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RESEARCH ARTICLE

Resistance to stem rot disease in groundnut (*Arachis hypogaea* L.) in inter-specific derivatives of wild *Arachis* species

Bangaru Kiranmayee · Hari Kishan Sudini · S. K. Bera · D. Shivani · Srinivasa Chary · H. V. VeerendraKumar · Anilkumar Vemula · Janila Pasupuleti

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Abstract Stem rot of groundnut, caused by a necrotrophic pathogen *Sclerotium rolfsii* Sacc., is an important soil-borne disease that can cause a pod yield loss of 20–80% depending on severity. Stem rot disease reaction of 160 groundnut genotypes was assessed by employing sick field screening at the International Crop Research Institute for the Semi-Arid Tropics (ICRISAT, Patancheru, India) and Indian Council of Agricultural Research - Directorate of Groundnut Research (ICAR-DGR) (under natural and high humidity conditions), pot screening under controlled conditions at ICRISAT and through oxalic acid assay. In the sick field at ICRISAT, percent mortality (PM) of the genotypes ranged from 13 to 80%, clearly discriminating the genotypes, while it was 8–58% at ICAR-DGR under natural conditions. The disease pressure was high in pot screening and under high humidity conditions at ICAR-DGR. Owing to a discrimination ability among the genotypes, ICRISAT sick field results were considered in selecting the resistant genotypes. Out of 160 genotypes, ten were found to be resistant (13–19% PM) and fourty were moderately resistant (20–29.43% PM) at ICRISAT sick field. Fourty four of these lines, (9 resistant and 35 moderately resistant) were developed from the wild *Arachis* species *A. villosa*, *A. correntina*, *A. helodes*, *A diogoi*, *A. cardenasii*, *A. stenosperma*, *A. paraguariensis, A. kempff-mercadoi*, *A. hoehnei.* The other 6 lines are not interspecific-derivatives to our best knowledge; one resistant and four moderately resistant lines are breeding lines derived from cultivated species at ICRISAT (4) and USA (1), and one moderately resistant is a land race from Nigeria. Interestingly, the stem rot resistant interspecific derivatives identified in the study, except *A. paraguariensis* (EE), originate from the AA genome of the wild *Arachis* species. The potential genotypes for stem rot disease resistance are three interspecific derivative lines, ICGR 161939, ICGR 162044, ICGR 162032,

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B. Kiranmayee · H. K. Sudini · H. V. VeerendraKumar · A. Vemula · J. Pasupuleti (✉)
International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru, Hyderabad, India
e-mail: Janila.Pasupuleti@icrisat.org

B. Kiranmayee · D. Shivani
Department of Genetics and Plant Breeding, Professor Jayashankar Telangana State Agricultural University (PJTSAU), Hyderabad, India

S. K. Bera
Indian Council of Agricultural Research-Directorate of Groundnut Research (ICAR-IIGR), Junagadh, Gujarat, India

S. Chary
Department of Statistics and Mathematics, PJTSAU, Hyderabad, India

H. V. VeerendraKumar
Department of Genetics and Plant Breeding, Acharya N G Ranga Agricultural University, Guntur, India

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and two advanced breeding lines, ICGV 10342 and ICGV 181045. Oxalic acid assay further confirmed the resistance of the three inter-specific derivatives, ICGR 161939, ICGR 162044 and ICGR 162032 with a low wilting score (1–2) and less lesion length (1–3 cm).The study suggests the use of comprehensive screening protocols employing both lab and sick field screening for assessing the components of host resistance to stem rot disease and find their use in breeding programs to develop stem rot resistant cultivars.

Keywords Soil-borne · Disease · Stem rot · Resistance · Inter-specific derivatives

Introduction

Groundnut (*Arachis hypogaea* L.), an allotetraploid (2n = 4x = 40), is an oilseed legume crop. Originating in South America, groundnut is grown primarily in Asia and Africa that account for > 90% of global groundnut area. It is grown in an area of 30.53 Mha with a production of 54.23 Mt and a productivity of 1.7 t/ha, which is variable across the growing countries of the world (FAOSTAT 2022). This crop is especially important to small-holder farmers in Africa and Asia who grow groundnuts under low-input conditions for food, oil, feed, and confectionary purposes. Groundnut is known for its rich nutritional composition. A variety of nutrients, including vitamin E, niacin, calcium, magnesium, phosphorus, zinc, iron, riboflavin, thiamine, and potassium, are present in groundnut kernels, in addition to edible oil (40–56%), protein (20–30%), and carbohydrates (10–20%) (Dean et al. 2009), thus contributing to human dietary needs. Apart from its nutritional value, groundnut also aids to human health. Groundnut oil contains a high concentration of plant sterols, particularly β-sitosterol, which has anticancer properties and can lower cholesterol levels up to 10–15% (Awad and Fink 2000). Furthermore, groundnut seed with high oil (50%) and oleic acid (75–80%) content can be an excellent substitute for canola and olive oil, which are currently 2.5 and 11 times more expensive, respectively (Shasidhar et al. 2017). The oil cake meal after oil extraction is utilized as a raw material for industry and as a protein additive in livestock feed regimens (Janila et al. 2016). The low-quality oil is used for making soaps, detergents, cosmetics, paints, candles, and lubricants. Thus, it is a multi-purpose crop.

Groundnut crop productivity is constrained by a variety of biotic and abiotic stresses (Bhawar et al. 2020; Patel et al. 2022). Among the stresses affecting groundnut production, diseases play a major role. Informal surveys, observations by scientists and farmers showed an increase in the incidence of soil-borne diseases and changes in disease incidence (Pande and Rao 2000). Due to the close association of pods with the soil, diseases caused by soil-borne pathogens are threatening groundnut production (Thiessen and Woodward 2012). Stem rot of groundnut, caused by a necrotrophic pathogen *Sclerotium rolfsii* is a devastating soil-borne disease. Owing to the changing cropping systems and climatic conditions, the incidence of this disease in groundnut has been increasing in several geographical areas over the past decade (Chen et al. 2018). The pathogen has a wide host range, it can infect around 500 plant species, including groundnut (Kasundra and Kamdar 2016). Under normal conditions, stem rot can cause a yield loss of 5% to 25% (Mayee and Datar 1988), and during heavy infection under congenial weather conditions (warm and moist environments), it can cause up to 80% pod yield loss (Mehan and McDonald 1990; Kasundra and Kamdar 2016).

Growth and survival of *Sclerotium rolfsii* are influenced by several factors including temperature, soil moisture and relative humidity. Fungal growth is aided by high humidity, good aeration, and light. The ambient temperature for the development of stem rot disease is 15–35 °C (Kasundra and Kamdar 2016). The pathogen seeks its entry into the host cells through the production of cell wall degrading enzymes such as, oxalic acid, pectic acid, poly galacturonases and cellulases (Bateman and Beer 1965; Aycock et al. 1966). The principal metabolite and the primary pathogenicity factor of stem rot disease is the oxalic acid (Bateman and Beer 1965; Bateman and Miller 1966; Aycock et al. 1966). It degrades the cell wall components, causing separation of intact cells from tissues, thus leading to tissue maceration (Bateman and Beer 1965; Punja 1985; Kuan and Tien 1993). On erect plants, light to dark brown lesions appear at the collar region of the stem, followed by yellowing and wilting of the plants. Clear symptoms of the disease on plants are visible in the infected fields. Besides, mats of white mycelia are seen on

the stem and soil surface, distinctive sclerotia, initially white, which later turn brown to black are also visible. After wilting, the plants eventually die by drying or shrivelling of the foliage (Kasundra and Kamdar 2016). Stem rot infection has an indirect effect on the dry weight and oil content of groundnut kernels, in addition to lowering the quality of the pods and fodder (Bera et al. 2016b).

