PREPRINT

PERTANIKA JOURNALS

TROPICAL AGRICULTURAL SCIENCE

Journal homepage: http://www.pertanika.upm.edu.my/

Performance Evaluation of Advanced Interspecific *Oryza* Spp. Crosses in Response to Varied *Meloidogyne graminicola* Inoculation Conditions

Emma Dorila Lombeida García[1], Luz Gómez Pando[2], Reina Concepción Medina Litardo[3], Iris Betzaida Pérez-Almeida[4]*, Pedro José García-Mendoza[5], Oscar Javier Navia Pesantes[4], Fernando Javier Cobos Mora[1], and Rosa María Álvarez Parra[6]

[1]*Faculty of Agricultural Sciences, Technical University of Babahoyo, Babahoyo, 120102 Los Ríos, Ecuador*
[2]*National Agrarian University La Molina, Perú, 15026 Lima Region, Peru*
[3]*Faculty of Agricultural Sciences, Guayaquil University, 090613 Guayas, Ecuador*
[4]*Research Department, Ecotec University, Samborondón, 092302 Guayas, Ecuador*
[5]*Faculty of Engineering, National Autonomous University of Tayacaja "Daniel Hernández Morillo" (UNAT), Tayacaja, 09156 Huancavelica, Perú*
[6]*Rice Unit, National Institute of Agricultural Research (INIA), Portuguesa, 2103 Aragua, Venezuela*

ABSTRACT

Rice is a vital staple crop for global food security; however, its production is constrained by the plant-parasitic nematode *Meloidogyne graminicola*. This study evaluated the responses of two advanced interspecific rice lines (L1 and L2) and the susceptible commercial cultivar INIAP 15 (VC3) to increasing initial inoculum densities (0, 500, 1,000, 2,000, and 3,000 J2 plant^{-1}). Nematode reproduction, plant growth, and grain yield were assessed to identify differences in resistance and yield tolerance among genotypes. Nematode infestation significantly reduced plant height, root length, grain number per plant, 1,000-grain weight, and grain yield, while increasing the percentage of empty grains. At the highest inoculum density, L2 exhibited the greatest yield tolerance, with an approximately 10% yield reduction, compared with 17% for L1 and 15% for VC3. Conversely, L1 developed fewer root galls and showed lower nematode reproduction indices, indicating a stronger resistance response. Multivariate analyses identified root length, grain yield, and nematode population density as the main drivers of phenotypic variation. Although cluster analysis grouped L2 with VC3 at the highest inoculum levels, L2 maintained superior overall

ARTICLE INFO

Article history:
Received: 02 December 2025
Accepted: 02 November 2026
Published: 02 December 2026

DOI: https://doi.org/10.47836/pjtas.49.6.02

E-mail addresses:
elombeida@utb.edu.ec (Emma Lombeida)
luzgomez@lamolina.edu.pe (Luz Gómez)
reina.medinal@ug.edu.ec (Reina Medina)
iperez@ecotec.edu.ec (Iris Pérez-Almeida)
pedrogarcia@unat.edu.pe (Pedro José García-Mendoza)
onavia@est.ecotec.edu.ec (Oscar Navia)
fcobos@utb.edu.ec (Fernando Cobos)
ralvarezp411@gmail.com (Rosa Alvarez)
* Corresponding author

© Universiti Putra Malaysia Press

performance and greater yield stability under severe infestation. These contrasting responses indicate that L1 and L2 represent complementary sources of resistance and tolerance, respectively. Their integration into national breeding programs and nematode-prone production systems could enhance rice resilience, support sustainable crop management, and contribute to food security.

Keywords: Genetic resistance, inoculum, *Meloidogyne graminicola*, nematode reproduction, rice, stress

INTRODUCTION

Rice (*Oryza sativa* L.) is a staple food for a significant portion of the world's population. However, the commercial production of this crop faces several biotic limits, such as plant-parasitic nematodes, which cause substantial yield losses in rice and represent an important constraint to crop productivity, particularly under favourable conditions for nematode development and infestation (Dutta et al., 2023; Jain et al., 2012). *Meloidogyne graminicola* has shown major value as a parasitic nematode affecting various crops in different parts of the world (Akhter et al., 2024; Mantelin et al., 2017). Even though being at low densities, this nematode causes significant yield losses in important crops such as sorghum (*Sorghum bicolor* (L.) Moench), wheat (*Triticum aestivum* L.), and rice (*O. sativa* L.) (Kumar et al., 2017; Singh Dhurwey et al., 2019).

M. graminicola is a species with a facultative meiotic parthenogenetic reproductive capacity. In the adult stage, females remain within the knots, depositing eggs in a matrix within the root cortex. The first-stage juveniles (J1) develop within the egg and molt to become J2. In the second juvenile stage (J2), it moves through the soil to find a suitable host and then penetrates the root to migrate intercellularly to the region of the meristem, where it establishes itself permanently, continuing its biological cycle until the adult stage, generating hyperplasia and hypertrophy in the surrounding cells, and causing the formation of hook-shaped knots in the root system, mostly at the root tips. High temperatures and humidity levels favour their development and reproduction, thereby increasing the degree of infestation. Under normal conditions, up to 50 adult females can be found in a single knot (Rusinque et al., 2021).

The knot nematode has been detected in rice fields located in Asia, America, South Africa and in recent years in Europe, which is why it was included in 2018 in the EPPO (European and Mediterranean Plant Protection Organisation) pest alert list, due to its potential damage to world production and its high survival capacity in environments with low oxygen levels (Rusinque et al., 2021; Ye et al., 2024).

In Ecuador, it was identified for the first time in the Guayas Province (1987), in a field planted with the Oryzica 1 cultivar, and thirteen years later (2000), it had already spread to all rice fields in that province, from where it continued to spread, being detected by 2002 in the Los Ríos Province, both in rainfed and irrigated rice fields, with high planting

densities (Rusinque et al., 2021). In this country, it has caused losses of more than 70% in irrigated rice crops, reaching populations of 4000 juveniles 2 of this nematode per plant (J2. P^{-1}), causing significant economic and social damage (Bridge & Starr, 2007; Lombeida García et al., 2022).

Due to its dispersion and its power to affect all commercial rice varieties, it is a cause for concern in regions where farmers use direct rice sowing systems, since high populations of this pathogen are recorded there (Buthanna Narasimhamurthy et al., 2023; Gilces et al., 2016).

This nematode alters root development and physiology, leading to the proliferation of very thin and spongy roots, resulting in substantial yield losses. The severity of the symptoms depends on the crop's susceptibility, the pathogen's population density in the soil at planting time, and environmental conditions. Therefore, it is recommended to perform laboratory, soil, and root analyses to verify the presence of the nematode under controlled conditions to ensure adequate monitoring (Luo et al., 2020; Rusinque et al., 2021).

Because *M. graminicola* has adapted to survive various environmental conditions, it can damage different rice production systems. Biotic and abiotic stresses can further influence the degree of host susceptibility (Lombeida García et al., 2021b). These researchers also report that high populations of *M. graminicola* in rice soils are attributed to low crop diversification, low vegetation cover, and minimal crop rotation.

Flooded soils are not effective for control, as although they prevent the pathogen from penetrating the rice roots, *M. graminicola* has a high survival capacity in such environments. According to Rusinque et al. (2021) under continuous flooding conditions, egg masses remain viable for up to 14 months, and the juveniles (J2) for at least five months, resuming activity when the fields are drained.

The lack of knowledge among farmers facilitates the development and reproduction of this pest (Avena-Arambul et al., 2016). Therefore, it is essential to use transfer technologies and develop various methodologies for effective control. Additionally, developing resistant or tolerant crops can help reduce losses and mitigate the impact on both crops and the environment. This approach allows for the implementation of early strategies for pest control and proper management (Bellé et al., 2019; Galeng-Lawilao et al., 2018).

The objective of this study was to evaluate the responses of two advanced interspecific rice lines (L1 and L2) and the susceptible commercial cultivar INIAP 15 (VC3) to increasing initial inoculum densities of *M. graminicola* (0, 500, 1000, 2000, and 3000 J2 $plant^{-1}$), considering nematode reproduction, plant growth, and grain yield, to assess differences in resistance-related responses and yield tolerance among the evaluated genotypes. We hypothesised that (i) increasing initial inoculum densities of *M. graminicola* would negatively affect rice growth and grain yield; (ii) the evaluated rice genotypes would differ in their capacity to support nematode reproduction; and (iii) genotypic differences in grain

yield tolerance would not necessarily correspond to differences in nematode reproduction. The findings aim to provide valuable insights for decision-making regarding the efficient management of the pathogen in the field and to guide future research projects focusing on knot nematode management in rice-growing areas of Ecuador.

