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Screening of Chilli (Capsicum annuum L.) Germplasm for Response to Root-Knot Nematode (Meloidogyne incognita) under Glasshouse Conditions

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Abstract

Root-knot nematodes (RKN), genus *Meloidogyne*, are important plant-parasitic nematodes that infect roots and induce gall formation, thereby impairing nutrient and water uptake and reducing crop productivity. Chilli (*Capsicum annuum* L.) is an economically important vegetable and spice crop, and identification of germplasm with reduced susceptibility to *Meloidogyne incognita* is a key step toward integrated nematode management. The present study screened eleven chilli lines, OHP-35 to OHP-45, for root-galling response to *M. incognita* under glasshouse conditions at the Plant Pathology Research Institute, Ayub Agricultural Research Institute (AARI), Faisalabad, Pakistan, in 2021. Two-week-old chilli seedlings were transplanted into 6-inch earthen pots containing sterilized silt–sand soil. Soil sterilization was carried out using a 5% formalin solution prepared at a formalin:water ratio of 5:95, followed by covering the treated soil with polyethylene for 48 h and airing for 24 h. Fifteen days after transplanting, plants were inoculated with 2,000 second-stage juveniles (J2) of *M. incognita* per pot. The nematode species was confirmed morphologically using female perineal patterns and juvenile characters. Plants were harvested 60 days after transplanting, roots were washed, and the number of galls per root system was recorded. Each chilli line was represented by five independent pot replicates. Mean root-gall counts varied significantly among genotypes, ranging from 1.4 in OHP-42 to 207.8 in OHP-36. OHP-45 also showed a low mean count of 4.2 galls per root system. Based on the Bridge and Page galling scale, OHP-42 was classified as resistant, OHP-45 as moderately resistant, OHP-40 as moderately susceptible, OHP-39, OHP-41 and OHP-43 as susceptible, and OHP-35, OHP-36, OHP-37, OHP-38 and OHP-44 as highly susceptible. Statistical analysis revealed highly significant differences among lines (one-way ANOVA: $F_{10,44} = 60.51$, $p < 0.001$; Kruskal–Wallis: $H = 50.21$, $df = 10$, $p < 0.001$). These results identify OHP-42 and OHP-45 as promising candidates for confirmatory resistance evaluation. However, because the study was conducted under a single glasshouse screening and did not include nematode reproduction parameters such as egg masses, final population density, or reproduction factor, the findings should be interpreted as preliminary evidence of reduced susceptibility rather than proof of durable field-level resistance.

Article Highlights

1. Eleven chilli (*Capsicum annuum* L.) lines, OHP-35 to OHP-45, were screened for root-galling response to *Meloidogyne incognita* under glasshouse conditions.

2. Each line was evaluated using five independent pot replicates, and plants were inoculated with 2,000 second-stage juveniles (J2) per pot.

3. Root galls were counted 60 days after transplanting, and mean gall counts ranged from 1.4 in OHP-42 to 207.8 in OHP-36.

4. OHP-42 and OHP-45 showed the lowest galling and were classified as resistant and moderately resistant, respectively, and are candidates for confirmatory testing.

1. Introduction

Chilli (*Capsicum annuum* L.) is one of the most important vegetable and spice crops worldwide. It is consumed fresh, dried, powdered, and processed, and is valued for its pungency, colour, nutritional quality, and medicinal properties. Chilli fruits contain vitamins A, B-complex, C, and E, as well as minerals and bioactive compounds such as carotenoids and capsaicinoids. In addition to culinary use, chilli and its extracts have been used in traditional medicine, analgesic preparations, oleoresin production, and as sources of natural antioxidants (Bosland and Votava, 2000).

Pakistan is among the major chilli-producing countries, and chilli is an important cash crop in several provinces. Production is concentrated mainly in Sindh and Punjab, with smaller area under cultivation in other regions. The crop contributes significantly to vegetable production, rural income, and export earnings. Despite its importance, chilli productivity is constrained by several biotic and abiotic stresses, including soil-borne pathogens and plant-parasitic nematodes.

