG2857—exhibited stable resistance, with less than 30% mortality against stem rot disease. Among these, ICG163, ICG875, and ICG111 displayed low mortality and consistent stability across multiple seasons in both the sick field and controlled conditions of the oxalic acid assay.

Conclusions The oxalic acid assay developed in this study effectively complements field phenotyping, as a reliable method for assessing stem rot resistance. Seven resistant genotypes identified through this assay can be utilized for the introgression of stem rot resistance into elite genotypes. Given the significant influence of the environment on stem rot resistance, it is essential to implement multi-season phenotyping to obtain precise results. Furthermore, the response to oxalic acid serves as a valuable supplement to traditional field phenotyping, since maintaining uniform disease pressure during field screenings is often challenging.

Keywords Resistant sources, Stem rot resistance, Oxalic acid assay, Precise phenotyping

*Correspondence:
Manish K. Pandey
Manish.Pandey@icrisat.org
Hari Kishan Sudini
Harikishan.Sudini@icrisat.org

[1]International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru 502324, India
[2]Acharya NG Ranga Agricultural University, Guntur, Andhra Pradesh 522034, India

Background

Groundnut (*Arachis hypogaea* L.) is an important oilseed crop and food legume, serving as a crucial source of proteins, vitamins, minerals, calories, and essential fatty acids in the human diet [1]. Globally, groundnut is cultivated over an area of 32.7 million hectares, producing 53.9 million tons with an average productivity of 1,648 kg per hectare (FAOSTAT, 2021). India holds the top position in terms of cultivation area (5.42 million hectares) and ranks second in production (10.1 million tons), with an average productivity of 1,863 kg per hectare (agricoop.nic.in). Groundnut production is adversely influenced by various biotic and abiotic factors [2]. Key biotic constraints include foliar and soil-borne diseases, among which stem rot, caused by the necrotrophic fungus *Sclerotium rolfsii* Sacc. (A.K.A: *Athelia rolfsii*), is particularly destructive. Depending on disease severity, stem rot can result in yield losses ranging from 20 to 80% [3]. This pathogen infects groundnut plants throughout their growth period, primarily targeting the crown region, leading to the wilting and eventual death of the entire plant. Given the aggressive nature of *S. rolfsii*, effective disease management strategies are imperative to prevent severe yield losses [4]. *S. rolfsii* belongs to the Basidiomycota phylum, Atheliales order, and Atheliaceae family. The pathogen initiates its life cycle as dormant sclerotia, which germinate under favorable environmental conditions. Upon germination, the pathogen produces mycelium that infects the groundnut stem, causing decay and rot of host tissues. As the fungus proliferates, it generates new sclerotia, which perpetuate its infection cycle in the soil. The pathogen thrives in warm and humid environments, and its spread is favored by high soil moisture, acidic soil, poor drainage conditions, and the presence of a susceptible host [5].

Management of stem rot in groundnut is typically achieved through chemical fungicides [6], cultural practices, and biocontrol agents [7]. Fungicides such as tebuconazole, prothioconazole, and penthiopyrad have shown efficacy against stem rot when applied multiple times (at least 2–3 sprays) [4]. However, the use of chemical fungicides is not economically and environmentally sustainabale for the majority of groundnut farmers in Asia and sub-Saharan Africa due to limited resources. Additionally, cultural management practices have proven to be less effective, as the pathogen is soil-borne and produces dark sclerotia that persist in the soil for prolonged periods [8]. In contrast, host-plant resistance offers a more sustainable and effective solution for managing stem rot [9]. The first step in developing resistant cultivars involves the identification of resistant sources that completely rely on precise phenotyping methods. The sick field phenotyping method is currently the most prevalent phenotyping method [10–14]. Due to the pathogen's erratic and non-uniform growth, multi-season and multi-location phenotyping is a more reliable approach for screening stem rot resistance. The absence of multi-seasonal phenotyping may lead to the selection of false-positive genotypes, thus emphasizing the need for comprehensive phenotyping approaches to identify true and stable resistance sources.

The production of oxalic acid (OA) by the stem rot pathogen is a critical determinant of its pathogenicity, as evidenced by the elevated levels of OA detected in the infected tissues of host plants [15]. The secretion of OA by fungi facilitates their growth and colonization of substrates [16]. Furthermore, OA, either independently or in combination with other fungal components, is known to effectively inhibit the host plant's oxidative burst response [17]. Traditionally, the identification of resistant sources has relied on field or glasshouse screenings. However, maintaining uniform disease pressure in these conditions is challenging due to variability in pathogen establishment, growth, and multiplication, which are influenced by microclimatic conditions [18]. Despite the application of same amount of inoculum to each plant, disparities arise, leading to inconsistent pathogen distribution and inaccurate germplasm evaluation [19]. To address these limitations, we developed the oxalic acid assay (OAA) to screen genotypes for stem rot resistance under uniform disease pressure, thereby increasing precision and saving time. Stem rot resistance is complex due to its polygenic nature and significant influence to environmental variations. Consequently, comprehensive evaluation of germplasm across multiple seasons and locations, coupled with the use of two or more screening methods, is essential to minimize disease escape and accurately categorize resistant sources.

With this context, three-year sick field screening was conducted for groundnut minicore accessions, supplemented by a laboratory-based OAA to ensure comprehensive evaluation and prevent disease escape.

Results

Evaluation of minicore lines for their reaction to stem rot in sick field

Phenotyping of a groundnut minicore set for stem rot resistance in a sick field trial revealed substantial genetic variability, significant at the 0.01% level of significance (LOS). Analysis of variance (ANOVA) indicated no significant differences between replications, but highly significant differences were observed across genotypes, time, and seasons (years) at the 0.01% LOS. This emphasize the impact of genetic parameters at different growth stages and environmental influences on the trait. Minimal variation was noted between experimental blocks (Table 1). These findings were consistent for both the component traits of stem rot: percent disease incidence

Table 1 ANOVA for two component traits of stem rot disease from field experiment showing the abundance variation for the disease at ICRISAT, Patancheru, Hyderabad

Trait	Source	Df	Mean Sq	F	Pr(> F)	Sig.
For PDI	Rep	1	602	2.254	0.133	
	Genotype	189	2297	8.595	< 2E-16	***
	Time	2	485,424	1816.685	< 2E-16	***
	Block	18	1104	4.132	< 2.5E-08	***
	Environment (Season)	2	124,978	467.727	< 2E-16	***
	Genotype X Environment	378	844	3.159	< 2E-16	***
	Residuals	2829	267			
For PM	Rep	1	77	0.368	0.544	
	Genotype	189	1625	7.751	< 2e-16	***
	Time	2	388,284	1852.35	< 2e-16	***
	Block	18	1204	5.74	4.51e-13	***
	Environment (Season)	2	72,775	347.18	< 2e-16	***
	Genotype X Environment	378	572	2.729	< 2e-16	***
	Residuals	2829	210			

Significance codes: 0 '***', 0.001 '**', 0.01 '*', 0.05 '.'

