

Evaluation of The Susceptibility of Rice Germplasms In Cambodia To The Rice Root-Knot Nematode, *Meloidogyne Graminicola*

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Abstract: Rice is the staple food and the most important agricultural crop in Cambodia, yet yields remain constrained by a range of biotic and abiotic stresses. Among these, the root-knot nematode *Meloidogyne graminicola* (M. *graminicola*) is a major soil-borne pest that causes considerable rice yield losses across Asia, including Cambodia. Sustainable and environmentally friendly management approaches are therefore essential, with resistant cultivars recognized as one of the most reliable strategies for long-term nematode control in heavily infested areas. This study evaluated the susceptibility of 37 rice cultivars cultivated in Cambodia to M. *graminicola* infection, with the highly susceptible cultivar IR64 included as a positive control. Plants were grown under controlled conditions and inoculated with 100 second-stage juveniles (J2) isolated from Cambodian rice fields. Nematode counts after one week post-inoculation provided a preliminary assessment of susceptibility, after which nine of the least susceptible cultivars were reinoculated with 140 J2 to evaluate reproduction factors. The results showed that six cultivars exhibited moderate susceptibility, three were susceptible, and IR64 was highly susceptible to M. *graminicola*. None of the evaluated cultivars demonstrated complete resistance, and nematode penetration and development were observed within roots as early as one day post-inoculation, highlighting the aggressiveness of the pest. These findings indicate that resistant germplasm is absent among the tested Cambodian rice cultivars, underscoring the urgent need for targeted breeding programs to incorporate nematode resistance. Such efforts will be essential to strengthen sustainable rice production and contribute to long-term food security in Cambodia.

Keywords: *Meloidogyne graminicola*, Reproduction Factor, Rice germplasm, Susceptibility, Sustainable Rice Production

1. INTRODUCTION*

Rice (*Oryza sativa* L.) is a staple food for many people around the world, especially in Asia, including in Cambodia. Rice production is the most important agricultural crop in Cambodia's economy, accounting for 34% of the country's gross domestic product (GDP), and more than half of the rural population relies on it for their livelihood. Cambodia cultivates two different types of rice, the photosensitive rice (long cycle), which is cultivated once a year, and photosensitive rice (short cycle), which is cultivated two or three

times a year [1]. Improving rice production is essential, as rapid population growth in recent years has impacted food security. However, rice is vulnerable to abiotic and biotic stress, such as diseases and pests. The root knot nematode *Meloidogyne graminicola* (M. *graminicola*) is a soil-borne pest that can cause up to an 80% loss in rice yield across Asia, including Cambodia [2,3,4], which accounts for 46% of all nematicide applications [5]. M. *graminicola* could infect the root within 24h and can reproduce 250 to 300 eggs in 30 days [6]. Moreover, M. *graminicola* has a wide host range, including rice, other cereal crops, grasses, and various dicotyledonous plants [7]. In order to manage this pest, several methods have been used, including the use of

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flooding fields and the application of chemical fertilizers and pesticide practices. However, the extensive use of chemical products and intensive farming methods has resulted in a multitude of long-term environmental and human health concerns [8,9,10]. These include soil degradation, water contamination, emerging pesticide-resistant pathogens, and biodiversity loss. The use of water flooding in the field also has limitations since the water needs to remain in the field for at least 22 months. Nevertheless, the water is limited in some regions. Moreover, the use of the flooding field could lead to methane emission [11], which leads to global warming. In response to these challenges, finding environmentally friendly alternative methods to control parasites is a priority for sustainable development and ensuring food safety. Biocontrol strategies were used worldwide for managing various pests and diseases. A study on resistant rice against plant-parasitic nematodes could be used as a strategy to reduce the impact of these pests on rice yields. Plant resistance is normally defined as the heritable ability of plants to escape attacks by the Root-knot nematode, partially or fully, thus minimizing the amount of damage experienced by the plant [12]. A tolerance plant is described as a plant's ability to minimize the negative impact of stress on its fitness, even when the stressor is present and causing damage [13]. At a world level, several rice cultivars have been reported to be resistant to *Mg*, including cultivars from *Oryza longistaminata* and *Oryza glaberrima* [14,15], *Oryza japonica* [16,17], and a few cultivars from *Oryza sativa* [18,19,20]. However, the evaluation of rice resistance to *Mg* in Cambodia has never been studied before. This study aimed to evaluate the susceptibility of a collection of Indica rice germplasm in Cambodia that could be naturally resistant to the Rice Root-Knot Nematode, *M. graminicola*, to be used in breeding programs contributing to sustainable rice production in Cambodia.

