Research Article

Response of Various Wheat Varieties Against Root-Knot Nematodes (*Meloidogyne graminicola*) Based on their Morphological Characters and Grain Yield

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Abstract | *Meloidogyne graminicola*, which is commonly referred to as the rice root-knot nematode (RKN), represents a substantial and prevalent challenge in Southeast Asia. This nematode species is recognized as one of the foremost biotic constraints in the region, making it a prominent and recurring issue for agriculture and crop management. This study investigated the response of fourteen different wheat plant varieties to root knot nematode and their impact on plant growth parameters. Impact of morphological plant characters on galling population and egg mass index was assessed. Results revealed that Ujala-16 yielded best crop stands with plant height (92.79 cm), root weight (0.94 g), tillering capacity (12.4), grain count per spike (49.7), grain weight (42.0 g), and grain yield per pot (9.3g). Ujala-16 and Faisalabad-86 had minimum galls per pot and egg mass index of 1.6:1.2 and 1.9:1.8, respectively. Mexipak-65 had maximum 9.4 galls per pot. There were significant variations observed among the varieties, indicating varying levels of resistance or susceptibility to nematode infestation. Ujala-16 and Sehar-06 demonstrated resistance (R) having maximum RGS (Root gall Severity) of 2.2 and 3.1 with minimum RI (Resistance Index) of 34.6 and 32.5, respectively. Faisalabad-85, Zardana-80, Morocco, and Mexipak-65 displayed moderate resistance (MR) while six varieties exhibited intermediate (IM) responses. Inqlab-91 and Iqbal-2000 were found susceptible (S) with maximum RI of 72.4 and 77.6, respectively. Significant negative correlations were found between nematode infestation and plant growth parameters. Plant height exhibited maximum impact of 59.09 and 45.24 % on galling index and egg mass index, respectively. The findings provide valuable insights for selecting resistant varieties and implementing nematode management strategies. Understanding the response of plant varieties to root knot nematodes is crucial for effective nematode control and ensuring optimal crop yield.

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Introduction

Wheat (*Triticum aestivum* L.) is highly important leading cereal crops worldwide, responsible for over 25% of global cereal production. In Pakistan, wheat plays a crucial role as the primary cereal crop and a fundamental element of the staple diet. It plays a substantial role in the agricultural sector, contributing 14.4% to the overall value-added and 3.1% to the country's GDP (Hassan *et al*., 2018). The productivity of rice and wheat in South-East Asia has experienced a decline in recent years. Comprehensive surveys and research have emphasized the significant influence of soil-borne pathogens and root health on the productivity of rice-wheat systems (Duxbury, 2001).

Meloidogyne graminicola, commonly known as root-knot nematode, are highly specialized parasites that inhabit the roots of plants. They present a significant obstacle to the cultivation of crop plantation in various tropical, subtropical, and temperate areas such as Bangladesh, Burma,

Laos, Thailand, Pakistan, Vietnam, India, China, Philippines, Nepal, and the United States (Munir and Bridge, 2003; Singh *et al*., 2007). The adult females of these nematodes possess a distinct pear-shaped or spheroid morphology, with a long neck that typically remains embedded in the root tissue. Unlike certain other nematode species, they do not undergo a transformation into cyst-like structures (Ravindra *et al*., 2017). It has emerged as a growing concern in both nurseries and upland rice cultivation. The second stage juveniles of this nematode exhibit a behavior of closely following the root tip during penetration into the roots. They migrate towards the plant's vascular cylinder, initiating the creation of multinucleated giant cells through a combination of endomitosis and cell hypertrophy processes, ultimately giving rise to the distinctive hook-shaped galls (Dutta *et al*., 2012). The primary harm inflicted on plants is a result of the disruption of vascular tissues, along with the extensive enlargement and proliferation of root cells. Infected plants exhibit stunted growth, weakened vitality, and symptoms of wilting. Studies have reported significant yield losses, ranging from 17 to 30%, attributed to poorly filled kernels in crops affected (Jain *et al*., 2012). Recent findings have emphasized the susceptibility of major crops in rice-based cropping systems, including wheat, onions, and bananas (Ravindra *et al*., 2013). Unfortunately, the use of nematicides and solarization methods is not a reliable option for controlling nematodes. Therefore, the most effective, cost-efficient, and sustainable approach to manage nematode infestations is through the use of nematode-resistant cultivars (Luc *et al*., 2005). However, there is a lack of information regarding the specific host-parasite relationships between the nematode on wheat. Acquiring such information would be valuable for identifying resistant germplasm that can be utilized effectively in breeding programs. Therefore, current study was aimed to Assess the impact of *Meloidogyne graminicola*, or the root-knot nematode, on wheat varieties in in terms of yield and plant health. Identify and characterize the host-parasite relationships between the nematode and wheat cultivars and to screen a diverse range of wheat varieties to pinpoint potential nematode-resistant germplasm and inform breeding programs, aiming to enhance crop resilience and productivity in the face of this significant agricultural challenge.

