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Screening of Advanced Chickpea Genotypes for Resistance to Ascochyta Blight under Field and Controlled Conditions

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ABSTRACT

Ascochyta blight caused by *Ascochyta rabiei* ranks the most important foliar disease of chickpea (*Cicer arietinum L.*) in Pakistan and other important chickpea growing areas. The most sustainable management option is host plant resistance, but screening must be ongoing, as pathogen populations evolve and formerly useful resistance can break down. This chickpea germplasm assessment included 94 advanced chickpea genotypes from the four-breeding series consisting of NKCS, CTD, CTK and CYT. In the 2024-25 Rabi season, we did the field in augmented design with one replication and controlled assessment for Ascochyta blight resistance at National Agricultural Research Centre, Islamabad. A disease severity rating was performed with a standardised rating scale of 1–9, after the plants were artificially infected with virulent *A. rabiei* strains. Genetic variation was widespread in this respect. Under field conditions, 74 genotypes (78.7%) showed resistant reactions, of which 23 were highly resistant (score = 1). Under controlled conditions, 63 (67.0%) maintained resistance. The results of controlled-environment and field screening were positively and strongly correlated (Pearson's $r = 0.799$, $p < 0.001$; $R^2 = 0.638$), indicating a significant level of agreement between them. One-way ANOVA indicated significant differences in mean disease severity among breeding series in both the field ($F(3, 90) = 7.17$, $p < 0.001$) and the controlled environment ($F(3, 90) = 10.35$, $p < 0.001$).The NKCS field showed the highest severity of 3.85 ± 1.69, while the CYT field showed 2.14 ± 1.49. Sixty-three genotypes were designated as resistant in both screening contexts, demonstrating consistent resistance under the conditions of this investigation.

Keywords: *Cicer arietinum*, *A. rabiei*, disease resistance, genetic screening, plant breeding, quantitative resistance, germplasm evaluation

INTRODUCTION

Chickpea (*Cicer arietinum* L.) is the second most valuable pulse crop in the world after common bean (*Phaseolus vulgaris* L.). It is grown on a land area of about 14.6 million hectares and yields an average of 14.2 million metric tons every year (FAOSTAT, 2025). Chickpea is a significant pulse crop in Pakistan, and it is significant in the economy of the agriculture sector. It is cultivated on an average of 1.073 million hectares with an average yield of 784 kg ha^{-1} which is a far cry compared to the global average of 973 kg ha^{-1} and far below the expected yield of 3–4 t ha^{-1} under ideal management (Anonymous, 2007; Ali and Kumar, 2005). The causes of this yield gap are the sum total of biotic stresses, insect pests, and abiotic limitations like drought, heat, and salinity (Sharma and Ghosh, 2016).

Ascochyta blight that is caused by the necrotrophic fungus *A. rabiei* (Pass.) Labr. Didymella rabiei (Kovachevski) v. Arx], is one of the major biotic

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constraints and the most severe foliar disease of chickpea in the world (Nene, 1982; Kaiser, 1997; Pande et al., 2005). The disease was initially reported in Italy and then to most of the major areas with chickpea (Sattar, 1933; Kaiser, 1973). Ascochyta blight can result in a loss of 50–70% of the yield and a total crop failure has been reported during epidemic years particularly in Thal and Pothohar regions in favorable conditions in Pakistan (Malik and Bashir, 1984; Iqbal et al., 2003). In Pakistan, the annual losses in the economy have been estimated to be over US$5 million (Malik et al., 1991).

This pathogen is a polycyclic infection with an optimal development ability at cool temperatures (15–25°C), high relative humidity (>80%) and long leaf wetness (>12 h) (Kaiser, 1973; Trapero-Casas and Kaiser, 1992). The manifestation of symptoms on leaves, stems, pods, and seeds is a dark brown to black necrotic lesion with pycnidia (Nene, 1984). The problem may result in severe infection leading to widespread defoliation, stem girdling, pod abortion, seed infection and ultimately plant death (Reddy and Kabbabeh, 1985).

Ascochyta rabiei has a high pathogenic variability. Various virulence patterns and physiological races have been reported in the various geographic locations (Vir and Grewal, 1974; Reddy and Kabbabeh, 1985; Singh, 1990; Jan and Wiese, 1991). Studies based on molecular markers have shown high genetic diversity among and within populations of pathogens (Sarwar et al., 2000; Moradi et al., 2012). It is said that the Pakistani and Indian isolates tend to be particularly violent and able to defeat the once effective sources of resistance (Singh et al., 1984; Iqbal, 2002). This variability makes it difficult to build resistance that is durable and highlights the importance of repeated screening of breeding material.