PDI: Percent disease incidence, PM: Percent mortality

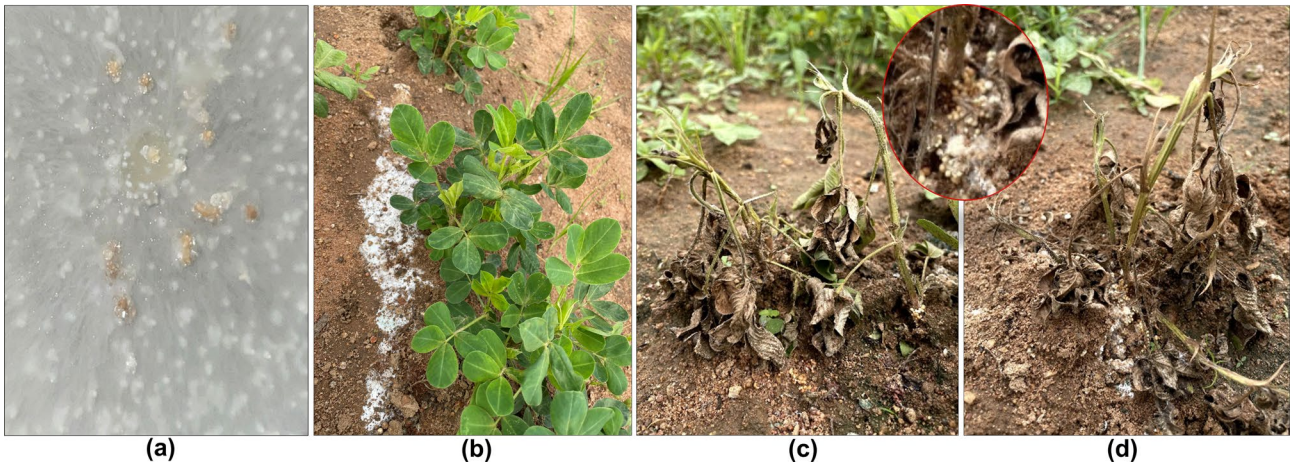


Fig. 1 Trend of disease from inoculation to symptoms development. (**A**) the isolated *S. rolfsii* grown in a petri plate, (**B**) initial development of mycelium in field after artificial inoculation, (**C**) initial symptoms like yellowing and wilting due to death of cells and clogging of vascular bundle, (**D**) final stage of plant before the plant falls off (whole plant turns brownish)

(PDI) and percent mortality (PM). The sick field displayed a complete range of disease severity, varying from 0 to 100% across the accessions for both PDI and PM (Fig. 1). The standard error of the mean was 17.19 for PDI and 17.98 for PM. For PDI, the phenotypic variance was 1615.84, with genotypic variance of 1024 and environmental variance of 591.27. Similarly, the phenotypic, genotypic and environmental variance was 911.9, 511.09 and 400.72, respectively for PM.

The phenotypic coefficient of variation (PCV) was observed to be high, with 82.97% for PDI and 89.8% for PM. Similarly, the genotypic coefficient of variation (GCV) was also found to be high, measuring 66.07% for PDI and 67.23% for PM. The environmental coefficient of variation (ECV) showed significant values as well, reaching 50.19% for PDI and 59% for PM. High heritability was recorded for PDI (63%), while PM exhibited a moderate heritability of 56% (Supplementary Table 1). A strong phenotypic correlation between the two component traits was noted, with coefficients of 0.77 in 2016, 0.90 in 2017, and 0.99 in 2022. Similarly, a high genotypic correlation was observed for PDI and PM across all the studied years, being 0.64 in 2016, 0.85 in 2017, and 0.93 in 2022 (Supplementary Table 2). A sick field experiment identified seven genotypes exhibiting resistance to moderate resistance against stem rot, each with an average mortality rate of less than 30% across all three seasons. These genotypes include ICG163, ICG721, ICG10479, ICG875, ICG11457, ICG111, and ICG2857 (Table 2). It was worth noting that the Spanish and Valencia type groundnut accessions were highly susceptible. Although not all genotypes from the Virginia type showed resistance, all the

Table 2 Disease values of component traits of stem rot disease for resistant genotypes in minicore from phenotyping in field and OAA

Sl. No.	Genotypes	Field screening		OAA(DS)
		PDI (Average of seasons) (%)	PM (Average of seasons) (%)	
1	ICG163	37.98	16.47	1
2	ICG721	27.04	21.35	1
3	ICG10479	35.81	22.33	2
4	ICG875	39.39	24.01	1
5	ICG11457	46.51	25.11	1
6	ICG111	36.44	25.43	2
7	ICG2857	37.50	25.71	1
8	RC (CS19)	48.2	35	2
9	SC (TMV2)	76.5	65	5

PDI: percent disease incidence, PM: percent mortality, OAA: oxalic acid assay, DS: disease score, RC: resistant check, SC: susceptible check

resistant and moderately resistant genotypes were from the Virginia type. PDI and PM were observed to increase progressively with the stage of crop (Fig. 2A). The pathogen *S. rolfsii* can infect the plant throughout its life cycle; however, the early stages of growth (<60 days after sowing (DAS)) are particularly sensitive to infection.

Phenotyping of minicore using OAA

The ANOVA results revealed the presence of highly significant differences among main stems and lateral stems at a 0.01% level of significance (Table 3), suggesting separate analyses. Significant variation was observed for disease score (DS) across genotypes, the concentration of OA used, and the time of observation for the main stems. Similarly, a significant difference was noted among genotypes at the 0.01% LOS, which was consistent with sick field screening results. Significant differences were also observed for the concentration of OA used and the time of observation at the 5% LOS for disease score. The interactions among genotype × time, genotype × concentration, time × concentration, and genotype × time × concentration were also found significant, indicating that all observations (DS and lesion length, LL) were related to stem rot disease under study. This observed variation is advantageous for crop improvement targeting stem rot resistance. For the component trait, lesion length, ANOVA demonstrated highly significant differences for genotypes, OA concentration, and the interaction between these two for both main stems and laterals (branches) (Table 3). The disease score for genotypes ranged from 1 to 6 on the full scale used in OAA. A high PCV of 87.93% for disease score and 69.08% for lesion length was observed. Additionally, high GCV was noted for both disease score (79.16%) and lesion length (51.55%) (Supplementary Table 3). The experiment was repeated twice for 30 genotypes (5 resistant, 10 moderately resistant, and 15 susceptible), yielding similar results for DS. High broad-sense heritability (81% for DS) was observed in this experiment, indicating minimal influence from micro-climatic variations. Approximately 80% (79.14%) of the evaluated genotypes exhibited a consistent (similar) disease reaction (Supplementary Table 4) as observed under sick field conditions (Fig. 2B). The high similarity in this diverse set (ICRISAT minicore) demonstrates the efficiency and reliability of this protocol for stem rot screening.