In groundnut and many other crops, a variety of strategies have been developed to manage *S. rolfsii* infection, including chemical application (Culbreath et al. 1995), cultural practices (Punja et al. 1986), and biological control (Benhamou and Chet 1996). Sprays of fungicides can reduce the production loss, but they are expensive and not environment friendly. In the USA, where the incidence of stem rot is high, chemical stem rot control in the years 2014 and 2015, costed estimated $16.5 million per year in year in Georgia alone (Little 2014, 2015). Further, the efficiency of chemical and cultural management of stem rot disease is frequently constrained by the pathogen's persistence in soil and vast host range (Shew et al. 1987). A potentially effective and environmentally friendly means to combat stem rot disease could be use of biological control methods along with development of disease resistant cultivars (Karthikeyan et al. 2006; Junsopa et al. 2017). Host plant resistance offers a more feasible and long-term solution for the management of stem rot disease. However, a reliable phenotyping method is a pre-requisite for the identification of resistant sources and for the development of resistant cultivars (Pande et al. 1994). The available limited investigations have reported stem rot susceptibility and resistance among groundnut genotypes (Smith et al. 1989; Grichar and Smith 1992; Shokes et al. 1993; Bera et al. 2014; Chen et al. 2018; Divya Rani et al. 2018), although only a few genotypes showed resistance. The non-uniform spatial distribution of the pathogen of the pathogen complicates the screening for resistance in the sick field (Shew et al. 1984b), besides the sensitivity of the pathogen to temperature, humidity, soil type, cropping system and host preference (Bera et al. 2014). Consequently, reliable screening that generates repeatable results is difficult. Evaluation in the field, microplots and greenhouse environments for resistance characterization helps in identifying resistant genotypes (Shew et al. 1987). Development of quick and efficient screening methods to identify resistant progenies or lines in the breeding programmes is essential. Oxalic acid assay is an indirect test to assess genotypes for stem rot disease resistance. It is independent of the environment and the pathogen. Earlier, the oxalic acid assay was employed to assess resistance to *Sclerotinia sclerotiorum,* also a soil-borne pathogen, in soybean (Wegulo et al. 1998), canola (Bradley et al. 2006), and groundnut (Bennett et al. 2015) cultivars.

Wild relatives of crop species are known to be the sources of desirable genes for a variety of biotic and abiotic stress resistance/tolerance. High levels of resistance/tolerance to groundnut stem rot are not available in the cultivated gene pool and have not been fully explored in the wild species. The two species in the primary gene pool *A. hypogaea* and *A. monticola* are tetraploids (AABB; 2n=4X=40) with A and B genomes. Most of the wild *Arachis* species belong to the secondary gene pool comprising of 30 most closely related diploid species with A, B, B(K), B(F), and D genomes, of which AA genome is the most common type (Moretzsohn 2014). These wild *Arachis* species possess resistance to diseases such as rust, early and late leaf spots, nematode, peanut mottle virus, peanut stripe virus, peanut bud necrosis virus, groundnut rosette disease and aflatoxin contamination (Dwivedi et al. 2008; Upadhyaya et al. 2011). A few resistant accessions to stem rot disease belonging to the wild *Arachis* species *A. appresipila* (PP), *A. pusilla* (TT), and *A. monticola* (AABB) have been identified earlier (Bera et al. 2016c) and there is scope to study the wild *Arachis* species and their derivatives to identify stem rot resistance.

Variability assessment also assists the peanut breeders in developing an appropriate breeding strategy based on the host's genetic resistance (Das et al. 2004). Hence, the present study has been conducted to evaluate and identify stable and reliable groundnut genotypes resistant to stem rot disease and to assess the genetic variability among the genotypes.

Materials and methods

Groundnut genotypes were screened for stem rot disease resistance during rainy (June to October 2021) season under different experimental conditions. The details of the experimental material and methods followed for each of the experiments are described under the following sections.

Plant material

The plant material comprised of 160 groundnut genotypes belonging to both Spanish (122) and Virginia (38) groups. These include 69 interspecific derivatives, 22 lines from a RIL population derived from an interspecific cross, 56 advanced breeding lines derived from interspecific derivatives, six advanced breeding lines from cultivated species, four lines from the International Crop Research Institute for the Semi-Arid Tropics (ICRISAT, Patancheru, India) mini-core collection (one landrace each from Nigeria and Eucador, one improved cultivar each from Sudan and USA), a high oleic line and two released groundnut cultivars (Supplementary (S) Table 1).

Experimental methods

Screening in the sick field at international crops research institute for the semi-arid tropics (ICRISAT), Patancheru

The ICRISAT's experimental plots are located at 17.51°N, 78.27°E, 545 m above mean sea level. During the screening period, the maximum and minimum temperatures ranged from 23.6 °C to 33.6 °C and 11.8 °C to 23.6 °C, respectively. Mean relative humidity (RH) was 89.57% (Fig. 1). The soil type at the site was red sandy loam soil with a pH of 7.0–7.5. The experiment was laid in an alpha-lattice design (16×10) with two replications. Sowing was done on broad-bed (1.5 m width) and furrow system. Each replication was divided into ten identical sized homogenous blocks, with sixteen genotypes per block. Each genotype was sown in a single row of 4 m each with a spacing of 30 cm between rows and 10 cm between the plants. No chemicals (herbicides, insecticides, fungicides) were applied and seed was not treated with any chemical. The field was regularly irrigated using sprinkler irrigation system to ensure sufficient soil moisture.

The stem rot pathogen, *S. rolfsii,* used in this study was obtained from the groundnut pathology laboratory culture collection at ICRISAT. The isolate was cultured on potato dextrose agar (PDA) medium at a temperature of 25 ± 2 °C and stored for future use. The fungal pathogen was further mass multiplied on sorghum grains. Sorghum grains were soaked overnight and autoclaved the next day for 15 min at a temperature of 121 °C and 15 lbs pressure. Sterile sorghum grains were filled in autoclavable polythene bags (500 g) and inoculated with mycelium of *S. rolfsii*. The inoculated bags were incubated at room temperature for 3–4 weeks to allow the fungus to grow. For artificially infecting the groundnut plants, pathogen inoculum was applied once at 20–25 days after sowing (DAS) and next at 50 DAS near the collar region (basal portion) of each plant by digging a furrow adjacent to each of the plant rows. 7–10 g of inoculum was applied to each groundnut plant. The field was irrigated on the day of inoculum application to ensure sufficient soil moisture for the pathogen establishment and growth. Irrigation was given every alternate day from the application of inoculum till 15 days to create congenial growth conditions for the pathogen (Fig. 2a). The total plant count (number of germinated plants) was recorded before inoculation. After the application of inoculum, the disease was assessed based on the number of plants that were infected and dead. The total number of plants that were infected and dead were noted at 30, 60 and 90 DAS. The dead plant count included the infected plant count. The number of infected and dead plants were then represented as percentage of infected plants (PDI) and percentage of dead plants (PM) for assessing the disease.