The management alternatives to Ascochyta blight are crop rotation, deep ploughing, removal of infected crop debris, seed treatment, foliar fungicides and host plant resistance (Bashir and Ilyas, 1983; Singh and Singh, 1990; Rauf et al., 1996). In as much as fungicides including mancozeb, chlorothalonil, and carbendazim may offer an effective solution to disease control (Tripathi et al., 1987; Kaiser et al., 1973), the consistent application of the chemicals across time has concerns that may include cost, environmental pollution, problem of food residue, and the emergence of fungicide-resistant populations of the pathogens (Kaur et al., 2009). This makes genetic resistance the best and most economically viable method of managing the disease (Reddy and Nene, 1987; Singh and Reddy, 1996). Recent in vitro work has also shown that fungicidal treatments can strongly suppress fungal growth in plant pathosystems (Aslam et al., 2025).

The Ascochyta blight resistance in chickpea is mostly quantitative and controlled by a number of genes that have additive and epistatic effects (Tewari and Pandey, 1986; Santra et al., 2000). As a result of genetic mapping, multiple quantitative trait loci (QTLs) linked to resistance have been identified, and each of them contributes to a fraction of the phenotypic variance (Sabbavarapu et al., 2013; Stephens et al., 2014; Deokar et al., 2019). This genetic complexity, combined with variability of the pathogen and environmental impacts, renders the development of durable resistance challenging to breed against. Recent post-2019 studies have expanded this framework by identifying new resistant sources in cultivated and wild Cicer germplasm and by refining marker-trait associations for Ascochyta blight resistance. Large-scale screening identified additional resistant donors (Gayacharan et al., 2020; Newman et al., 2021), while GWAS and high-density mapping studies detected resistance regions on chromosomes Ca1, Ca3, Ca4, Ca6, and Ca7 and further narrowed key chromosome 4 intervals linked with candidate genes such as CaAP2 and CaCNGC1 (Alo et al., 2022; Raman et al., 2022; Sahin et al., 2023; Singh et al., 2023; Dariva et al., 2024).

Previous screening exercises at national and international levels had found several resistant sources of chickpea. In Pakistan, Iqbal and Ghafoor (2005) screened 448 genotypes and found 30 resistant lines in the seedlings and at the adult plant stage. According to Ghazanfar et al. (2010), NIAB lines carrying seven resistant genotypes were identified against NARC lines carrying seven genotypes. There is also the identification of resistant germplasm by international screening programs such as ILC and FLIP materials (Singh and Reddy, 1990; Reddy and Singh, 1996). Nevertheless, further screening of developed breeding lines is still essential since the populations of pathogens evolve, environmental-specific resistance might be deliberate and long-term resistance might necessitate pyramiding of complementary resistance loci.

With the purpose to: (1) screen advanced chickpea genotypes systematically in four breeding series in terms of their resistance to Ascochyta blight; (2) compare disease responses under field and controlled screening conditions; (3) determine which genotypes are resistant in both screening systems; (4) estimate the relationship between field and controlled screening environments to enhance screening efficiency; and (5) describe the distribution of resistance across breeding series, the current study was carried out.

Recent studies published in 2025 and 2026 have further highlighted that durable resistance to Ascochyta blight in chickpea requires both accurate

phenotypic evaluation and improved genetic understanding. Under high disease pressure, genotypes previously regarded as moderately resistant may show clear differences in disease development, indicating that routine field screening alone may not always capture the full extent of useful variation in breeding material (Crociara et al., 2025) reported that several moderately resistant chickpea genotypes performed better than resistant checks when assessed through disease severity and AUDPC. Their results also suggested that some previously reported marker-trait associations were not sufficiently stable for direct use in selection. These findings are relevant to the present study, as they emphasize the importance of evaluating chickpea germplasm under both field and controlled conditions to identify dependable sources of resistance.

At the same time, current breeding approaches are moving beyond reliance on single major loci and are increasingly recognizing the polygenic basis of resistance. (Lin et al., 2025) reported moderate to high prediction accuracies for genomic selection of Ascochyta blight resistance in chickpea, indicating its potential to accelerate the development of resistant germplasm. This is particularly important because *A. rabiei* is highly variable, and resistance based on a limited genetic background may not remain effective over time. Similarly, (Alaskar et al., 2026) demonstrated that gene stacking improved chickpea responses to Ascochyta blight, providing further evidence that combining multiple resistance factors may strengthen breeding outcomes. Collectively, these studies suggest that future breeding programs should integrate phenotypic screening, molecular information, and multi-gene approaches rather than depending on a single selection strategy.

