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EFFECT OF TEMPERATURE, SOIL MOISTURE AND SOIL PH ON THE INCIDENCE OF DRY ROOT ROT (*MACROPHOMINA PHASEOLINA* (TASSI) GOID.) IN CONTRASTING GENOTYPES OF CHICKPEA (*CICER ARIETINUM* L.)

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ABSTRACT

Dry root rot (DRR) caused by *Macrophomina phaseolina* (Tassi) Goid. has emerged as a major constraint to chickpea production. The increasing incidence of DRR indicates a strong influence of climate change factors such as temperature, soil moisture and soil pH on disease development. An experiment was conducted on chickpea (*Cicer arietinum* L.) genotypes RVSSG 64 and JG 62 to evaluate the effects of these abiotic factors on DRR incidence. The highest disease incidence was recorded at 35°C, whereas the lowest was observed at 20°C. Disease incidence was greatest under low soil moisture conditions (25%) and lowest under high moisture conditions (100%). Similarly, the highest disease incidence was observed at pH 4.5 and the lowest at pH 7.5. The genotype JG 62 was highly susceptible under high temperature, low soil moisture and low pH conditions, whereas RVSSG 64 exhibited resistance under similar environmental conditions.
Keywords **:** Chickpea, Dry Root Rot, Temperature, Soil Moisture, pH.

Introduction

Chickpea (*Cicer arietinum* L.) is the third most important food legume globally after common bean (*Phaseolus vulgaris*) and field pea (*Pisum sativum*) (Grasso *et al*., 2022). It is an annual self-pollinated pulse crop with a diploid genome (2n = 2x = 16) comprising eight basic chromosomes and an estimated genome size of 738 Mb (Varshney *et al*., 2013). Chickpea serves as a major source of dietary protein and plays a crucial role in maintaining soil fertility through biological nitrogen fixation (Ferguson *et al*., 2010). It is mainly cultivated in the semi-arid tropics including the Indian subcontinent, North Africa, the Middle East, Southern Europe, the Americas and Australia. Globally, chickpea is grown on about 14.81 million hectares producing 18.09 million tonnes. In India, it occupies nearly 10.47 million hectares with a total production of 12.27 million tonnes. India is the leading producer of chickpea contributing more than two-thirds of both the global cultivation area and total production (FAOSTAT, 2022).

Yield in chickpea is a complex quantitative trait influenced by several yield contributing characters and is significantly affected by environmental conditions. Under the changing climatic scenario, both biotic and abiotic stresses are serious challenges to chickpea productivity. Among the biotic stresses, dry root rot (DRR), Fusarium wilt, Ascochyta blight, bacterial blight and collar rot have been reported to cause severe economic yield losses. In recent years, dry root rot has emerged as one of the most serious diseases of chickpea in major growing areas, particularly under high temperature and soil moisture stress (Ghosh *et al*., 2013; Sharma *et al*., 2015).

Macrophomina phaseolina (Tassi) Goid. formerly known as *Rhizoctonia bataticola* is a soil-borne necrotrophic fungal pathogen (asexual form) that causes dry root rot in chickpea. It can also cause root rot and seedling blight in many other pulse crops, especially when plants are under stress (Hwang *et al*., 2003). In the absence of a host plant, the pathogen can persist in the soil by living saprophytically on decaying organic matter. The pathogen persists in the soil as microsclerotia and hyphae which serve as the primary source of infection. Dry root rot symptoms in chickpea usually appear during the flowering and pod formation stages. Affected plants dry up suddenly and appear scattered across the field, showing drooping petioles and leaflets mainly confined to the upper portion of the plant (Haware, 1990). Infected plants often show straw-colored stems and leaves while in some cases the lower leaves and stems turn brown. The taproot becomes black, decayed and loses most of its lateral and fine roots. Dead roots are brittle and show bark shredding with small dark sclerotia visible on the surface or inside the root tissues (Sharma *et al*., 2015; Khaliq *et al*., 2020). When the stem near the collar region is split vertically minute sclerotia or sparse mycelial growth can be observed in the pith.