MATERIALS AND METHODS

The study was carried out at the Faculty of Agricultural Sciences (FACIAG) of the Technical University of Babahoyo (UTB), situated at km 7 ½ of the Babahoyo-Montalvo Road in Los Ríos province, 1º47'47.56''S and 79º28'53.40''W latitude and longitude, respectively. The experiments were conducted under controlled conditions within a greenhouse with a mean temperature of 26.3 °C and a relative humidity of 78.8%, and nematode population density assessments were carried out in the Phytopathology laboratory of FACIAG.

The rice genotypes utilised in the study included a susceptible commercial type, INIAP 15 (VC3), employed as a control, along with two advanced F_5 rice lines derived from interspecific crosses between *Oryza rufipogon* G. and *O. sativa* L. ssp. *japonica*, designated as L1= PUYON/JP002-P11-10 and L2= PUYON/JP002-P8-20.

Nematode Source, Multiplication, and Inoculation Procedure

The *M. graminicola* population was obtained from rice plants of the susceptible cultivar INIAP 15 grown under greenhouse conditions at UTB, Ecuador. The nematode was propagated on the same susceptible cultivar, with species identity verified through morphological traits. Second-stage juveniles (J2) were harvested from egg masses incubated in water until hatching, and quantified via a counting chamber to establish the target inoculum densities.

The experiment was carried out in undrained nursery bags with 1 kg of solarised soil. Five rice seeds were sown per bag and thinned to a single seedling 15 days post-germination; therefore, each bag was an independent experimental unit. At 25 days post-germination, the plants were inoculated with *M. graminicola* at five initial densities: 0, 500, 1000, 2000, and 3000 J2 plant^{-1}. Previously to inoculation, the soil was carefully cleared around each plant to expose the root circumference. A 100 mL aqueous suspension containing the designated J2 count was then slowly distributed around the exposed root zone to ensure uniform coverage instead of localised application. Although J2 viability was not explicitly quantified prior to inoculation, only freshly isolated inoculum was utilised.

Experimental Design, Experimental Unit and Variables Analysed

The experiment was conducted using a completely randomized design (CRD) with a 3 × 5 factorial arrangement and five replicates. Factor A consisted of three rice genotypes (INIAP 15, PUYON/JP002-P11-10, and PUYON/JP002-P8-20), while factor B comprised

five levels of *M. graminicola* inoculum (0, 500, 1,000, 2,000, and 3,000 second-stage juveniles [J2] per plant). The experimental unit consisted of a nursery bag containing 1 kg of solarised soil and five rice plants subjected to the same treatment combination. A total of 75 experimental units were established, corresponding to 15 treatment combinations, each with five independent replicates. As previously stated, fifteen days after germination, the plants were thinned, leaving one plant per bag, which served as the experimental unit.

Pathogen population density in the root (DPPR) and pathogen population density in 100 cm^3 of soil (DPP100) were evaluated at three phenological stages of the crop: flowering, grain filling, and harvest.

Extraction and Quantification of *Meloidogyne graminicola* Populations in Roots and Soil

The population densities of *M. graminicola* were assessed at three phenological stages of rice: flowering, grain filling, and harvest. Root and rhizosphere soil samples collected from each plot were processed independently to determine nematode population densities in the roots (DPPR) and per 100 cm^3 of soil (DPP100).

Nematode Extraction and Quantification from Roots

Nematodes were isolated from rice roots following the grinding and sieving protocol described by Lombeida García et al. (2021a). Roots were thoroughly washed, cut into 1-cm fragments, and a 10-g fresh subsample was homogenised in a blender at low speed for 20 s. The suspension was then passed through a sieve cascade (250, 150, and 25 µm mesh). Material retained on the 25 µm sieve was collected, and the final volume was adjusted to 100 mL.

Following homogenisation, a 2-mL aliquot was transferred to a counting chamber for quantification under a stereomicroscope. Total nematode abundance was determined by extrapolating counts to the final 100 mL volume, with DPPR ultimately expressed as the number of nematodes per 10 g of fresh roots.

Nematode Extraction and Quantification from Soil

Rhizosphere nematodes were isolated using the Baermann tray-based incubation method described by Lombeida García et al. (2021a). Following homogenisation of the root-zone soil samples, a 100-cm^3 subsample was transferred to filter paper on a mesh within a water-filled incubation tray. After a 72-h incubation period to allow motile nematodes, primarily second-stage juveniles (J2), to migrate into the water, the suspension was recovered, concentrated, and adjusted to a final volume of 100 mL.

After homogenisation, a 2-mL aliquot was transferred to a counting chamber and then examined under a stereomicroscope to determine the J2 count, which was extrapolated to

the total suspension volume. Soil nematode population density (DPP100) was expressed as the number of J2s per 100 cm³ of soil.

Evaluated crop characteristics comprised the number of tillers (NT), plant height (PH), root length (RL), number of knots per plant (NKP), number of panicles per plant (NPP), number of grains per plant (NGrP), empty grains per panicle (GrV), thousand-grain weight (WK1000), and grain yield (Y) adjusted to 15% moisture. These measurements followed the evaluation scales (Table 1 and Table 2) previously established by Gómez et al. (2008) and Taylor & Sasser (1978).

Table 1
M. graminicola incidence and severity rating scale

Degree	Number of Knots	Nematodes × 100 cm^{-3} of Soil	Nematodes × 10 g^{-1} of Roots	Qualification
0	0	0	0	Low
1	1 - 10	1 - 40	1 - 300	Medium
2	11 - 30	41 - 120	301 - 1000	Moderated
3	31 - 75	121 - 150	1001 - 3000	High
4	>75	> 150	> 3000	Very high

Source: Gómez et al. (2008)

Table 2
Reproductivity of Meloidogyne *spp. rating scale*

Knot Index	Reproduction Index IR = Pf/Pi	Response
0	0	Inmune
1	1-2	Resistant
2	3-10	Mildly resistant
3	11-30	Mildly sensitive
4	31-100	Sensitive
5	>100	Highly sensitive

Note. IR = Index; Pi = initial population; Pf = Final population
Source: Taylor & Sasser (1978)

Statistical Analysis

The experiment was conducted using a completely randomized design with a 3 × 5 factorial arrangement, comprising three rice genotypes (L1, L2, and VC3) and five initial inoculum densities of *M. graminicola* (0, 500, 1000, 2000, and 3000 J2 $plant^{-1}$), resulting in 15 treatment combinations. Each nursery bag containing one rice plant constituted an independent experimental unit.

The data were analysed using the following statistical model as in Equation 1:

$$Y_{ijk} = \mu + G_i + P_i + GP_{ij} + \varepsilon_{ijk} \quad [1]$$

where Y_{ijk} represents the observation obtained from the kth experimental unit of the ith genotype subjected to the jth inoculum density; μ is the overall mean; (G_i) is the fixed effect of the ith genotype; (P_i) is the fixed effect of the jth inoculum density; (GP_{ij}) is *the interaction effect between genotype and inoculum density; and* (ε_{ijk}) is the random experimental error associated with each observation.

Analysis of variance (ANOVA) was performed using InfoStat software, version 2017. The assumptions of normality of residuals and homogeneity of error variances were evaluated using the Shapiro- Wilk and Bartlett tests, respectively. Count variables and variables that did not meet the assumptions of ANOVA were transformed using the square-root transformation $\sqrt{(x + 1)}$. Treatment means were compared using Tukey's multiple-comparison test at a significance level of 5% ($\alpha = 0.05$).

Principal component analysis (PCA) and hierarchical cluster analysis were performed using variables standardised to Z-scores (mean = 0 and standard deviation = 1) to minimise the influence of differences in measurement scales. The scores and loadings of the first two principal components (PC1 and PC2) were used to construct a biplot illustrating the relationships among the evaluated observations and the contributions of the original variables to the principal components.

Hierarchical cluster analysis was performed using a Euclidean distance matrix calculated from the standardised variables and Ward's agglomerative method. The number of clusters was determined by visual inspection of the resulting dendrogram.