2. METHODOLOGY

2.1 Nematode population

In this study, the Meloidogyne graminicola C21 population [21] originally isolated from a naturally infested rice field in Kampong Thom province in Cambodia, was maintained and propagated on the susceptible *O. sativa indica* rice cultivar IR64. First, the rice was sown for one week in 10 ml of sterile sand, and then the rice was inoculated with 100 second-stage juveniles (J2) larvae that had been isolated from the infested rice field, and another inoculation was performed two days after. One month after infection, pre-parasitic J2 were collected from the inoculation column by sieving it through three layers of mesh with the sizes of 250 μm , 100 μm , and 25 μm consecutively. Juveniles and eggs were collected on the 25 μm mesh, rinsed several times with sterile distilled water (dH₂O), and collected into a hatching column filled with mineral water. After 24 h of

incubation, the juveniles present in the beaker were used as inoculum.

2.2 Rice genotype

The rice cultivars used in this study mostly originated from Cambodia, and a few cultivars from Laos and Thailand. Most of the Cambodian rice germplasms were previously studied for genetic variation by Orn et al. [22]. The rice belongs to *Oryza sativa* L. and is an *Indica* genotype. The rice seeds were obtained from the Cambodia Agriculture Research and Development Institute (CARDI) and the General Directorate of Agriculture (GDA), Cambodia. Out of thirty-seven Indica rice germplasm, twenty rice germplasm were photosensitive (long cycle) and seventeen rice germplasm were insensitive (short cycle). The rice germplasms used in this study were selected based on different types, such as a collection of fragrant and white rice, colored rice, and sticky rice, as shown in **Table 1**. The nematode susceptible rice cultivar, IR64, was used as a positive control [14,15,17]. The rice germplasms were then grown and tested for their susceptibility to the plant parasitic nematode *M. graminicola*.

2.3 Nematode inoculation

For screening the susceptibility of the 38 cultivars, including susceptible rice IR64, each rice cultivar was tested three times, with each test consisting of two samples. For the evaluation of the nematode reproduction factor of the nine cultivars after screening, 10 samples of each cultivar x three replications were conducted. First, each rice cultivar was disinfected with 3% sodium hypochlorite (NaOCl) for five minutes, then the seeds were rinsed three times with sterile distilled water. Each cultivar was then pre-germinated on a plate filled with sterile sand for three days before being transferred into an inoculation column (5.5 cm high, 3 cm diameter) containing 10 ml of sterilized sand. Seven days after transferring to the inoculation column, the rice plants were inoculated with 100 J2 of *M. graminicola* suspension around the rice root apex by using non-sterile pipetting. The inoculated plants were maintained in the control room at a temperature range between 26-28°C (night-day), with a 12h photoperiod.

2.4 Evaluation of the penetration and development of *M. graminicola* on susceptible rice

The penetration and development of nematodes were evaluated by using fuchsin staining on two rice cultivars, Sen Kraob and IR64. Sen Kraob is a non-seasonal fragrant rice that was released in 2019 by CARDI, and it has gained recognition for winning the gold award two times in 2023 and 2024. In addition to its high yield and market price, this variety has been recommended and encouraged to farmers by

the Ministry of Agriculture, Forestry, and Fisheries, making it particularly relevant for evaluation. The rice plants were inoculated with 100 J2. Infected root tips were then stained with Acid fuchsin modified from Bybd *et al.* [23] at different times: 1 day post inoculation (dpi), 2 dpi, 4 dpi, and 6 dpi. The plants without nematode inoculation served as a control. First, the infected rice roots were thoroughly washed with tap water to remove the soil. Then, the roots were cut into small segments (2 to 5 cm) and placed in a 150 ml beaker containing 50 ml of 0.6% NaOCl. The roots were left at room temperature for seven minutes, with occasional shaking. Next, the roots were washed three times with 150 mL of tap water and soaked for an additional 10 minutes to remove residual NaOCl. The roots were then stained in a 30-fold dilution of fuchsin solution (0.35% of acid fuchsin (C₂₀H₁₇N₃Na₂O₉S₃) and 25% of acetic acid (CH₃COOH). The root was then boiled in acid fuchsin for 3 minutes at low temperature, then the root was cool down at room temperature for one hour before washing with distaining solution containing 100% acetic acid (glacial), lactic acid (90% of S-lactic acid) and distilled water at the ratio of 1:1:1 v/v/v (milliliter), then rinsed twice with distilled water. The stained roots were then placed on a glass slide with a drop of glycerol for observation and taking pictures under a stereomicroscope (Leica).