Materials and Methods

Experiment was conducted at the Department of Plant Pathology, University of Agriculture, Faisalabad, during the 2020-21 to evaluate fourteen wheat (*Triticum aestivum* L.) varieties for their resistance against *M. graminicola*. The evaluation was based on various morphological plant characteristics, including plant height, root weight, tillering capacity, grain count per spike, grain weight, and grain yield. Diseased plants were collected from wheat fields located in Jhumra, Jaranwala, and the University of Agriculture, Faisalabad. Nematode was isolated from both root and soil samples using the Baermann funnel technique with incubation periods of 24, 48, and 72 hours. This methodology was chosen to gain insights into the presence and distribution of nematodes within a particular environment, potentially of agricultural or ecological significance. The Baermann funnel technique is a widely employed method for nematode isolation, as it allows these microscopic organisms to migrate out of the soil or root samples and collect in a container through a water-based system, facilitating their subsequent examination. The use of varied incubation periods, such as 24, 48, and 72 hours, suggests an interest in capturing nematodes at different life stages or with varying behaviors, as nematodes may migrate out of their habitat at different rates. Longer incubation times can provide a

more comprehensive representation of the nematode community in the studied area The soil used for filling the pots was sterilized at 120°C for 20 minutes and stored in plastic bags at 25°C before the inoculation. The wheat varieties were planted in pots with a diameter of 20 cm using sterilized soil. After one month, when the plants established their root systems, they were inoculated with *M. graminicola* by creating holes in the soil around each plant and covering them to prevent moisture loss. Data was collected after 5 to 6 weeks of inoculation, including the number of nematode galls per pot and the egg mass index, based on the morphological plant characteristics of the different varieties. The varieties were screened on the basis of their RI, RGS and RF.

$RI^a = RGS^b + RF^c$ a=Resistance Index; Where RI<2.0 = Immune, RI 2.1-4.0 = Highly Resistant (HR), RI 4.1-18 = Resistant (R), RI 18.1-50 = Moderately Resistant (MR), RI 51-71= Intermediate (IM), RI 72-98 = Susceptible(S), RI >99 = Highly Susceptible (HS), b= Root Galling Severity; Where Scale1= No symptoms observed, healthy, 9=>80 % of the roots were galled, c= Reproductive Factor is 101 number of eggs recorded from roots divided by number of eggs used to inoculate the roots (Mullin *et al.*, 1991). Data recorded was analyzed using Analysis of Variance (ANOVA) and their means were compared using Tukey's (HSD) (Steel and Torrie, 1960). Correlation and regression analysis were computed by using Minitab 13 (Minitab, 2013). Graphical representation was made by using Microsoft Excel program.

$$\text{Relative frequency of Nematode (\%)} = \frac{\text{Number of samples with RKN}}{\text{Total number of samples}} \times 100$$

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

$$\text{Disease Severity (\%)} = \frac{\text{Sum of all diseasse rating}}{\text{total number of rating} \times \text{maximum disease grade}} \times 100$$

Results and Discussion

The varying responses of different plant varieties in terms of their morphological plant characters were found as shown in Figure 1. These variations encompass plant height, root weight, tillering capacity, grain count per spike, grain weight, and grain yield. Among the plant height measurements, Ujala-16 stands out as the tallest variety, reaching an impressive 92.79 cm, while Punjab-96 shows the shortest plants at 68.4 cm. Turning our attention to root weight, Sehar-06 exhibits the highest value of 0.94 g, indicating a robust and well-developed root system, whereas Mexipak-65 displays the lowest weight at 0.6 g. When considering the number of tillers per pot, both Ujala-16 and Sehar-06 demonstrated the highest counts, recording 12.4 and 11.6, respectively. This suggests their potential for prolific tillering and consequently higher grain yield. In contrast, Punjab-96 exhibits the fewest tillers per pot, totaling 7.4. Analyzing grains per spike, Ujala-16 exhibits the greatest count of 49.7, indicating its potential for a higher yield per spike.