Root-knot nematodes of the genus *Meloidogyne* are among the most damaging plant-parasitic nematodes globally. *Meloidogyne incognita* is a major species affecting solanaceous crops, including tomato, pepper, and chilli. Infective second-stage juveniles (J2) enter root tips and establish specialized feeding sites called giant cells. This induces hypertrophy and hyperplasia of root tissues, resulting in characteristic root galls or knots. Severe infection disrupts water and nutrient transport, reduces photosynthate allocation, stunts plant growth, lowers yield, and in young plants may cause mortality. It is estimated that root-knot nematodes affect thousands of plant species and contribute significantly to global crop losses (Taylor and Sasser, 1978; Bridge and Page, 1980).

Host-plant resistance is one of the most sustainable components of integrated management of root-knot nematodes. Genetic variation in galling response exists among pepper and chilli genotypes, and germplasm screening is therefore an essential first step toward identifying resistant or moderately resistant lines for breeding and production systems. Recent studies have continued to evaluate pepper and chilli germplasm against *M. incognita*. Brahmani et al. (2024) screened bell-pepper genotypes and identified a small group with resistant reactions. Gowda et al. (2025) evaluated a larger chilli germplasm panel using multiple phenotypic and nematode-related traits, along with multivariate and marker-based approaches. Kaur et al. (2025) reported variation in galling and related responses among chilli genotypes under screen-house conditions. These studies demonstrate that root-gall counts can be informative, but robust resistance claims generally require confirmation of experimental design, verified nematode identity, appropriate controls, and additional endpoints such as egg masses, final nematode population, reproduction factor, and plant growth parameters.

The objective of the present study was to compare root-gall counts among eleven chilli lines (OHP-35 to OHP-45) following artificial inoculation with *M. incognita* under glasshouse conditions. The study was conducted as part of a BSc (Hons) Plant Pathology internship project in 2021. The manuscript reports the recorded observations, performs descriptive and inferential statistical analysis, and identifies lines that warrant further confirmatory evaluation.

2. Materials and Methods

2.1 Experimental site and plant material

The experiment was conducted in 2021 in the nematology section/glasshouse of the Plant Pathology Research Institute, Ayub Agricultural Research Institute (AARI), Faisalabad, Pakistan. Eleven chilli lines, designated OHP-35 to OHP-45, were screened for response to *M. incognita*. Two-week-old seedlings of each line were raised and used for transplanting. The study was undertaken as a BSc (Hons) Plant Pathology internship project.

2.2 Soil sterilization and potting

Silt–sand soil was collected for the experiment. The soil was sterilized using a formalin solution prepared by mixing formalin and water at a ratio of 5:95, corresponding to approximately 5% (v/v) formalin. The solution was applied uniformly to the soil heap, which was then covered with a polyethylene sheet for 48 h to confine the fumigant fumes. After 48 h, the polyethylene sheet was removed and the soil was aired for 24 h. The sterilized soil was thoroughly mixed and filled into 6-inch earthen pots. One two-week-old chilli seedling was transplanted per pot.

2.3 Nematode inoculum production, identification, and inoculation

Inoculum of *Meloidogyne incognita* was obtained from chilli/tomato plants growing in the experimental area of the nematology section at AARI. Infested root material was collected, and nematodes were recovered using a standard extraction procedure. Roots were chopped into small pieces and treated with sodium hypochlorite solution to release eggs and juveniles. The extract was filtered through sieves, and second-stage juveniles (J2) were recovered by Baermann funnel extraction/centrifugal flotation. The juveniles were counted under a stereomicroscope, and the suspension was adjusted to deliver 2,000 J2 per pot.

The nematode species was identified morphologically. Females were examined for perineal pattern characters typical of *M. incognita*, including the high vulval slit, rounded to slightly indented dorsal arch, and simple striae. Juveniles were also examined for stylet length, lateral field incisions, and median bulb shape, following Taylor and Sasser (1978). Only active motile J2 were used for inoculation.

Fifteen days after transplanting, each pot was inoculated with 2,000 J2 of *M. incognita*. The nematode suspension was applied into small holes made around the base of each plant to ensure even distribution in the root zone. Uninoculated control pots received sterile water without nematodes, and a susceptible check was maintained in parallel to validate nematode pathogenicity. The control and check validation data are not shown in the germplasm comparison table but confirmed that the inoculum was viable and that galling observed in test lines was nematode-induced.

2.4 Experimental design and replication

The experiment was arranged in a completely randomized design. Each chilli line was represented by five independent pots, and each pot served as one biological replicate. Thus, five root-gall observations (R1–R5) were recorded for each line. The use of independent pots ensured that the replicates were experimental units rather than subsamples from the same pot.