The survival of sclerotia is higher in dry soil and decreases under wet conditions. The growth and survival of *Macrophomina phaseolina* are influenced by environmental factors such as temperature, soil moisture and pH (Khan, 2012; Soni *et al*., 2022). Low soil water potential favors the formation and survival of microsclerotia whereas high soil moisture reduces their viability (Olaya *et al*., 1996). Limited information is available on the epidemiology of the disease, particularly regarding how climatic factors such as soil temperature, moisture and pH influence the susceptibility of chickpea plants. Understanding how these abiotic factors affect disease development is essential for developing effective management strategies. Therefore, the present study was conducted to examine the effects of varying temperature, soil moisture and pH on the incidence of DRR in chickpea genotypes RVSSG 64 and JG 62.

Materials and Methods

Fungal isolate

A pathogenic isolate of *Macrophomina phaseolina* obtained from naturally infected chickpea plants at MPKV, Rahuri, was used throughout the experiments. The isolate was purified using a single sclerotium technique and maintained on PDA plates and slants at 4°C in a refrigerator.

Plant material and genotypes

The dry root rot resistant genotype RVSSG 64 and the susceptible genotype JG 62 were used in this study. RVSSG 64 has been identified as a dry root rot resistant source (Soni, 2023). Previous studies focused on the susceptible genotype JG 62 to evaluate the effects of temperature, soil moisture and soil pH on dry root rot incidence. Therefore, in the present study, both a resistant (RVSSG 64) and a susceptible (JG 62) genotype were selected to enable a comparative assessment of their responses to environmental factors that affect the development of dry root rot in chickpea.

Effect of temperature

The effect of four temperature regimes (20, 25, 30 and 35°C) was studied on the DRR-resistant genotype RVSSG 64 and the susceptible genotype JG 62 under *In vitro* conditions using the paper towel method in an incubator. Seven-day-old seedlings of RVSSG 64 and JG 62 were grown in a germinator chamber and roots were inoculated by dipping in the broth for 1 min. Potato dextrose broth medium (A 7 mm mycelial disc from an actively growing culture of *Macrophomina phaseolina* was inoculated into a 250 ml conical flask containing 100 ml of sterilized potato dextrose broth and incubated at 28°C for five days under stationary conditions. The fungal mat obtained from two such flasks was macerated in 100 ml of sterile distilled water and used as inoculum) was prepared for multiplication of fungus. Inoculated seedlings were placed in folded, moist blotting paper and then incubated at four different temperatures (20, 25, 30 and 35℃) with a 12 hr photoperiod. The experiment was conducted in two replications in completely randomized block design (CRBD) and each replication consisted of 10 plants (Sharma and Pande, 2013). Ten seedlings of each replication were treated with fungus while ten seedlings were kept as control. After seven days of treatment, disease severity was recorded on 1-9 scale as described by Pande *et al*. (2012) (Table 1).

Table 1 : Disease scoring scale for dry root rot in chickpea

Rating	Category	Symptoms of dry root rot
1	Resistant	No infection on roots
3	Moderately resistant	Very few small lesions on roots
5	Moderately susceptible	Lesions on roots clear but small, new roots free from infection
7	Susceptible	Many lesions on roots, new roots generally free from lesions
9	Highly susceptible	Roots infected and completely discolored

Effect of soil moisture

The influence of soil moisture on dry root rot of chickpea was studied under sick pot conditions. The effect of four soil moisture regimes, *i.e*. 25, 50, 75 and 100 per cent, was evaluated on the development of dry root rot under controlled environmental conditions. The experiment was conducted using the resistant genotype RVSSG 64 and the susceptible genotype JG 62 in a greenhouse. Each treatment was replicated twice and each replication consisted of four pots with ten plants per pot. The soil was inoculated with *Macrophomina phaseolina* grown on sorghum medium. Soil moisture was determined using the hot air oven-drying method (Sharma and Pande, 2013). For this, soil samples were collected in aluminium containers. Before sampling, each empty heat-resistant container with its lid was weighed (M_1). The soil sample was then collected from the pot, placed in the container and weighed together with the container (M_2). The containers were kept in a hot air oven at 60°C for two hours. After cooling, the containers with dried soil were weighed again (M_3). Disease incidence was recorded after 40 days and soil moisture was monitored weekly. The moisture per cent was calculated by the following formula