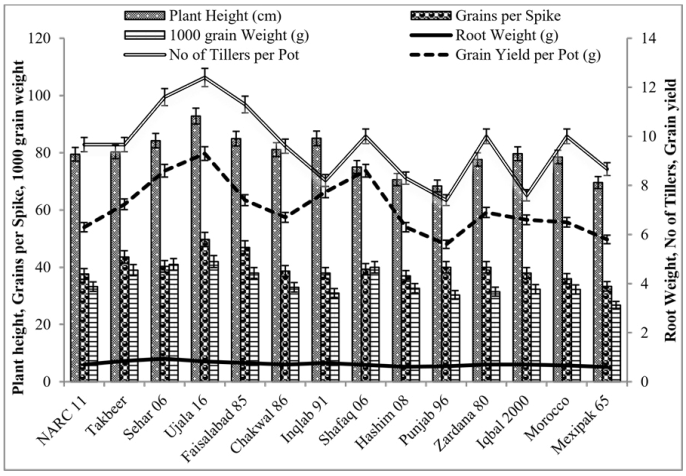

Figure 1: *Responses of different varieties to their morphological plant characters.*

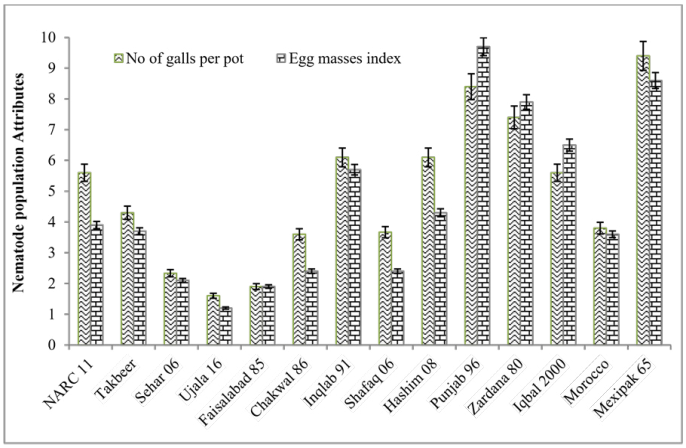

Figure 2: *Responses of different varieties to root knot population parameters.*

Conversely, Mexipak-65 displays the lowest grain count per spike, amounting to 33. In terms of 1000 grain weight (g), Ujala-16 stands out once again with the heaviest grains, weighing 42 g, while Mexipak-65 showcases the lightest grains, weighing 26.73 g. In the assessment of grain yield per pot (g), Ujala-16 achieves the highest yield at 9.3 g, signifying its potential for an overall higher yield, while Punjab-96 exhibits the lowest yield at 5.6 g. Figure 2 presents the responses of different plant varieties to root knot

nematodes, specifically focusing on the number of galls per pot and the egg masses index. The results revealed significant variations among the varieties, indicating varying levels of resistance or susceptibility to nematode infestation. Notably, Ujala-16 and Faisalabad-85 exhibit the lowest numbers of galls per pot, suggesting a higher degree of resistance to nematodes, with values of 1.6 and 1.9, respectively. In contrast, Punjab-96 and Mexipak-65 display the highest numbers of galls per pot, indicating a greater susceptibility to nematode infestation, with values of 8.4 and 9.4, respectively. The egg masses index provides insights into the reproductive success and abundance of nematodes. Ujala-16 stands out once again with the lowest index value of 1.2, indicating a lower presence of nematode egg masses and potentially a reduced nematode population. In contrast, Punjab-96 and Mexipak-65 exhibit higher index values of 9.7 and 8.6, respectively, indicating a higher abundance of nematode egg masses and a more significant nematode presence. Overall, Ujala-16 demonstrates a higher level of resistance or tolerance to root knot nematodes based on both the number of galls per pot and the egg masses index. Conversely, Punjab-96 and Mexipak-65 exhibit greater susceptibility to nematode infestation.