2.5 Evaluation of the susceptibility of the rice cultivars

The evaluation of the susceptibility of rice in Cambodia was conducted through two consecutive screening trials. The first screening was conducted on 38 rice cultivars, including susceptible rice IR64, based on counting the number of galls observed one week after infection to assess the level of infestation. The second evaluation was conducted on nine rice cultivars that showed lower susceptibility in the previous screening. IR64 was used as a susceptible control. For the first screening, the inoculated rice plants were collected to count the number of galls after seven days of inoculation. This test was conducted with three independent repetitions, each consisting of two replications.

For the second evaluation, three independent experiments were carried out with 10 plants of each cultivar for each experiment. The rice plants were inoculated with 140 J2 as the initial population (Pi). After 30 days, the plants were assessed for infection. Infected plants were then collected for the extraction of eggs and juveniles, which were counted under a stereomicroscope. The susceptibility of rice cultivars to the *M. graminicola* was rated according to the reproduction factor (Rf), modified by Sasser *et al.* [30] and Shekari *et al.* [31]. The total number of juveniles and eggs per plant was recorded as the final population (Pf), and the

reproduction factor (Rf) was calculated as the equation below:

$$(Rf = Pf / Pi) \quad (\text{Eq. 1})$$

Plants with an Rf < 0.1 were rated as highly resistant, Rf = 1 was rated as resistant, Rf > 1 was rated as moderately susceptible, Rf > 5 was rated as susceptible, and Rf > 10 was rated as highly susceptible.

2.6 Statistical analysis

The descriptive statistics and statistical analysis of significant differences were performed using Microsoft Excel and RStudio software version 4.4.2 in 2024. The confidence interval of 95% was selected for all statistical tests. After verifying the normality by the Shapiro-Wilk range test, the comparison of the root galling index and reproduction factor between each rice cultivar was performed using one-way ANOVA followed by Turkey's post hoc test with a *p*-value threshold of 0.05. Below this threshold, the observed differences were considered significant.

Table 1. Information of the 38 rice cultivars in Cambodia

Rice cultivars	Photoperiod	Origin	Cycle	References
Collection of fragrant and white rice				
White Phka Rumduol	Sensitive	Cambodia	6 months	[24]
Kraches	Sensitive	Cambodia	-	
Neang Om	Sensitive	Cambodia	6 months	[25]
Neang Ork	Sensitive	Cambodia	6 months	[22]
Phka Romdeng	Sensitive	Cambodia	6 months	[24]
Phka Mealadei	Sensitive	Cambodia	6 months	[26]
Phka Romeat	Sensitive	Cambodia	6 months	[24]
Prich	Sensitive	Cambodia	6 months	[27]
Russey	Sensitive	Cambodia	-	-
Sen Pidao	Sensitive	Cambodia	4 months	[24]
Thnoat	Sensitive	Cambodia	6 months	[22]
Angkrang	Sensitive	Cambodia	-	-
CAR14	Insensitive	Cambodia	3 months	[24]
CAR15	Insensitive	Cambodia	4 months	[24]
CAR16	Insensitive	Cambodia	4 months	[24]
Sen Kraob	Insensitive	Cambodia	3 months	[28]
Champe Sar 70	Insensitive	Cambodia	3 months	[26]
SBT254	Insensitive	Laos	-	-
SBT26	Insensitive	Laos	-	-
SBT65	Insensitive	Laos	-	-
SBT7	Insensitive	Laos	-	-
Phka Rumduol Prang	Insensitive	Cambodia	4 months	[24]
Sragae Sral	Insensitive	Cambodia	3 months	-
Line 6.OTY.9036	-	Cambodia	-	-
Color rice				
Neang Kouy	Insensitive	Cambodia	3 months	[29]
Red Phka Romdoul	Insensitive	Cambodia	4 months	[24]
Preto L	Insensitive	Laos	-	-
TC12	Insensitive	-	-	-
TC13	Insensitive	-	-	-
TC14	Insensitive	-	-	-
SCS 119 Rubi	Sensitive	-	-	-
ROJO SEMA	Sensitive	-	-	-
Sticky rice				
Sbai Mongkol	Sensitive	Cambodia	6 months	[24]
TDK10	Sensitive	Thailand	-	-
RD6	Sensitive	Laos	-	-
HS	Insensitive	Laos	-	-
PGHD	Insensitive	Laos	-	-
Susceptible control				
IR64	Insensitive	IRRI	4 months	

