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Screening and identification of cucumber germplasm and rootstock resistance against the root-knot nematode (*Meloidogyne incognita*)

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ABSTRACT. Root-knot nematodes (*Meloidogyne* spp) are destructive agricultural pests that reduce the productivity of cultivated vegetables worldwide, especially when vegetables are cropped continuously in greenhouses. Cucumbers (*Cucumis sativus* L.), in particular, suffer extensive damage due to root-knot nematodes, and only a few wild species are known to be resistant. Grafting of cultivated plants to rootstocks of known resistant germplasms could be an effective method to resolve this problem. In this study, 21 cucumber germplasms and seven rootstocks were evaluated for resistance based on the growth of cucumber seedlings and resistance indexes to *Meloidogyne incognita*, which were surveyed 25 days after inoculation with *M. incognita*. Cluster analysis and principal component analysis (PCA) were used to investigate the resistance of 21 cucumber germplasms and seven rootstocks based on their growth and resistance indexes after inoculation with *M. incognita*. These analyses showed that the 21 germplasms and seven rootstocks could be divided into three groups based upon

their resistance levels: moderately resistant, susceptible, and highly susceptible to *M. incognita*. All 21 cucumber germplasms exhibited susceptibility or high susceptibility to *M. incognita* and most rootstocks exhibited moderate resistance. The PCA results were consistent with those of the clustering analysis. The Jinyou No.1 cultivar had the highest resistance to *M. incognita* among the 21 cucumber germplasms, and Huangzhen No.1 cultivar had the highest resistance among the seven rootstock cultivars.

Key words: Cucumber; Rootstock; Root-knot nematodes; Resistance identification; Clustering analysis; Principal component analysis

INTRODUCTION

Cucumber (*Cucumis sativus* L.) is an important vegetable worldwide and is the second most popular crop planted in greenhouses (Sebastian et al., 2010; Mao et al., 2016). In China, cucumber has been cultivated for at least 3000 years, with cultivated acreage in 2012 estimated at 240,000 ha (Lv et al., 2012). As the result of continuous cropping over many years, the severity of root-knot nematode infestation has increased, especially when cucumbers are cropped in greenhouses (Huang et al., 2016). *Meloidogyne incognita* is the most common root-knot nematode species in Shaanxi province (Zhang et al., 2011; Liu et al., 2014). Surveys conducted by Dong et al. (2003) showed that approximately 67.6% of greenhouses contained plants infested with root-knot nematodes, with 95% of greenhouses containing crops over 4-years-old suffering from infestation. *M. incognita* infestation has important effects on the growth of cucumber roots and causes the formation of root galls, yellowed and stunted leaves, and even the destruction of whole plants (Pandey et al., 2003; Escobar et al., 2015). In addition, infestation by *M. incognita* also increases the occurrence of soil-borne diseases such as Fusarium wilt (Wang and Roberts, 2006). These issues result in severe damage to the yield and quality of vegetables (Echeverrigaray et al., 2010; Huang et al., 2014; Qiao et al., 2014).

Researchers have attempted to identify cultivated cucumber resources with resistance to *M. incognita* (Ye et al., 2011; Ma et al., 2014). Winstead and Sasser (1956) discovered that all 50 of their cucumber varieties were resistant to *M. hapla*, but were susceptible to *M. incognita* and *M. javanica*. Fassuliotis and Rau (1963) evaluated cucumber germplasm from the US Department of Agriculture, none of which exhibited resistance to *M. incognita*. For many years after these studies, researchers were unable to identify any cucumber materials with resistance to *M. incognita* (Walters and Wehner, 1997; Jia and Wu, 2011). However, several wild species that are highly resistant to *M. incognita* have been reported in recent years, such as *C. metuliferus* Naud., *C. heptadactylus* Naud., *C. longipes* Hook, and *C. hystrix* Chakr (Chen et al., 2001; Shen et al., 2007; Ma et al., 2014).

Owing to the lack of cucumber germplasm identified with resistance to *M. incognita*, chemical nematicides have now become the major means of controlling root-knot nematodes (Li et al., 2013; Xi et al., 2013). However, the chemical nematicides used have severely polluted the soil and aroused substantial public concern about food safety, especially considering that cucumbers are mostly eaten fresh. Unfortunately, wild species have always been difficult to crossbreed with cultivated varieties; therefore, wild resistant species have been used as

rootstocks for grafting as an effective method to impart the disease resistance of the resistant varieties to the cultivated varieties (Li et al., 2014; Ma et al., 2014; Wang et al., 2014; Liu et al., 2015). Liu et al. (2015) screened the resistance of 53 wild cucumber germplasms to nematodes and selected a *Meloidogyne incognita*-resistant rootstock suitable for cucumber, melon, and watermelon scions. Subsequently, Wang et al. (2013) collected 20 national and international cucumber rootstocks to evaluate their capacity for resistance to *M. incognita*. Those authors found that Figleaf gourd, Kurotane, and Eibulu plants had stronger capacity for resistance.