The number of galls per pot and egg mass index had a negative correlation with various plant growth parameters, including plant height, root weight, number of tillers per pot, grains per spike, 1000 grain weight, and grain yield per pot (Table 2). All of these parameters had a strong negative correlation which suggests that higher infestations of root knot nematodes are associated with decreased performance in these growth parameters. The relationships between the independent variables (plant height, root weight, number of tillers per pot, grains per spike, grain weight, and grain yield per pot) and the dependent variables (number of galls per pot and egg mass index) were examined using regression equations, as shown in Table 3. The number of galls per pot (Y_1), the regression equations indicated that plant height had the most significant role in per unit decrease in the number of galls with a maximum impact of 59.09% ($p = 0.001$). This was followed by grain yield per pot, number of tillers per pot, and grain weight, with impact of 11.89, 10.2 and 7.63%, respectively. Grains per spike and root weight had non-significant impact of 1.37 and 1.92%, respectively. Similarly, the impact of the independent variables on the egg mass index (Y_2) showed significant results based on the provided

Table 1: *Classification of varieties based on their resistance response.*

S. No	Varieties	Root gall severity (RGS)		Reproductive factor (RF)	Resistant index (RI)	
		Mean ±S.E	Scale		Score	Reaction
1	NARC 11	6.9±0.4	8.0	43.4	50.3	IM
2	Takbeer	5.4±0.7	6.0	48.6	54.0	IM
3	Sehar 06	2.7±0.1	4.0	15.2	17.9	R
4	Ujala 16	2.2±0.2	4.0	15.6	17.8	R
5	Faisalabad 85	3.9±0.1	6.0	37.6	41.5	MR
6	Chakwal 86	6.8±0.5	7.0	45.6	52.4	IM
7	Inqlab 91	5.3±0.3	6.0	66.4	71.7	S
8	Shafaq 06	7.1±0.9	7.0	57.4	64.5	IM
9	Hashim 08	8.3±0.5	7.0	46.5	54.8	IM
10	Punjab 96	6.9±1.0	6.0	55.6	62.5	IM
11	Zardana 80	5.5±0.3	5.0	42.6	48.1	MR
12	Iqbal 2000	6.4±0.8	6.0	71.4	77.8	S
13	Morocco	6.3±0.7	7.0	38.7	44.9	MR
14	Mexipak 65	5.9±0.4	6.0	39.9	45.8	MR
Standard deviation (SD)		1.6	2.1	5.6	7.6	
P value (0.05)		0.042*	0.001**	0.002**	0.00**	
Regression equation			R_2	Impact (%)	P value	
$^*Y_1=26.5 -0.2729 X_1^*$			59.09	59.09	0.001	
$^*Y_1=25.74-0.213 X_1^*-5.38 X_2^{ns}$			61.01	1.92	0.005	

R, Resistant; IM, Intermediate; MR, Moderately Resistant; S, Susceptible.

regression equations and associated p-values. The regression equations revealed that plant height had the most significant role for decrease in the egg mass index with a maximum impact of 45.24% ($p = 0.000$). Grain weight and number of tillers per pot followed with impact of 24.44 and 14.72% respectively. On the other hand, root weight and grains per spike had non-significant impact of 0.73 and 0.47% respectively. Table 1 shows the classification of different varieties based on their response to root knot nematodes. Two varieties, Sehar-06 and Ujala-16, are classified as resistant, as evidenced by their low root gall severity (RGS) values of 2.7 and 2.2, respectively. Their reproductive factor (RF) values of 15.2 and 15.6, and resistant index (RI) values of 17.9 and 17.8, respectively. Four varieties display an intermediate response to root knot nematodes. Faisalabad-85, Zardana-80, Morocco, and Mexipak-85 are classified as moderately resistant (MR), with RI scores of 41.5, 48.1, 44.9, and 45.8, respectively. Six varieties, namely

Table 2: *Correlation of root knot nematodes development with morphological plant characters.*