Mean performance vs. stability (GGE) analysis for assessing stem rot disease reaction of genotypes

GGE biplot is the most precise/reliable way of assessing the performance of genotypes over different environments (seasons/locations). An ideal genotype should have a good mean performance and low environment interaction. The mean vs. stability bi-plot graph was generated as given by Yan et al. (2002) [20]. This biplot view has been plotted using the Best Linear Unbiased Predictors (BLUPs) of PM values of 30 representative genotypes across the three seasons to predict the performance of the genotypes (Fig. 3). PC 1 explained 83.69% and PC 2 explained 11.46% of the total variation based on PM values. The single arrowhead line, the AEC abscissa, passing through the origin, indicated a highly resistant genotype with a lower PM value. Considering so, the genotypes falling to the leftmost position of the arrow are considered promising for stem rot disease resistance and genotypes falling towards the right side are promising high susceptibility. Genotypes: ICG163, ICG10479, ICG721, ICG875, ICG111, ICG11457, ICG2857 were positioned downstream the bi-plot origin, and had less PM value. The stability of the genotypes was assessed based on the length of the projection in both the directions from the AEC coordinate. Genotypes possessing more projection from the AEC coordinate are considered to be less stable. The Fig. 3 shows that the genotypes ICG163, and ICG875 were more stable, whereas ICG10479, ICG163 and ICG2857 were less stable. When it comes to susceptibility genotypes, genotypes ICG4543, ICG3673, ICG8567, and ICG5195 were found highly susceptible

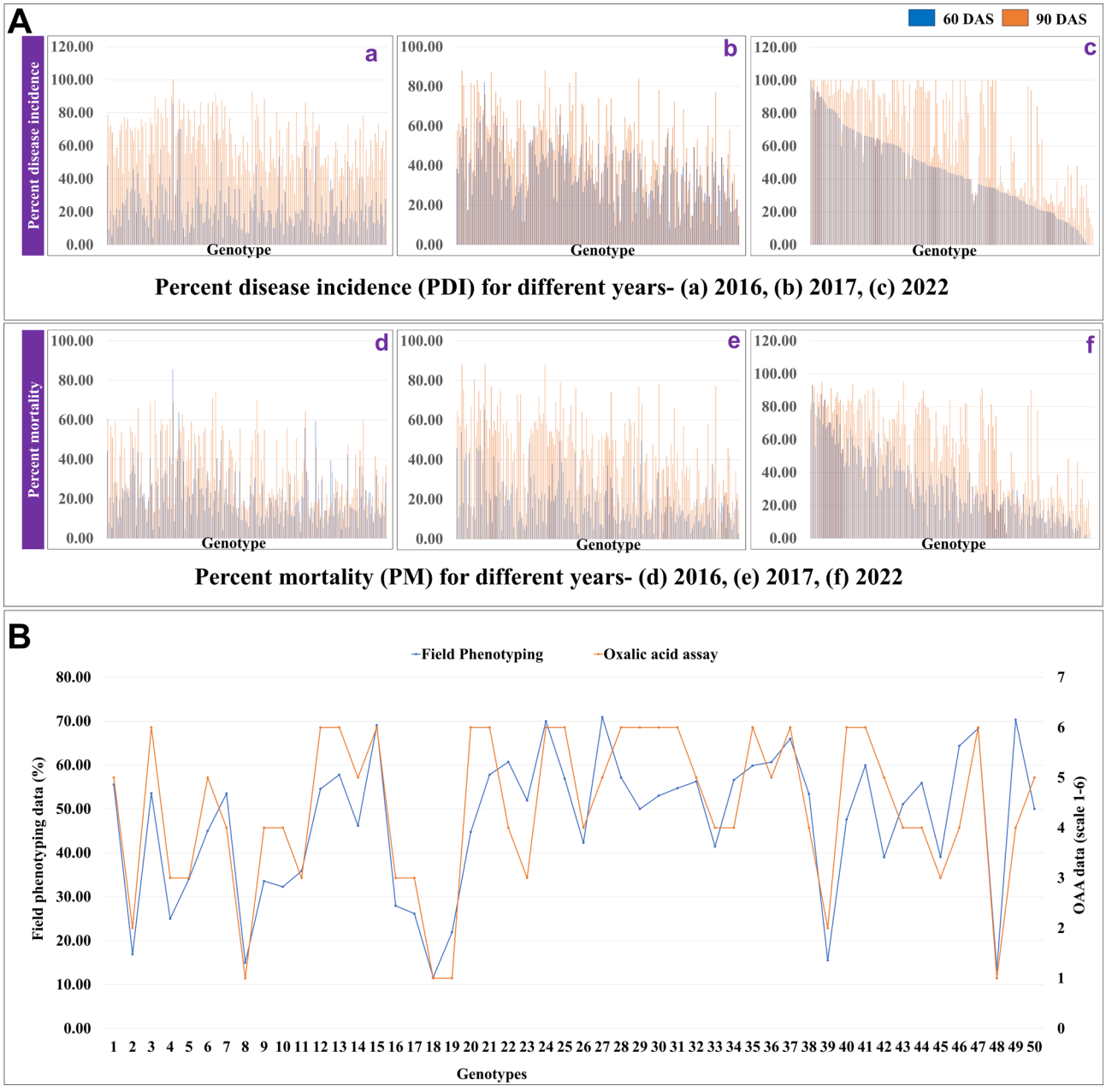


Fig. 2 Disease trend in sick-field screening and its proportional similarity with lab-based oxalic acid assay. (**A**) Disease trend in sick plot phenotyping, at ICRISAT, Patancheru showing a significant increase in disease with time. (**B**) Representing the high proportion of similarity between the field screening and OAA among the diverse set (groundnut mini core) of genotypes

while ICG4543, and ICG3673 being highly stable across the seasons. Considering both the mean performance and stability, the genotypes ICG163 and ICG875 can be considered ideal as they have less PM and high stability. The genotypes that are closer to the ideal genotype are considered to be desirable [20]. Therefore, the genotypes, ICG163, ICG875, ICG10479, ICG721, ICG2857, ICG111, and ICG11457 were identified as desirable genotypes with less PM for use in further genetics and breeding research.