Recent physiological and histopathological investigations have also contributed to a better understanding of resistance mechanisms in chickpea. (Sharma et al., 2025) observed that resistant genotypes possessed stronger structural barriers that restricted pathogen infection and development, suggesting that anatomical traits may serve as useful complementary indicators during resistance assessment. These findings indicate that disease scores should not be interpreted solely as visual expressions of symptom severity, but rather as the outcome of multiple interacting defense responses that may vary among genotypes. From a breeding perspective, this implies that genotypes showing similar field reactions may still differ in the mechanism, stability, and durability of resistance. Therefore, screening under both natural field conditions and controlled inoculation remains essential for the identification of robust breeding material.

Another important development is the recognition that resistance to Ascochyta blight should be evaluated within a broader environmental context (Hubbard et al., 2025) showed that drought stress and varietal background can influence plant performance and may also modify disease expression. This is particularly relevant for chickpea-growing regions such as South Asia, where variable moisture conditions can affect both host vigor and epidemic development. Overall, recent literature indicates that effective management of Ascochyta blight will depend on the identification of stable resistance sources, their validation across contrasting environments, and the integration of field observations with controlled screening and modern breeding tools. The present study contributes to this objective by evaluating advanced chickpea genotypes under both field and controlled conditions and by identifying promising breeding series and individual entries for future resistance improvement programs.

Materials and Methods

Experimental Site and Climatic Conditions

The field experiment took place in Pulses Research Field, National Agricultural Research Centre (NARC) at Islamabad, Pakistan (33°42′N, 73°04′E; 683 m above sea level) in the 2024–25 Rabi (winter) season. The climate of the area is subtropical continental climate, which has four seasons. Winter (December–February) is usually cool and humid with average temperatures of 12 to 20°C, morning fogs, extended wetness of the leaves, and the relative humidity is usually above 60%. It is these conditions that are conducive to the growth and propagation of Ascochyta blight. The region is receiving a yearly rainfall of about 1,143 mm and a good percentage of it is received during the winter seasons. The data on temperature, humidity and rainfall were taken during the experiment with the help of an automated weather station installed near the field.

Material and Design of the Experiments

The number of genotypes of advanced chickpea that were tested was 94. The material was four breeding series produced at NARC: NKCS (n = 40), CTD (n = 18), CTK (n = 15), and CYT (n = 21). The NKCS series was composed of lines of Kabuli type and was mainly chosen in terms of large seed size, cream color of seed-coat and adaptation to irrigated soils. The CTD series consisted of desi-type advanced yield-trial with excellent agronomic performance and desirable cooking quality. The CTK series was a series of superior lines that were chosen due to disease resistance and agronomic stability. The CYT series consisted of high-end lines when subjected to coordinated yield test, which reflects different genetic background and adaptation patterns. Recording Pedigree and breeding history of each genotype was done to be further interpreted.

The seed was planted on 5 November 2024 with a hand-pulled seeding drill. The extremely vulnerable cultivar AUG-424 was planted after each five test entries to act as a spreader row and monitor of the disease, thus facilitating even spread of inoculum and disease pressure throughout the experimental area. The experiment was set up in an augmented block design comprising of 19 blocks and each block being constituted of five unreplicated test genotypes and one replicated susceptible check. Plots 4 m in length, 30 cm in between rows, and 10 cm in between plants in rows were used in single rows. The traditional agronomic use of chickpea was adhered to such as managing the weeds with herbicides before emergence and hand weeding during vegetative stage. The development of diseases in the absence of any application of fungicides was to be observed in order to indicate the genetic resistance of the sampled material.

Pathogen Isolation, Culture and Inoculum Preparation

The isolates of *A. rabiei* were collected in the fields of NARC in the 2023–24 season using naturally infected chickpea plants that had typical blight symptoms, including necrotic lesions with prominent pycnidia. Pathogen viability and virulence were ensured using fresh samples of several infected plants in active disease progression. With aseptic conditions, 3–5 mm^2 pieces of tissue were excised at the margin of the healthy tissue and diseased tissue.

Sterility of tissue sections was done by placing them in 0.1% sodium hypochlorite, washing three times with sterile distilled water, and drying them on sterile filter paper and placing on potato dextrose agar (PDA) and streptomycin sulfate (50 µg mL^{-1}) to inhibit bacterial growth. Seventh to ten days were incubated in sealed parafilm-sealed petri dishes at 20 ± 2°C at a 12 h photoperiod until pycnidia grew.

The isolations of single-pycnidium were done in order to achieve genetic uniformity in pure cultures (Trapero-Casas and Kaiser, 1992). Each pycnidium was transferred onto a sterile PDA plate under a dissecting microscope and crushed in sterile water to release conidia, and streaked. Following Kaiser (1973), morphological identification was done based on pycnidial size, color and the conidial morphology. Pathogenicity was verified on susceptible check cultivars in the greenhouse before the isolates were admitted to large-scale production of inoculating.

