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Screening of Chickpea (*Cicer arietinum* L.) Genotypes for Dry Root Rot Resistance

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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Short Research Article

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Abstract

Dry root rot in chickpea causes up to 100% loss in yield in favourable environmental conditions. Identification of resistant cultivars would be highly economical, eco-friendly and a viable solution for rainfed condition for combating the devastating pathogen. The present study was conducted to evaluate 30 chickpea genotypes for resistance to dry root rot caused by *Macrophomina phaseolina* using sick pot technique and roll towel method. The comparative evaluation of chickpea genotypes for dry root rot resistance revealed both consistent and contrasting resistance reactions. Genotypes such as Super Annigeri 1 and COC 24 02 consistently exhibited susceptibility across both methods, validating them as reliable checks. Jaki 9218 was resistant at the seedling stage (roll towel) but highly susceptible in soil (sick pot), indicating stage-specific resistance. In contrast, P2278 and NBeG 857 were susceptible in roll towel but resistant in sick pots, reflecting strong Adult Plant Resistance (APR). COC 24 04 showed variable performance, shifting from seedling tolerance to moderate resistance in soil assays, supporting the polygenic nature of DRR resistance.

Keywords: Chickpea; dry root rot; roll towel screening; sick plot screening.

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1. Introduction

Chickpea (*Cicer arietinum* L.) is a nutrient-rich legume crop which is widely used for human consumption and cultivated as Rabi season cool annual crop under rainfed condition. The crop is vulnerable to several diseases, some of which may be devastating. Chickpea suffers from about 172 pathogens consisting of fungi, bacteria, viruses and nematodes. The major root diseases are Fusarium wilt (Causal organism *Fusarium oxysporum*) and dry root rot (Causal organism *Macrophomina phaseolina*). Dry root rot of chickpea caused by *Macrophomina phaseolina* is emerging as a serious threat to the chickpea production worldwide. Economic loss due to root damage is mainly caused by root diseases. Dry root rot in chickpea causes up to 100% loss in yield in favourable environmental conditions (Gupta and Sharma, 2015). The disease is aggravated by drought conditions in rainfed cultivation The drought conditions becomes still severe under prevailing global climate change. Consequent to this the productivity would decrease considerably in developing countries like India, Pakistan and Ethiopia (Foyer et al., 2016).

M.phaseolina is a polyphagous soil borne pathogen infecting over 500 plant species worldwide causing huge losses. Though, the fungus is seed and soil borne; soil borne inoculum is more important in causing infection and disease development. Extensive root rotting is reported with most of the lateral roots destroyed. These rotten roots are brittle and contain microscopic sclerotial forms in the outer tap root surface. Integrated disease management comprises seed treatment with biocontrol agents like Rhizobium and Trichoderma, fungicidal application, crop rotation with non-host species and irrigation management. Crop rotation for dry root rot management is limited as the pathogen has wider host range (Sinha et al., 2021). The pathogen thrives in the form of sclerotia which can live in soil for more than twelve months thus withstands crop rotation. The crop rotation with cotton, bajra, soybean, mungbean aggravates the inoculum. The application of fungicides is of high cost and limited to irrigated condition and is partially effective due to presence of sclerotia. Integrated disease management also includes soil solarisation and fumigation which are detrimental to beneficial organisms.

Identification of resistant cultivars would be highly economical, eco-friendly and a viable solution for rainfed condition for combating the devastating pathogen. The screening of genotypes is the primary prerequisite for identifying resistant cultivars. Various screening methods have been reported for different diseases like Phytophthora root rot using hydroponics (Amalraj et al., 2019) and dry root rot using roll towel method, blotter paper technique, sick pot technique (Irulappan & Senthil-Kumar, 2021) and sick plot technique. Comparing the advantages and disadvantages this study aims at screening of advanced cultures and varieties of chickpea for dry root rot using roll towel method and sick pot culture.

2. Materials and Methods

Seeds of 30 chickpea genotypes (twelve F_6 progeny families and eighteen varieties) were screened in this study. Two screening methods were employed namely roll towel method and sick pot culture. The roll towel method was done under laboratory and sick pot culture under greenhouse condition. Seeds were surface sterilized in 1% sodium hypochlorite (NaOCl) for 3 min, subsequently washed with deionised water and air-dried before being used in experiments to avoid fungal contamination.