2.5 Harvesting and root-gall assessment

Plants were harvested 60 days after transplanting. Roots were carefully excavated, washed under running water to remove adhering soil, and examined for root galls/knots. The total number of galls per root system was recorded for each replicate. Galling was used as the primary screening parameter because it reflects the ability of the nematode to establish feeding sites in the host root.

2.6 Galling scale and reaction classification

The number of galls per root system was converted into a reaction category using the galling scale of Bridge and Page (1980). The scale was corrected to remove the gap between 13 and 31 galls; the appropriate category for 11–30 galls is moderately susceptible. The scale used in the present study is shown in Table 2.

Scale value	Number of galls/knots per root system	Reaction
0	0	Immune
1	1–2	Resistant
2	3–10	Moderately resistant
3	11–30	Moderately susceptible
4	31–100	Susceptible
5	> 100	Highly susceptible

2.7 Statistical analysis

Data were subjected to one-way analysis of variance (ANOVA) to test differences among chilli lines. Because root-gall counts are count data and variances were heterogeneous among lines, the results were also confirmed using the Kruskal–Wallis non-parametric test. Means, standard deviations, and standard errors were calculated from the five independent replicates per line. Statistical analyses were performed using R software (R Core Team, 2024). Differences were considered significant at $p < 0.05$.

3. Results

Root-gall counts varied markedly among the eleven chilli lines. The lowest mean gall count was recorded for OHP-42 (1.4 ± 0.9 SD), followed by OHP-45 (4.2 ± 1.9 SD). The highest mean count was recorded for OHP-36 (207.8 ± 15.0 SD). OHP-35 (177.0 ± 9.9 SD) and OHP-44 (175.6 ± 46.7 SD) also showed very high mean gall counts. OHP-37 and OHP-38 had mean counts of 131.6 and 136.2 galls per root system,

respectively. Intermediate responses were observed in OHP-39 (58.0), OHP-41 (45.0), OHP-43 (48.8), and OHP-40 (22.6).

One-way ANOVA showed that genotype had a highly significant effect on root-gall number ($F_{10,44}$ = 60.51, $p < 0.001$). The Kruskal–Wallis test also confirmed highly significant differences among lines (H = 50.21, df = 10, $p < 0.001$). These results indicate that the observed variation in galling was not due to random variation alone.

Based on the corrected Bridge and Page scale, OHP-42 was classified as resistant, OHP-45 as moderately resistant, OHP-40 as moderately susceptible, OHP-39, OHP-41 and OHP-43 as susceptible, and OHP-35, OHP-36, OHP-37, OHP-38 and OHP-44 as highly susceptible. The uninoculated control remained gall-free, and the susceptible check developed severe galling, confirming the validity of the assay.

Line	R1	R2	R3	R4	R5	Mean	SD	SE	Scale	Reaction
OHP-35	178	165	169	188	185	177.0	9.9	4.4	5	Highly susceptible
OHP-36	191	196	221	225	206	207.8	15.0	6.7	5	Highly susceptible
OHP-37	115	134	117	141	151	131.6	15.5	6.9	5	Highly susceptible
OHP-38	147	122	134	156	122	136.2	15.1	6.8	5	Highly susceptible
OHP-39	28	45	87	76	54	58.0	23.7	10.6	4	Susceptible
OHP-40	22	56	11	9	15	22.6	19.3	8.6	3	Moderately susceptible
OHP-41	78	45	32	58	12	45.0	25.1	11.2	4	Susceptible
OHP-42	2	0	1	2	2	1.4	0.9	0.4	1	Resistant
OHP-43	21	45	88	34	56	48.8	25.5	11.4	4	Susceptible
OHP-44	112	223	156	167	220	175.6	46.7	20.9	5	Highly susceptible
OHP-45	2	3	5	4	7	4.2	1.9	0.9	2	Moderately resistant

Figure 1. Mean root-gall counts of eleven chilli lines inoculated with *Meloidogyne incognita* under glasshouse conditions. Error bars represent ±1 standard deviation across five independent replicates.

