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Evaluation of Wild and Cultivated Cucurbitaceous Rootstocks for Resistance to Root-knot Nematode (*Meloidogyne incognita*)

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ABSTRACT: Cucumber (*Cucumis sativus*) is extensively cultivated in both open fields and under protected conditions in Andhra Pradesh. Among biotic constraints, root knot nematode (*Meloidogyne incognita*) are considered as important production limiting factors causing 30 – 50% yield losses in cucumber. Among phytonematodes, particularly the genus *Meloidogyne*, commonly known as root-knot nematode, poses a significant challenge to cucumber production. These plant-parasitic nematodes rely on root sap for survival, disrupting the proper uptake of water and minerals by the plant. Symptoms of infestation include root galling, leaf yellowing, and stunted plant growth. Utilizing resistant cultivars/species is a crucial strategy for managing root-knot nematodes compared to other control methods. This study aimed to assess thirteen wild and cultivated cucurbitaceous rootstocks along with two cucumber scions for their resistance to root-knot nematode (*Meloidogyne incognita*) under protected conditions, employing artificial inoculation at the College of Horticulture, Anantharajupeta, Annamayya District. Andhra Pradesh. The experiment was laid out in completely randomized block design with two replications. The results revealed that higher highest number of days taken for germination in *Citrullus colocynthis*, shoot length, shoot fresh weight, root dry weight was observed in pumpkin whereas low number of egg masses, rootknot females and final nematode population was observed in pumpkin followed by *Citrullus colocynthis* and sponge gourd.

Keywords: Cucumber, *Meloidogyne incognita*, Screening, resistance, Artificial inoculation.

INTRODUCTION

India's diverse climate and soil conditions allow for the cultivation of a wide array of horticultural crops, earning the country its reputation as the fruit and vegetable basket of the world. Among these crops, cucumber (*Cucumis sativus*), a member of the Cucurbitaceae family, is widely cultivated in both open fields and protected environments, particularly in Andhra Pradesh. In India, cucumber is grown on 116 hectares with a production of 1608 metric tons per hectare (NHB 2021-2022 First Advance Estimates).

Pests, both insect and non-insect, including plant-parasitic nematodes, cause approximately 21.3% crop losses, amounting to Rs. 102,039.79 million (1.58 billion USD) annually in India. Among these nematodes, the root-knot nematode (*Meloidogyne* spp.) is responsible for 75.83% of the losses. It causes a 12% reduction in cucumber yield, leading to an estimated financial loss of Rs. 110.46 million per year in open-field cultivation (Kumar *et al*., 2017). However, as vegetable cultivation shifts to greenhouses, the risk of soil-borne diseases and nematode infestations increases due to monocropping and intensive farming practices.

Cucumbers are vulnerable to several serious fungal diseases and nematode infections, resulting in significant losses in both yield and quality. *Meloidogyne*, the most prevalent nematode genus in greenhouse-grown vegetables (Ti leubayeva *et al*., 2021), is a major concern. These pests infect a wide range of cultivated plants, causing substantial agricultural losses globally (Eder *et al*. 2018). *Meloidogyne incognita* attacks some of the most economically important crops, such as watermelon (*Citrullus lanatus*), tomato (*Solanum lycopersicum*), and cucumber (*Cucumis sativus*), leading to extensive damage worldwide (Bello *et al*., 2021; Eder *et al*., 2021; Kayani *et al*., 2017; Jones *et al*., 2013). The agricultural damage caused by root-knot nematodes has prompted significant research interest, with global yield losses estimated to range between 22-30% or higher (Janati *et al*., 2018). These losses are often exacerbated by the repeated cultivation of the same crop cultivars on the same land, which promotes the buildup of nematode populations (Hussain *et al*., 2016; Mukhtar *et al*., 2017a).

Nematode management typically involves a combination of resistant plant varieties, crop rotation, cultural practices, and chemical nematicides. Common control methods include chemical treatments, cultural interventions, and planting resistant cultivars (Sasanelli *et al*., 2021). Despite progress in breeding root-knot nematode-resistant varieties and hybrids for many horticultural crops, advancements have been limited for cucumber. Attempts to develop interspecific hybrids between cucumber and resistant wild *Cucumis* species have been challenging due to differences in chromosome numbers, which complicates traditional hybridization efforts.