To induce the multiplication of inoculum, the pathogen was subcultured in chickpea seed extract agar (CSEA), which aids a lot in sporulation (Ilyas and Khan, 1986). The medium was made; 20 g of finely ground chickpea seeds, 20 g of glucose, and 20 g of agar were autoclaved in 1 L of distilled water at 121°C for 20 min.

After incubating cultures at 20 ± 2°C temperature under 12–12 light–dark cycles in 15 days, the pycnidial growth and conidial production were maximized. To prepare conidial suspensions, the cultures that were 15 days old were flooded with sterile distilled water with 0.01% Tween-20. It was then filtered by four layers of sterile muslin cloth to eliminate mycelial debris and agar pieces. A hemocytometer was used to determine spore concentration and sterile water was used to adjust the spores to a concentration of 5×10^5 spores mL^{-1} . Each inoculation occurrence had fresh inoculum as a result of the significant loss of conidial viability that happens during storage.

Inoculation Procedures and Development of Diseases

Field Conditions

The presence of disease pressure in the field was achieved and sustained by a staged inoculation approach. Inoculation of spreader rows of the sensitive cultivar AUG-424 was done with a backpack pressurized sprayer (capacity 20 L) that was calibrated to produce about 500 L ha^{-1}. Field inoculations were initiated on 20 December 2024 (45 days after sowing, at the 6–8 leaf stage) and were repeated on 27 December 2024, 3 January 2025, and 10 January 2025. Test entries were spray-inoculated on 27 December 2024 and again on 3 January 2025 to ensure uniform disease pressure. This method promoted the progressive disease formation and secondary infection by splash-dispersed and wind-borne conidia.

Foliar spray was used to inoculate test genotypes to provide full coverage of all leaf surfaces which included adaxial and abaxial, stem, and developing reproductive organs. Inoculation was conducted during the evening (after 17:00 h) in order to limit the effects of ultraviolet on conidia and to enter naturally rising humidity and falling temperatures. This was facilitated by natural winter conditions of winter December–February, heavy dew, frequent fog, and the light rainfall which prolonged the wet condition of the leaves to enable conidial germination and infection (Trapero-Casas and Kaiser, 1992). In rainless periods that lasted longer, supplemental overhead mist irrigation was used in the early morning and late evening hours to maintain conducive disease development in the environment.

Controlled Conditions

During the controlled environment screening, seedlings were cultivated in plastic pots, measuring 20 cm x 25 cm, filled with a mixture of autoclaved loam soil, sand, and well-decomposed compost, mixed in a 2:1:1 volume ratio. Five seeds of each genotype were sown in one pot, and then reduced to three homogeneous seedlings per pot. Thus, one pot contained one genotype, and the three seedlings per

pot were considered subsamples rather than replications. Pots were placed under transparent polyethylene sheet enclosures in humid chambers in the greenhouse of the NARC Plant Pathology, with a relative humidity of more than 95%. A fine spray system was used to maintain high humidity. Inoculation of seedlings with the conidial suspension was done 3–4 weeks after sowing, when seedlings were at the vegetative branching stage, using hand-held atomizers that created small droplets of the conidial suspension. Inoculation was done at 20 ± 2°C, and a relative humidity of >95%, to ensure successful infection, while later greenhouse conditions were set to 18–22°C, >70% RH. To ensure a consistent disease pressure and reduce infection escape inoculum was reapplied after 7 days.

Disease Assessment and Data Entry

Severity of diseases was initially determined at the appearance of symptoms on the susceptible checks, which occurred at 10–14 days following inoculation under field and 7–10 days following inoculation under controlled condition. Tests were done at a 1-week interval until susceptible checks had an entire foliar necrosis and over 75% of plant mortality. In cases where the difference in scores was greater than one unit, the plants were re-investigated and a unanimous score was given. The mean severity scores were determined by plant, evaluator and assessment date. The genotypes were further categorized as highly resistant (score = 1), resistant (scores 2–3), moderately resistant (scores 4–5), susceptible (scores 6–7), highly susceptible (scores 8–9). Documentation was done by retaining representative photographs of every resistance class.

The standardized 1-9 rating scale of Singh et al. (1981) was used to assess disease reaction.

Score	Disease Reaction	Description
1	Highly Resistant (HR)	No visible symptoms or only minute flecks.
2	Resistant (R)	Small hypersensitive flecks; no sporulation.
3	Moderately Resistant (MR)	Small pustules surrounded by necrosis or chlorosis.
4	MR–MS	Small to medium pustules with chlorosis.
5	Intermediate (M)	Medium pustules with some chlorosis.
6	Moderately Susceptible (MS)	Medium pustules with little chlorosis.
7	MS–S	Medium to large pustules, limited chlorosis.
8	Susceptible (S)	Large pustules with abundant sporulation.
9	Highly Susceptible (HS)	Very large pustules with profuse sporulation and severe infection.