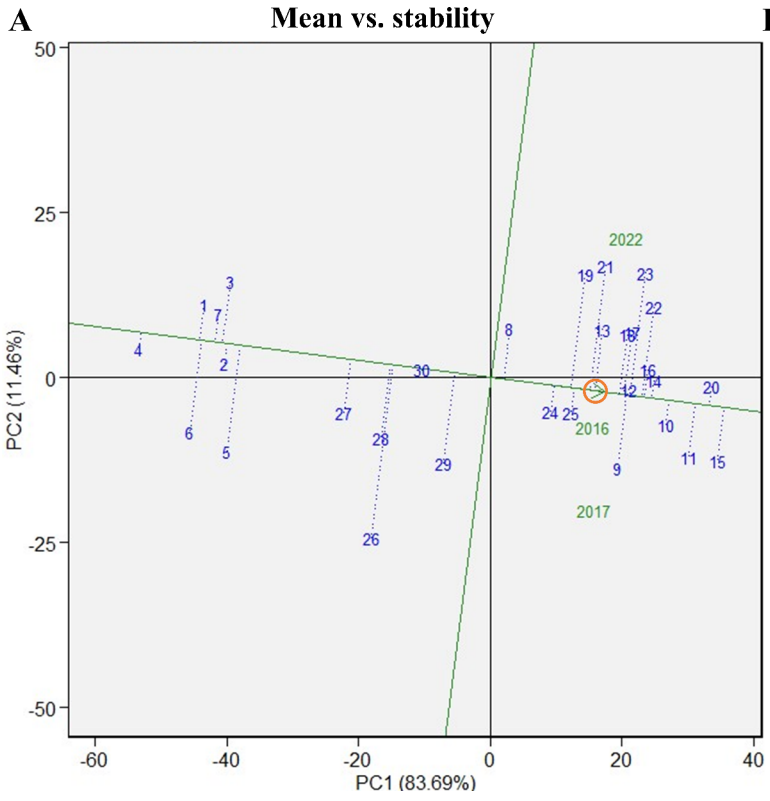
Discussion

Stem rot is a significant disease with the potential to cause up to 80% yield loss in groundnut [10, 21]. It is widespread in major groundnut-growing regions, including the USA, Argentina, China, Australia, and India. The disease symptoms are primarily associated with the production of OA [22] at elevated concentrations, in addition to other cell wall-degrading enzymes, such as polygalacturonases. These factors contribute to the softening and degradation of the crown region, leading to girdling

Table 3 ANOVA of stem rot screening through OAA for traits such as disease score and lesion length of main stem and lateral samples

Traits	Models	Df	Main stem			Df	Lateral stem		
			MSS	F value	Pr (> F)		MSS	F value	Pr (> F)
Disease Score	Replication	2	0.029	1.34	0.262	2	0.06	1.735	0.177
	Concentration	1	1453.5	64966.62	< 2e-16***	1	1051.5	29257.802	< 2e-16***
	Time	2	1245.5	55677.61	< 2e-16***	2	1003.5	27921.21	< 2e-16***
	Genotypes	134	18.8	838.91	< 2e-16***	138	18.4	512.154	< 2e-16***
	Concentration: time	2	36.4	1628.02	< 2e-16***	2	48.2	1340.324	< 2e-16***
	Concentration: genotypes	268	12.5	559.83	< 2e-16***	276	13.8	383.633	< 2e-16***
	Time: genotypes	268	2	87.56	< 2e-16***	276	1.5	42.436	< 2e-16***
	Concentration: time: genotypes	268	1.5	65.54	< 2e-16***	276	1.4	39.204	< 2e-16***
	Residuals	1466	0.022			1666	0.035		
Lesion Length	Replication	2	2.3	0.579	0.561	2	0.6	0.468	0.626
	Concentration	1	9408	2267.801	< 2e-16***	1	2615	2058.566	< 2e-16***
	Genotypes	134	93	22.469	< 2e-16***	134	30.7	24.175	< 2e-16***
	Concentration: genotypes	268	18	4.362	< 2e-16***	134	9.5	7.47	< 2e-16***
	Residuals	398	4.1			562	1.3		

Significance codes: 0 '***', 0.001 '**', 0.01, '*', 0.05 '.'



B

Genotype	Genotype code	Percent mortality (BLUPs)		
		2016	2017	2022
ICG10479	1	19	20	37
ICG111	2	26	26	32
ICG11457	3	17	22	43
ICG163	4	14	20	26
ICG2857	5	23	39	24
ICG721	6	18	35	23
ICG875	7	16	25	39
ICG3343	8	55	39	63
ICG13491	9	54	72	63
ICG4543	10	66	65	70
ICG8567	11	64	73	70
ICG5494	12	53	64	72
ICG12189	13	53	53	75
ICG6201	14	57	64	75
ICG5195	15	51	86	77
ICG1137	16	50	65	77
ICG1274	17	56	55	78
ICG10474	18	50	59	78
ICG10566	19	51	45	79
ICG3673	20	57	71	82
ICG2019	21	48	49	83
ICG397	22	53	56	83
ICG3584	23	49	54	87
ICG14630	24	59	53	59
ICG9249	25	66	51	60
ICG10479	26	41	57	29
ICG111	27	33	43	40
ICG11457	28	41	46	40
ICG163	29	52	50	42
ICG2857	30	44	41	51

Fig. 3 "Mean vs. Stability" view of the GGE biplot considering percent mortality of groundnut accessions against *Sclerotium rolfsii* incidence across three seasons. (**A**) shows the mean percent mortality vs. stability graph. (**B**) showing the genotypes their percent mortality data across the seasons

of individual branches or the entire plant, ultimately resulting in wilting and rotting of the crown region. The infection mechanism of *Sclerotium rolfsii* in the field is complex and influenced by multiple factors (Supplementary Fig. 1). As a soil-borne pathogen, *S. rolfsii* primarily establishes contact with plant tissues at the crown region. Upon reaching this area, it secretes OA, a major virulence factor [22], into the infected tissue. This secretion weakens the plant, making it vulnerable, and facilitates the pathogen to use the plant tissue as a nutrient source. Disease progression is characterized by the formation of a dense layer of white mycelium over the plant surface. Although cell wall-degrading enzymes typically cannot function at the normal pH of plant tissues [23], the mycelium of *S. rolfsii* produces OA at higher concentrations, which decreases the cellular pH, enabling these enzymes

to act more effectively [24]. OA primarily targets pectin, a complex carbohydrate that is a crucial component of the plant cell wall. Pectin provides structural support and regulates the flow of water and nutrients. One mechanism by which OA disrupts pectin is through chelation of calcium ions, which compromises the cross-linking network that imparts strength and rigidity to the cell wall [25]. Additionally, OA increases cell membrane permeability, resulting in leakage of cellular contents and disruption of cellular functions. The binding of OA to calcium ions also leads to the formation of calcium oxalate crystals, which can accumulate and disrupt cell wall integrity. This accumulation can clog vascular tissues, impeding water and nutrient transport resulting in wilting. At high concentrations, OA can induce the generation of reactive oxygen species (ROS) within cells, which cause oxidative damage to membranes, proteins, and DNA, ultimately leading to cell death and tissue damage. Furthermore, OA can trigger programmed cell death (apoptosis) in plant cells [26], contributing to the breakdown of cell wall components and weakening of structural integrity leading to cell death.