Standardized screening methods have been developed to assess resistance to root-knot nematodes and various diseases (Fassuliotis, 1979). Biochemical responses during nematode invasion also support resistance mechanisms. Host tissues or cells may exhibit non-cooperative actions, and chemical inhibitors within the host can neutralize the giant-cell-inducing effects of nematode salivary secretions (Barons, 1939). This study aims to evaluate cucumber rootstocks for their resistance to root-knot nematodes.

MATERIAL AND METHODS

Wild and cultivated cucurbitaceous species rootstocks *viz.,* Pumpkin (*Cucurbita moschata*), Bottle gourd (*Lagenaria siceraria*), Sponge gourd (*Luffa cylindrica*), Ridge gourd (*Luffa acutangula*), Snake gourd (*Trichosanthes cucumerina*) Ash gourd (*Benincasa hispida*), *Citrullus colocynthis, Cucumis melo, Cucumis melo* sub *sps. Cocnomon, Cucumismelo sub sps Agrestis, Momordica charantia, Cucumis prophetarum, Momordica cymbalaria.*

The experiment was carried out during2022-23 at Department of Vegetable Science, College of Horticulture, Anantharajupeta, A.P. the experimental site was located geographically between 13^{o} 59′ North latitude and 79^{o} 19′ East longitude. Seedlings of rootstocks were raised in portrays. Pot culture culture experiment was carried out in CRD design with three replications in the shade net at Horticultural Research Station, Anantharajupeta during *kharif*, 2022-23**.** Plastic sterilized pots, 15 cm in diameter, were filled with a mixture of denematised, sterilized sand, soil, and FYM in a 2:1:1 ratio at 1 kg per pot. Seeds of each variety were sown in these pots containing steam-sterilized soil.

A pure culture of *M. incognita* was initiated from single egg masses and propagated on roots of a highly susceptible tomato genotype in the greenhouse. Eggs were collected from galled roots of tomato plants and inoculated into the potted plants, which had been maintained as a pure culture. This inoculation process was conducted two months prior to the start of the experiment.

Nematode inoculation. The inoculation method recommended by Sasser *et al*. (1957) was employed for introducing nematodes into the experimental setup. Infested roots from the pure culture were cut into approximately 2 cm long pieces and submerged in a 0.5% sodium hypochlorite (NaOCl) solution. The container was shaken for about three minutes to dissolve the gelatinous matrix and release the eggs from the egg mass. After this, the mixture was incubated for 48 hours under laboratory conditions. The concentration of the inoculum was adjusted to a known number by adding water. The eggs were then transferred to petri dishes and aerated frequently using an aerator to facilitate hatching.

For inoculation, the juvenile stage (J2) of *M. incognita* was placed at a depth of 2 cm near the rhizosphere and covered with sterile sand. Each pot containing 15-day-old seedlings of rootstocks and scions was inoculated with *M. incognita* J2 at a rate of two juveniles per gram of soil.

Screening of cucurbitaceous rootstocks for resistance against root-knot nematode. At 30 days after inoculation, the plants that had been inoculated were carefully removed from the pots. The roots were then washed thoroughly to remove soil and other adhering particles using a gentle stream of water. Subsequently, the roots were examined under a stereoscopic microscope, and the number of galls formed on each plant root was counted.

The cucurbit rootstocks and scions were then categorized according to the Root-knot Index Scale. This scale likely involves assessing the severity of root-knot nematode infestation based on the number and size of galls present on the roots, ranging from minimal (low severity) to severe (high severity). Each plant would be assigned a score or category on this scale to quantify the level of resistance or susceptibility to the nematodes.

Table 1: Assessment of root knot index (Taylor and Sasser 1978).

Percentage of galls	Grade	Degree of resistance
0	0	Immune (I)
1 – 2	1	Highly resistant (HR)
3 – 10	2	Resistant (R)
11 – 30	3	Moderately resistant (MR)
31 – 100	4	Susceptible (S)
101 and above	5	Highly susceptible (HS)

Evaluation of cucurbitaceous rootstocks and scions against root-knot nematode. Observations were recorded on shoot length, fresh shoot weight, dry shoot weight, root length, fresh root weight, dry root weight, number of galls/plant, final nematode population on each rootstock/scion in soil as well as in root and reactions of the cultivars to the test nematode, *Meloidogyne incognita*.