Five representative plants in each plot or pot were evaluated individually by two experienced evaluators.

Statistical Analysis

Disease severity data were summarized as genotype means and then analyzed at the breeding-series level. One-way analysis of variance (ANOVA) was used separately for field and controlled conditions to test differences among NKCS, CTD, CTK, and CYT series. Mean separation was performed with Tukey's honestly significant difference (HSD) test at alpha = 0.05. Pearson correlation and simple linear regression were used to quantify agreement between field and controlled screening results. Analyses were conducted in R software, and descriptive values in the tables are presented as mean ± standard deviation (SD).

RESULTS

Disease Severity Distribution and Descriptive Statistics

A total of 94 advanced chickpea genotypes were screened and it was found that the genetic variation of resistance to Ascochyta blight was significant in the field-screening and controlled-screening conditions, which demonstrated that there was useful diversity in the germplasm that was assessed. The severity of the diseases was 1–9 in field condition and 3–9 in controlled condition. All the genotypes were scored 0 under controlled conditions. The general mean disease intensity (2.06–3.61) was greater in the field (3.13 ± 2.72) than in the controlled setting (3.93 ± 2.54) across all the genotypes, with a lower standard deviation and more homogeneous disease pressure in the controlled environment.

The total proportion of genotypes with a 3 or less score in the field (74 out of 94) was 78.7%, which were therefore identified as resistant or highly resistant, and of 63 genotypes with a 3 or less score under controlled conditions (67.0%). In the field, resistance classes were distributed as 23 highly resistant (24.5%), 51 resistant (54.3%), 14 moderately resistant (14.9%) and 6 susceptible or highly susceptible (6.4%) genotypes. In controlled surroundings, there were no genotypes in highly resistant range; 63 genotypes (67.0%) were resistant, 24 (25.5%) moderately resistant and 7 (7.5%) susceptible or highly susceptible.

Table 1. Descriptive statistics for disease severity across breeding series under field and controlled conditions

Breeding series	n	Field conditions (Mean ± SD)	Field range	Controlled conditions (Mean ± SD)	Controlled range
NKCS	40	3.85 ± 1.69	1-7	4.70 ± 1.67	3-9
CTD	18	2.67 ± 1.41	1-5	3.56 ± 1.15	3-7
CTK	15	2.73 ± 0.70	1-3	3.00 ± 0.00	3-3
CYT	21	2.14 ± 1.49	1-7	3.19 ± 0.87	3-7
Overall	94	3.13 ± 1.72	1-9	3.93 ± 1.54	3-9

The frequency distribution under field conditions demonstrated: 23 (24.5) highly resistant (score=1), 51(54.3) resistant (scores 2-3), 14 (14.9) moderately resistant (scores 4-5), 6 (6.4) susceptible or highly susceptible (scores ≥6). The dominance of the resistant genotypes can be explained by the improved breeding level of the materials under evaluation that has been previously selected on the disease resistance. The distribution changed under controlled conditions: 0 (0%) highly resistant, 63 (67.0%) resistant, 24 (25.5%) moderately resistant, and 7 (7.5%) susceptible or highly susceptible, which showed the more stringent screening of controlled environments with optimum infection conditions and repeated inoculations (Figure 1).