$$\text{Percent Disease Incidence (PDI)} = \frac{\text{Number of infected plants}}{\text{Total number of plants}} \times 100$$

$$\text{Percent Mortality (PM)} = \frac{\text{Number of dead plants}}{\text{Total number of plants}} \times 100$$

Based on the PM, genotypes were classified according to the following scale (Kasundra and Kamdar 2016):

Percent mortality (PM)	Classification
< 10	Highly resistant (HR)
10–19	Resistant (R)
20–29	Moderately Resistant (MR)
≥ 30	Susceptible (S)

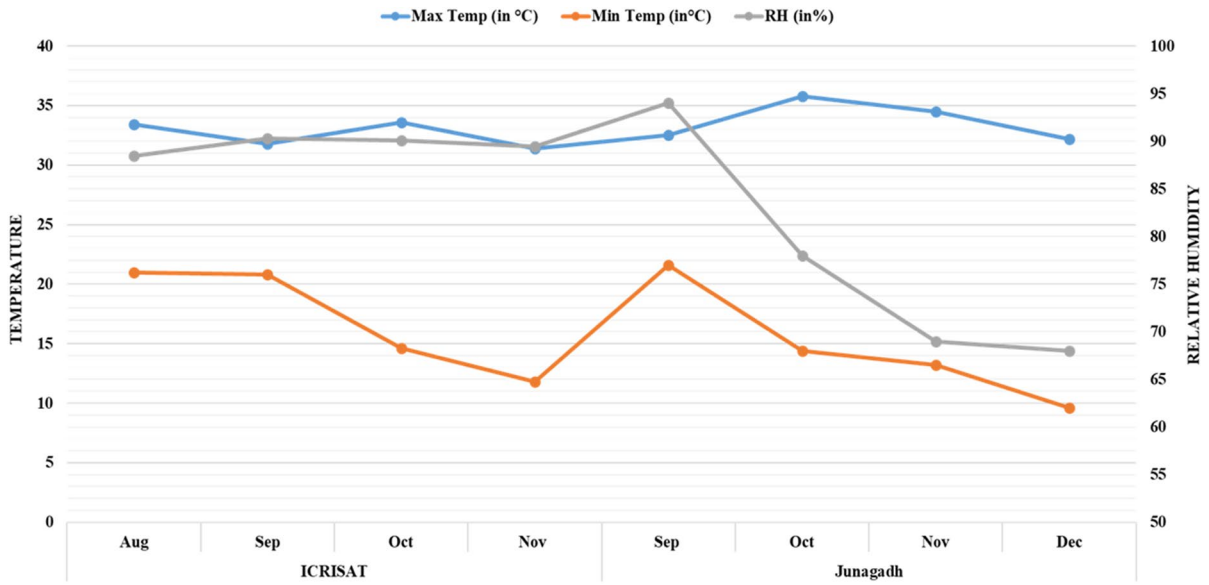

Fig. 1 Temperature and Relative humidity during the experimental period at International Crop Research Institute for the Semi-Arid Tropics (ICRISAT) (August to November 2021) and Junagadh under normal humid conditions (September to December, 2021)

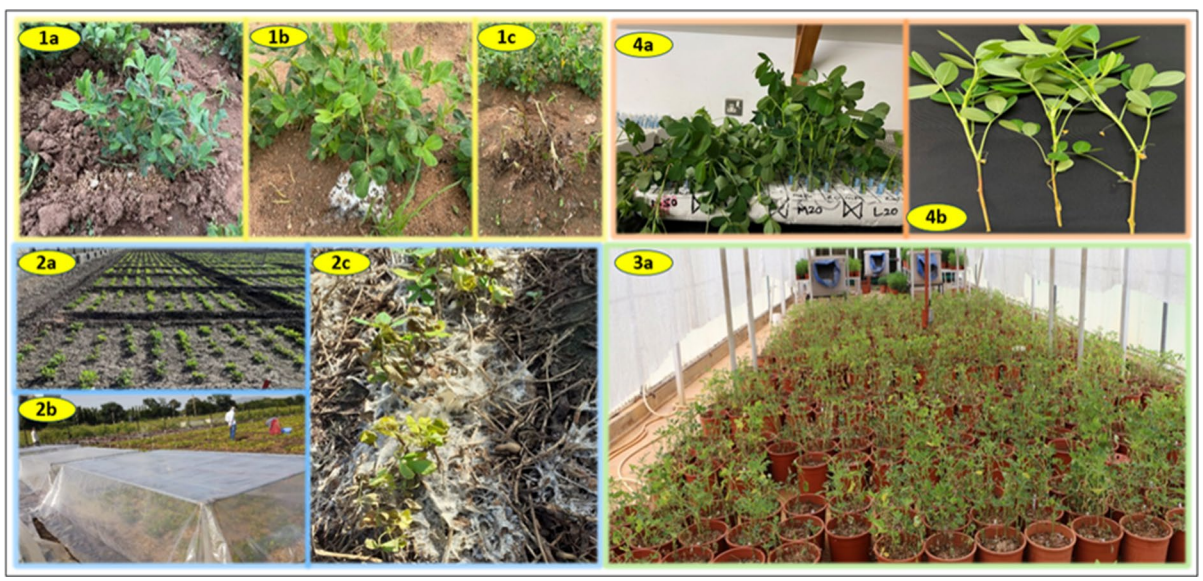

Fig. 2 Stem rot disease screening experiments: 1a-1c Sick field screening at the International Crop Research Institute for the Semi-Arid Tropics (ICRISAT). **1a** Depicting infection to healthy plant by artificial inoculation, **1b** Multiplication of the pathogen and formation of white mycelia, Fungus attacking the collar region of the stem and infecting the healthy plants, **1c** Progressed stage of infection leading to death of groundnut plants; **2a–c** Sick field screening at the Indian Council of Agricultural Research - Directorate Groundnut Research (ICAR-DGR) **2a** Showing healthy groundnut plants before inoculation, **2b** Covering the screening block with polythene sheet, **2c** White fungal mat developed all along the plant row, showing infected and dead plants; **3a** Showing the screening of groundnut lines under controlled conditions in the polyhouse; **4a–b** Oxalic acid assay **4a** The detached stems of 42-day old plants were placed in 50 mM and 20 mM oxalic acid solution, showing the difference among the same genotypes in 50 mM and 20 mM solution, **4b** Depicting stem lesions (discolouration of stems). (color figure online)

Disease screening in the pots under polyhouse conditions at ICRISAT

The 160 groundnut genotypes screened in the sick field, were also assessed for stem rot disease resistance in pots under controlled conditions in the polyhouse (23.8 m × 6.1 m) made of polycarbonate sheets. Temperature of 18–25 °C and relative humidity of 80–90% were maintained inside the polyhouse. The temperature was regulated by two coolers and a cooling pad, while humidity was preserved by the screens. The experiment was conducted in a randomized complete block design (RCBD) with two replications. Plastic pots of 22.8 cm were used. The pots were filled with a sterilized soil mixture of sand and red soil in the proportion of 3:2 and five seeds were sown per pot.