RESULTS AND DISCUSSION

The analysis of variance revealed significant differences ($p \leq 0.01$) for the interaction effect of genotypes by pathogen levels (G*P) in all the variables analysed, except for the number of tillers (NT), number of panicles per plant (NPP), and plant height (PH) (Table 3). This suggests that the levels of pathogen inoculum used did not affect the genotypes under study uniformly. Nevertheless, the main effects of genotypes and pathogens were highly significant for NT and NPP, while significant effects for PH were only detected for pathogen inoculum levels. This indicates that at least two genotypes showed different responses for NT and NPP, and at least two inoculum levels differed in their effects on the number of tillers, number of panicles per plant, and plant height.

RL, NGrP, WK1000, GrV and Y, mean, respectively, root length, number of grains per plant, number of empty grains per panicle, thousand-grain weight, and grain yield.

Table 3
Mean squares of ANOVA for different variables evaluated in three rice genotypes subjected to 5 levels of infestation with M. graminicola

Source of Variance	DF	DPPR ($\times 10^3$)	DPP100 ($\times 10^6$)	NT	NPP	PH	NKP
Genotypes (G)	2	3.17	72.13**	12.81**	7.84**	20.52	1725.88**
Pathogen (P)	4	141.97**	101.50**	5.01**	11.80**	145.50**	4106.62**
G*P	8	5.22**	17.74**	0.71	0.69	15.37	196.58**
Error	60	1.29	0.12	0.39	0.46	23.50	10.03
Total	74						
	Mean	38278.13	4336.26	7.787	7.00	90.00	22.96
	CV	21.11	8.06	7.986	9.69	5.39	13.80
	R2	0.89	0.99	0.69	0.71	0.35	0.97
Source of variance	**DF**	**RL**	**NGrP**	**WK1000**	**GrV**	**Y**	
Genotypes (G)	2	758.57**	576.01**	30.91**	229.20**	0.79**	
Pathogeno (P)	4	172.01**	3732.55**	26.41**	1116.76**	0.68**	
G*P	8	61.62**	187.10**	2.55**	42.49**	0.07**	
Error	60	3.54	56.90	0.21	8.58	0.01	
Total	74						
	Mean	16.55	71.65	23.86	12.13	3.82	
	CV	11.37	10.53	1.92	24.14	1.92	
	R2	0.93	0.84	0.94	0.91	0.94	

Note. **indicates significant differences at the 1% level for the effect being evaluated. DF, DPPR, DPP100, NT, NPP, PH, and NKP mean, respectively, degrees of freedom, pathogen population density in roots, the pathogen population density in 100 cm3 of soil, number of tillers, number of panicles per plant, plant height, and number of knots per plant

Pathogen Population Density at Root Level (DPPR) and in Soil Volume at 100 cm3 (DPP100)

The highest root-level population density of M. *graminicola* was observed in Line PUYON/JP002-P11-10 (L1), followed by the commercial variety INIAP 15. At the soil level, the commercial variety recorded the highest pathogen population, exceeding the two advanced lines. Considering the main effect of the inoculum levels, all four inoculum levels used significantly surpassed the control level in both roots and soil volume analysed. Regarding DPPR, the inoculum levels of 1000, 2000, and 3000 J2 plant^{-1} resulted in populations significantly ($p \leq 0.05$) higher than those recorded for the inoculum level of 500 J2. plant^{-1}. When counting the pathogen populations in the soil volume analysed, the level of 3000 J2. plant^{-1} was higher than the populations recorded at the other inoculum levels, but all inoculum levels were significantly ($p \leq 0.05$) higher than the control level.

Effect of Inoculum Density on the Development of Number of Tillers (NT) and Number of Panicles Per Plant (NPP)

The analysis of variance results indicated that at least two of the genotypes and two of the inoculum levels used produced significant differences in the number of tillers per plant (Table 3). However, no significant interaction effects were observed between the two factors, suggesting their independence.

The number of tillers varied from 6 to 9, with the lowest value observed in PUYON/JP002-P11-10 at the highest inoculum levels (2000 and 3000 J2. plant^{-1} of *M. graminicola*), and the highest value in PUYON/JP002-P8-20 at level 0 (without inoculation) (Table 4). The number of tillers counted at different pathogen inoculum levels was significantly lower than the number of tillers recorded at level zero. In the genotype x inoculum interaction, genotype 1 (L1) showed a lower number of tillers at inoculum levels of 1000, 2000, and 3000 J2. plant^{-1} compared to levels of 500 and zero J2. plant^{-1}. Genotype 2 (L2) had fewer tillers at all four inoculum levels (500, 1000, 2000, and 3000 J2. plant^{-1}) compared to the control level, while the control variety showed no significant differences in the number of tillers across different inoculum levels and the control.

Table 4
Average values of population density in root and soil and in different biometric variables evaluated in three rice genotypes, subjected to different levels of infestation with M. graminicola

Genotypes	Pathogen Levels (J2. plant^{-1})	DPPR	DPP100	NT	NPP	PH	NKP	RL
L1		44480 a	2399 c	7.00 c	6.44 c	89.16 a	14.88 c	15.08 b
L2		31432 c	5036 b	8.40 a	7.56 a	90.96 a	22.52 b	11.92 c
VC_3		38923 b	5573 a	7.96 b	7.00 b	89.88 a	31.48 a	22.64 a
LSD		414.49	197.78	0.35	0.38	2.74	1.79	1.06
	0	0.00 c	0.00 e	8.73 a	8.53 a	95.07 a	0.00 e	21.60 a
	500	32861 b	5175 c	7.93 b	6.93 b	90.80 b	15.73 d	17.33 b
	1000	52617 a	4015 d	7.53 bc	6.73 bc	88.87 b	24.20 c	15.47 c
	2000	48479 ab	5958 b	7.40 c	6.40 c	87.60 b	30.53 b	16.07 bc
	3000	57434 a	6534 a	7.33 c	6.40 c	87.67 b	44.33 a	12.27 d
LSD		17964	255.34	0.45	0.50	3.54	2.31	1.37
L1	0	0.00 f	0.00 j	8.00 bc	8.00 bc	95.00 ab	0.00 h	24.40 bc
L1	500	22772 ef	2698 h	7.60 c	6.80 de	90.80 bde	7.80 g	13.80 d
L1	1000	78273 a	1539 i	6.80 d	6.20 ef	87.80 cde	15.80 f	13.00 de
L1	2000	53078 abcde	1885 i	6.40 d	5.80 f	86.80 cde	20.60 e	12.00 de
L1	3000	68275 ab	5876 f	6.20 d	5.40 f	85.40 e	30.20 cd	12.20 de
L2	0	0 f	0.00 j	9.80 a	9.40 a	97.40 a	0.00 h	13.60 d
L2	500	27203 def	4334 g	8.20 bc	7.00 de	89.20 bde	20.00 e	12.00 de
L2	1000	51861 abcde	4417 g	8.00 bc	7.20 cd	89.00 bde	22.80 e	9.80 f

Table 4 (*continued*)

Genotypes	Pathogen Levels (J2. plant^{-1})	DPPR	DPP100	NT	NPP	PH	NKP	RL
L2	2000	35277 cde	9223 a	8.00 bc	7.20 cd	89.60 bde	27.60 d	13.00 de
L2	3000	42816 bcde	7208 c	8.00 bc	7.00 de	89.60 bde	42.20 b	11.20 ef
VC_3	0	0 f	0.00 j	8.40 b	8.20 b	92.80 abc	0.00 h	26.80 a
VC_3	500	48607 abcde	8492 b	8.00 bc	7.00 de	92.40 abd	19.40 ef	26.20 ab
VC_3	1000	27717 def	6091 ef	7.80 bc	6.80 de	89.80 bde	34.00 cd	23.60 c
VC_3	2000	57082 abcd	6765 d	7.80 bc	6.20 ef	86.40 de	43.40 b	23.20 c
VC_3	3000	61211 abc	6517 de	7.80 bc	6.80 de	88.00 cde	60.60 a	13.40 de
LSD		31115	442.19	0.79	0.86	6.13	4.01	3.54

Note. L1, L2, and VC3 represent the second-stage pathogen juveniles per plant. DPPR, DPP100, NT, NPP, PH, NKP, and RL denote the pathogen population density in roots, the pathogen population density in 100 cm3 of soil, the number of tillers per plant, the number of panicles per plant, plant height, the number of knots per plant, and root length, respectively
Means with the same letter do not show significant differences at 5%, according to Tukey's test

The number of panicles per plant ranged from 5 to 9, with the lowest value observed in L1 and the highest in L2. Analysing the main effect of inoculum levels, the number of panicles recorded at the control inoculum level was significantly higher than at the other inoculum levels used in the study. Regarding the interaction effect, all pathogen levels used resulted in a lower number of panicles per plant compared to the control across the three genotypes.