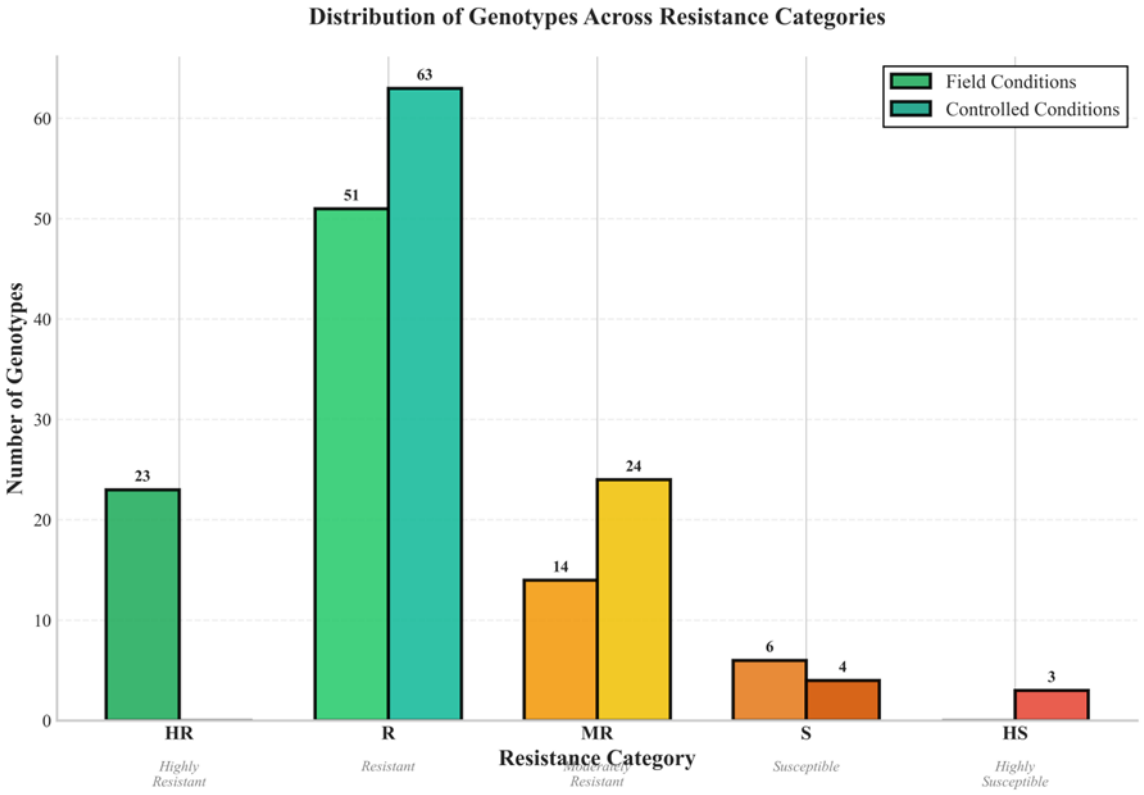

Figure 1. Frequency distribution of genotypes across resistance categories under field and controlled screening conditions. Categories: HR=Highly Resistant (score 1), R=Resistant (scores 2-3), MR=Moderately Resistant (scores 4-5), S=Susceptible (scores 6-7), HS=Highly Susceptible (scores 8-9).

Performance of Breeding Series

There were significant changes in breeding series (Table 1; Figure 2). The CYT series had the lowest mean severity of disease measured in the field (2.14 ± 1.49) whereas CTK showed the most uniform response because every genotype in that series scored 3 or less in the field and exactly 3 under controlled conditions. In contrast, NKCS was the most sensitive series, with mean disease severities of 3.85 ± 1.69 in the field and 4.70 ± 1.67 under controlled circumstances. Even yet, a subset of NKCS entries still performed better than the susceptible check AUG-424, which acted only as a disease spreader and pressure indicator rather than as a prospective resistant entry.

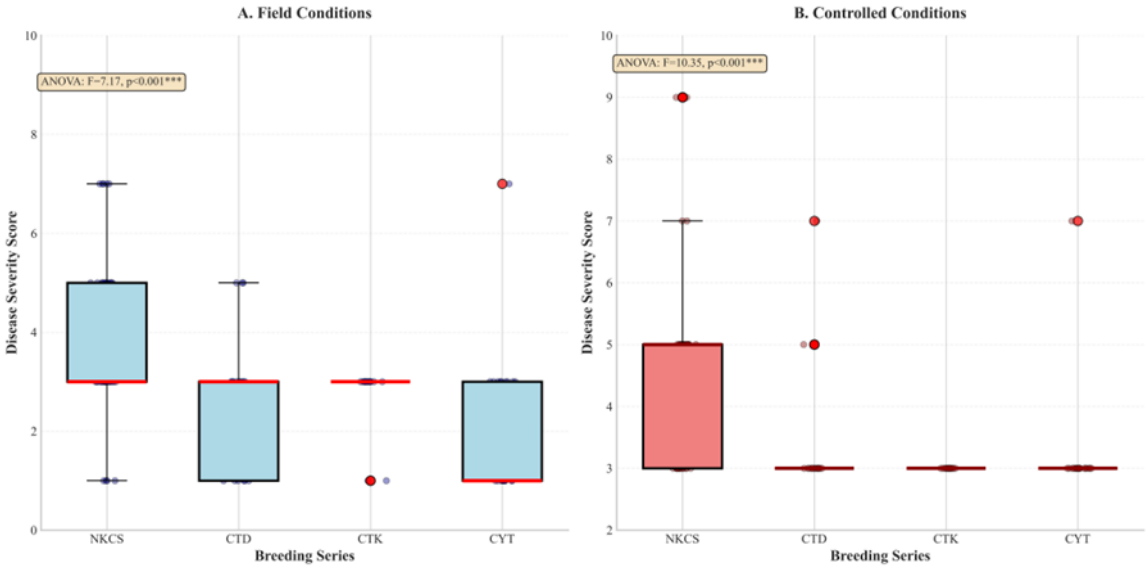

Figure 2. Disease severity distribution in breeding series in (A) field and (B) controlled conditions.