The pathogen inoculum preparation remains the same as the field screening experiment. At 20–25 DAS, the inoculum was applied to the collar region of each plant (~ 7 g per plant) in all the pots (Fig. 2c). Before the inoculum application, total plant count was taken for all the genotypes in both replications. For the disease assessment, observations namely, number of infected and dead plants were taken at 30, 45, 60 and 90 DAS. At 90 DAS (final stage), disease score was also recorded using a rating scale. The disease rating scale ranged from 1–5, where 1 denotes a healthy plant (resistant), 2 denotes lesions only on the stems (moderately resistant), 3 denotes up to 25% of the plant branches are symptomatic (moderately susceptible), 4 denotes 26–50% of the plant branches are symptomatic (susceptible), and 5 denotes > 50% of the plant branches are symptomatic (highly susceptible) (Shokes et al. 1996). PDI and PM were calculated from the number of infected and dead plants respectively as done for the field experiment.

Disease screening at ICAR-DGR, Junagadh

All the groundnut genotypes were assessed for stem rot disease in the sick field at the Indian Council of Agricultural Research - Directorate of Groundnut Research (ICAR-DGR; 21.52°N latitude and 70.47°E longitude, 107 m above mean sea level). During the screening period, the maximum and minimum temperatures ranged from 23 °C to 35.8 °C and 9.6 °C to 26.5 °C, respectively. Mean relative humidity (RH) was 77.2% (www.jau.in) (Fig. 1). The soil type was black (vertisol).

The experiment was laid in an alpha-lattice design (16 × 10) with two replications. Genotypes were planted in a single row on a 2-m-wide bed, with 30 cm between lines and 10 cm between plants. The experimental method, the preparation of inoculum, and the field screening protocol was conducted as described by Kasundra and Kamdar (2016). Apart from the plant protection measures against stem rot, the crop was grown in accordance with the prescribed package of practices for groundnut. The experiment was conducted under two conditions in the same sick field. One, wherein the entire screening plot was covered after inoculation with 1 mm thick low density polyethylene sheet (to increase the humidity up to 90% and prevent moisture loss), and another, without the covering of polythene sheet under natural conditions (Fig. 2b). For disease assessment, percent mortality (PM) was recorded 15 days after inoculation (or 75 days after sowing) and expressed as a percentage.

Response to oxalic acid assay under laboratory conditions

Of the 160 genotypes, twenty-nine selected resistant/moderately resistant (14) and susceptible (15) genotypes were subjected to the oxalic acid assay in laboratory. The groundnut genotypes were raised in 8-inch pots in the polyhouse for 42 days at 28 ± 2 °C. Oxalic acid (obtained from Qualigens Chemicals & Reagents by ThermoFisher Scientific) was prepared in two concentrations, 20 mM and 50 mM. For the preparation of one litre oxalic acid solution, 2.52 g and 6.3 g of oxalic acid was dissolved in distilled water and made up to one litre for 20 mM and 50 mM solutions, respectively. A 0 mM concentration was used as control to compare the symptoms. On the 42nd day (6-week-old plants), the main stems and lateral branches were detached and immediately immersed indistilled water. Later, the main stems and lateral branches were dried with tissue papers and immersed in 0-, 20- and 50 mM oxalic acid solutions in 5 ml tubes. The tubes were filled with 3.5 ml of each concentration of oxalic acid solution and refilled whenever required. Three main stems and three lateral branches of each genotype were used *i.e.,* replicated three times and the whole setup was kept in incubator at 22 ± 2 °C up to 36 h. Observations were taken at

12, 18, 24, 30 and 36 h based on a 1–6 disease rating scale (Bennett et al. 2015) 1 = No wilt symptoms, 2 = one leaf wilted, 3 = two leaves wilted, 4 = one petiole collapsed, 5 = two petioles collapsed, 6 = branch stem collapsed and lesion length was also measured at final hour observation (after 36 h) (Fig. 2d).

Data analysis

Arcsine transformation was applied for both PDI and PM. For ICRISAT field and pot experiment data, repeated measures analysis using SAS mixed procedure (SAS Institute, Inc. 2018, SAS V 9.4) was applied. Genotypes were considered as between-subject factors, the DAS (repeated observations at 30, 60, and 90-day intervals) were considered as within-subject factor, DAS and replication were taken as fixed factors, and genotype as a random factor. A first-order auto regressive [AR (1)] covariance structure was used for the data, which was selected based on the AIC (Akaike Information Criteria). BLUPs (Best Linear Unbiased Predictors) were calculated for genotype effects from the analysis of variance (ANOVA).

The genetic parameters such as genotypic coefficient of variation (GCV), phenotypic coefficient of variation (PCV), genetic advance as a percentage of mean (GAM) and repeatability were calculated. The heritability was estimated as repeatability (Falconer 1989). Based on the values obtained for PCV and GCV, they were categorized into low (< 10%), medium (10–20%) and high (> 20%) based on the scale given by Sivasubramanian and Madhavamenon (1973). The estimates of heritability were categorized as high (> 60%), moderate (30–60%) and low (0–30%) (Robinson et al. 1949). Genetic advance was calculated and classified as high (> 20%), moderate (10–20%) and low (< 10%) as given by Johnson et al. (1955).

ANOVA was performed using SAS mixed model procedure (SAS Institute, Inc. 2018, SAS V 9.4) for ICAR-DGR sick field experiment, to assess the effect of genotypes. Genotypes and nested effect of block within replication were considered as random effects and replication as fixed effect. BLUPs were estimated for genotype effects from the ANOVA.

A combined ANOVA was performed for the oxalic acid experiment using a mixed model analysis approach to assess the main and interaction effects of concentration, time, and genotype effects which were considered as fixed effects. The individual variance of concentration is modelled to error distribution using the residual maximum likelihood (REML) method by the SAS mixed procedure (SAS Institute, Inc. 2018, SAS V 9.4). BLUEs were estimated for all main and interaction effects of all factors from the combined ANOVA.

Pearson's correlation coefficients were estimated between stem rot disease assessment traits using the SAS PROC CORR procedure.

Results

Observations from the stem rot disease sick field at ICRISAT

Analysis of variance (ANOVA) showed that the genotypes did not show significant differences for percent disease incidence (PDI), while for percent mortality (PM), highly significant differences ($p < 0.01$) were observed for the genotypes and DAS × genotypes interaction effect (Table 1). For PM, moderate heritability of 57% and high GAM of 46.05% was observed (Table 2). PDI at 30 DAS showed a highly significant ($p < 0.01$) and positive correlation with PDI at 60 DAS (r = 0.44**) and 90 DAS (r = 0.36**). PM at 30 DAS showed a highly significant ($p < 0.001$) and positive correlation with PM at 60 DAS (r = 0.67***) and 90 DAS (r = 0.70***). A highly significant ($p < 0.001$) and positive correlation was found between PDI and PM at 30 DAS (r = 0.67***) and between PDI and PM at 60 DAS (r = 0.4***) (Table 3). The stem rot disease progressively increased with time. At 30 and 60 DAS, percent disease incidence (PDI) was 14–28% and 68–83%, respectively, and at 90 DAS, 100% PDI was observed. Percent mortality (PM) ranged between 0–18%, 2–37% and 13–80% at 30, 60 and 90 DAS respectively (S Table 2). Out of the 160genotypes,, 10 were resistant, 40 were moderately resistant and 110 were susceptible as per the scale given by Kasundra and Kamdar (2016). The ten resistant genotypes are, ICGR 161954, ICGR 161940, ICGV 181045, ICGV 11447, ICGR 162036, ICGR 162035, ICGR 161932, NRCGCS-224, VG 1007, and ICGR 161951 with their PM varying from 13 to 19%. Nine of these genotypes (except ICGV 181045), have been derived from interspecific derivatives of wild