Identification of genotypes with stable resistance to stem rot is a critical step for incorporating resistance genes into elite cultivars through breeding programs. In this study, groundnut minicore accessions were evaluated for stem rot disease resistance using two distinct methodologies: field conditions and controlled laboratory conditions (OAA). Previous studies have utilized various methodologies for disease screening [10–14]. To address the lack of a standardized field screening protocol for stem rot, we have developed a flexible and robust phenotyping method that enabled the evaluation of a large number of accessions, thereby generating a substantial data for meaningful analysis and interpretations. This method ensured that the screening process closely mimicked the pathogen's natural behavior in its field habitat. Upon analysis, a significant and noteworthy variation was observed among the genotypes, indicating that the population utilized in this study was well-suited for drawing conclusions about stem rot resistance. ANOVA revealed significant seasonal variations, suggesting that the trait under study was highly influenced by environmental factors, considering its polygenic nature [9]. Disease infection and development was observed at all stages of growth, and the severity increased over time until 60 DAS. Thereafter, the disease progression slowed due to an increase in the plant's tolerance capacity to withstand stress. This trend highlights the polygenic nature of stem rot resistance and underscores the importance of genotype, environment, and their interactions in influencing disease incidence, establishment, and dissemination durations (Fig. 2). The level of disease progression varied with the crop's growth stage.

In contrast, the absence of a significant difference between replications across genotypes suggests that the experimental setup was highly reliable. Variability parameters indicated a wide range of disease scores, spanning from 0 to 100, reflecting complete variation among the genotypes for both component traits, such as PDI and PM. PM is a more reliable trait compared to PDI, as PDI includes every small level of visible infection (symptom) on the plant, while PM directly measures the number of dead plants, a primary determinant of yield loss. Moreover, high heritability values [27] were noted, emphasizing the substantial influence of genetic factors on the expression of stem rot resistance. The genetic advance for this trait was notably high, indicating significant potential for improvement through plant breeding. A substantial contribution (36%) of environmental variance to the phenotypic variance was observed. Given the significant role of the environment in stem rot resistance, it is advisable to evaluate genotypes across multiple years to achieve reliable results [28]. It is important to note that *S. rolfsii*, the pathogen responsible for this disease, exhibits irregular growth patterns [19] and variability in growth across different parts of the field due to the strong influence of microclimate factors [18] such as plant architecture, soil temperature, and soil moisture [29]. Despite providing each plant with the same inoculum dosage, variations in pathogen growth and the resulting disease pressure were observed, leading to diverse responses among the genotypes [14]. Furthermore, due to the pathogen's erratic growth behavior, certain genotypes were able to escape disease incidence [30], making it challenging to maintain uniform disease pressure across all genotypes in the field. Consequently, multi-season screening becomes essential when the objective is to identify the most reliable resistant genotypes against stem rot. Through field screening, which exhibited high genotype × environment interaction (G×E), we were able to identify three genotypes with low percent mortality and high stability across different seasons (Fig. 3).

Microclimate plays a significant role in influencing disease growth and development (disease pressure). Genotypes with a bushy growth habit (i.e., denser foliage) tend to increase the humidity of the microclimate, that promotes pathogen multiplication. Consequently, a robust phenotyping protocol is needed to maintain uniform disease pressure across all genotypes, reducing environmental influence and enabling precise identification of stable, resistant genotypes. This is where our OAA proves to be effective, as it ensures uniform disease pressure and highly reliable screening results. Resistance to stem rot disease, is significantly influenced by environmental factors, which can lead to replication differences. If pathogen spread is reduced due to an unfavorable microclimate, the genotype may recover during early

and mid-stages of its lifecycle. Thus, screening genotypes through OAA is crucial for identifying disease-resistant genotypes. In OAA experiment, we evaluated plant responses to varying OA concentrations: 0 mM, 20 mM, and 50 mM. A 50 mM concentration was the maximum applied, which none of the cultivated varieties could tolerate beyond 24 h. We used a 20 mM solution as a standard concentration for screening, as it represents the highest level of OA produced by *S. rolfsii* under natural conditions [31]. Of the two key traits evaluated (disease score and lesion length), disease score proved to be the most reliable, as it exhibited greater consistency across replicates and repetitions. Visual assessment categorized scores are as follows: scores of 1 and 2 indicated no or low wilting, score 3 indicated moderate wilting, while scores 4, 5, and 6 represented high wilting.

The symptoms observed in OAA closely resemble those seen in sick field conditions, such as cell death and lesion formation on the stem. However, in OAA, lesions are more prominently visible compared to field conditions due to the absence of soil particles and other physical disturbances. Yellowing of leaves was rarely observed in OAA, as the exposure to OA is limited to a short duration. In OAA, symptoms developed more rapidly than in the field due to the direct application of a specific concentration and sufficient quantity of OA. In contrast, symptom development in the field is slower due to the gradual production and distribution of OA. The lesion length in OAA is significantly greater compared to field experiments, as the transfer of OA through the vascular bundle is faster. OAA is a simple, highly beneficial, reliable, and precise technique that requires less time and effort compared to field methods. Maintaining plants in a greenhouse for a short duration of 42 days is straightforward and less labor-intensive compared to field maintenance. The entire experiment requires minimal resources, including pots, OA, 5 ml glass tubes for OA treatment, and an incubator to maintain a temperature of 24–26 °C. The seed requirement for each test entry is also minimal. In our experiment, we used only 9 seeds to obtain 9 plants from each genotype (3 treatments × 3 replicates). In contrast, sick field screening involves substantial costs, including land preparation, sowing 20–40 seeds per test entry, irrigation, and weeding. Therefore, the key advantages of this protocol include easy maintenance of uniform disease pressure, shorter time requirement (completed in 45 days), high precision with minimal environmental influence, consistent and reproducible results, reduced labor, and high cost-effectiveness. These are essential attributes for an effective disease screening technique, making OAA a reliable protocol for stem rot screening and a supplementary approach to sick-field screening. The identification of stable resistant genotypes is a critical objective for any disease screening protocol. In field screening, however, there is a risk that host plants may escape disease exposure due to the uneven distribution of mycelium, despite the uniform application of inoculum [10]. In contrast, uniform disease pressure is imposed across all genotypes and replications in case of OAA, eliminating the possibility of escape and ensuring more accurate results compared to field screening. Our analysis demonstrated significantly higher consistency in genotype categorization across all seasons of sick field screening and OAA. Moreover, OAA is also suitable for screening genotypes that do not produce seeds and for vegetatively propagated species, such as *Arachis glabrata*. Through OAA, we identified seven resistant groundnut lines (Table 3). Sometimes, it is possible to see genotypes showing resistance in OAA even if they do not exhibit resistance in the field. This discrepancy is likely due to the interaction of the pathogen with other minor chemical substances in the environment. Therefore, it is recommended to use both OAA and field screening in tandem to complement each other and enhance the accuracy of phenotyping. The optimal approach would be to first employ OAA screening, as it is faster and more cost-effective, to reduce the sample size of the population. Subsequently, field evaluations can be conducted on genotypes that demonstrate resistance in OAA, ensuring a comprehensive and reliable assessment of disease resistance.