RESULTS AND DISCUSSION

Effect of nematode inoculation with *Meloidogyne incognita* on shoot and root parameters in wild and cucurbitaceous rootstocks and cucumber scions. Table 2 depicted that among the thirteen wild and cucurbitaceous rootstocks and two cucumber scions highest shoot length was recorded in followed by Pumpkin (165.33 cm) followed by *Citrullus colocynthis* (147.67 cm) and the lowest shoot fresh weight *Cucumis melo subsps agrestis* (84.67 cm). shoot fresh weight was significantly higher in sponge gourd (30.20 g) followed by Pumpkin (28.19 g) and the lowest shoot fresh weight *Cucumismelo* (9.42 g), shoot dry weight was significantly higher in Snake gourd (3.58 g) followed by Pumpkin (3.56 g) and the lowest shoot dry weight *Cucumismelo* sub *spsconomon* (0.70 g). Root length was significantly higher in sponge gourd (14.58 cm) followed by Ash gourd (14.08cm) and the lowest root length *Cucumis melo* (8.98cm). the root fresh weight was significantly higher in Ash gourd (4.30 g) followed by Bottle gourd gourd (2.15g) and the lowest shoot dry weight *Cucumis melo* sub sps *conomon* (0.70 g). Root dry weight was significantly higher in pumpkin (0.54g) followed by Bottle gourd (0.33g) and the lowest shoot dry weight *Cucumis melo* sub sps *conomon* (0.13g). The results indicated that *Meloidogyne* spp. suppressed cucumber growth when applied at the standard inoculum level.

Some of the rootstocks *viz*., ridge gourd, ash gourd and oriental pickling melon resulted in shorter shoots due to the plants impaired ability to support and sustain elongation. The damage to the roots means that less water and nutrients are available for shoot elongation. As the infected plants face insufficient supply of nutrients, photosynthates, energy and water leading to stunted and reduced growth of foliar parts subsequently results in reduced biomass and productivity which was in approval with Hussain *et al*. (2016); Kayani *et al*. (2013); Ismael and Mahmood (2020); Chahar *et al*. (2021) in cucumber.

Tamilselvi (2013) also reported that among the nematode inoculated cucurbits, mean root fresh weight were not reduced due to low nematode reproduction on species like colocynth, African horned cucumber and pumpkin roots. In susceptible species the deformed root system is due to infestation by *M. incognita* which causes development of giant cells and block age of xylem vessels. This interferes with nutrient uptake and results in impaired plant growth. These findings agree with Ploeg and Phillips (2001) in melons species and Krishnaveni and Subramanian (2002) in cucumber where more than 1000 J2 of *M. incognita* as initial inoculation caused significant reduction in shoot length, root length and shoot weight.

The reduction in plant growth might be attributed to severe root galling and an arrested root system caused by nematode infection. This decrease is likely due to the improper uptake and transport of elements, nutrients, and water resulting from the infection. Infected plants face an insufficient supply of nutrients, photosynthates, energy, and water, leading to stunted and reduced growth of foliar parts, which subsequently results in reduced biomass and productivity. These findings are consistent with the studies by Hussain *et al*. (2016); Kayani *et al*. (2013); Ismael and Mahmood (2020); Chahar *et al*. (2021) in cucumber.

The deformed root system, caused by infestation by *M. incognita*, leads to the development of giant cells and blockage of xylem vessels. This interference with nutrient uptake results in impaired plant growth. These observations are in agreement with the findings of Krishnaveni and Subramanian (2002); Thangamani *et al*. (2018); Siddique *et al*. (2020) in cucumber.

Effect of nematode inoculation on gall production. Table 3 depicted of gall parameters of different wild and cultivated cucurbitaceous species.

Among the rootstocks, the number of egg masses were significantly lower in pumpkin (4.53 g/root) followed by *Citrullus colocynthis* (10.57 g/root) and the sponge gourd (13.63 g/root).

Formation of fewer galls in resistant rootstocks was probably due to failure of nematode juveniles to produce functional feeding site in the host after invasion and to develop subsequently as reproducing females where these findings were similar to Sobezak (2005); Thangamani *et al.* (2018) in bitter gourd, Punithaveni *et al.* (2015); Ismael and Mahmood (2020) in cucumber.