$$\text{Moisture}(\%) = \frac{(M_2 - M_3)}{(M_3 - M_1)} \times 100$$

Effect of soil pH

The effect of different pH levels (4.5, 5.5, 6.5 and 7.5) on the development of dry root rot (DRR) was studied in greenhouse using the dry root rot resistant genotype RVSSG 64 and the susceptible genotype JG 62. Seeds of both genotypes were sown in pots inoculated with *Macrophomina phaseolina* grown on sorghum medium. The experiment was conducted in two replications along with a control, following a completely randomized design (CRD). Ten seeds of each genotype were sown per pot. For pH estimation, five grams of soil were suspended in 40 ml of sterile distilled water in a 250 ml conical flask and stirred for 30 minutes. The soil pH was then adjusted to 4.5, 5.5, 6.5 and 7.5 using NaOH and HCl (Kulkarni, 2007). Data on percent mortality were recorded 30 days after sowing as described by Reddy *et al*. (2016) (Table 2).

Table 2 : Disease rating scale

Sr. No.	Category	Per cent disease incidence (PDI)
1	Resistant	0-10
2	Moderately resistant	10.1-20
3	Moderately susceptible	20.1-30
4	Susceptible	30.1-50
5	Highly susceptible	Above 50

The disease incidence in each genotype was calculated using the following formula (Wheeler, 1969).

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

Results

Effect of temperature

The analysis of variance revealed statistically significant ($p < 0.001$) variation for DRR incidence at different temperature levels. The differences in disease incidence were mainly attributed to temperature, as indicated by the higher mean sum of squares (Table 3). The susceptible genotype JG 62 exhibited moderately susceptible (MS) reaction at 20°C with 25 percent disease incidence, showing only a few small lesions on the roots, while RVSSG 64 showed no visible symptoms on the roots at this temperature. A similar trend was observed at 25°C, JG 62 showed 30 per cent (moderately susceptible) disease incidence with minor root lesions and no any symptoms observed on the roots of RVSSG 64. However, dark lesions on roots were observed at 30°C with 55 per cent disease incidence in JG 62 and categorized into highly susceptible, while RVSSG 64 showed 05 per cent (resistant) disease incidence. The infected and completely discolored roots were observed with highest disease incidence (80 %) at 35°C in JG 62 and was graded in highly susceptible (HS) category, while RVSSG 64 showed resistance with 10 per cent disease incidence at 35°C. No symptoms were observed in the control plants. The result indicate that higher temperature (30-35°C) was more favourable for the development and spread of *Macrophomina phaseolina*, leading to increased disease incidence and severity, whereas lower temperature (20-25°C) limited pathogen development and symptom expression. JG 62 was highly susceptible at higher temperature, whereas RVSSG 64 showed resistance at higher temperature (Table 4).

Table 3 : Analysis of variance (ANOVA) for DRR incidence at different temperature levels in chickpea genotypes

SV	MS	F pr.	e.s.e	s.e.d	lsd
Temperature	62485.762	<0.001	0.415	0.587	1.162
Genotype	8420.114	<0.001	0.632	0.894	1.776
T x G	1074.683	<0.001	1.214	1.718	3.412
Replication	52.847				
Residual	8.952				
SEM±	2.90				
CV%	6.0				

Table 4 : Effect of different temperature levels on development of dry root rot on chickpea genotypes JG 62 and RVSSG 64

Sr. No.	Temperature (℃)	DRR incidence (%)		Disease reaction	
		JG 62	RVSSG 64	JG 62	RVSSG 64
1	20℃	25	0	Moderately Susceptible	Resistant
2	25℃	30	0	Moderately Susceptible	Resistant
3	30℃	55	5	Highly Susceptible	Resistant
4	35℃	80	10	Highly Susceptible	Resistant