2.1 Media and Inoculum Preparation

Sand and maize meal were mixed in a 2:1 ratio and filled into polythene bags. Sufficient water was added to moisten the mixture, ensuring it is not soggy. The bags were then sterilized in an autoclave at 121°C for 30 minutes. After autoclaving, the medium was inoculated with five mycelial discs (5–7 mm in diameter) taken from the actively growing edge of a well-grown *Macrophomina phaseolina* culture plate. The inoculated bags were incubated at 28 ± 2°C until the entire medium was completely colonized by mycelial growth.

2.2 Sick Pot Culture

The seeds of each genotype were sown in pots filled with sterilized sand to ensure a pathogen-free growing medium at the initial stage. Each genotype was tested using four replications, consisting of one un-inoculated control and three inoculated treatments. The seedlings were allowed to grow until they reached the two-leaf stage (approximately 10–12 days after sowing). At this stage, the three treatment pots were inoculated with *Macrophomina phaseolina* inoculum, prepared using the sand-maize meal medium (2:1 ratio) and pre-incubated for 10–15 days to allow for sufficient fungal growth. The inoculum was applied by placing a measured quantity (e.g., 10–15 g per pot) near the root zone to ensure direct contact with the developing root system. The control pots remained un-inoculated to provide a baseline for healthy plant development. Irulappan & Senthil-Kumar, 2021 has detailed the above methodology.

2.3 Roll Towel Method

The pathogen *Macrophomina phaseolina* was cultured on potato dextrose agar (PDA) for 7 to 10 days. The fungal culture was macerated in sterile distilled water to prepare a mycelial or spore suspension at a concentration of approximately 1×10^6 spores per ml. Chickpea seeds were surface sterilized and soaked in this fungal suspension for 4 to 6 hours under aseptic conditions. Sterile blotting or filter paper sheets were spread on a clean surface, and the inoculated seeds were evenly placed on them and then covered with another sheet. The sheets were roll tightly and positioned vertically in a plastic container which was filled with water to maintain humidity. The rolls were incubated at 28 to 30°C for 7 to 10 days. After incubation, the rolls were unwrapped, and the seedlings were assessed for root rot symptoms, including browning, lesions, or tissue rotting. The method was suggested. Talekar et al., 2021 has utilized the method to screen chickpea genotypes in controlled condition. Durgadevi et al., 2026 has provided a improved blotting paper technique to screen large number of genotypes.

Fig. 1. Symptoms of dry root rot in chickpea genotypes under roll towel and sick plot screening methods

3. Results and Discussion

Table 1. Disease rating scale

Rating	Category	Symptoms of DRR	DRR%
1	Resistant	No infection on roots	0.0–10
2–3	Moderately resistant	Very few small lesions on roots	10.1–20
4–5	Tolerant	Lesions on roots clear but small, new roots free from infection	20.1–30
6–7	Susceptible	Lesions on roots many, new roots generally free from lesions	30.1–40
8–9	Highly susceptible	Roots infected and completely discoloured	> 40.1

3.1 Disease Incidence

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

A direct comparison of the genotypes showed that some behaved the same in both methods. This indicates stable resistance or susceptibility. For example, the genotype Super Annigeri 1, which is a known susceptible check, was rated Susceptible in the sick pot culture and highly susceptible in the roll towel method. This consistent reaction shows that it is a reliable benchmark line and proves that the inoculum and disease pressure were effective in both methods, as also reported earlier. In the same way, COC 24 02 was susceptible in both tests, which again confirms the reliability of the screening process and the virulence of the fungal isolate.

Table 2. Disease rating scale based on percent infection and host reaction

Percent infection	Disease rating	Reaction
0	0	Immune
1-20	1	Resistant
20.1-40	2	Moderately Resistant
40.1-50	3	Moderately Susceptible
50.1-75	4	Susceptible
>75	5	Highly Susceptible

(El – Bramawy and Wahid, 2007)