Effect of Inoculum Density on Plant Height (PH) Development

For the main effect of genotypes, no significant differences were shown in plant height (PH) among the three genotypes. However, for the main effect of inoculum levels, the zero level showed the greatest PH, significantly exceeding the other four inoculum levels. Meanwhile, when analysing the interaction effect, a general trend of decreasing PH was observed as the inoculum level increased, which was more pronounced in L1 and L2 than in VC3.

Effect of Inoculum Density on the Number of Knots Per Plant (NKP)

The average number of knots per plant was highest in the commercial variety (VC3), significantly exceeding the values recorded for the other two evaluated genotypes. With respect to the main effect of inoculum levels, the 3000 J2 plant^{-1} density induced the highest number of knots per plant, significantly surpassing all other evaluated treatments (Table 4). This pattern remained consistent across all three genotypes. These data indicate that the highest inoculum density amplified *M. graminicola* reproduction, consequently driving a greater formation of root knots as a direct parasitic response. Furthermore, highly

developed knots were observed on the roots, reflecting optimal conditions for pathogen establishment within these hosts.

Effect of Inoculum Density on Root Length (RL) Development

The commercial variety INIAP 15 exhibited the longest average root length (RL), significantly greater than the two advanced lines. Conversely, line 1 (L1) significantly outperformed line 2 (L2) in RL. Similarly, when examining the primary effect of inoculation, as anticipated, the zero level attained the greatest root length, statistically outperforming the other inoculation levels considered in the study, while the highest inoculation level (3000 J2. plant^{-1}) registered the shortest RL (Table 4). Overall, all examined genotypes demonstrated a tendency for decreased root length with higher inoculation levels, with this trend being more pronounced in VC3 and L1.

Effect of Inoculum Density on the Number of Grains/Plant (NGrP)

L1 exhibited the highest NGrP, statistically surpassing the two other genotypes. Similarly, in terms of the main effect of inoculum level, level zero demonstrated the highest average NGrP, (Table 5). Regarding the interaction effect, both L1 and VC3 displayed average NGrP values statistically equal to each other when compared with zero inoculum levels, but were higher than the other combinations of these factors. The lowest mean NGrP values were observed with the highest inoculum level utilised across all three genotypes.

Table 5
Average values for grain yield and some yield components obtained in the evaluation of three rice genotypes subjected to different levels of infestation with M. graminicola

Genotypes	Pathogen Levels (J_2. plant^{-1})	NGrP	WK1000 (g)	GrV (%)	Y (t/ha)	Relationship with Control (%)
L1		76.44 a	24.66 a	8.92 c	3.61 c	-
L2		71.68 b	24.34 b	12.53 b	3.89 b	-
VC_3		66.84 c	22.59 c	14.94 a	3.95 a	-
LSD		4.27	0.26	1.66	0.04	
	0	95.27 a	26.03 a	2.99 e	4.165 a	100.00
	500	77.20 b	23.73 b	7.75 d	3.796 b	91.14
	1000	68.60 c	23.61 b	9.99 c	3.778 b	90.71
	2000	63.93 c	23.55 b	14.14 b	3.769 b	90.49
	3000	53.27 d	22.39 c	25.79 a	3.582 c	86.00
LSD		5.51	0.34	2.14	0.054	
L1	0	98.00 a	24.48 c	2.58 g	3.917 c	100.00
L1	500	82.80 bc	23.02 hg	4.58 efg	3.683 gh	94.03

Table 5 (*continued*)

Genotypes	Pathogen Levels (J_2. plant^{-1})	NGrP	WK1000 (g)	GrV (%)	Y (t/ha)	Relationship with Control (%)
L1	1000	74.00 cd	22.64 h	6.34 ef	3.622 h	92.47
L1	2000	70.00 de	22.50 h	10.60 cd	3.600 h	91.91
L1	3000	57.40 fgh	20.32 i	20.52 b	3.251 i	83.00
L2	0	88.20 b	25.79 b	3.64 fg	4.126 b	100.00
L2	500	85.20 b	24.51 c	7.60 de	3.921 c	95.03
L2	1000	66.20 def	24.24 cd	13.54 c	3.878 cd	93.99
L2	2000	65.20 defg	23.90 de	11.10 cd	3.824 de	92.68
L2	3000	53.60 h	23.26 fg	26.78 a	3.722 fg	90.21
VC_3	0	99.60 a	27.82 a	2.76 fg	4.451 a	100.00
VC_3	500	63.60 efg	23.66 ef	11.06 cd	3.785 ef	85.04
VC_3	1000	65.60 defg	23.96 cde	10.10 cd	3.834 cde	86.14
VC_3	2000	56.60 gh	24.26 cd	20.72 b	3.882 cd	87.22
VC_3	3000	48.80 h	23.59 efg	30.06 a	3.774 efg	84.79
LSD		9.54	0.58	3.70	0.093	

Note. NGrP, WK1000, GrV and Y mean, respectively, number of grains per plant, thousand-grain weight, empty grains and grain yield
Means with the same letter do not show significant differences at 5%, according to Tukey's test

Effect of Inoculum Density on Thousand-kernel Weight (WK1000)

Similar to the previous observation, L1 and the zero-inoculum level recorded the highest average values of WK1000, significantly surpassing the other genotypes and inoculum levels used in the study. VC3 with zero inoculum level exhibited the highest average thousand kernel weight, statistically outperforming the other combinations of genotype and inoculum levels. Additionally, L2 and L1 with zero inoculum level registered the highest averages for WK1000, surpassing their respective remaining combinations of genotype and inoculum levels. All three genotypes studied exhibited the lowest averages for the variable WK1000 when the highest inoculum level used in the study was applied.

Effect of Inoculum Density on the Number of Empty Grains (GrV)

The variable GrV exhibited its highest average value at the highest inoculum level (3000 J2. P^{-1}), statistically surpassing the other inoculum levels used. The zero-inoculum level registered the lowest average GrV (Table 5). VC3 recorded the highest number of empty grains, significantly ($p \leq 0.05$) outperforming the two remaining lines (L1 and L2). L2 and VC3, with the maximum inoculum level (3000 J2. plant^{-1}), exhibited the highest average values for this variable, with no significant differences between the two factor interactions. However, it statistically surpassed the rest of the combinations of both factors.

Effect of Inoculum Density on Grain Yield (Y)

The commercial variety (VC3) obtained the best average productivity, statistically exceeding the two advanced lines used in the study. Likewise, the zero-inoculum level recorded the highest average yield, significantly exceeding the rest of the inoculum levels used in this study. The use of inoculum levels of 500, 1000 and 2000 J2. plant $^{-1}$ generated a reduction in average productivity levels of about 10 %, while with 3000 J2. plant $^{-1,}$ the reduction rose to 14 % (Table 5). When analysing the interaction effect, it can be seen that the commercial variety INIAP 15 (VC3) registered the highest average productivity with the zero-inoculum level, which was statistically superior to the rest of the inoculum levels used in this cultivar, as well as to the rest of the combinations of genotypes by inoculum level. It is worth mentioning that the two advanced lines (L1 and L2) obtained their lowest productivity levels when the highest inoculum level was used (3000 J2. plant^{-1}), which in the case of L1 represented a decrease in average productivity of 17 % with respect to the zero-inoculum level, while in L2 the reduction in yield was only 10 %. As for VC3, the average productivity was reduced by about 15 % when the inoculum level of (3000 J2. plant^{-1}) was used, compared to the zero-inoculum level used in this genotype.

Reproduction Index of *M. graminicola*

Table 6 displays the population density of the nematode *M. graminicola* in flowering plants of the improved lines and in the susceptible reference control, which were inoculated at the time of transplanting. Line 2 (L2) exhibited the highest average density with an inoculum level of 1000 J2 per plant, significantly outperforming the genotype combinations at other inoculum levels. The combinations of L2 with 500 J2 per plant and VC3 with 3000 J2 per plant recorded the second-highest population density levels in the study, with no significant differences between the two averages. The lowest population density levels were observed in genotype L1 with 3000 J2 per plant and 500 J2 per plant.

The commercial variety (VC3), susceptible to the nematode, showed a gradual increase in population density values as the inoculation level was raised from 500 to 3000 J2. plant^{-1}. In line 2, the nematode reproduced approximately 75 and 85 times compared to the initial population (500 and 1000 J2. plant^{-1}), respectively; in VC3, *M. graminicola* reproduced 46 and 24 times, while in line 1 it multiplied 25.5 and 23.8 times more with the mentioned inoculum levels. The lowest reproduction rates were observed in L1 and L2 with 3000 J2. plant^{-1}.