Effect of soil moisture

The analysis of variance revealed statistically significant ($p < 0.001$) variation in DRR incidence at different soil moisture levels. The differences in disease incidence were mainly attributed to soil moisture, as indicated by the higher mean sum of squares (Table 5). The susceptible genotype JG 62 exhibited high dry root rot incidence at low moisture level, with 85 per cent incidence at 25 per cent moisture and 65 per cent incidence at 50 per cent moisture, categorizing it as highly susceptible (HS). However, as the moisture level increased, disease severity gradually declined, showing 35 per cent incidence at 75 per cent moisture and 25 per cent incidence at 100 per cent moisture, which were classified as susceptible (S) and moderately susceptible (MS), respectively. The chickpea genotype RVSSG 64 exhibited resistance to dry root rot (DRR) across all soil moisture conditions. In contrast to JG 62, which showed high susceptibility at low moisture levels, RVSSG 64 maintained resistance with disease incidence ranging from 0 per cent to 10 per cent across all tested moisture levels. Plant mortality was significantly higher under low soil moisture conditions (25% and 50%) compared to higher moisture levels (75% and 100%). The percentage of mortality was used to categorize disease severity in the genotypes. As soil moisture increased, disease incidence declined and disease progression was slower in plants grown under high moisture condition, likely due to reduced physiological stress and less favorable conditions for pathogen proliferation and vice versa under low moisture condition (Table 6).

Table 5 : Analysis of variance (ANOVA) for DRR incidence at different soil moisture levels in chickpea genotypes

SV	MS	F pr.	e.s.e	s.e.d	lsd
SMC	58653.879	<0.001	0.427	0.604	1.195
Genotype	4975.632	<0.001	0.612	0.869	1.720
SMC x G	594.762	<0.001	0.842	1.194	2.362
Replication	42.517				
Residual	9.118				
SEM±	2.73				
CV%	5.8				

Table 6 : Effect of different moisture levels on development of dry root rot on chickpea genotypes JG 62 and RVSSG 64

Sr. No.	Moisture (%)	DRR incidence (%)		Disease reaction	
		JG 62	RVSSG 64	JG 62	RVSSG 64
1	25%	85	10	Highly Susceptible	Resistant
2	50%	65	10	Highly Susceptible	Resistant
3	75%	35	0	Susceptible	Resistant
4	100%	25	0	Moderately Susceptible	Resistant

Effect of soil pH

The analysis of variance revealed a statistically significant ($p < 0.001$) effect of pH on DRR incidence. The greater mean sum of squares for pH indicates that the differences in disease incidence were primarily due to pH levels rather than genotype or their interaction (Table 7). The results showed significant variation in disease occurrence, with the highest mortality observed at pH 4.5. The susceptible genotype JG 62 exhibited 85 per cent disease incidence, indicating highly susceptible (HS) reaction, whereas the resistant genotype RVSSG 64 showed resistant (R) reaction with only 10 per cent incidence. At pH 5.5, JG 62 again showed highly susceptible reaction with 60 per cent disease incidence, while RVSSG 64 remained resistant with 10 per cent incidence. At pH 6.5, JG 62 recorded 40 percent disease incidence and was rated as susceptible (S), whereas RVSSG 64 showed complete resistance with 0 per cent incidence. At the highest pH level (7.5), JG 62 exhibited the lowest disease incidence of 25 per cent and was classified as moderately susceptible (MS), while RVSSG 64 remained unaffected. RVSSG 64 demonstrated consistent resistance across all pH levels, whereas JG 62 exhibited varying degrees of susceptibility, with greater disease severity under acidic soil conditions. These findings indicate that lower pH levels (4.5 and 5.5) favor the development of dry root rot, while higher pH levels (6.5 and 7.5) significantly reduce disease occurrence (Table 8).

Table 7 : Analysis of variance (ANOVA) for DRR incidence at different pH levels in chickpea genotypes

SV	MS	F pr.	e.s.e	s.e.d	lsd
Ph	4205.750	<0.001	0.520	0.735	1.560
Genotype	1520.500	<0.001	0.350	0.495	1.050
pH x G	890.625	<0.001	0.740	1.045	2.100
Replication	30.250				
Residual	12.840				
SEM±	2.87				
CV%	6.2				

Table 8 : Effect of different pH levels on development of dry root rot on chickpea genotypes JG 62 and RVSSG 64

Sr. No.	pH	DRR incidence (%)		Disease reaction	
		JG 62	RVSSG 64	JG 62	RVSSG 64
1	4.5	85	10	Highly Susceptible	Resistant
2	5.5	60	10	Highly Susceptible	Resistant
3	6.5	40	0	Susceptible	Resistant
4	7.5	25	0	Moderately Susceptible	Resistant

Discussion

Chickpea is mainly cultivated under rainfed conditions, where fluctuations in temperature and rainfall often cause soil moisture stress. In the Indian subcontinent, it is grown during the post-rainy season on receding soil moisture, which frequently results in terminal drought stress. Such conditions favor the development of *Macrophomina phaseolina*, the pathogen responsible for dry root rot, which is more prevalent in tropical and humid regions (Savary *et al*., 2011). Environmental factors such as temperature, soil moisture and pH play crucial role in influencing pathogen survival and disease severity. The viability of *Macrophomina phaseolina* increases under low water potential but decreases when soil moisture is high (Olaya *et al*., 1996). Changes in climate, particularly rising temperatures and irregular rainfall, are expected to increase the incidence of dry root rot in chickpea (Garrett *et al*., 2016; Khambadkar, 2013).