Among the rootstocks, the root knot females was significantly lower in Pumpkin (11.48 g/root) followed by ridge gourd (19.81 g/root), *Citrullus colocynthis* (21.38 g/root).

In the highly susceptible cultivars, the production of the maximum egg masses on the roots explain that a maximum number of juveniles were successful in completing their life cycles after entering the host. Whereas, in contrast, in the resistant cultivars, only a few juveniles were able to infect the roots and become mature, which is reflected by their reproductive factors and the number of egg masses (Khan *et al*., 2019).

Very few egg masses were formed in the resistant lentil accessions, suggesting that the post penetration development was affected by the plant root system (Khan *et al*., 2017). Differences in egg-mass formation rates may be in part, due to genetic factor in the host which confers susceptibility or resistance (Jacquet *et al*., 2005; Castagnone-Sereno, 2006).

Resistance reaction might be due to the presence of nematode resistant gene (Hadisoeganda and Sasser, 1982; Roberts and May, 1986) making plants less attractive to attack by nematodes. The compatible and incompatible reactions might be due to presence of resistant genes, which were activated as a result of nematode invasion (Williamson, 1999; Davis *et al*., 2000; Williamson and Kumar 2006).

Among the rootstocks, reproduction factor was significantly lower in pumpkin (0.84) followed by *Citrullus colocynthis* (0.90) and the highest reproduction factor found in honey dew melon (2.58). These results are in confirmity with findings of Pandey and Nayak (2018) who tested fifty two varieties of ridge gourd and found that only six varieties were resistant reaction, seven varieties were moderately resistant, thirty two varieties were susceptible and five varieties were highly susceptible. These findings were similar to Sobezak (2005); Thangamani *et al.* (2018) in bittergourd and Punithaveni *et al.* (2015); Anjali *et al*. (2022) in cucumber.

Reaction of wild and cultivated cucurbitaceous species to test nematode. Among the rootstocks, least count of root knot index was found in pumpkin followed by *Citrullus colocynthis*attaining the reaction category of moderately resistant. A root knot index of 4 was observed in Ridge gourd, Ash gourd, *Cucumis melo* which attained the category of Susceptible and wild melon, honey dew melon recorded root knot index of 5 which could be under highly susceptible.

The biochemical mechanism of invasion supports this process, which occurs due to the non-cooperative action of host tissue or cells. Chemical inhibitors in the host tissue counteract or neutralize the giant cell-inducing effect of the nematode's salivary secretions (Barrons, 1939). Rootstocks and scions susceptible to nematode infection support higher populations compared to resistant rootstocks. These findings are similar to those reported by Sobezak (2005); Thangamani *et al*. (2018) in bitter gourd, and Punithaveni *et al*. (2015) in cucumber.

Nevertheless, cucurbit crops vary in their efficiency to host RKN. There was a lower Pf/Pi ratio for *M. incognita* on *C. pepo* (pumpkins) than on *M. charantia*, followed by *C. sativus* and *L. siceraria* (Chandra *et al*., 2010). The Pf/Pi for *M. incognita* was lower on zucchinis than cucumbers (De Souza Galatti, 2013; Lopez-Gomez and Verdejo-Lucas 2013).

Cultivars with lower reproductive factors will be appropriate for the management of root-knot nematodes. The host status of any crop is determined by the reproductive factor of the nematode, which quantifies its reproductive potential on a specific crop plant (Windham and Williams 1988). When the reproductive factor of a nematode on a selected host is less than one, it indicates that the nematode is unable to reproduce on that host. Conversely, if the reproductive factor exceeds one, the nematode can successfully multiply on that host (Pofu *et al*., 2010).

Table 2: Reaction of wild and cultivated cucurbitaceous rootstocks to *M. incognita* on growth parameters at 45 days after inoculation.