Methods

Groundnut mini core subset comprising of 184 accessions, representing 10.8% and 1.29% of the core collection (1704 accessions) and the entire collection (14,310 accessions) respectively, was used in the study [32].

Sick-field screening of mini core accessions for stem rot

Field screening of the groundnut mini core collection was conducted in a sick plot for stem rot at ICRISAT, Patancheru, Hyderabad (geographic coordinates: 17.51161°N, 78.28000°E) during three rainy seasons (June/July to September/October) in 2016, 2017, and 2022. Screening trials (Supplementary Fig. 2) were arranged in an alpha lattice design with two replications to minimize inter-block effects and reduce experimental heterogeneity. Each replication consisted of 19 blocks, with a block size of 10 plots and a plot size of 4 m x 1.5 m. Single-row plots of 4 m length were planted, with an inter-row spacing of 30 cm and an intra-row spacing of 10 cm. Sowing was performed on a broad bed furrow with four rows per bed. All genotypes, along with the resistant check (CS-19) and susceptible check (TMV2), were sown in four-meter rows, with 40 plants per replication. Plant count for each genotype was recorded at

15 DAS. Artificial inoculation was performed at 22 DAS using previously mass-multiplied *S. rolfsii* pathogen, which was manually applied at the base of each plant, ensuring contact with the stem. The pathogen was mass-multiplied on sorghum seeds that had been soaked overnight and autoclaved for two cycles [33]. Soil moisture (in field) was maintained throughout the experiment to create conducive conditions for both pathogen proliferation and crop growth. A second inoculation was carried out at 45 DAS, using the same procedure and quantity of inoculum. The first set of observations was recorded at 30 DAS, assessing two disease component parameters: PDI and PM. Subsequent observations were recorded at 60 and 90 DAS. ANOVA was performed to evaluate the presence of significant variation for each trait among the genotypes, seasons, and time points.

$$\mathrm{PDI} = \frac{\text{number of plants infected}}{\text{total number of plants}} \times 100$$

$$\mathrm{PM} = \frac{\text{number of plants dead}}{\text{total number of plants}} \times 100$$

Response to OAA

The plants were grown in a greenhouse using 10-inch pots filled with a 2:1:1 mixture of red soil, sand, vermicompost, and maintained under favorable conditions. Genotypes were sown to ensure the development of nine main stems and nine lateral branches for each genotype. Different concentrations of OA solutions (0 mM, 20 mM, and 50 mM) were prepared on the day of sampling, which was carried out at 42 DAS [19]. The main stems and laterals were transversely cut to prevent any splitting of the stem samples. The detached stem portions were immediately immersed in a beaker containing reverse osmosis (RO) water and kept submerged until sampling of all genotypes was completed. The detached stem samples were then carefully blotted with blotter paper to remove excess RO water from the stem surface. These stem samples were subsequently placed in individual 5 ml tubes containing different concentrations of OA (0, 20, 50 mM) in three replications, arranged in a completely randomized design (CRD). This experimental setup (Fig. 4A) was transferred to an incubator maintained at a temperature range of 21–24 °C. The plant response to OA treatment were recorded at 12, 24, and 36 h of incubation using a 1–6 rating scale [34], where 1 = no wilt symptoms, 2 = one leaf wilted, 3 = two leaves wilted, 4 = one petiole collapsed, 5 = two petioles collapsed, and 6 = whole stem collapsed [19] (Fig. 4B). The length of stem discoloration (lesion length) resulting from OA treatment was also measured. The tubes were refilled with fresh OA solutions based on the requirement after each observation. ANOVA and other genetic parameters were analyzed using R software. The proportion of similarity between OAA and field screening was then assessed to determine the efficiency of OAA in supplementing sick field phenotyping. Specifically, the response of main stems to 20 mM concentration at 36 h was compared with the PM data obtained from sick field screening at 90 DAS. PM from sick field screening and disease scores from the OAA were used for assessment, as these parameters are more accurate and closely associated with yield. In sick field screening, genotypes with PM < 10% were classified as highly resistant, 10–19% as resistant, 20–29% as moderately resistant, and > 30% as susceptible [35].

Statistical analysis

ANOVA, variability parameters, and correlation analyses were conducted using the 'variability' package in R software. ANOVA was performed to determine the presence of significant variation within the minicore set for stem rot resistance and to evaluate the influence of environmental factors (seasons) on the trait. Variability parameters were analyzed to assess the effectiveness of the generated data for future plant breeding applications. Correlation analysis was conducted to identify relationships between the component traits of stem rot. Best linear unbiased predictors (BLUPs) were derived using the 'Phenotype' package in R software. Additionally, GGE biplot analysis was performed using the 'ggplot' package in R software [36] to check the stable performance of each genotype across the seasons.