The first two principal components (PCs) accounted for over 80% of the observed variability in both genotypes and variables studied. Specifically, the first PC alone explained 63.12% of this variation, while the first three PCs collectively explained approximately 90% of the total phenotypic variation observed in the rice genotypes under varying levels of *M. graminicola* infestation.

In the first component (CP1), all variables significantly contributed to the total observed phenotypic variability. However, in CP2, only NT, NKP, DPP100, NGrP, WK1000, GrV, and Y made significant contributions to this variation. Similarly, in CP3, only the RL variable significantly contributed to the total observed phenotypic variation among the 11 evaluated traits (Figure 1).

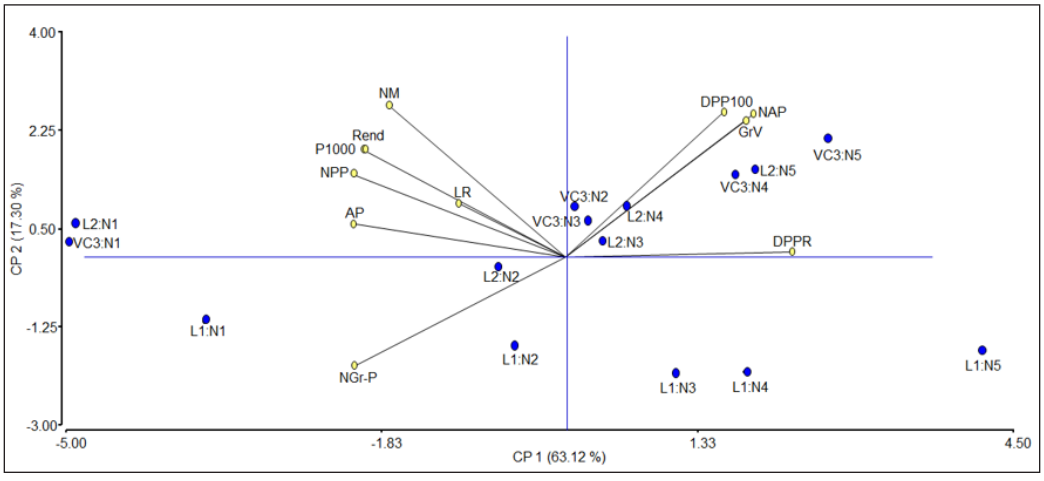

Figure 1. Principal component analysis for the characterisation of genotype performance as a function of the levels of *M. graminicola* applied

The biplot, structured with the first two axes (CP), illustrates genotype performance under varying levels of *M. graminicola* (Figure 1). Treatments (combinations of genotypes with *M. graminicola* levels) are grouped into four distinct categories. In the first quadrant, line 2 (L2) is clustered with levels N2, N3, and N5, along with the commercial variety (VC3) with levels N2, N3, N4, and N5, exhibiting the highest average pathogen populations in roots (DPPR) and 100 cm3 of soil (DPP100). Additionally, these genotypes are grouped in the second quadrant, but with the lowest pathogen level (N1), demonstrating the highest average productivity and yield components NT, NPP, and WK1000, as well as greater plant heights (PH) and root lengths (RL). In the third quadrant, lines 1 (L1) with pathogen levels N1 and N2, and L2 with level N2, show the highest average values for the number of grains per panicle. The fourth quadrant contains genotype L1 with the highest pathogen levels (N3, N4, and N5), characterised by the lowest average yield values, yield components NT, NPP, and WK1000, plant height, and root length (Figure 1).

The cluster analysis also revealed the formation of four groups based on the combination of genotypes and levels of infestation with the pathogen (Figure 2). The first group comprised all three evaluated genotypes at the lowest pathogen level (0 J2. plant^{-1}). The second group included line 1 (L1) at the intermediate pathogen level (500 J2. plant $^{-1}$) and line 2 (L2) at both intermediate and high levels of infestation (500 and 1000 J2.

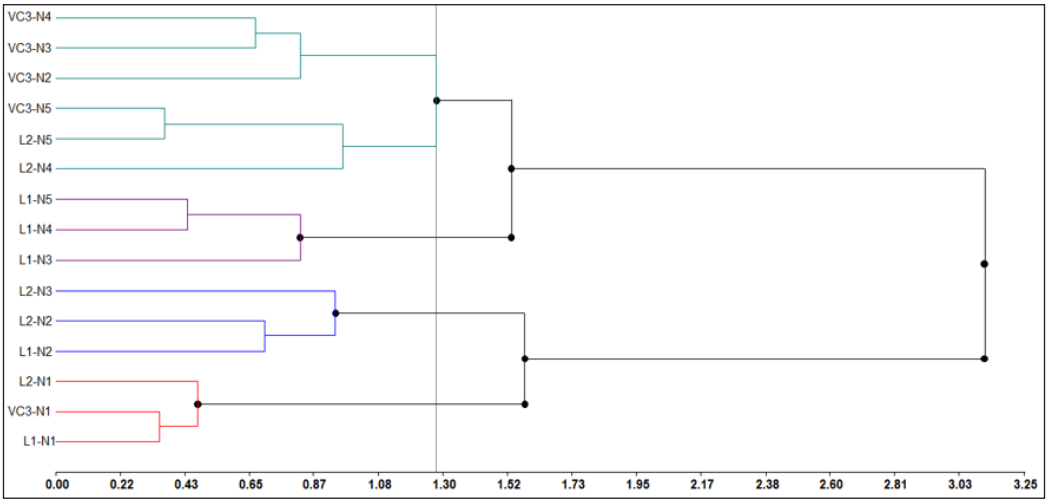

Figure 2. Dendrogram for the classification of three rice genotypes evaluated under different levels of infestation with *M. graminicola*, using 11 biometric characters. Cophenetic correlation = 0.992

plant $^{-1}$). The third group consisted solely of line 1 at the highest pathogen levels (1000, 2000, and 3000 J2. plant $^{-1}$). Finally, the largest group, the fourth, comprised line 2 (L2) at its highest infestation levels (2000 and 3000 J2. plant $^{-1}$) and the control variety (VC3) at its highest pathogen levels (500, 1000, 2000, and 3000 J2. plant $^{-1}$).

Nematode Inoculum

The nematode *M. graminicola* exhibited the ability to survive and reproduce under the environmental conditions of the experiment. In all treatments, there was a higher population during flowering compared to the original population (applied at the time of transplanting). However, as the level of infestation increased, the population growth rate of the pathogen was affected, as evidenced by a decrease in the Reproduction Index (IR). This decline could be attributed to heightened intraspecific competition for resources.

The reproduction index (IR = Pf/Pi) of *M. graminicola* generally decreased across all evaluated rice genotypes, while the initial inoculum density increased. This inverse relationship indicates a reduction in nematode multiplication relative to the initial population, a trend likely driven by density-dependent and limiting processes such as intraspecific competition for available feeding sites and root resources. Similar population dynamics have been documented in other *Meloidogyne* species (Giné et al., 2014; Noling & Ferris, 1986). Although these specific mechanisms were not directly measured in the present study, they provide a robust explanation for the observed population constraints.

Notably, the rice genotypes exhibited differential responses to shifting initial inoculum levels (Table 6). In L1, the final nematode population increased up to an inoculum level

Table 6
Population density and reproduction rate of M. graminicola *(J2. plant^{-1}) at flowering, inoculated at transplanting stage*

Rice Cultivars	Population Density of *M. graminicola* (J2. plant -1) at Transplantation	Population Density of *M. graminicola* (J2. plant -1) at Flowering	Reproduction Index (IR=Pf/Pi)
L1	500	12742 f	25.48 d
	1000	23796 d	23.80 d
	2000	22434 d	11.22 f
	3000	19230 e	6.41 g
	Mean		16.73
L2	500	37493 b	74.99 b
	1000	84923 a	84.92 a
	2000	23238 d	11.62 f
	3000	24349 d	8.12 g
	Mean		44.91
VC3	500	23211 d	46.42 c
	1000	24127 d	24.13 d
	2000	32832 c	16.42 e
	3000	36616 b	12.21 f
	Mean		24.79
	LSD	2387	2.64

Note. Pi = initial population; Pf = Final population
Means with the same letter do not show significant differences at 5%, according to Tukey's test

of 1000 J2 plant^{-1}, followed by a significant reduction at 3000 J2 plant^{-1}. Conversely, L2 displayed a more pronounced threshold response; its peak population density was recorded at 1000 J2 plant^{-1} (84,923 J2 plant^{-1}), dropping sharply at 2000 J2 plant^{-1} (23,238 J2 plant^{-1}). The susceptible control (VC3) did not show a comparable decline in final population density under high inoculum pressure, contrasting with the advanced lines. Despite these variations in final absolute populations, the reproduction index decreased at higher inoculum levels across all three genotypes. Throughout the experiment, L1 consistently maintained lower reproduction indices than L2 and VC3 at all inoculum levels, showing a comparatively lower host suitability for *M. graminicola* multiplication.