Comparison of ANOVA and Post-hoc

Table 2. ANOVA summary and Tukey HSD pairwise comparisons among breeding series

Comparison	Field mean difference	Field p-value	Controlled p-value
ANOVA	-	< 0.001	< 0.001
CTD vs CTK	0.07	0.999	0.599
CTD vs CYT	-0.52	0.688	0.809
CTD vs NKCS	1.18	0.030	0.011
CTK vs CYT	-0.59	0.640	0.971
CTK vs NKCS	1.12	0.067	0.0002
CYT vs NKCS	1.71	< 0.001	0.0002

Note: Tukey HSD comparisons were interpreted at alpha = 0.05. Because each genotype was represented by one field plot or one pot, no separate treatment-wise standard errors or significance letters are reported.

Correlation and Regression Analysis

Screening results in the field and controlled environment were highly positively correlated (Figure 3; Table 3). The correlation analysis undertaken on the full dataset yielded a Pearson's correlation coefficient of r = 0.799 (n = 94, p < 0.001, which reflects strong correlation between the tests. The Spearman rank correlation was also rather strong (ρ = 0.724, p < 0.001) indicating that the genotype rankings were largely consistent between environments. The regression equation using simple linear regression obtained was Controlled score=0.717 (Field score) +1.674 (R2=0.638, standard error=0.056, p<0.001). This means that field scores explained 63.8% of controlled-environment scores variation. The positive slope indicates that the greater the disease severity observed in the field, the greater the disease severity under controlled conditions. In breeding series, NKCS (r = 0.822), CTD (r = 0.700), and CYT (r = 0.746) had positive correlations. Correlation of CTK was not estimable as all CTK lines had an identical control-condition score, and hence zero variance.

Table 3. Correlation coefficients between field and controlled screening by breeding series

Series	n	Pearson r	p-value	R2
NKCS	40	0.822	< 0.001	0.676
CTD	18	0.700	0.001	0.490
CTK	15	NA	NA	NA
CYT	21	0.746	< 0.001	0.556
Overall	94	0.799	< 0.001	0.638

Note: Correlation for CTK was not estimable because the controlled-condition score was constant for all CTK genotypes.

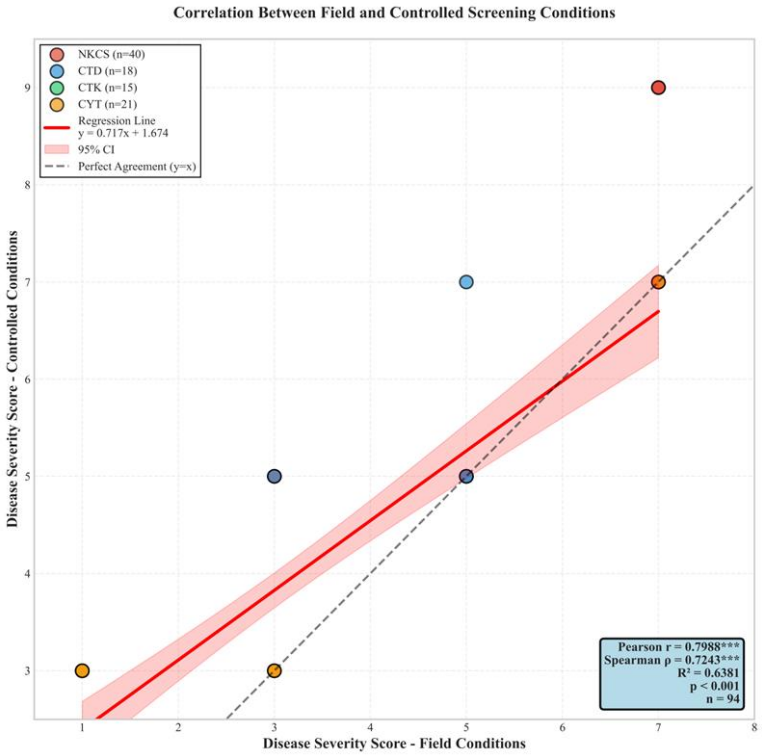

Figure 3. Relationship between field and controlled screening based on correlation and linear regression.