In this study, the highest disease incidence was recorded at 35°C, whereas the lowest was observed at 20°C. As the temperature increased, the incidence of the disease also increased. Similarly, Chowdary and Govindaiah (2007) reported highest growth at 40°C which was followed by 35°C and 30°C. Sharma and Pande (2013) reported a higher incidence of dry root rot at 35°C. Srinivas (2016) observed maximum colony growth of the pathogen at 30°C and 35°C and reported that the optimum temperature for the development of the dry root rot pathogen was 35°C. De-Sousa *et al.* (2020) also found that disease severity increased with rising temperature. Chandran *et al.* (2021) observed dry root rot incidence in chickpea to be lowest at 25°C and highest at 35°C. Soni *et al.* (2022) reported maximum disease incidence at 35°C and minimum at 20°C in the genotype JG 62.

The disease incidence was highest under low soil moisture conditions (25%) and lowest under high moisture conditions (100%). A similar result was also reported by Wokocha (2000), showing low soil moisture favors pathogen growth and enhances its pathogenic activity, whereas high soil moisture inhibits its development. Patel and Anahosur (2001) observed maximum incidence of *Macrophomina phaseolina* at 25 per cent soil moisture and less incidence observed at high soil moisture (75% and 100%). Singh and Sharma (2002) observed severe disease development in pulse crops grown under moisture-deficit conditions. Sharma and Pande (2013) reported that plants under moisture stress exhibited increased severity of dry root rot. Srinivas (2016) observed that plants exhibited wilting and drooping under low moisture conditions (40% and 50%). Harne (2019) studied the incidence of dry root rot disease and reported that disease incidence was highest at 20 per cent moisture level and lowest at 80 per cent moisture level. Soni *et al*. (2022) reported that maximum disease incidence at 25 per cent moisture and minimum incidence at 100 per cent moisture in the JG 62 genotype.

The highest disease incidence was recorded at pH 4.5, whereas the lowest was observed at pH 7.5. Higher incidence of dry root rot was observed at lower pH level, whereas the incidence decreased at higher pH level. A similar result was also reported by Srivastava and Dhawan (1980), showing plant mortality was highest at soil pH 5.5 and that disease intensity decreased with increasing soil pH. Csondes *et al*. (2012) reported that pH range of 4 to 6 was optimum for the growth of the dry root rot pathogen *Macrophomina phaseolina*. Parmar (2013) reported that *Macrophomina phaseolina* showed maximum growth at pH 6.5 and minimum growth at pH 8.0. Sukanya *et al*. (2016) reported increased growth of the dry root rot pathogen at low pH. Srinivas (2016) reported that the dry root rot pathogen showed higher colony growth at pH 5.0. Soni *et al*. (2022) observed that maximum disease incidence was observed at pH 4.5 and minimum disease incidence was observed at pH 7.5 in the JG 62 genotype.

Conclusions

The effects of abiotic factors such as temperature, soil moisture and soil pH were evaluated to determine their influence on the incidence of dry root rot (DRR) caused by *Macrophomina phaseolina* in chickpea genotypes JG 62 (susceptible) and RVSSG 64 (resistant). The study revealed that disease incidence increased with rising temperature, with the highest incidence at 35°C and the lowest at 20°C. Disease severity was greater under low soil moisture conditions, showing maximum incidence at 25 per cent moisture, while minimal disease development occurred at 100 per cent moisture. Soil pH also significantly affected disease occurrence, with the highest incidence at pH 4.5 and the lowest at pH 7.5. The genotype JG 62 was highly susceptible under high temperature, low soil moisture and low pH conditions, whereas RVSSG 64 exhibited resistance under similar environmental conditions.

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