Table 1 Repeated measures analysis for the International Crop Research Institute for the Semi-Arid Tropics (ICRISAT) sick field and pot experiments for stem rot disease assessment traits

Source of variation	Sick field screening at ICRISAT		Pot screening at ICRISAT		
	PDI	PM	PDI	PM	DS
	Random effect (Variance component)				
Genotype	0.001	0.014**	0	0.009	0.005*
DAS × Genotype	0.004	0.011**	0.009**	0.001	
AR (1)	0.29	0.32	0.49	0.74	
Residual	0.04	0.03	0.07	0.15	0.02
	Fixed effect F-statistic (Type-III)				
Replication	2.04	4.24*	4.86*	0.3	0.39
DAS	1959.74***	399.72**	531.9**	275.37**	
DAS × Replication	1.31	9.94**	3.18*	9.92**	

*, **, *** are significant at $p < 0.05$, $p < 0.01$, $p < 0.001$ respectively

DAS, Days after sowing; PDI, Percent disease incidence; PM, Percent mortality; DS, Disease score; AR, Auto-regressive model.

Table 2 Genetic parameters for stem rot disease assessment traits at the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) and Indian Council of Agricultural Research - Directorate Groundnut Research (ICAR-DGR)

Variability parameters	ICRISAT sick field		ICRISAT pot			ICAR-DGR	
	PDI	PM	PDI	PM	DS	PM _1	PM _2
GCV	3.43	29.67	0.00	16.46	3.50	10.39	24.43
PCV	9.71	39.32	8.48	29.27	6.59	20.33	42.18
Repeatability (%)	12.00	57.00	0.00	32.00	28.00	17.00	43.00
GA% of Mean	2.49	46.05	0.00	19.04	3.82	6.95	36.95

PDI—Percent disease incidence; PM—Percent mortality; GCV—Genotypic coefficient of variance; PCV—Phenotypic Coefficient of variance; GA—Genetic advance as percent of mean; PM_1—Percent mortality recorded under condition 1- with the covering of polythene sheet (high humidity conditions); PM_2—Percent mortality recorded under condition 2- without the covering of polythene sheet (natural conditions)

Table 3 Association between the stem rot disease assessment traits for the International Crop Research Institute for the Semi-Arid Tropics (ICRISAT) sick field experiment

	PDI (30 DAS)	PDI (60 DAS)	PDI (90 DAS)	PM (30 DAS)	PM (60 DAS)	PM (90 DAS)
PDI (30 DAS)		0.44**	0.36**			
PDI (60 DAS)			−0.03		0.4***	
PDI (90 DAS)						0.06
PM (30 DAS)	0.67***				0.67***	0.70***
PM (60DAS)						0.66**

PDI—Percent disease incidence; PM—Percent mortality; DAS—Days after sowing

*, **, *** are significant at p < 0.05, p < 0.01, p < 0.001 respectively

Arachis species namely, *A. diogoi* (all ICGR lines), *A. cardenasii* (ICGV 11447), *A. villosa* (NRCGCS 224), *A. correntina* (VG 1007).. One line that was resistant (ICGV 181045), was derived from cultivated groundnut species. Among the 40 moderately resistant lines, PM ranged between 20 and 29.43%, thirty-five of these lines were developed from the interspecific derivatives. The other six lines (1 resistant and 5 moderately resistant) are not interspecific- derivatives to our best knowledge. Four of these were derived

from cultivated species (ICGV 03043, ICGV 06188, ICGV 00440, ICG 721), one is a mini-core line which is a land race from Nigeria (ICG 14482), the pedigree details of these lines are given in the S Table 1.

Observations from the pot experiment at ICRISAT

Under the controlled conditions in pots, every plant was infected. The disease progressively increased with time to reach 100% PDI by 60 DAS. From the ANOVA (Table 1), non-significant effect of genotype for both PDI and PM was observed. DAS was highly significant ($p<0.01$) for both PDI and PM. DAS × genotype interaction effect was significant ($p<0.01$) for PDI and non-significant for PM. At 30 and 45 DAS, PDI was 28.73–62.51% and 89.90–98.55%, respectively, whereas by 60 DAS, PDI was 100%. The PM at 30, 45, 60 and 90 DAS was 1.49–8.40%, 18.61–33.72%, 33.50–52.32%, and 48.59–67.14% respectively (S Table 2). The disease score ranged from four to five for all the genotypes, and none of the genotypes could be considered resistant based on the disease score scale by Shokes et al. (1996). Low heritability for PDI (0%), disease score (28%) and moderate heritability for PM (32%) was observed in the pot experiment (Table 2). PDI at 30 DAS showed a positive significant correlation ($p<0.01$) with PM at 30, 45, 60 and 90 DAS (r = 0.46** to r = 0.51**), while PDI at 45 DAS did not show significant correlation with PM. Significant correlations were observed between PM at 30 DAS with PM at 45, 60, 90 DAS (r = 0.93** to r = 0.94**), and PM at 45 DAS with PM at 60 and 90 DAS (r = 0.96**) and PM at 60 DAS with PM at 90 DAS (r = 0.91**) (Table 4).

Table 5 Analysis of variance for Indian Council of Agricultural Research - Directorate of Groundnut Research (ICAR-DGR) sick field experiment for percent mortality at 75 days after sowing (DAS)

Source of variation	Condition 1	Condition 2
Block	0.001	0.001
Genotype	0.009	0.01**
Replication	11.28**	41.76***
Residual	0.05***	7.16

*, **, *** are significant at $p<0.05$, $p<0.01$, $p<0.001$ respectively

Condition 1- with the covering of polythene sheet (high humidity conditions); Condition 2-without the covering of polythene sheet (natural conditions)

Observations from the experiments conducted at DGR, Junagadh

The same set of 160 groundnut genotypes were screened at ICAR-DGR, Junagadh to assess their disease reaction under two different humidity conditions, viz., normal (natural conditions) and high humidity (> 90%) conditions. Percent mortality (PM) was recorded 15 days after inoculation (or 75 DAS). For condition 1 (with the covering of the polythene sheet), which has high humidity, ANOVA indicated non-significant genotypic variation (Table 5) and PM ranged from 51.22 to 80.58% at 15 days after inoculation (S Table 2) indicating severe disease pressure. A low heritability (17%) and low GAM (6.95%) was observed. None of the genotypes recorded a score that could categorise them as resistant due to high disease pressure (Table S2). For condition 2 (without the covering of polythene sheet), which represent the

Table 4 Association between the stem rot disease assessment traits for the International Crop Research Institute for the Semi-Arid Tropics (ICRISAT) pot experiment under controlled conditions

	PDI (45DAS)	PDI (60DAS)	PM (30DAS)	PM (45DAS)	PM (60DAS)	PM (90DAS)
PDI (30DAS)	−0.29**	−0.14	0.49**	0.50**	0.51**	0.46**
PDI (45DAS)	1	−0.08	−0.11	−0.1	−0.08	−0.14
PDI (60DAS)		1	−0.12	−0.11	−0.09	−0.12
PM (30DAS)			1	0.93**	0.93**	0.94**
PM (45DAS)				1	0.96**	0.96**
PM (60DAS)					1	0.91**

PDI—Percent disease incidence; PM—Percent mortality; DAS—Days after sowing

*, **, *** are significant at p < 0.05, p < 0.01, p < 0.001 respectively

natural conditions, ANOVA revealed highly significant ($p < 0.01$) genotypic differences (Table 5). PM ranged between 8.24 and 58.62%. Two genotypes, ICGL 17105 and ICGR 162010 (both inter-specific derivatives) with PM of 8% were identified as resistant sources under this condition (Table S2). A moderate heritability (43%) and high GAM (36.95%) was observed (Table 2).