Stability of Resistance to Different Screening Conditions

One of the most prominent results of the research was the discovery of genotypes that were resistant in both screening conditions. Sixty-three genotypes were categorized as resistant in both screening environments, showing the consistency of resistance in the conditions used in this study.Sixty-three genotypes, representing 67.0% of the total set, had ≤3 in both the field and controlled conditions, showing the consistency of the resistance response in the conditions used in this study.

Distribution

These uniformly resistant genotypes occurred as follows: CYT, 18 genotypes (85.7%); CTK, 15 genotypes (100%); CTD, 15 genotypes (83.3%); and NKCS, 15 genotypes (37.5%). The entire list of 63 resistant genotypes is to be presented as a supplementary material; several of them are presented in Table 4.

Among the 23 genotypes that were highly resistant in the field (score = 1), all genotypes were resistant to moderately resistant under controlled conditions (scores 3–5), though none of the genotypes retained a score of 1 under controlled screening. The average of the control-condition scores were 0.80 ± 0.98 units higher than field scores as would be expected since pressure of the disease is stronger and more uniform in the controlled environment.

Table 4. Examples of genotypes showing consistent resistance across both screening environments

Genotype	Series	Field score	Controlled score
NKCS121	NKCS	1	3
NKCS122	NKCS	1	3
CTD2403	CTD	1	3
CTD2407	CTD	1	3
CTK2601	CTK	1	3
CTK2602	CTK	2	3
CYT2102	CYT	1	3
CYT2116	CYT	1	3
CYT2117	CYT	1	3
CYT2119	CYT	1	3

DISCUSSION

This study demonstrates that a significant portion of elite chickpea germplasm possesses considerable levels of resistance to Ascochyta blight. Here, 74 out of 94 genotypes (78.7%) have shown resistance during field evaluation, while 63 genotypes (67.0%) have shown resistance during both field and controlled screening.

This study demonstrates that breeding material already contains considerable variation that can be

exploited to enhance resistance levels. Evaluation of CYT and CTK breeding lines shows considerable levels of resistance, where CYT breeding lines show low average disease score, high number of uniformly resistant lines, while CTK breeding lines show remarkable consistency.

Good association between results of field and controlled screening (r = 0.799) indicates that controlled screening could be used effectively during early stages of selection, while field evaluation is critical because natural variations in canopy microclimate, plant architecture, etc., cannot be replicated during controlled screening.

The fact that the scores of the disease are distributed continuously, combined with variation across breeding series, is in line with the quantitative nature of Ascochyta blight resistance in chickpea. This has been interpreted in line with previous genetic studies that have identified multiple loci which have small to moderate effects on resistance (Santra et al., 2000; Sabbavarapu et al., 2013; Stephens et al., 2014; Deokar et al., 2019). In this regard therefore, the genotypes that were highly resistant and the ones that were always resistant, as this paper has identified, are valuable resources of pyramiding resistance as well as of mapping populations.

The variability of the pathogen is still a significant issue in the implementation of enduring resistance. Earlier investigations have demonstrated that the populations of *A. rabiei* vary significantly in their geographical regions in terms of their virulence (Vir and Grewal, 1974; Reddy and Kabbabeh, 1985; Singh, 1990; Jan and Wiese, 1991; Iqbal, 2002). Mixed inoculum in the current study probably gave a general test of the resistance at the local conditions, though additional confirmation of the resistant origin against the characterized isolates and between the locations would enhance their importance in the long-term breeding and development in varieties.

These results are also consistent with recent post-2019 studies showing that Ascochyta blight resistance is highly quantitative and often isolate- or population-specific, with multiple small- to moderate-effect loci now being tracked through GWAS, haplotype analysis, and fine mapping for marker-assisted breeding (Raman et al., 2022; Sahin et al., 2023; Singh et al., 2023; Dariva et al., 2024).

All in all, our data propose a way forward: employing the best-performing resistant genotypes, notably those derived from CYT and CTK, as donor parents, and testing their resistance against various populations of the pathogen and conditions. This would help in resistance pyramiding, giving a platform for additional QTL identification.

CONCLUSION

Field and controlled evaluations of 94 elite chickpea genotypes found a large degree of variability for resistance to Ascochyta blight. Seventy-four of these genotypes were resistant to Ascochyta blight in the field, while sixty-three remained resistant under controlled conditions as well. The CYT and CTK series were consistently the strongest and best sources of resistance.

There was a significant difference ($p<0.01$) among the breeding lines using ANOVA, and there is a strong correlation between field and controlled environments which suggests that controlled environment screening will be a valuable tool for selecting for resistance when used in conjunction with confirmatory field testing.

These resistant genotypes from this study are an excellent source of potential parents for use in resistance pyramiding or QTL analysis. Their incorporation into breeding programs should lead to the development of chickpea cultivars that have a greater range of and longer lasting resistance to Ascochyta blight.

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