Resistance mechanisms involving restricted nematode development and impaired feeding-site formation have been documented in rice infected by *M. graminicola* (Phan et al., 2018). However, the present findings do not provide direct evidence of such mechanisms in L1 or L2, and further histological and molecular investigations are needed to elucidate the biological basis of their responses.

As is known, plants influence the composition of the soil microbiome around their roots by releasing chemicals (metabolites). Therefore, this result is significant, as resistance to *M. graminicola* has been identified in only a few rice varieties (*O. sativa*), aside from those found in *O. glaberrima* and *O. glumaepatula*, where accessions with high resistance reactions at the nematode penetration site have been reported (Petitot et al., 2020).

Effect of Inoculum Density on Root Length (RL) Development

Both the commercial variety and the lines showed decreased root development (RL) in the presence of *M. graminicola*, consistent with findings reported by Cabasan et al. (2018) who noted significant damage to the root system of rice crops in the Medio Piura valley caused by this pathogen. However, varying responses to nematode presence were observed among the materials. Specifically, the control variety (VC3) exhibited minimal variation in response to different infestation levels, experiencing a drastic 50% reduction in root length only at the highest infestation level (3000 J2. plant^{-1}), in contrast to the response of line 1 (L1), which showed a 43.44% reduction at level 1 (500 J2. plant $^{-1}$). Notably, line 2 (L2) exhibited less impact on root growth compared to other materials, experiencing an 18% reduction at the maximum infestation level (3000 J2. plant $^{-1}$).

Effect of Inoculum Density on the Number of Knots Per Plant (NKP)

Knot development was observed in all evaluated materials, indicating morphological alterations caused by pathogen attack, as previously noted by Bellafiore et al. (2015). They pointed out that knots caused by *M. graminicola* in rice plant roots occur across all developmental stages and in various rice agroecosystems. The presence of well-developed hook-shaped or spiral knots at the root apex indicates successful completion of the pathogen's life cycle (Haque & Khan, 2022; Hernández-Ochandía et al., 2012). In this study, an increase in knot numbers was observed with higher infestation levels. However, in the control variety (VC3), nematodes developed the highest number of knots, reaching an average maximum of 60 knots per plant, followed by line 2 (L2) with a maximum of 42 knots per plant, and finally line 1 (L1) with a maximum of 30.20 knots per plant. This differential effect in knot development among materials indicates the presence of defense mechanisms affecting nematode survival within plants. Dash et al. (2021) found that after juvenile nematodes penetrate resistant rice plant roots, defense-related genes are activated, triggering plant immune responses mediated by phytoalexins, defense proteins, diterpenoids, etc., inhibiting nematode growth and reproduction. Additionally, Petitot et al. (2020) found that varietal resistance in rice against *M. graminicola* involves, among other factors, the gene silencing pathway of nematode Mg-VAP1 genes in J2, reducing its ability to colonize roots. In the case of *M. graminicola*, once introduced into the plant, it undergoes molting to reach the adult stage and reproduce. The molt from J2 to J3 involves

the expression of 85 genes encoding cuticle proteins or enzymes related to the molting process, with 21 of these genes being deficiently activated (when the nematode feeds on resistant plants), affecting larval development to J3. Another defense mechanism reported in feeding sites is tissue degeneration before J2 becomes an adult.

The lower number of root galls observed in the advanced rice lines (L1 and L2) relative to the susceptible control (VC3) probably suggests genotype-dependent differences in response to *M. graminicola* infection. Similar variations in root galling patterns and nematode susceptibility among rice genotypes have been thoroughly documented (Cabasan et al., 2018; Zhan et al., 2018). These findings place both advanced lines as promising genetic resources for rice breeding programs targeting enhanced performance under nematode pressure, although further research is required to elucidate the underlying physiological and genetic mechanisms driving these differential responses.

The structural alterations induced by *M. graminicola* within the root vascular system typically alter water and nutrient transport, leading to stunted vegetative development. Furthermore, this pathogenesis impairs the photosynthetic apparatus, causing leaf chlorosis and substantial yield losses (Amarasinghe & Hemachandra, 2020; Gómez et al., 2008; Jain et al., 2012; Mantelin et al., 2017). In the present study, infestation precipitated a significant reduction in root length, tillering, and plant height (Tables 3 and 4). Yield components, including NPP, NGrP, and WK1000, were concurrently suppressed alongside an elevated percentage of empty grains (GrV), directly impacting overall grain yield.

The magnitude of these detrimental effects correlated with both *M. graminicola* population density and total knot counts relative to the uninfested control. These outcomes align with the observations of Chen et al. (2022), who reported that *M. graminicola* densities between 500 and 2000 J2 severely compromised rice morphology, suppressing plant height, panicle growth rates, and panicle length. Similarly, the altered grain characteristics observed in our trials are similar to the findings of Patil & Gaur (2014), who noted lighter grains and reduced nutritional quality under nematode pressure. Gilces et al. (2016) also demonstrated that an inoculum of 1000 J2 per plant restricted plant height, tillering, and final yield. This critical threshold behavior underscores Triviño's recommendation to implement targeted management strategies upon detecting 200 J2 per 100 cm^3 of soil, as pathogenic damage escalates sharply with increasing initial populations.

The commercial variety (VC3) exhibited the highest susceptibility, characterised by extensive root knotting. This heavy infection triggered severe vascular disruptions and depleted photoassimilate levels, compromising overall plant productivity. Notably, this physiological collapse manifested as a 51% decrease in grains per panicle, a 15% reduction in grain weight, and an overall yield loss of 16%.

Line 1 (L1) responded to the pathogen by significantly reducing RL at level 1 (500 J2. $plant^{-1}$). Despite apparent minimal impact on nematode population in the rhizosphere

(indicated by the highest value for DPPR), it effectively controlled pathogen population within the plant, evident in fewer knots. The maximum effect of *M. graminicola* on this material primarily reduced grains per panicle by 41% and grain weight by 16%, leading to a 17% yield reduction.

Line 2 (L2) exhibited the lowest DPPR value, suggesting an impact on nematode population in the rhizosphere and reduced root growth compared to other materials. At the maximum infestation level (3000 J2. plant^{-1}), it experienced only an 18% reduction, along with intermediate knot formation between L1 and VC3. This implies possession of defense mechanisms against nematode presence within the plant, likely inherited from the parental PUYON (*O. rufipogon*). Similar to other materials, L2 displayed significant effects in the presence of *M. graminicola*, including a 39.20% reduction in grains per panicle, an 8% reduction in grain weight, resulting in a 10% yield reduction.

It is evident that despite line 2 having a lower yield potential than the control variety (VC3), as it produces fewer grains per plant (88 vs 99.6) and has lower grain weight (27 vs. 25), it exhibited greater efficiency in grain filling in the presence of *M. graminicola*, resulting in yields comparable to those of VC3. In general, line 2 experienced the lowest percentage of yield loss, ranging from 4.97% (with 500 J2. plant^{-1}) to 9.79% (with 3000 J2. plant^{-1}), compared to the other two genotypes evaluated. This makes it a promising candidate for evaluation in areas with high *M. graminicola* infestation.

The evaluated rice lines exhibited contrasting responses to *M. graminicola* infection, highlighting the distinction between resistance and tolerance. Line L2 (PUYON/JP002-P8-20) demonstrated superior yield tolerance, sustaining the lowest relative yield reduction (approximately 10%) at the highest inoculum density (3000 J2 plant^{-1}). However, this line also exhibited the highest mean nematode reproduction index (44.91), indicating that its capacity to maintain grain yield did not involve a restriction of nematode multiplication. Conversely, L1 (PUYON/JP002-P11-10) exhibited the lowest mean reproduction index (16.73), suggesting a lower host suitability for nematode reproduction despite experiencing a higher yield reduction (17%). These contrasting phenotypes confirm that yield maintenance under nematode pressure operates independently of the mechanisms controlling pathogen suppression. Consequently, L2 functions as a tolerant genotype, whereas L1 exhibits true resistance traits under the evaluated experimental conditions.