The oxalic acid assay to assess stem rot disease resistance

Selected resistant (14) and susceptible (15) genotypes from the ICRISAT sick field experiment were subjected to the oxalic acid assay. For disease score, there were highly significant ($p < 0.001$) differences for main effects (concentration, time, and genotypes) and their interaction effects for both main stems and laterals in response to the disease. For lesion length, ANOVA revealed highly significant differences ($p < 0.001$) for main (concentration and genotypes) effects and their interaction effects for both main stems and laterals. (Table 6). Disease score is the indicator of wilting of genotypes. No wilting or lesions appeared at any time for lateral and main stems immersed in water (0 mM oxalic acid), hence the data was not reported. Wilting of laterals and main stems progressed with time from 12 to 36 h and was more severe in the stems immersed in 50 mM oxalic acid solution as compared to 20 mM oxalic acid solution. Since the thickness of the laterals is lesser than the main stems, the oxalic acid solution could seep into the laterals faster than the main stems, thus causing earlier and more severe wilting of the laterals in both concentrations. During the final observation recorded at 36 h of immersion, stems of all the genotypes immersed in 50 mM solution, showed a higher disease score compared to the ones immersed in 20 mM solution. For all the genotypes, in both main stems and laterals, the disease score was ~ 6 in 50 mM oxalic acid solution, while in 20 mM solution, the disease score varied between one and six (S Table 3). Lesion length was 0.53–7.50 cm and 1.00–4.25 cm for main stems and laterals respectively for the 20 mM solution, whereas for 50 mM solution, it was 3.00–11.73 cm and 4.20–11.93 cm for main stems and laterals respectively (S Table 4). Seventeen of the twenty-nine genotypes with a disease score of one, four genotypes with a score of two and two genotypes with a score of three were observed, when their main stems were immersed in 20 mM oxalic acid solution. However, the lesion length varied among them. Twelve of these seventeen genotypes (with score 1) showed a smaller lesion length (0.53–1.80 cm) compared to other genotypes.

Stem rot resistant sources

Percent mortality (PM) from the ICRISAT sick field screening, had a good differentiating ability (13–80%) among the genotypes. From the screening under natural conditions at DGR, where the PM was 8–58% (75 DAS), the genotypes ICGL

Table 6 Analysis of variance of fixed effects (Type-III) of oxalic acid concentration (mM), duration of immersion (time) on the disease score and lesion length

	Effect	Lateral stem Fixed effect (F Value)	Main stem
Disease score	Concentration	16,582.90***	13,648.50***
	Time	265.34***	465.62***
	Concentration*Time	45.16***	36.06***
	Genotype	65.59***	47.96***
	Concentration*Genotype	59.60***	35.49***
	Time*Genotype	6.80***	6.96***
	Concentration*Time*Genotype	6.79***	7.3***
	Residual		
Lesion length	Concentration	923.05***	1676.78***
	Genotype	6.21***	32.39***
	Concentration*Genotype	4.82***	8.95***
	Residual	0.747***	0.4805***

***significant at $p < 0.001$

17105 and ICGR 162010 were identified as resistant sources. Pot screening in controlled conditions at ICRISAT and high humidity condition screening at ICAR-DGR, indicated heavy disease pressure and all the genotypes succumbed to disease. The genotypes ICGR 161939, ICGR 162044, ICGR 162032, and ICGV 10342 and ICGV 181045 were found to be the best sources of stem rot resistance from this study. They have shown moderate resistance under ICRISAT sick field conditions, and less infection compared to the other genotypes even under heavy disease conditions. Also, through the oxalic acid assay, it was revealed that the genotypes ICGR 161939, ICGR 162044 and ICGR 162032 recorded low wilting disease score (1–2) and less lesion length (1–3 cm).

Discussion

The primary gene pool of *Arachis s*pecies consists of two tetraploid species viz., wild species *A. monticola* (AABB) and the cultivated groundnut *A. hypogaea* (AABB), and the secondary gene pool comprises of 30 different diploid wild species with AA, BB, DD, FF genomes. The wild diploid species of *Arachis* are an important source of resistant genes against several traits of agronomic importance, particularly resistance to diseases. An example of transfer of resistance from secondary gene pool into cultivated species involved use of an accession, GKP10017 (PI 262141) belonging to wild diploid species, *A. cardenasii* in the US breeding programmes for transferring resistance to both early and late leaf spots (Moretzsohn 2014). ICRISAT, through the mechanism of sharing improved genetic material to the collaborators in Asia and Africa shared improved foliar fungal disease resistant germplasm of groundnut with the genomic regions from wild *A. cardenassi* resulting in commercialization of several cultivars with moderate resistance to foliar fungal diseases (Bertioli et al. 2021). For stem rot disease resistance, wild *Arachis* species, *A. appresipila* (PP), *A. pusilla* (TT) and *A. monticola* (AABB) (Bera et al. 2016b), *A. batizocoi* (Guclu et al. 2020) were reported to be potential sources.

Stem rot in groundnut is an economically important disease affecting the production across the groundnut growing regions of Asia, Africa and America. Research has shown that the pod yield reduction can go up to 80% when the disease incidence is high (Thiessen and Woodward 2012; Bera et al. 2016c). Application of fungicides can reduce the yield loss, but they are expensive and not eco-friendly. The cost estimated was $16.5 million per year in Georgia alone in 2014 and 2015 in the USA, where the prevalence of stem rot is high (Little 2014, 2015). Managing the stem rot disease by achieving host-plant resistance is highly desirable given the soil-borne nature of the disease and is an environmentally sustainable option. Earlier, some cultivars with moderate resistance to stem rot including, Bailey (Isleib et al. 2011), NC 3033 (Beute et al. 1976), York (Gorbet & Tillman 2011) and Florida-07 (Gorbet & Tillman 2009) have been released in the United States through field screening.

Sources of resistance for groundnut stem rot disease were identified using different screening methods that assess disease incidence and severity and the most common method is screening under sick field conditions (Santosh kumar et al. 2011; Revankar et al. 2018; Bennett and Chamberlin 2020; Fan et al. 2020). Screening for stem rot resistance has remained a challenge in making progress towards host-resistance to stem rot disease in groundnut owing to cost involved in maintaining sick plot and screening in sick plot, and the variability within the sick plot because of inoculum load, microclimate and environmental conditions. Among the methods of stem rot screening used by researchers, the stage of inoculum application, disease assessment criteria and time of recording observations vary. The disease incidence was studied both at harvest (Gorbet et al. 2004b) and seedling stage (35 days after sowing) (Bera et al. 2016c). In the present study, different screening methods, experimental and disease pressure conditions were employed to assess genetic variability among 160 groundnut genotypes that include 147 interspecific derivatives.