4. Discussion

The present screening revealed substantial variation in root-galling response among the eleven chilli lines. OHP-42 showed the lowest mean gall count (1.4 galls per root system), and OHP-45 also showed a low mean count (4.2). In contrast, OHP-36, OHP-44, and OHP-35 exhibited very high mean gall counts, exceeding 175 galls per root system. These observations suggest that OHP-42 and OHP-45 may possess reduced susceptibility to *M. incognita* under the tested glasshouse conditions and deserve priority for confirmatory evaluation.

The identification of low-galling genotypes is consistent with previous reports showing genetic variation in pepper and chilli responses to root-knot nematodes. Brahmani et al. (2024) evaluated bell-pepper germplasm and identified resistant genotypes after screening. Gowda et al. (2025) screened a larger chilli germplasm collection and used multiple traits along with multivariate and molecular-marker approaches. Kaur et al. (2025) also reported differential galling responses among chilli genotypes under screen-house conditions. These studies support the value of germplasm screening as an initial step in nematode management. However, they also emphasize that strong resistance claims require well-defined experimental designs, verified nematode identification, appropriate controls, and measurement of nematode reproduction in addition to gall counts.

Root-gall number is a useful screening parameter because galling reflects the ability of the nematode to establish feeding sites in roots. However, gall count alone does not fully define resistance. A genotype may restrict gall formation but still allow some nematode reproduction, or it may show low galling due to environmental or experimental variation. More complete characterization of resistance should include egg masses per root system, final population density, reproduction factor (Pf/Pi), juvenile production, plant height, biomass, root architecture, and yield-related traits where applicable. The present dataset primarily includes gall counts, so conclusions regarding durable resistance are necessarily limited.

The statistical analysis confirmed that the differences among lines were highly significant. Both ANOVA and the Kruskal–Wallis test supported the conclusion that genotype influenced galling response. Nevertheless, significance in a single glasshouse experiment does not automatically translate into field-level resistance. Environmental factors, nematode population pressure, soil type, temperature, and cultivar background can all influence the expression of resistance. Therefore, OHP-42 and OHP-45 should be considered promising candidates rather than proven resistant cultivars.

The corrected Bridge and Page scale was important for proper classification. The originally supplied scale contained a gap between 13 and 31 galls, which prevented confident classification of OHP-40. After correcting the moderately susceptible range to 11–30 galls, OHP-40 was classified as moderately susceptible. This correction is consistent with standard galling classification systems and prevents misinterpretation of intermediate responses.

The inclusion of an uninoculated control and a susceptible check strengthened the interpretation of the results. The absence of galling in the uninoculated control and severe galling in the susceptible check confirmed that the observed symptoms were caused by *M. incognita* and that the inoculum was virulent.

However, for publication in a higher-ranked journal, the numerical data for controls and the susceptible check should be included in a supplementary table or mentioned explicitly with means and replication.

Future work should repeat the screening of OHP-42 and OHP-45 with a clearly documented experimental design across seasons or locations. The study should include verified J2 inoculum, adequate independent replication, an uninoculated control, and a susceptible check. In addition to gall counts, researchers should record egg masses, final nematode population, reproduction factor, plant growth parameters, and yield components if the study is extended to field conditions. Molecular marker analysis, where feasible, could also help determine whether known resistance loci such as *Me* genes are associated with the low-galling phenotype (Gowda et al., 2025).

5. Conclusion

Among the eleven chilli lines evaluated, OHP-42 showed the lowest mean root-gall count (1.4 galls per root system), followed by OHP-45 (4.2 galls per root system). OHP-36 had the highest mean count (207.8), while OHP-35 and OHP-44 also showed high galling. Statistical analysis confirmed highly significant differences among genotypes. Based on the corrected Bridge and Page scale, OHP-42 was classified as resistant and OHP-45 as moderately resistant. These lines are promising candidates for confirmatory testing against *Meloidogyne incognita*. However, the current evidence is based on a single glasshouse screening and does not include nematode reproduction parameters. Therefore, OHP-42 and OHP-45 should be regarded as preliminary resistant/moderately resistant candidates, and repeat experiments with egg mass counts, final population density, reproduction factor, and field validation are required before any definitive resistance recommendation or farmer-level recommendation can be made.

Declarations

Competing interests

The author declares no competing interests.

Ethics approval

Not applicable to this plant glasshouse study.

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Author contributions

Muhammad Ans: conceptualization, methodology, investigation, data curation, formal analysis, writing—original draft, writing—review and editing.