Identifying genetic material with the agronomic stability of L2 remains a major breeding challenge due to the scarcity of high-tolerance rice lines. For instance, Cabasan et al. (2018) identified only a few IRRI lines capable of keeping yield reductions below 20% among their evaluated genotypes. Coyne et al. (2014) and Chen et al. (2022) underscore the necessity of developing such tolerant genotypes to mitigate pathogen-induced damage under high infestation pressure, particularly since initial nematode field densities heavily modulate the phenotypic expression of rice tolerance.

The multivariate analysis led to the formation of four groups based on the combinations of genotypes and the levels of pathogen inoculum applied, considering the 11 traits assessed in the plants. In this study, the first two principal components (PCs) accounted for slightly over 80% of the total phenotypic variation observed in the three genotypes subjected to varying levels of *M. graminicola* infestation, which is considered quite satisfactory.

Gautam et al. (2024) reported a variance of 67.5% for PC1 and 13.7% for PC2 in their study characterizing the metabolites produced by *M. graminicola* as defense mechanisms against the pathogen's action, a proportion similar to that found in this study for the first two PCs. However, as noted by Lombeida García et al. (2021b) discrepancies in such studies may stem from various factors, including the use of different genotypes, diverse agroecological environments, and the evaluation of distinct traits. Both the PC and cluster analyses facilitated a clear distinction between the treatments most affected by the levels of inoculum used, in comparison to the control.

In the principal component (CP) analysis, it was observed that both L2 and VC3 exhibited the highest levels of the pathogen, as evidenced by their grouping in the first quadrant. This grouping was attributed to their elevated average population densities in both the root and soil (DPPR and DPP100), resulting in the highest number of knots per plant among all treatments. Conversely, genotypes L2 and VC3, unaffected by the pathogen (0 J2. plant^{-1}), were positioned in the opposite quadrant.

The third quadrant housed genotype L1, which exhibited zero pathogen levels, while L1 with the highest levels of *M. graminicola* infestation (1000, 2000, and 3000 J2. plant^{-1}) was located on the opposing side, in the fourth quadrant.

However, the cluster analysis provided a clearer separation of the control with the highest levels of the pathogen. Group 1 comprised the three genotypes evaluated, without pathogen affectation, while the fourth group included L2 and VC3 with the highest pathogen levels. This analysis revealed that the level of affectation of L1 with 500 J2. plant^{-1} was similar to the affectation experienced by L2 with 500 and 1000 J2. plant^{-1} (Figure 2), suggesting that both lines (L1 and L2) behave similarly at low pathogen levels. Similarly, levels of 1000, 2000, and 3000 J2. plant^{-1} affected L1 in the same way, forming the third group, demonstrating the greater susceptibility of L1 compared to L2. The dendrogram also shows that the 2000 and 3000 J2. plant^{-1} levels of the pathogen affected L2 similarly, while VC3 showed a similar response from 500 to 3000 J2. plant^{-1}, grouping these treatments together.

These results confirm the elevated susceptibility of the commercial variety (VC3), which displayed a degree of damage comparable to that of line L2 under peak nematode pressure. Furthermore, multivariate analyses effectively segregated the experimental treatments (genotype × inoculum level combinations) based on the 11 evaluated phenotypic traits, clearly delineating how each genotype responds across the shifting pathogen gradient.

CONCLUSION

In the evaluated rice genotypes, increased inoculum levels of *M. graminicola* significantly affected characteristics such as plant growth, root development, yield components, and grain yield. Nonetheless, the magnitude of these effects varied among genotypes, indicating differential responses to nematode infestation.

The advanced rice lines L1 (PUYON/JP002-P11-10) and L2 (PUYON/JP002-P8-20) exhibited fewer root galls than the susceptible commercial variety INIAP 15 (VC3), which suggests differences in the host's response to nematode infection. But nematode multiplication was observed in both lines, indicating that neither of them completely prevented the pathogen from reproducing.

Line L2 showed the greatest tolerance in terms of yield under high inoculum pressure, with a yield reduction of approximately 10% at 3,000 J^2 per $plant^{-1}$, compared to 17% in L1 and 15% in VC3. Meanwhile, L1 exhibited the lowest average number of root galls and consistently lower nematode reproduction indices during flowering, thus suggesting differences between the two advanced lines in their response to nematode infection.

These findings highlight the potential value of both advanced lines for rice breeding programs aimed at achieving a better response to *M. graminicola*. However, although these lines were derived from interspecific crosses involving *O. rufipogon*, the present study does not determine the parental origin or the specific mechanisms underlying their responses. Therefore, further research involving the parental genotypes and genetic analyses is needed to clarify these aspects.

ACKNOWLEDGEMENT

The authors acknowledge the institutional and technical support provided by the research group throughout the development of this study.

REFERENCES

Akhter, N., Howlader, M. T. H., & Khokon, M. A. R. (2024). Phylogenetic analyses and pathogenic diversity of *Meloidogyne graminicola* of rice (cv. BRRI Dhan28) from different agro-ecological zones of Bangladesh. *Journal of King Saud University - Science*, *36*(10), Article 103472. https://doi.org/10.1016/j.jksus.2024.103472

Amarasinghe, L. D., & Hemachandra, K. H. D. J. K. (2020). *Meloidogyne graminicola* infestation in selected Sri Lankan rice varieties *Oryza sativa* L. and nemato-toxic effect of *Trichoderma* to reduce infectivity. *Journal of Science of the University of Kelaniya*, *13*, 18-34. https://doi.org/10.4038/josuk.v13i0.8021

Avena-Arambul, B., Ceceña-Durán, C., González-Mendoza, D., Grimaldo-Juárez, O., & Durán-Hernández, D. (2016). *Conducta poblacional de fitonematodos en cultivos agrícolas en el valle de Mexicali, Baja California* [Population behavior of phytonematodes in agricultural crops in the Mexicali Valley, Baja California]. OmniaScience. https://doi.org/10.3926/oms.350

Bellafiore, S., Jougla, C., Chapuis, É., Besnard, G., Suong, M., Vu, P. N., De Waele, D., Gantet, P., & Thi, X. N. (2015). Intraspecific variability of the facultative meiotic parthenogenetic root-knot nematode (*Meloidogyne graminicola*) from rice fields in Vietnam. *Comptes Rendus Biologies*, *338*(7), 471-483. https://doi.org/10.1016/j.crvi.2015.04.002

Bellé, C., Ramos, R. F., Balardin, R. R., Kaspary, T. E., & De Brida, A. L. (2019). Reaction of rice cultivars to *Meloidogyne graminicola* as a function of irrigation management. *Communications in Plant Sciences, 9*. https://doi.org/10.26814/cps2019020

Bridge, J., & Starr, J. (2007). *Plant nematodes of agricultural importance: A colour handbook* (1st ed.). CRC Press. https://doi.org/10.1201/b15142

Buthanna Narasimhamurthy, H., Sehgal, M., & Ganesha Naik, R. (2023). Rice root-knot nematode (*Meloidogyne graminicola*): A major menace in rice production. In M. Huang (Ed.), *Sustainable rice production: Challenges, strategies and opportunities*. IntechOpen. https://doi.org/10.5772/intechopen.107752

Cabasan, M. T. N., Kumar, A., & De Waele, D. (2018). Host response of rice genotypes from crosses of high-yielding and drought-tolerant *Oryza sativa* advanced breeding lines to the rice root-knot nematode *Meloidogyne graminicola*. *Archives of Phytopathology and Plant Protection, 51*(1-2), 81-94. https://doi.org/10.1080/03235408.2018.1438820

Chen, B., Sikandar, A., Ahmad, S., Luo, M., & Wu, H. (2022). *Meloidogyne graminicola*'s effect on growth performance of rice under low population density. *Agronomy, 12*(3), Article 587. https://doi.org/10.3390/agronomy12030587

Coyne, D. L., Nicol, J. M., & Claudius-Cole, B. (2014). *Practical plant nematology: A field and laboratory guide* (2nd ed.). SP-IPM Secretariat, International Institute of Tropical Agriculture. https://hdl.handle.net/10568/81172

Dash, M., Somvanshi, V. S., Budhwar, R., Godwin, J., Shukla, R. N., & Rao, U. (2021). A rice root-knot nematode *Meloidogyne graminicola*-resistant mutant rice line shows early expression of plant-defence genes. *Planta, 253*, Article 108. https://doi.org/10.1007/s00425-021-03625-0