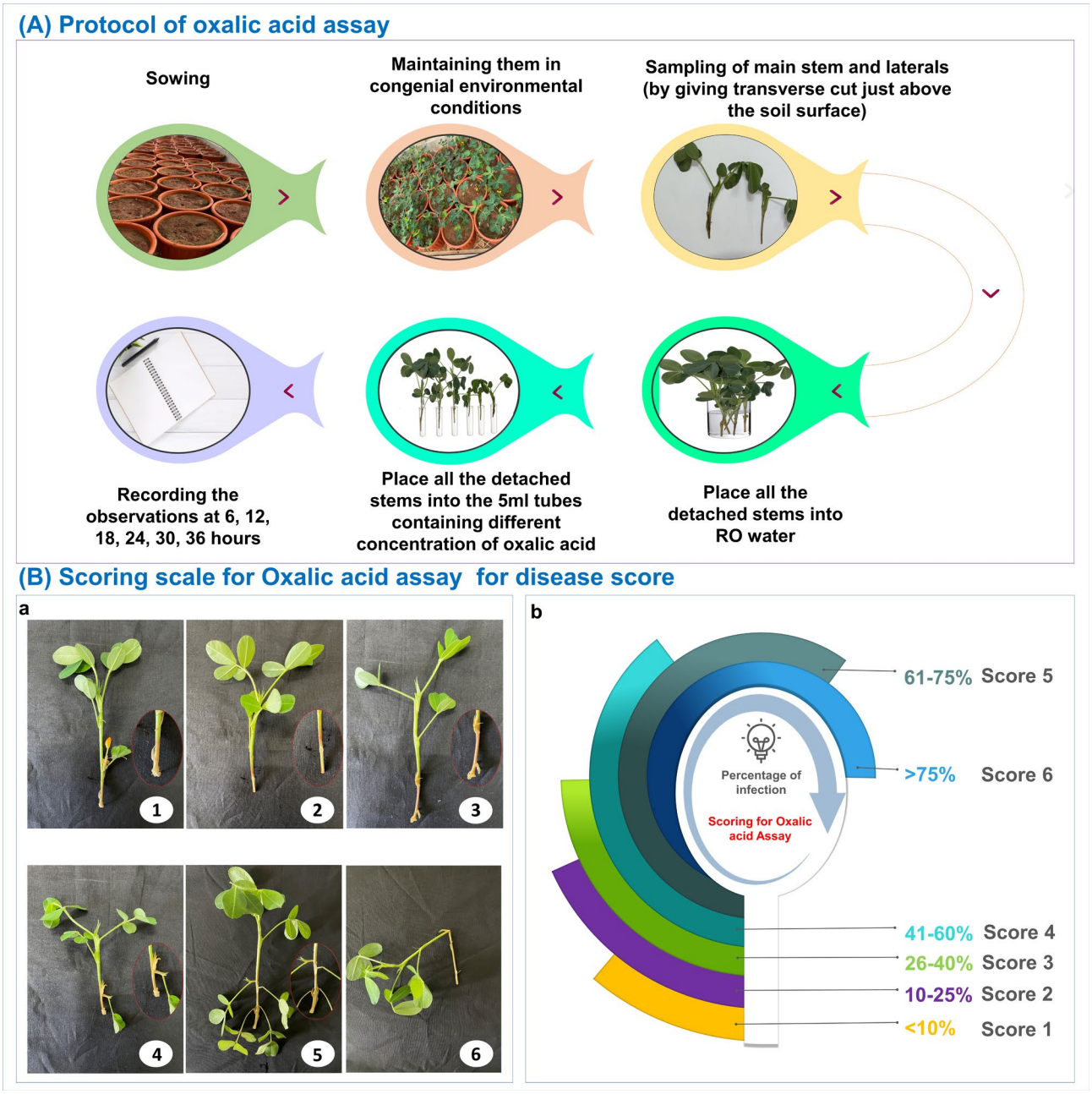


Fig. 4 Oxalic acid assay protocol and disease scoring scale. (**A**) Detailed protocol followed for OAA experiment, (**Ba**) Scoring scale used for recording observation in OAA experiment, (**Bb**) the percentage of infection seen on the plant according to scoring scale

Conclusions

A total of 184 groundnut minicore accessions were evaluated for their reaction to stem rot disease comprehensively over a span of three years using sick field and oxalic acid assay. Subsequently, seven genotypes that consistently exhibited resistance to stem rot were selected. As the pathogen's growth pattern displayed irregularity across the groundnut fields, influenced by different environmental factors, it becomes imperative to employ a multi-season phenotyping approach to comprehensively understand and characterize this particular trait. In addition to multi-season phenotyping, employing diverse screening protocols is essential. It is well understood that response to OAA is a useful tool to cross check the lines that were categorised as resistant under sick field conditions. It exhibited high proportion of similarity (nearly reaching 80%) with sick field screening in addition to increasing trait heritability from 63 to 80%. Therefore, it is always advisable to consider using OAA as a reliable supplement to field screening.

Abbreviations

OAA	Oxalic Acid Assay
PDI	Percent Disease Incidence
PM	Percent Mortality
GCV	Genotypic Coefficient of Variation
PCV	Phenotypic Coefficient of Variation
ECV	Environmental Coefficient of Variation
DS	Disease Score
LL	Lesion Length
USA	United States of America
ROS	Reactive Oxygen Species
DAS	Days After Sowing
mM	Milli Molar
OA	Oxalic Acid
ICRISAT	International Crops Research Institute for the Semi-Arid Tropics
RO	Reverse Osmosis
CRD	Completely Randomised Design
ANOVA	Analysis of Variance
LOS	Level of significance

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12870-024-05706-0.

Supplementary Material 1

Supplementary Material 2

Acknowledgements

HVV would like to thank Council of Scientific and Industrial Research (CSIR), Government of India for the financial assistance in the form of CSIR-NET-JRF. Authors would like to thank ICRISAT-Genebank for providing minicore seed material for the experimentation.

Author contributions

Conceptualization; funding acquisition; investigation; project administration; resources; supervision; writing-review & editing: M.K.P; validation; writing-original draft; software; formal analysis; validation; writing-original draft; methodology; validation; writing original draft; writing-review & editing: H.V.V; Data curation; investigation; methodology; formal analysis; writing review & editing: H.K.S; Data curation; investigation; methodology: B.K.; Data curation; validation; formal analysis: R.P.V; Data curation; formal analysis: A.R.N.K.

Funding

Thanks for partial financial assistance from Indian Council of Agricultural Research (ICAR)-ICRISAT collaborative projects and Bill & Melinda Gates Foundation (BMGF), USA.

Data availability

The data generated is being made available in Supplementary File 2.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

Received: 7 February 2024 / Accepted: 15 October 2024
Published online: 05 November 2024