At ICRISAT stem rotsick field, the results showed that the disease slowly progresses with the time. The incidence of stem rot increases gradually towards the maturity of the crop (Bera et al. 2016b), however, the disease development is slower in older plants than in younger plants (Pande et al. 1994; Bekriwala et al. 2016). As the plants mature, their stems thicken and thick stems inhibit the entry and establishment of the pathogen, thus the development of disease is slower in older and mature plants. The appearance of

the disease is clearly noticeable at 30–45 DAS and at the time of harvest (Bera et al. 2014) and earlier research employed this (Santosh Kumar et al. 2011; Revankar et al. 2018). Hence in the present study, the pathogen inoculum was applied at 20–25 DAS under both ICRISAT field and pot screening. At ICRISAT, stem rot was assessed at 30, 60 and 90 DAS through PDI and PM in terms of number of infected and dead plants, respectively. In previous research, maximum phenotypic variability and high heritability were estimated for disease incidence (%) at 30, 60, 90 DAS and selection for stem rot resistance based on field screening employed PDI (Santosh Kumar et al. 2011; Revankar et al. 2018) and PM (Fan et al. 2020; Bera et al. 2016b). ICRISAT sick field screening results have indicated that PDI and PM at 30, 60, 90 DAS showed significant differences at different DAS, suggesting that the level of disease varied at different time periods (days). However, by 90 DAS, all the genotypes had shown 100% PDI and no significant differences were not observed among the genotypes. For PM, significant differences were observed among the genotypes and genotypes x DAS; PM was 0–18%, 2.24–36.93% and 13.16–79.59% at 30, 60, 90 DAS, respectively, indicating that the resistance of a particular genotype varied with time period. A genotype resistant at 30 or 60 DAS, succumbed to the disease at 90 DAS, which has been the case with many genotypes included in the study. Differences among groundnut genotypes for their response to stem rot disease have been earlier reported (Branch and Csinos 1987; Branch and Brenneman 1993; Gorbet 2004a; Bera et al. 2016b; Fan et al. 2020; Guclu et al. 2020).

Differences in PM were observed for the same genotypes between replications for both ICRISAT sick field and ICAR-DGR (both conditions) screenings, a consequence of non-uniform spatial distribution of the pathogen under field conditions (Shew et al. 1984b). Even when the inoculum is applied uniformly, individual plants might escape the disease (Shokes et al. 1998). This disease shows a clustered spatial pattern/irregular pattern under field conditions (Shew and Beute 1984a), and hence stem rot results would vary from one plot to another. Similar results were also reported by Bowen (2003). Also, the microclimate for the same genotype under different replications might be different. Microclimate includes factors such as plant architecture, soil moisture and soil temperature (Munir et al. 2021). Soil moisture and soil temperature have been reported as important factors that influence the development of stem rot disease (Punja 1985). Low soil moisture is often associated with a greater number of dead plants (Bowen 2003). Disease incidence is usually high in well-drained sandy soils (Weerapat 1964), so is the ICRISAT sick soil. Fluctuating soil moisture and temperature levels between the replications could have affected PM counts for the same genotype. Plant architecture, including plant density, canopy structure and growth habit of the genotypes can influence plant microclimates and thus the development of disease (Bailey and Brune 1997; Shokes et al. 1998; Kora et al. 2005). Under field conditions, a genotype showing different PM under two different replications could be due to any of the plant architectural features. A dense canopy increases the humidity compared to a sparse canopy and can cause a greater multiplication of the pathogen. When a genotype under study (bunchy/runner) is adjacent to a runner genotype in one replication and bunchy type in the other, the disease incidence might vary for the same genotype. When adjacent to a runner, it would have higher disease as the runners have more plant tissue coming in contact with the soil, thus causing spread of the disease to the genotypes around it (Agmon et al. 2022). Thus, when assessing for *S. rolfsii* resistance, several replications in field plots are required to overcome the effects of the pathogen and the microclimatic factors (Shokes et al. 1998).

The infection rate is much higher in the controlled conditions than the field conditions at ICRISAT, as all the congenial conditions for the pathogen growth coexist in controlled condition. Infection was observed in all the genotypes. As the pathogen inoculum had a restricted surface area for growth, it could grow well and no plant could escape the disease, consequently, the disease pressure was high. By 60 DAS, 100% PDI was observed in most genotypes. The genotypes recorded a PM of 48–67% at 90 DAS indicating all the genotypes are susceptible (PM of > 30%). Similar results were observed by Pande et al. (1994) and Bekriwala et al. (2016), where highest susceptibility of 79–100% PDI was noticed by 45 DAS, beyond which there was not much differentiability. Highly significant differences obtained for the trait PDI for DAS and DAS x genotype indicates that disease incidence varied with the time periods and a differential response was seen over the days among the

genotypes. Both PDI and PM showed significant differences for replications and DAS x replications. PDI and PM could have varied between the replications due to the variable growth of the pathogen over the days. Also, the microclimate factors (soil moisture, soil temperature) might have influenced the disease (Punja et al. 1985; Munir et al. 2021). For research in controlled conditions (glasshouses/polyhouses), PDI (Divya Rani et al. 2018), PM (Pande et al. 1994) and disease scores (Guclu et al. 2020) were used to assess the disease.

Screening experiment conducted at ICAR-DGR examined the disease reaction of the genotypes under two different conditions. The first condition was created by covering with polythene sheet to ensure maximum growth and proliferation of the pathogen by providing hot, humid and moist conditions that are highly congenial for the pathogen, while the second represented normal environment without any steps to manipulate humidity, temperature and moisture. Under polythene sheet, the disease pressure was high, and all the genotypes succumbed to the disease and no significant differences for PM among them. Low heritability (17%) values for PM indicated that screening under heavy disease pressure conditions by disturbing plant innate immunity systems is not an appropriate method to select resistant genotypes. Under second condition, the PM was 8–58% at 75 days after sowing with moderate heritability (43%). Screening is most reliable and efficient when conducted under conditions similar to those in which the crop is grown along with adequate inoculum load favouring full symptom expression (Hahn et al. 1989). Under such high humid screening conditions, the environmental conditions vary and such variations from the optimum levels weaken plant's natural immunity (Velásquez et al. 2018).