(-) means missing information

3. RESULTS AND DISCUSSION

3.1 Nematode penetration and development

The Fuchsin staining showed the penetration and development of root-knot nematode *M. graminicola* in susceptible rice IR64 and Cambodia rice cultivar, Sen Kraob (Fig. 1). In control rice, IR64 and Sen Kraob have shown no gall formation in both rice cultivars. The nematodes were shown to penetrate the root as early as 1 day post-inoculation (dpi) in both IR64 and Sen Kraob. The nematode migrated vertically to the root tip at 1 dpi. In 2 dpi, the nematodes were seen to turn and move horizontally through the root rip in both IR64 and Sen Kraob rice. The J2 of Mg induces the formation of a specialized feeding site, or the giant cell. This giant cell is a source of nutrition for the nematode's development inside the root. The gall formation was detected in 2 dpi in IR64, while Sen Kraob was delayed until 4 dpi. The delay in gall formation suggests that Sen Kraob is less susceptible than IR64. The Fuchsin staining confirmed that *Meloidogyne graminicola* penetrated both IR64 and Sen Kraob within 1 dpi, suggesting that early nematode attraction and invasion are not hindered in either cultivar. However, gall formation was observed earlier in IR64 (2 dpi) than in Sen Kraob (4 dpi), indicating that post-penetration

development, particularly the establishment of giant cells, was delayed in Sen Kraob. Such a delay has been described as a hallmark of partial resistance or tolerance in rice against root-knot nematodes [3]. The restriction of gall initiation in Sen Kraob could result from localized defense responses that interfere with giant cell differentiation, such as cell wall reinforcement or altered transcriptional reprogramming [32]. By contrast, the rapid gall formation in IR64 reflects a high level of compatibility with *M. graminicola*. Similarly, Feng et al. [20] demonstrated that nematode migration was hindered in the resistant rice cultivar Huaidao 5, resulting in lower nematode establishment. These findings suggest that Sen Kraob, though not fully resistant, may exhibit a moderately resistant phenotype that slows down nematode development.

From an agronomic perspective, such partial resistance could reduce nematode multiplication and crop damage in the field, making Sen Kraob a valuable genetic resource for rice improvement in Cambodia and other nematode-prone regions [33,34]. Further studies, including histological and transcriptomic analyses, are needed to clarify the molecular mechanisms underlying delayed gall formation and to confirm whether these responses confer durable resistance under field conditions [3].