References

1. Willett W, Rockström J, Loken B, Springmann M, Lang T, Vermeulen S, Garnett T, Tilman D, DeClerck F, Wood A, Jonell M. Food in the Anthropocene: the EAT–Lancet Commission on healthy diets from sustainable food systems. Lancet. 2019;393(10170):447–92.
2. Krishna G, Singh BK, Kim EK, Morya VK, Ramteke PW. Progress in genetic engineering of peanut (*Arachis hypogaea* L.)-A review. Plant Biotechnol J. 2015;13(2):147–62.
3. Kokalis-Burelle N, Mahaffee WF, Rodriguez-Kabana R, Kloepper JW, Bowen KL. Effects of switchgrass (*Panicum virgatum*) rotations with peanut (*Arachis hypogaea* L.) on nematode populations and soil microflora. J Nematology. 2002;34(2):98.
4. Standish JR, Culbreath AK, Branch WD, Brenneman TB. Disease and yield response of a stem-rot-resistant and-susceptible peanut cultivar under varying fungicide inputs. Plant Dis. 2019;103(11):2781–5.
5. Paparu P, Acur A, Kato F, Acam C, Nakibuule J, Nkuboye A, Musoke S, Mukankusi C. Morphological and pathogenic characterization of *Sclerotium Rolfsii*, the Causal Agent of Southern Blight Disease on Common Bean in Uganda. Plant Dis. 2020;104(8):2130–7.
6. Grichar WJ. Management of stem rot of peanuts (*Arachis hypogaea*) caused by *Sclerotium rolfsii* with fungicides. Crop Prot. 1995;14(2):111–5.
7. Spadaro D, Gullino ML. Improving the efficacy of biocontrol agents against soilborne pathogens. Crop Prot. 2005;24(7):601–13.
8. Ganesan S, Kuppusamy RG, Sekar R. Integrated management of stem rot disease (*Sclerotium Rolfsii*) of groundnut (*Arachis hypogaea* L.) using Rhizobium and *Trichoderma Harzianum* (ITCC-4572). Turkish J Agric Forestry. 2007;31(2):103–8.
9. Dodia SM, Joshi B, Gangurde SS, Thirumalaisamy PP, Mishra GP, Narandrakumar D, et al. Genotyping-by-sequencing based genetic mapping reveals large number of epistatic interactions for stem rot resistance in groundnut. Theor Appl Genet. 2018;132:1001–16.
10. Agmon S, Kunta S, Dafny Yelin M, Moy J, Ibdah M, Harel A, Rabinovitch O, Levy Y, Hovav R. Mapping of stem rot resistance in peanut indicates significant effect for plant architecture locus. Crop Sci. 2022;62(6):2197–211.
11. Bera SK, Kamdar JH, Kasundra SV, Thirumalaisami PP. Identification of groundnut genotypes and wild species resistant to stem rot using an efficient field screening technique. Electron J Plant Breed. 2016;7(1):61–70.
12. Fan P, Song W, Kang Y, Wan L, Lei Y, Huai D, Chen Y, Wang X, Jiang H, Yan L, Liao B. Phenotypic identification of peanut germplasm for resistance to southern stem rot. Oil Crop Sci. 2020;5(4):174–9.
13. Guclu V, Aydogdu M, Basak M, Kizil S, Uzun BÜ, Yol EN. Characterization of a groundnut collection to stem rot disease caused by *Sclerotium Rolfsii*. Australas Plant Pathol. 2020;49:691–700.
14. Shokes FM, Weber Z, Gorbet DW, Pudelko HA, Taczanowski M. Evaluation of peanut genotypes for resistance to southern stem rot using an agar disk technique. Peanut Sci. 1998;25(1):12–7.
15. Godoy G, Steadman JR, Dickman MB, Dam R. Use of mutants to demonstrate the role of oxalic acid in pathogenicity of Sclerotinia sclerotiorum on Phaseolus vulgaris. Physiol Mol Plant Pathol. 1990;37(3):179–91.
16. Dutton MV, Evans CS. Oxalate production by fungi: its role in pathogenicity and ecology in the soil environment. Can J Microbiol. 1996;42(9):881–95.
17. Sennoi R, Jogloy S, Gleason ML. Development of an oxalic acid assay to evaluate *Sclerotium rolfsii* resistance in *Jerusalem artichoke*. SABRAO J Breed Genet. 2021;53(1):70–8.
18. Shew BB, Wynne JC, Beute MK. Field, microplot, and greenhouse evaluations of resistance to *Sclerotium Rolfsii* in peanut. Plant Dis. 1987;71(2):188–91.
19. Bennett RS. Growth chamber assay for evaluating resistance to *Athelia Rolfsii*. Peanut Sci. 2020;47(1):25–32.
20. Yan W, Tinker NA. (2002). Biplot analysis of multi-environment trial data: Principles and applications. Can J Plant Sci. 2006;86(3):623–45.
21. Praveen A, Kannan C. Disease incidence and severity of *Sclerotium rolfsii* on *Arachis hypogaea* L. Plant Archives (09725210). 2021;21(1).
22. Kritzman G, Chet I, Henis Y. The role of oxalic acid in the pathogenic behavior of *Sclerotium Rolfsii* Sacc. Exp Mycol. 1977;1(4):280–5.
23. Cessna SG, Sears VE, Dickman MB, Low PS. Oxalic acid, a pathogenicity factor for *Sclerotinia Sclerotiorum*, suppresses the oxidative burst of the host plant. Plant Cell. 2000;12(11):2191–9.
24. Noyes RD, Hancock JG. Role of oxalic acid in the Sclerotinia wilt of sunflower. Physiol Plant Pathol. 1981;18(2):123–32.
25. Bateman DF. Simultaneous production and synergistic action of oxalic acid and polygalacturonase during pathogenesis by *Sclerotium Rolfsii*. Phytopathology. 1965;55:204–11.
26. Williams B, Kabbage M, Kim HJ, Britt R, Dickman MB. Tipping the balance: *Sclerotinia sclerotiorum* secreted oxalic acid suppresses host defenses by manipulating the host redox environment. PLoS Pathog. 2011;7(6):e1002107.

27. De Resende MDV. Biometric genetics and statistics in the breeding of perennial plants. Emprapa Technological Information. 2002.
28. Branch WD, Brenneman TB. Stem rot disease evaluation of mass-selected peanut populations. Crop Prot. 1999;18(2):127–30.
29. Munir M, Kirk KR, Plumblee MT, Anco D, Munir M, Kirk KR, Plumblee MT, Anco DJ. Late Leaf Spot Defoliation, Microclimate Modification, and Southern Stem Rot Development in Peanut (*Arachis hypogaea* L.) in South Carolina. Peanut Sci. 2021;48(2):76–86.
30. Shokes FM, Weber Z, Gorbet DW, Pudelko HA, Taczanowski, M. Evaluation of peanut genotypes for resistance to southern stem rot using an agar disk technique. Peanut Sci. 1998;25:12–17.
31. Amaro JK, Xavier LV, Ribeiro MM, Vieira BS, Mendes GD. Optimization of oxalic acid production by fungi for biotechnological solubilization of rock phosphate. Scientia Agricola. 2022;80:e20210076.
32. Upadhyaya HD, Bramel PJ, Ortiz R, Singh S. Developing a mini core of peanut for utilization of genetic resources. Crop Sci. 2002;42(6):2150–6.
33. Tarafdar A, Rani TS, Chandran US, Ghosh R, Chobe DR, Sharma M. Exploring combined effect of abiotic (soil moisture) and biotic (*Sclerotium Rolfsii* Sacc.) Stress on collar rot development in chickpea. Front Plant Sci. 2018;9:1154.
34. Kolkman JM, Kelly JD. An indirect test using oxalate to determine physiological resistance to white mold in common bean. Crop Sci. 2000;40(1):281–5.
35. Bera SK, Radhakrishnan T, Kasundra SV, Kamdar JH. Phenotyping technique for stem rot disease in groundnut under field conditions. ICAR-Directorate of Groundnut Research, Gujarat, India, Technical Bulletin. 2016;24.
36. Yan W, Kang MS, Ma B, Woods S, Cornelius PL. GGE Biplot vs. AMMI Analysis of Genotype-by-Environment Data. Crop Sci. 2007;47(2):643–53. https://doi.org/10.2135/CROPSCI2006.06.0374.

Publisher's note