Rootstocks		Shoot length (cm)	Shoot fresh weight (g)	Shoot dry weight (g)	Root length (cm)	Root fresh weight (g)	Root dry weight (g)	Number of galls/plant (5g^{-1} of root)
T_1	Ridge gourd (*Luffa acutangula*)	98.00	12.86	2.26	13.48	2.26	0.26	56.33
T_2	Ash gourd (*Benincasa hispida*)	85.00	18.49	2.30	14.08	2.30	0.22	72.67
T_3	Sponge gourd (*Luffa cylindrica)*	130.00	30.20	3.26	14.58	2.96	0.15	28.33
T_4	Pumpkin (*Cucurbita moschata*)	165.33	28.19	3.56	11.27	3.56	0.54	22.75
T_5	Bottle gourd (*Lagenaria siceraria*)	81.00	16.73	2.57	13.11	2.57	0.33	29.45
T_6	Snake gourd (*Trichosanthes cucumerina*)	97.67	18.81	3.18	12.14	3.32	0.21	61.04
T_7	Honey dew melon (*Cucumis melo*)	85.00	9.42	0.98	8.98	1.98	0.44	128.13
T_8	Oriental pickling melon (*Cucumis melo* sub sp. *conomon*)	99.00	13.98	1.70	13.67	0.70	0.13	74.25
T_9	Bitter apple (*Citrullus colocynthis*)	147.67	13.87	1.24	12.82	1.24	0.25	15.33
T_{10}	Wild melon (*Cucumismelo sub sps Agrestis*)	84.67	14.81	1.57	11.28	1.57	0.26	116.33
T_{11}	Bitter gourd (*Momordicacharantia*)	98.67	15.95	1.62	11.73	1.62	0.18	53.67
T_{12}	Wild gourd (*Cucumisprophetarum*)	87.67	16.40	1.81	12.30	1.81	0.28	41.00
T_{13}	Kasarakayee (*Momordicacymbalaria*)	92.67	22.62	2.04	12.15	2.04	0.31	42.67
	SEd±	2.94	2.33	0.51	0.92	0.50	0.09	1.26
	CD at 5%	6.00	1.93	1.04	1.87	1.02	0.18	2.57

Table 3: Reaction of wild and cultivated cucurbitaceous rootstocks to *M. incognita* on nematode resistance parameters at 45 days after inoculation.

Rootstocks		Number of root knot females (5g^{-1} of root)	Number of egg masses (5g^{-1} of root)	Final Nematode Population (pf =A+B)			Reprod-uction Factor Rf = p final/pinitial	Root knot Index	Reaction
				Initial population (Pi)	Number of eggs/g^{-1} of root A	Number of Juveniles (J_2/ (kg of soil) B			
T_1	**Ridge gourd (*Luffa acutangula*)**	19.81	18.17	1000	285.11	1284.78	1.57	4	S
T_2	**Ash gourd (*Benincasa hispida*)**	29.44	19.79	1000	281.10	1490.43	1.77	4	S
T_3	**Sponge gourd (*Luffa cylindrica*)**	23.78	12.78	1000	255.37	748.37	1.00	3	MR
T_4	**Pumpkin (*Cucurbita moschata*)**	11.48	6.48	1000	160.67	683.00	0.84	3	MR

T_5	**Bottle gourd (*Lagenaria siceraria*)**	24.57	21.93	1000	273.67	967.67	1.24	3	MR
T_6	**Snake gourd (*Trichosanthes cucumerina*)**	35.94	22.59	1000	321.67	1312.67	1.63	4	S
T_7	**Honey dew melon (*Cucumis melo*)**	31.67	24.50	1000	301.38	2286.33	2.58	5	HS
T_8	**Oriental pickling melon (*Cucumis melo* sub sp. *conomon*)**	34.98	17.55	1000	351.67	1293.67	1.64	4	S
T_9	**Bitter apple (*Citrullus colocynthis*)**	21.38	11.38	1000	297.00	612.00	0.90	3	MR
T_{10}	**Wild melon (*Cucumismelo sub spsagrestis*)**	25.80	20.94	1000	303.67	2177.33	2.48	5	HS
T_{11}	**Bitter gourd (*Momordica charantia*)**	31.59	21.71	1000	305.00	1301.67	1.61	4	S
T_{12}	**Wild gourd (*Cucumis prophetarum*)**	33.31	19.68	1000	311.67	1403.00	1.76	4	S
T_{13}	**Kasarakayee (*Momordica cymbalaria*)**	33.64	22.66	1000	314.67	1314.00	1.67	4	S
	SEd±	4.46	1.25		7.27	9.44			
	CD at 5%	9.10	3.63		14.85	19.27			

MR –Moderately resistant S –Susceptible HS – Highly susceptible (Tayler and Sasser 1978); Pf – Final population Pi – Intial Population

Table 3 Screening wild and cucurbitaceous rootstocks and cucumber scions for growth parameters inoculated with root knot nematode (*Meloidogyne incognita*) at 45 days after inoculation

Fig. 1. Tomato plant infested with *Meloidogyne incognita* (pure culture).