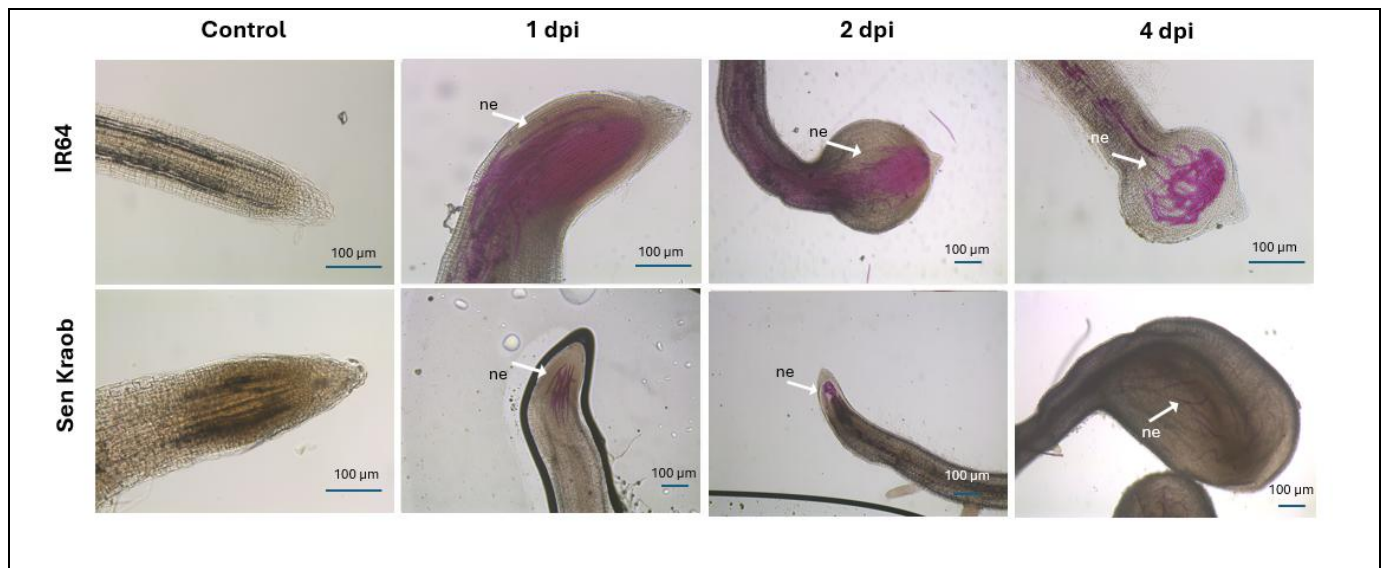


Fig. 1. Nematode penetration and development in susceptible rice IR64 and Sen Kraob

3.2 Evaluation of susceptibility of different rice cultivars in Cambodia to *M. graminicola*

A total of 37 rice cultivars in Cambodia were screened for susceptibility to *M. graminicola*. A bar graph representing the number of gallions after seven days of inoculation in different rice cultivars is shown in Fig. 2. It shows the difference in susceptibility of 37 rice cultivars to

Mg compared to the control susceptible rice IR64. The error bar indicates the difference between the cultivars. The susceptible rice cultivar IR64 has the highest gall number, with a mean of 7 ± 0.69 galls based on six replicates. The *p*-value showed no statistical difference ($p=0.15$) in gall number compared to the other rice germplasms. The rice cultivars that have the lowest gall number were from Laos, HS (2 ± 0.57) and TC14 (3 ± 0.66). Among 37 screening rice cultivars, 13 rice cultivars showed 4 number of gall seven days after inoculation, including TDK10, TC13, Phka Promdeng, Sen Kraob, Preto L, SBT254, Sen Pidao, PGHD, CAR14, CAR15, Prich TC14, and HS. However, there was no statistically significant difference among the rice cultivars ($p=0.15$). Gall formations on roots indicate the induction of nematode feeding sites that were essential for nematode development and show the damage caused by the root-knot nematode. However, the gall formation does not always correlate with nematode reproduction [35]. The formation of galls is a characteristic symptom of infection by the root-knot nematode (*Meloidogyne* spp.) and reflects the reprogramming of plant root development. Within these galls, giant cells rise through repeated nuclear division without cytokinesis. These cells act as metabolic sinks, supplying the nutrients essential for the nematode to grow and reproduce [36]. The process of gall initiation begins when infective juveniles release effectors that alter host cell cycles and hormone signaling. Nematode release secreted

effectors such as hormones, auxins and Cytokinins, which can cause the formation of a gall [37]. Auxin redistribution, mediated by transporters such as AUX1, LAX3, and PINs, stimulates abnormal cell division. Meanwhile, the action of auxin is counterbalanced by the promotion of cell proliferation and vascular differentiation by the action of the hormone cytochrome, creating a meristem-like state favorable for giant cell expansion [38,39]. Resistant cultivars may develop galls that are small, malformed, or non-functional, limiting nematode development despite visible symptoms [40,41]. These decouplings underscore that gall induction and reproductive success are partly distinct processes influenced by host defense and environmental conditions. Physiologically, galls disrupt root architecture, reduce water and nutrient uptake, and predispose plants to secondary infections, contributing to stunting and yield loss. Understanding the molecular basis of gall formation provides opportunities for crop protection. For example, Overexpression of cytokinin oxidase, which reduces cytokinin levels, has been shown to suppress gall initiation, while breeding for resistance genes that impair giant cell maintenance may offer durable nematode resistance [42,43]. The nematode reproduction factor is necessary in order to determine whether the rice is resistant. The resistance to nematodes is usually developed by selection of plants with reduced rates of nematode reproduction, typically lower following a resistant cultivar than a susceptible cultivar [44].