Field and greenhouse screening methods despite being the most widely used screening methods, are time consuming and resource intensive. Breeding for stem rot resistant groundnut cultivars would advance more quickly with rapid phenotyping procedures. An alternate and indirect screening method for stem rot disease is the oxalic acid assay (OAA). However, the correspondence of OAA with field screening results must be ascertained. OAA is a laboratory-based assay in which many plants can be screened within a weeks' time and has the benefit of screening without the presence of the pathogen. Oxalic acid is released by the pathogen *Sclerotinia* spp. (Godoy et al. 1990; Livingstone et al. 2005) and *S. rolfsii* (Bateman and Beer 1965; Kritzman et al. 1977) as a major pathogenic metabolite and the OAA identifies the genotypes based on their physiological resistance mechanisms (Kolkman and Kelly 2000) in oxalic acid solution. OAA was earlier used to screen crops such as, common bean (Kolkman and Kelly 2000), sunflower (Noyes and Hancock 1981), soybean (Cunha et al. 2010) and groundnut (Bennett et al. 2015) for resistance to *Sclerotinia sclerotiorum.* By inoculating detached shoots from 6-week-old plants with *S. rolfsii* mycelial plugs, Akem and Dashiell (1991) discovered variations in resistance among soybean genotypes. In the current study, after the immersion of detached stems in oxalic acid solution, disease ratings were taken at regular intervals of 6 h (from 12–36 h), and this reduced the variability due to environmental conditions (Kolkman and Kelly 2000). Based on an earlier study it was reported that the pathogen *S. rolfsii* produces highest concentration of oxalic acid of 20 mM (Amaro et al. 2022). In our study, two different concentrations of oxalic acid, 20 mM and 50 mM along with a control (0 mM oxalic acid solution) were used to test the response of genotypes. No symptoms appeared in control, thus validating the results. Variance analysis revealed highly significant differences for the traits wilting severity and lesion length of both main stems and laterals over the time periods in both the concentrations, significant differences were observed among the genotypes with respect to wilting of the main stems and laterals. The laterals of all genotypes succumbed to the disease at an earlier duration than the main stems, however, the level of wilting (disease score) varied at different time periods among the genotypes. Some genotypes wilted earlier than the others. The interaction effects of all these factors (concentration, time, genotypes) showed significant differences, indicating the dependency of one factor on the other. Similar results were obtained by Bennett et al. (2015), genotypes when placed in 20- and 50 mM oxalic acid solutions, showed variations over time periods and significant three way interaction was observed for laterals. Wilting of stems gradually progressed with time. To assess the disease response of the genotypes, wilting score in 20 mM solution is more appropriate than the scores for 50 mM solution as 50 mM solution screening was comparable to heavy disease pressure screening and

all the genotypes succumbed to the disease by the end of the experiment. The best performing genotypes were decided based on the final disease score at 36 h of main stems immersed in 20 mM solution. However, lesion length and disease score are not correlated, hence the genotypes with lower disease score (1–3) and less lesion length can be considered are resistant.

It is important to screen groundnut genotypes for stem rot disease resistance under field conditions with artificial inoculation of the pathogen as the crop has to be ultimately produced in the field (Bowen 2003). But, results from one field to another, between field and controlled environments vary. Certain genotypes show resistance in the field but fail under controlled conditions (Singh et al. 1997). The time, place and experimental conditions are very important for stem rot disease development, as the incidence and onset of stem rot varies depending on planting date, and weather conditions (Bowen 2003). The inheritance of stem rot disease resistance is complex, which is governed by the cumulative effects of several genes (Bera et al. 2016a, b, c a; Dodia et al. 2019) and is greatly influenced by environmental conditions (temperature, humidity, moisture). Hence, it is important to evaluate the genotypes in the field and controlled environments to characterize stem rot resistance (Shew et al. 1987), so as to find stable sources of resistance. As PM appeared to be more useful than PDI for differentiating the genotypes, results of PM from all the experiments were compared to identify the best genotypes. Sick plot screening at ICRISAT has been used for making selections due to a good genetic variability.

In our study, through the ICRISAT sick field screening, 50 out of 160 genotypes showed PM < 30%, of which 10 were resistant with PM ranging from 13 to 19% and 40 were moderately resistant with PM of 20–29.43%. Among the 50 genotypes, 44 (9 resistant and 35 moderately resistant) were bred using inter-specific derivatives of wild *Arachis* species namely, *A. villosa*, *A. correntina*, *A. helodes*, *A diogoi*, *A. cardenasii*, *A. stenosperma* and *A. paraguariensis, A. kempff-mercadoi* and *A. hoehnei* all of which except *A. paraguariensis* (EE), comprise of the AA genome. Singh and Oswalt (1991) reported that the accessions of *A. stenosperma, A. cardenasii* and *A. villosa* showed resistance to rust and early or late leaf spots, tomato spotted wilt virus, thrips and/or aphids. The other six lines (1 resistant and 5 moderately resistant) are not interspecific-derivatives to our best knowledge. One line that was resistant (ICGV 181045), was derived from cultivated groundnut species. Among the moderately resistant lines, four were derived from cultivated species (ICGV 03043, ICGV 06188, ICGV 00440, ICG 721), one is a mini-core line which is a land race from Nigeria (ICG 14482). Under heavy infection conditions/pathogen congenial conditions (controlled screening in pots and high humidity conditions of ICAR-DGR), none of the genotypes showed resistance, indicating that disease screening experiments must be conducted under natural conditions with optimum pathogen load. Under natural conditions screening at ICAR-DGR, ICGL 17105 and ICGR 162010 were identified as resistant sources at 75 DAS. The genotypes ICGR 161939, ICGR 162044, ICGR 162032 and ICGV 10342 showed moderate resistance under ICRISAT sick field, and less infection compared to other genotypes even under heavy disease conditions. The mechanisms of disease resistance among the resistant and moderately resistant lines must be separately studied and explored in the future. The OAA results indicate the resistance of the lines, ICGR 161939, ICGR 162044 and ICGR 162032 that recorded low wilt score (1–2) and small lesion length (1–3 cm). The identified ICGR lines are derived from a cross between ICGV 03057 X CS 19 and CS 19 is an interspecific derivative between the cross TMV2 × *Arachis diogoi*(AA). CS19 has been identified as a stem rot resistant line in earlier research and has been used as a parent in crossing programs (Bera et al. 2014, 2016b). Resistant genes/alleles to stem rot from these derivatives of wild accessions can be transferred into cultivated species using back-cross breeding procedures, and identification of resistant QTLs followed by development of diagnostic markers aid in foreground selection for transferring these QTLs into cultivated groundnut (Bera et al. 2016b).

Conclusion

The stem rot resistant sources identified from the study are largely the derivatives from the wild diploid *Arachis* species with AA genome. Exploring AA genome wild species for stem rot resistance is valuable. Of the10 resistant and 40 moderately resistant

genotypes identified, 44 (nine resistant and thirty-five moderately resistant) are inter-specific derivatives of wild *Arachis* species *A. villosa*, *A. correntina*, *A. helodes*, *A diogoi*, *A. cardenasii*, *A. stenosperma*, *A. paraguariensis*, *A. kempff-mercadoi* and *A. hoehnei*, allwhich except *A. paraguariensis* (EE), comprise of the AA genome. The other 6 lines are not interspecific-derivatives to our best knowledge; one resistant and five moderately resistant lines are breeding lines derived from cultivated species at ICRISAT (4) and USA (1), and one moderately resistant line is a land race from Nigeria. The inter specific derivatives, ICGR 162032, ICGR 161939, ICGR 162044 and advanced breeding lines ICGV 10342, and ICGV 181045 were identified as promising genotypes, which can be used for developing bi-parental populations for QTL mapping. The identified lines are in elite genetic background which can be directly used in breeding programs as parents. Stem rot disease screening at ICRISAT in disease sick plot under natural conditions with optimal disease pressure discriminated the genotypes for stem rot resistance effectively. Under severe disease pressure in pot and field is not useful as all the genotypes succumbed to disease. Oxalic acid assay (OAA), a robust and quick method is helpful in phenotyping for stem rot disease. OAA can be used for initial selection of population that can reduce the number of lines to be taken for sick field screening. OAA is a potential tool to rapidly assess large number of gene bank accessions or breeding populations.

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Declarations

Conflict of interest The authors have no relevant financial or non-financial interests to disclose.

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