Nematodes development	Plant height (cm)	Root weight (g)	No of tillers per pot	Grains per spike	1000 grain weight (g)	Grain yield per pot (g)
Number of galls per Pot	-0.769 (0.001)*	-0.682 (0.007)*	-0.783 (0.001)*	-0.659 (0.010)*	-0.859 (0.000)*	-0.745 (0.002)*
Egg masses index (1-10) scale	-0.673 (0.008)*	-0.573 (0.032)*	-0.751 (0.002)*	-0.514 (0.060)*	-0.805 (0.001)*	-0.694 (0.006)*

Table 3: *Impact of different morphological plant characters with nematode population.*

Y_1=24.34-0.136 X_1- 2.07 X_2^{ns}-0.741 X_3	71.21	10.2	0.001
*Y_1=24.76-0.124^{ns} X_1-1.81 X_2^{ns}-0.694 X_3^{ns}-0.049 X_4^{ns}	72.58	1.37	0.052
Y_1=26.96-0.2172 X_1+9.19 X_2^{ns}-0.151 X_3^{ns}+0.142 X_4^{ns}-0.455 X_5*	80.21	7.63	0.002
Y_1=29.49 0.2971 X_1+10.18 X_2*-0.159 X_3*+0.207 X_4^{ns}-0.652 X_5*+1.038 X_6^{ns}	92.10	11.89	0.000
Y_2=25.98 -0.2708 X_1	45.24	45.24	0.003
*Y_2=25.40-0.229 X_1^{ns}-3.76 X_2^{ns}	45.97	0.73	0.005
Y_2=23.49-0.124 X_1^{ns}- 0.74 X_2^{ns}-1.009 X_3	60.69	14.72	0.021
Y_2=22.95-0.138^{ns} X_1-0.41 X_2^{ns}-1.070 X3^{ns}+0.063 X_4^{ns}	61.16	0.47	0.064
Y_2=26.02-0.267 X_1+15.72 X_2*-0.315 X_3^{ns}+0.330 X_4*-0.633 X_5*	85.60	24.44	0.003
Y_2=29.10- 0.365 X_1+16.92 X_2*-0.325 X_3^{ns}+0.409 X_4*-0.873 X_5*+1.267 X_6^{ns}	90.56	4.96	0.000

Y_1= Number of galls per pot, Y_2=Egg mass index, X_1= Plant Height (cm), X_2= Root Weight (g), X_3= No of Tillers per Pot, X_4= Grains per Spike, X_5= grain Weight (g), X_6= Grain Yield per Pot (g)

NARC-11, Takbeer, Chakwal-86, Shafaq-06, Hashim-08, and Punjab-96, exhibit an intermediate response as well, with RI scores of 50.3, 54.0, 52.4, 64.5, 54.8, and 62.5, respectively. Two varieties, Inqlab-91 and Iqbal-2000, are found to be susceptible to root knot nematodes, with RI values of 71.7 and 77.8, respectively. This classification provides valuable information about the resistance or susceptibility of each variety, helping in making informed decisions regarding their use in managing root knot nematode infestation.

Different varieties gave varying degrees of responses to their morphological plant characters. Plant height (cm), root weight (g), tillering capacity, grain count per spike, grain weight (g), grain yield (g) ranged from 69.6-79.4, 0.60-0.94, 7.6-9.6, 33.3-49.7, 26.7-42.0, 5.6-9.3, respectively. Hussain *et al.* (2012) supported these findings as he found plant height (cm), tillering capacity, and grain count per spike ranged from 92-110, 342-399, 49-52, respectively. However, there is a big difference of tiller count. Because he conducted field studies, our experimental data is based on pot basis. Various wheat cultivars demonstrate distinct yield potential under identical or varying growing conditions owing to their unique genetic makeup (Alignan *et al.*, 2009; Sial *et al.*, 2010). Shahwani *et al.* (2014) measured plant height 96.75 cm, with an average of 6.03 tillers per plant. The spike length was recorded at 11.07 cm, and there were approximately 41.96 grains per spike. The seed index was determined to be 42.99 g. The biological yield reached 10,434 kg/ha, while the grain yield amounted to 4,956 kg/ha. Old varieties showed susceptible behavior against *M. graminicola* as compared to the latest approved varieties in the last few years (Shukla and Chand, 2018). Number of galls per pot and egg mass index ranged from 1.6-9.4 and 1.2-9.7, respectively among different varieties. Further, Devi *et al.* (2016) reported total number of gall and eggs per plant ranged from 1.5-38.7 and 26-3100 based on their different crops under study. The root-knot nematode (*M. graminicola*) is recognized as the most significant pest, widely present in major rice-producing countries worldwide, leading to substantial losses of up to 90% (Jain *et al.*, 2012). Out of total 14 tested varieties, two showed resistance (RI<18) four marginal resistance (RI<50), six intermediate (RI<70), and two susceptible responses (RI<90) based on their resistance index (RI) value. Hada *et al.* (2020) found genetic resistance against *M. graminicola* based on egg masses and number of galls. He found R_2 (%) of egg masses and galls ranged from 8.32-10.89 and 9.24-9.991, respectively of different plant traits. The number of galls per pot and egg mass index had negative correlation with various plant growth parameters. All