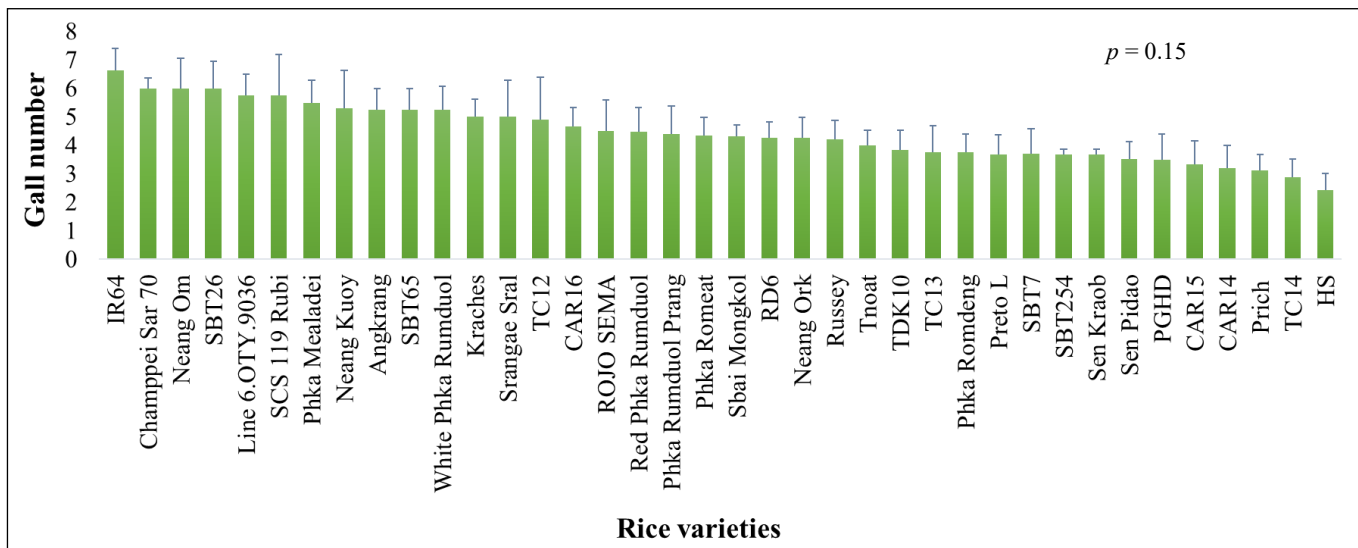


Fig. 2. Screening of susceptibility of different rice cultivars in Cambodia to *Mg* seven days after inoculation based on the number of galls. The bars in the graph represent the mean \pm SE of the data from six replications. The $p = 0.15$ indicates no statistical significance ($p > 0.05$) across isolates using the Tukey HSD posthoc test.

The reproduction factor of nine cultivars with the lowest gall number was evaluated in Fig. 3 compared to the susceptible rice IR64. All the rice cultivars had the reproduction factor higher than 1 ($R_f > 1$), which indicates susceptibility. However, compare to the susceptible rice IR64

($R_f = 11.8 \pm 2.4$), six rice cultivars had statistically significant while their reproduction factors were lower than five ($R_f < 5$) such as Sen Pidao (4.57 ± 1.35), PGHD (4.33 ± 0.77), TC14 (3.52 ± 0.3), SBT254 (2.72 ± 0.23), HS (2.71 ± 0.66) and Prich (2.07 ± 0.34). The reproduction factor of

Prich, HS, and SBT254 was almost 10 times lower than that of the susceptible rice IR64. The reproduction factor of the cultivars such as CAR14, Sen Kraob, and CAR15 was two times lower than that of IR64.

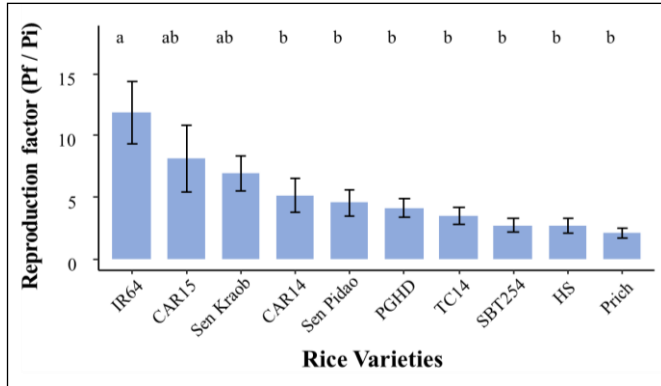


Fig. 3. Average reproduction factor of *M. graminicola* population on rice cultivars. The bars in the graph represent the mean ± SE of the data from ten replications. The same letters above each bar within each district indicate no statistical significance ($p > 0.05$) across isolates using the Tukey HSD posthoc test.