Fig. 2. Life stages of root knot nematode (*Meloidogyne incognita*).

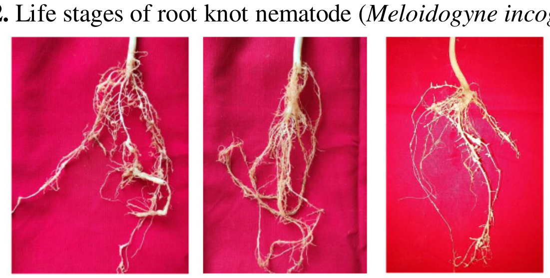

Fig. 3. Roots of cucurbitaceous rootstocks with reaction of moderate resistance.

CONCLUSIONS

Our findings are similar to previous research about the highly susceptible response of cucurbitaceous species against RKNs. Screening is a continuous process because sexual reproduction in RKNs possesses the capability to break any type of resistance by adjusting themselves against the subjected crop species (Gulzar *et al*., 2022). This study indicated that the moderately resistant cultivars, such as pumpkin and *Citrullus* *colocynthis*, are recommended for cultivation under integrated production systems. These cultivars present a profitable alternative for producing healthy, toxin-free cucurbits for consumers and for developing new resistant cultivars. Screening for root-knot resistant germplasm and developing resistant rootstocks can provide an environmentally friendly method for managing soil borne diseases in cucurbitaceous species. Given the rising cost of nematicides and their restrictions on commercial use, grafting may become an economically feasible method. *C. moschata* rootstocks may be particularly useful for low-input sustainable horticulture and could benefit home gardeners, especially those in areas with high infestations of southern root-knot nematode.

FUTURE SCOPE

The future scope for screening cucurbits for nematode resistance involves several promising avenues of research and innovation:

1. Molecular Breeding and Genetic Engineering: Advances in molecular breeding techniques, including marker-assisted selection (MAS) and genome editing (e.g., CRISPR-Cas9), offer new possibilities for developing nematode-resistant cucurbit varieties. Identifying and introducing nematode resistance genes from wild cucurbit species into cultivated varieties could be key to overcoming the challenges posed by *Meloidogyne* spp.

2. High-Throughput Screening Technologies: The use of high-throughput phenotyping and screening systems will enable faster and more precise identification of nematode-resistant varieties. Automated systems that combine imaging, robotics, and data analysis could streamline the screening process in large-scale breeding programs.

3. Exploiting Wild Relatives: Research into the resistance traits of wild *Cucumis* species and other cucurbit relatives holds great potential. By overcoming the challenges of interspecific hybridization (e.g., chromosomal differences), breeders could introduce durable resistance genes from wild relatives into commercial cultivars.

4. Transcriptomics and Proteomics: Investigating the molecular mechanisms behind nematode resistance through transcriptomics, proteomics, and metabolomics could provide insights into the biochemical pathways involved in resistance. Understanding these pathways may lead to the development of nematode-resistant varieties with enhanced durability.

5. Biological Control Agents: Leveraging biocontrol agents, such as nematode-parasitic fungi or bacteria, could complement genetic resistance in cucurbits. Screening for and developing cucurbit varieties that are more compatible with biocontrol agents could provide an integrated pest management approach to nematode control.

6. Exploration of Natural Compounds: Identifying natural compounds or plant-derived secondary metabolites that inhibit nematode activity could lead to the development of eco-friendly nematode management strategies. Screening cucurbits for the production of such compounds or their ability to interact with nematodes could be an area of focus.

7. Multi-Disease Resistance Breeding: Since cucurbits face multiple biotic stresses, including nematodes and fungal diseases, future research should focus on developing varieties with combined resistance to multiple pathogens. Screening for multi-disease-resistant rootstocks or scions can enhance crop resilience and sustainability in diverse growing environments.

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