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Data availability

The raw root-gall counts supporting the conclusions of this study are included in Table 1. Additional original records, including control data and nematode identification images, are available from the corresponding author on reasonable request.

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Figures

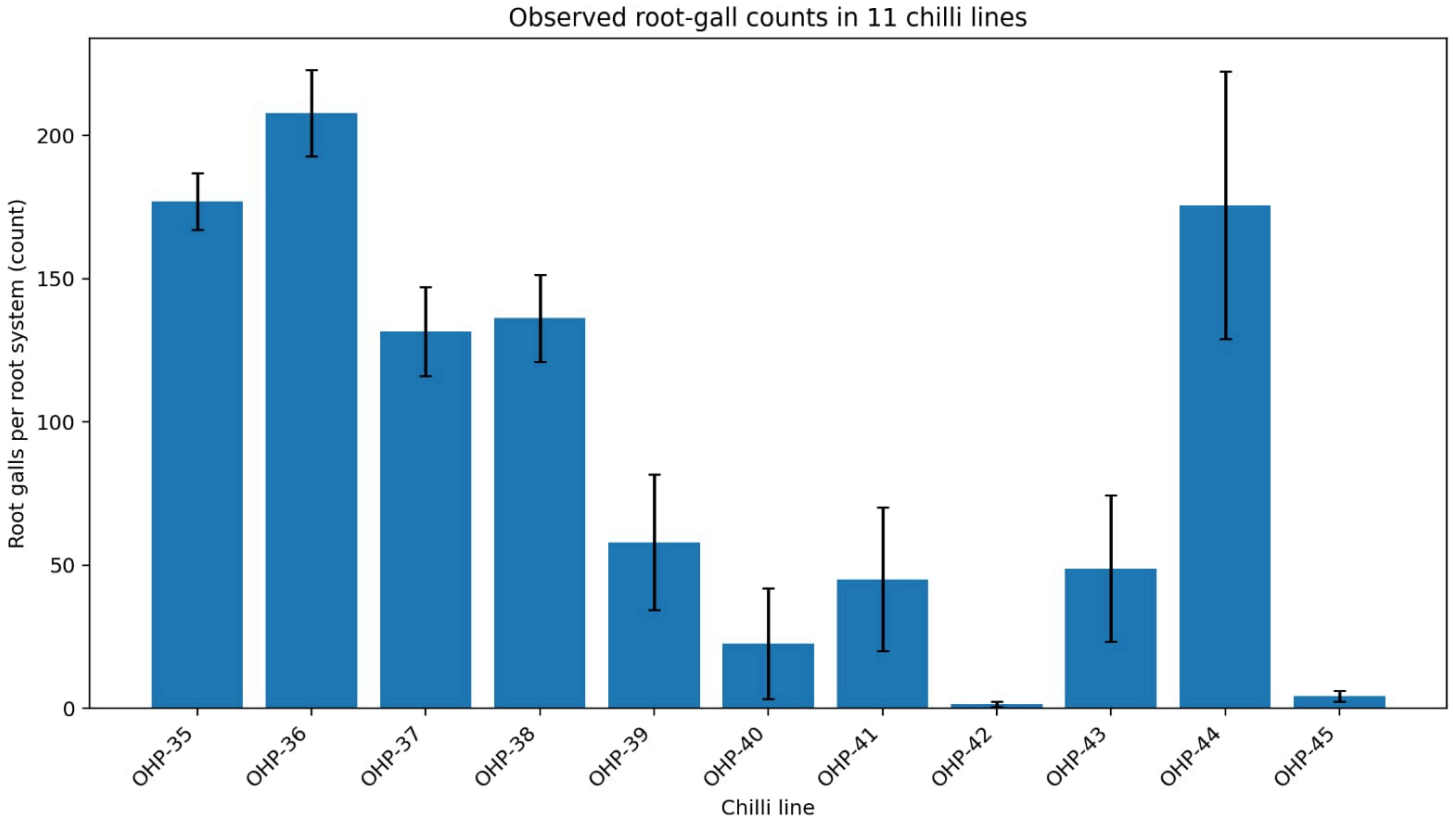


Figure 1

Mean root-gall counts per line; error bars show ±1 sample standard deviation across the five recorded observations. These error bars describe observed variability and do not indicate statistical significance.