Dutta, S., Mondal, S., Hazra, A., Ghosh, S., Panja, B., Chakrabarti, M., & Mukherjee, A. (2023). Evaluation of root-gall-associated fungal endophytes for the control of *Meloidogyne graminicola* infecting rice. *Biological Control, 186*, Article 105341. https://doi.org/10.1016/j.biocontrol.2023.105341

Galeng-Lawilao, J., Kumar, A., & De Waele, D. (2018). QTL mapping for resistance to and tolerance for the rice root-knot nematode, *Meloidogyne graminicola*. *BMC Genetics, 19*, Article 53. https://doi.org/10.1186/s12863-018-0656-1

Gautam, V., Nagar, R., Barai, P., Garg, V., Singh, S., Singh, H., Patel, S., Mukesh, Kumar, A., & Singh, R. K. (2024). Exploring the rice root metabolome to unveil key biomarkers under the stress of *Meloidogyne graminicola*. *Plant Stress, 14*, Article 100620. https://doi.org/10.1016/j.stress.2024.100620

Gilces, C. T., Santillán, D. N., & Velasco, L. (2016). Plant-parasitic nematodes associated with rice in Ecuador. *Nematropica, 46*(1), 45-53. https://journals.flvc.org/nematropica/en/article/view/88224/0

Giné, A., López-Gómez, M., Vela, M. D., Ornat, C., Talavera, M., Verdejo-Lucas, S., & Sorribas, F. J. (2014). Thermal requirements and population dynamics of root-knot nematodes on cucumber and yield losses under protected cultivation. *Plant Pathology, 63*(6), 1446-1453. https://doi.org/10.1111/ppa.12217

Gómez, L., Rodríguez, M. G., Noval, B. de la, Miranda, I., & Hernández, M. A. (2008). *Interacción entre el Ecomic® y una población cubana de Meloidogyne incognita en tomate* [Interaction between Ecomic® and a Cuban population of *Meloidogyne incognita* in tomato], *Revista de Protección Vegetal*, *23*(2), 90-98. http://scielo.sld.cu/scielo.php?script=sci_arttext&pid=S1010-27522008000200005

Haque, Z., & Khan, M. R. (2022). Organic management of rice root-knot nematode, *Meloidogyne graminicola*. In K. K. Chaudhary & M. K. Meghvansi (Eds.), *Sustainable management of nematodes in agriculture* (Vol. 1, pp. 247-267). Springer. https://doi.org/10.1007/978-3-031-09943-4_10

Hernández-Ochandía, D., Arias, Y., Gómez, L., Peteira, B., Miranda, I., & Rodríguez, M. G. (2012). Elementos del ciclo de vida de población cubana de *Meloidogyne incognita* (Kofoid y White) Chitwood en *Solanum lycopersicum* L. [Life-cycle characteristics of a Cuban population of *Meloidogyne incognita* (Kofoid and White) Chitwood in *Solanum lycopersicum* L.]. *Revista de Protección Vegetal, 27*(3), 188-193. http://scielo.sld.cu/scielo.php?script=sci_arttext&pid=S1010-27522012000300008

Jain, R. K., Khan, M. R., & Kumar, V. (2012). Rice root-knot nematode (*Meloidogyne graminicola*) infestation in rice. *Archives of Phytopathology and Plant Protection, 45*(6), 635-645. https://doi.org/10.1080/03235408.2011.588059

Kumar, V., Kumar, A., & Verma, K. K. (2017). Effect of different initial population densities of *Meloidogyne graminicola* on the plant growth of sorghum. *Journal of Entomology and Zoology Studies, 5*(5), 1906-1908. https://www.entomoljournal.com/archives/2017/vol5issue5/PartY/5-5-305-196.pdf

Lombeida García, E., Gómez Pando, L., Reyes Borja, W. O., Triviño Gilces, C., Hasang Moran, E., Duran Canare, P. L., & Hufana Duran, D. (2021a). Densidad de población de *Meloidogyne graminicola* en plantaciones comerciales de arroz y comportamiento de líneas avanzadas en la provincia de Los Ríos, Ecuador [Population density of *Meloidogyne graminicola* in commercial rice plantations and behaviour of advanced lines in the province of Los Ríos, Ecuador]. *Revista de la Sociedad Científica del Paraguay, 26*(1), 64-81. https://doi.org/10.32480/rscp.2021.26.1.64

Lombeida García, E., Gómez Pando, L., Reyes Borja, W., Medina Litardo, R., & Caicedo Camposano, O. (2021b). Evaluation the sustainability of rice production systems under different conditions of infestation with *M. graminicola*. *Neutrosophic Sets and Systems, 46*, 479-487. https://fs.unm.edu/nss8/index.php/111/article/view/2054

Lombeida García, E. D., Medina Litardo, R., Uvidia Vélez, M., & Pazmiño Pérez, Á. (2022). Caracterización de un sistema de producción de arroz (*Oriza sativa* L.) en el cantón Babahoyo [Characterisation of a rice production system in the canton of Babahoyo]. *Revista Científica y Tecnológica UPSE, 9*(2), 39-47. https://doi.org/10.26423/rctu.v9i2.686

Luo, M., Li, B. X., & Wu, H. Y. (2020). Incidence of the rice root-knot nematode, *Meloidogyne graminicola*, in Guangxi, China. *The Plant Pathology Journal, 36*(3), 297-302. https://doi.org/10.5423/PPJ.NT.02.2020.0034

Mantelin, S., Bellafiore, S., & Kyndt, T. (2017). *Meloidogyne graminicola*: A major threat to rice agriculture. *Molecular Plant Pathology, 18*(1), 3-15. https://doi.org/10.1111/mpp.12394

Noling, J. W., & Ferris, H. (1986). Influence of alfalfa plant growth on the multiplication rates and ceiling population density of *Meloidogyne hapla*. *Journal of Nematology, 18*(4), 505-511. https://pmc.ncbi.nlm.nih.gov/articles/PMC2618574/

Patil, J., & Gaur, H. S. (2014). The effect of root-knot nematode, *Meloidogyne graminicola*, on the quality and vigour of rice seed. *Nematology, 16*(5), 555-564. https://doi.org/10.1163/15685411-00002787

Petitot, A.-S., Dereeper, A., Da Silva, C., Guy, J., & Fernandez, D. (2020). Analyses of the root-knot nematode (*Meloidogyne graminicola*) transcriptome during host infection highlight specific gene expression profiling in resistant rice plants. *Pathogens, 9*(8), Article 644. https://doi.org/10.3390/pathogens9080644

Phan, N. T., De Waele, D., Lorieux, M., Xiong, L., & Bellafiore, S. (2018). A hypersensitivity-like response to *Meloidogyne graminicola* in rice (*Oryza sativa*). *Phytopathology, 108*(4), 521-528. https://doi.org/10.1094/PHYTO-07-17-0235-R

Rusinque, L., Maleita, C., Abrantes, I., Palomares-Rius, J. E., & Inácio, M. L. (2021). *Meloidogyne graminicola*—A threat to rice production: Review update on distribution, biology, identification, and management. *Biology, 10*(11), Article 1163. https://doi.org/10.3390/biology10111163

Singh Dhurwey, D., Bhatt, J., & Singh, S. N. (2019). Effect of different levels of root-knot nematode (*Meloidogyne graminicola*) on the plant growth of wheat. *Journal of Entomology and Zoology Studies, 7*(3), 1370-1373. https://www.entomoljournal.com/archives/2019/vol7issue3/PartW/7-3-227-778.pdf

Taylor, A. L., & Sasser, J. N. (1978). *Biology, identification, and control of root-knot nematodes (Meloidogyne species)*. Department of Plant Pathology, North Carolina State University, and United States Agency for International Development.

Ye, S., Zhou, S., Ma, Y., Yang, J., Shi, X., Zhang, R., Yang, Z., Peng, D., & Ding, Z. (2024). Biocontrol activity and potential mechanism of *Bacillus cereus* G5 against *Meloidogyne graminicola*. *Pesticide Biochemistry and Physiology, 204*, Article 106079. https://doi.org/10.1016/j.pestbp.2024.106079

Zhan, L. -P, Ding, Z., Peng, D. -l., Peng, H., Kong, L. -an, Liu, S. -m, Liu, Y., Li, Z. -c., & Huag, W. -k. (2018). Evaluation of Chinese rice varieties resistant to the root-knot nematode *Meloidogyne graminicola*. *Journal of Integrative Agriculture, 17*(3), 621-630. https://doi.org/10.1016/S2095-3119(17)61802-1