Table 3. Genotypes studied under roll towel method and sick plot culture

Category	Genotypes under roll towel method	Genotypes under sick plot culture
Resistant (R)	Jaki 9218; P2254; COC 21-06; DC-17111;P2259	P2278; NBeG857
Moderately Resistant (MR)	COC-2101; JG 36; COC 24 04; COC 24 01; Nagavalli Local	P2254; P4928; COC 24 04; COC-2102; P2259; ICCV 181674; Nagavalli local; COC 1901
Moderately Susceptible (MS)	COC 1902; ICPL 414; RG 2016-134; CO 4; COC 21-05; COC 1901; CO 3; JG 11	COC 1902; RG 2016-134; COC 24 01; COC 21-06; DC-17111
Susceptible (S)	COC 24 02; NBeG857; P2278; COC-2102; Bannekuppa Local-1; COC 24 03;ICCV 181674	ICPL 414; Super Annigeri 1; COC 24 02; Bannekuppa Local-1; CO 4; COC 21 01; JG 11; JG 36; COC 24 03; COC 21-05; CO 3
Highly Susceptible (HS)	Super Annigeri 1, P4928	Jaki 9218

The most important finding of this study is the difference seen between the two methods. Some genotypes showed different reactions depending on the method used. This means that resistance may change with the growth stage of the plant or the environment. For example, Jaki 9218 was rated Resistant in the roll towel method but Highly Susceptible in the sick pot culture. Similar results for Jaki 9218 was obtained by Dinesh et al., 2025. This big difference suggests that Jaki 9218 has strong early-stage resistance, which may come from seed coat chemicals, fast germination, or early defence enzymes like chitinases and β-1,3-glucanases (Sharma et al., 2016). These defences work well at the seedling stage but may fail later when the fungus attacks mature roots under soil and drought stress. This supports the idea that vigorous seedlings can “escape” early infection but remain unprotected in later stages (Ghosh et al., 2013).

In contrast, P2278 and NBeG 857 showed the opposite trend. Both were Resistant in the sick pot method but Susceptible in the roll towel test. This means they lack resistance at the seedling stage but show strong Adult Plant Resistance (APR), which is well known in plant pathology. Such resistance may come from stronger root tissues, larger root systems that reduce damage, or the late activation of defence pathways like ISR or SAR. For breeding, these genotypes are useful for durable field resistance, but seed treatments may be needed to support early growth.

The case of COC 24 04 shows this complexity clearly. It performed well with high seedling vigour and tolerance in the roll towel method, but in the sick pot culture it was only classified as Moderately Resistant. This shows that resistance to DRR is controlled by many genes and depends on traits expressed at different growth stages (El-Bramawy & Wahid, 2007). Such differences also highlight the risk of overestimating resistance if only one screening method is used.

The possibility of disease escape must also be considered, particularly in the sick pot culture where uniform inoculum distribution is challenging. Plants that happen to grow in areas with lower pathogen density may appear resistant despite being susceptible. However, because the roll towel test offers a highly uniform and controlled environment, escape is less likely. Thus, genotypes like P2278 and NBeG857, which were resistant in sick pots but susceptible in roll towels, warrant re-study under stricter replication and possibly multi-location sick field trials to confirm whether their resistance is genuine or a result of escape. If results are consistent across replicates and environments, confidence increases that these lines possess true genetic resistance.

Environmental conditions are also key to interpreting these findings. *R. bataticola* is most aggressive under high temperatures (>30°C) and drought stress (Sharma & Pande, 2013). The sick pot culture, conducted under soil and often with imposed stress, selects for resistance effective in field-like conditions. In contrast, the roll towel test is performed under constant humidity and optimum temperature, which favours seedling diseases like seed rot and damping-off. Thus, the methods do not only differ by plant growth stage but also by environmental paradigm, which helps explain the contrasting genotypic responses.

4. Conclusion

The comparative evaluation of chickpea genotypes for dry root rot resistance revealed both consistent and contrasting resistance reactions. Genotypes such as Super Annigeri 1 and COC 24 02 consistently exhibited susceptibility across both methods, validating them as reliable checks. Jaki 9218 was resistant at the seedling stage (roll towel) but highly susceptible in soil (sick pot), indicating stage-specific resistance. In contrast, P2278 and NBeG 857 were susceptible in roll towel but resistant in sick pots, reflecting strong Adult Plant Resistance (APR). COC 24 04 showed variable performance, shifting from seedling tolerance to moderate resistance in soil assays, supporting the polygenic nature of DRR resistance.

Disclaimer (Artificial Intelligence)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

Competing Interests

Authors have declared that no competing interests exist.

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