According to Shekari Mahoonaki et al. [31], rice with a reproduction factor (Rf) > 1 is considered moderately susceptible, while rice with Rf > 5 and a root galling index equal to 4 is considered susceptible. Therefore, in **Table 2**. Sen Pidao, PGHD, TC14, SBT254, HS, and Prich were classified as moderately susceptible, while CAR15, CAR14, and Sen Kraob were classified as susceptible. The differential susceptibilities among rice cultivars can be attributed to multiple factors, including the absence of major resistance genes, weaker structural barriers such as the cell wall, and induced defense responses like immune system activation upon nematode invasion [3,38]. Resistance to root-knot nematodes (*Meloidogyne* spp.) has been reported predominantly in African rice species, particularly *Oryza longistaminata* and *O. glaberrima* [14,15,39]. These species exhibit resistance at different stages of the infection process. Some genotypes deploy pre-infection mechanisms by reducing nematode attraction and penetration, while others restrict post-infection stages such as migration, development, and reproduction, or combine both strategies [47]. Pre-infection resistance is often associated with pre-existing structural barriers that limit nematode entry [48]. Dimkpa et al. [49] further demonstrated that certain *Oryza sativa* genotypes showed resistance to *M. graminicola* at very early infection stages, with reduced penetration and gall formation compared to susceptible varieties. In resistant japonica rice (*O. sativa* cv. *Zhonghua 11*), *M. graminicola* infection induces a rapid cell death response, particularly in the root mesoderm during nematode migration, thereby completely inhibiting nematode development and gall formation [17,19].

Table 2. Susceptible rating scale of Cambodia rice cultivars to *M. graminicola*

Rice cultivars	Reproduction factor (Rf)	Host status
IR64	11.87 ± 2.47	HS
CAR15	8.13 ± 3.38	S
Sen Kraob	6.93 ± 1.21	S
CAR14	5.11 ± 1.32	S
Sen Pidao	4.57 ± 1.35	MS
PGHD	4.33 ± 0.77	MS
TC14	3.52 ± 0.3	MS
SBT254	2.72 ± 0.23	MS
HS	2.71 ± 0.66	MS
Prich	2.07 ± 0.34	MS

Rf = Pf/Pi. Rf = reproduction factor, Pf = final population, Pi = initial population, HS = Highly susceptible, MS = moderate susceptible, and S = susceptible.

Recent studies have advanced our understanding of the molecular basis of rice resistance to nematodes. Quantitative Trait Loci (QTL) mapping revealed major resistance loci on chromosome 11 in *O. sativa* [50,51], while the MG1 resistance gene was identified as a key factor conferring durable resistance to *M. graminicola* [13]. Collectively, these findings suggest that the interaction between rice and root-knot nematodes involves both structural and molecular defenses, and that resistant germplasms from African rice and select Asian rice cultivars can provide valuable genetic resources for rice breeding programs.

4. CONCLUSIONS

The 37 rice germplasms cultivated in Cambodia were evaluated for their susceptibility to the root-knot nematode *Meloidogyne graminicola*. None of the tested cultivars exhibited complete resistance, as determined by gall formation. However, six cultivars Prich, HS, SBT254, TC14, Sen Pidao, and PGHD demonstrated moderate susceptibility. These findings provide valuable information for rice breeding programs aimed at improving yield and crop quality, even in fields naturally infested with *M. graminicola* in Cambodia. For future research, additional Cambodian rice cultivars, including Phka Knhey, Phka Chansensar (premium white rice), Neang Khon, Reang Chey, Ponla Pdao, Neang Minh (white rice), Leak Sleuk, Neng Sar, and Phka Mlis (fragrant rice), should be assessed to identify potential candidates with enhanced resistance. Such efforts will support the development of rice varieties better adapted to diverse agroecosystems across Cambodia and contribute to the sustainable management of *M. graminicola*.

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