of these parameters had strong negative correlation which implies that higher infestations of root knot nematodes are associated with decreased performance in these growth parameters. Contrary to the findings of Bashir *et al.* (2013), our research indicates that there is no positive correlation between gall infestation and grain yield. It is important to highlight that the observed difference can be attributed to the variations in the location and the specific crop being studied. However, the research conducted by (Smiley *et al.*, 2004) confirmed an inverse correlation between root nematode density in winter wheat and grain yield. The number of galls per pot indicated that plant height had the most significant role in per unit decrease in the number of galls as well as egg mass index with a maximum impact of 59.09 and 45.24% ($p = 0.001$ and 0.000). Two varieties, Sehar-06 and Ujala-16, are classified as resistant, as evidenced by their low root gall severity (RGS) values of 2.7 and 2.2, respectively. Their reproductive factor (RF) values of 15.2 and 15.6, and resistant index (RI) values of 17.9 and 17.8, respectively. RGS, RF and RI values ranged from 2.2-8.3, 15.2-71.4, and 17.8-77.8, respectively. All the varieties were susceptible to root knot nematodes however only difference is aggressiveness among different varieties (Pokharel *et al.*, 2007). Pokharel *et al.* (2011) supported our findings and revealed that *M. graminicola* isolates affected the susceptibility of wheat cultivars, including those obtained from prestigious international centers such as IRRI and CIMMYT. The measured root gall severity index (RGS) ranged from 5.8 to 65.8, while the reproductive factor (RF) ranged from 3.5 to 8.5 for the evaluated wheat cultivars. This composite index, combining RGS and RF, exhibited a relatively consistent pattern with limited variation. To develop a reliable screening protocol (Pokharel *et al.*, 2012), to investigate the effects of incubation period and inoculum level on the severity of root galling (RGS) and reproductive factor (RF) on *Meloidogyne graminicola*. The findings revealed that *M. graminicola* displayed notably higher reproductive activity on wheat. The measured RGS values ranged from 5.3 to 8.3, and the resistance index covered from 117 to 162, indicating that all the tested cultivars exhibited a highly susceptible reaction to the nematode which confirmed our findings. Padgham *et al.* (2004) also reported root-galling severity ratings in wheat cultivars a range of 1.5 to 7.0 were observed. Based on these findings, it was determined that all wheat varieties exhibited a significant susceptibility to *M. graminicola*, indicating their role as excellent hosts for the nematode.

Conclusions and Recommendations

Study revealed significant variations in the response of plant varieties to root knot nematodes. Ujala-16 and Sehar-06 exhibited resistance to nematodes while Inqlab-91 and Iqbal-2000 were responded as susceptible. There was negative impact of nematodes on plant development. Plant height (cm) implied a significant relation having maximum impact on per unit population fluctuations. These findings provide valuable insights for breeders and farmers in selecting resistant varieties and implementing effective nematode management strategies.

Novelty Statement

These findings provide valuable insights for breeders and farmers in selecting resistant varieties and implementing effective nematode management strategies.

Author's Contribution

Ahmad Khan, Muhammad Zubair Akram, Muhammad Arslan Azmat and Muhammad Tahir: Data collection and manuscript writing.
Muhammad Amjad Ali: Supervise the research work and project
Samreen Nazeer: Manuscript writing.
Sabina Asghar: Data collection.

Conflict of interest
The authors have declared no conflict of interest.

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