Currently, various species of cucumber and cucumber rootstock are used in production. Their ability to resist root-knot nematode lacks systematic evaluation. Previously, the resistance of vegetables to root-knot nematodes has been compared using disease index (Huo et al., 2008). However, a single evaluation index is often not accurate enough, and there is a need for the comprehensive consideration of multi-indicators (Wang et al., 2013). In this study, 21 cucumber germplasms and seven rootstock cultivars that are widely used for production were collected and their resistance to nematodes was evaluated using a multiple evaluation index. The results indicate that cucumber germplasm and rootstock cultivars had high diversity in terms of resistance to *M. incognita,* and are expected to provide the basis for further application in production.

MATERIAL AND METHODS

Source of root-knot nematodes

The root-knot nematodes were collected from tomato roots that had obvious galls and were maintained using the susceptible tomato cultivar Dongfen No.3 in a greenhouse at 22-26°C. The roots of tomato plants were chopped and sterilized with 0.5% sodium hypochlorite for 3 min, and then rinsed with water. Root-knot nematode eggs were isolated from the tomato roots with water on a 25-μm sieve. Eggs were subsequently collected and cultivated on a moist Petri dish containing distilled water at 28°C for 24 h to hatch second-stage juveniles (J2), which were then collected and used for species identification and plant inoculation.

Preparation of plant material

We used 14 cucumber cultivars, six inbred lines, one wild germplasm, and seven cucumber rootstocks in this study (Table 1). Seeds of all cultivars and inbred lines were washed in water at 55°C for 15 min, and then cultivated at 28°C on wet filter paper in Petri dishes. Seeds prepared for germination were sown in autoclaved sand in 8 x 8-cm plastic pots, and watered with 1/4 Hoagland's nutrient solution twice weekly. All pots (one plant per pot) were arranged in an illumination incubator (RXZ-5COB-LED, Ningbo, Zhejiang, China) under the following cycle: 26°C and 14-h light/18°C and 10-h darkness, with relative humidity maintained at 88%.

Identification of root-knot nematode

Based on morphology, the root-knot nematodes were identified by Guanqu Zhang from the Nematode Research Group at the Plant Protection College. For PCR identification, mixed DNA was extracted from the hatched J2s using the method reported by Cenis (1993).

Table 1. Twenty-eight germplasms used in this study.

No.	Cultivar	Source of collection	No.	Cultivar	Source of collection
1	No.14	Yangling, Shaanxi	15	Lisha	Beijing
2	No.26	Yangling, Shaanxi	16	Zhongnong No.16	Beijing
3	Q_{24}	Yangling, Shaanxi	17	Zhaibuwan	Jilin
4	Q_{8}	Yangling, Shaanxi	18	Gaochanwang Yapajia	Jilin
5	Q_{16}	Yangling, Shaanxi	19	Yanziru	Jinzhou, Liaoning
6	Commum CAT	Yangling, Shaanxi	20	Cuixiang No.6	Zhuzhou, Hunan
7	Jinyou No.1	Tianjin	21	Liuyangbai	Hunan
8	Jinchun No.4	Tianjin	22	Qianglishi F1	Shandong
9	Jinchun No.5	Tianjin	23	Ganfeng No.1	Qingdao, Shandong
10	Jinyan No.4	Tianjin	24	Guozhen No.2	Beijing
11	Luyangxinsi	Shandong	25	Banzhen No.3	Shouguang, Shandong
12	Baisite No.8	Guangzhou	26	Liangba	Shouguang, Shandong
13	Changchunmici	Jilin	27	Huangzhen No.1	Beijing
14	Laolaishao	Shandong	28	Xiuli	Shouguang, Shandong

PCR amplification was performed using specific primers as described by Hu et al. (2011). Specific primers were synthesized by Sangon Biotech (Shanghai, China) and are listed in Table 2. Of these, MF/MR were diagnostic primers designed based on 28S rRNA D2D3 of *Meloidogyne* nematodes. Mi-F/Mi-R, Me-F/Me-R, and Fjav/Rjav were diagnostic primers designed based on the alignment of ribosomal intergenic spacer 2 (IGS2) sequences of *M. incognita*, *M. enterolobii*, and *M. javanica*, respectively. The final volume of the PCR mixture was 20 µL, and included 60 ng DNA, 10 µL Mix, 1 µL forward and reverse primers, and 7 µL ddH_2O. The PCR program was as follows: 4 min at 95°C, followed by 35 cycles of 30 s at 94°C, 30 s at 60°C, 1 min at 72°C, and 10 min at 72°C. PCR products were stored at 4°C.

Table 2. Primers sequences used for root-knot nematode identification.

Forward primer	Sequence (5'-3')	Reverse primer	Sequence (5'-3')
MF	GGGGATGTTTGAGGCAGATTTG	MR	AACCGCTTCGGACTTCCACCAG
Mi-F	GTGAGGATTCAGCTCCCCAG	Mi-R	ACGAGGAACATACTTCTCCGTCC
Fjav	GGTGCGCGATTGAACTGAGC	Rjav	CAGGCCCTTCAGTGGAACTATAC
Me-F	AACTTTTGTGAAAGTGCCGCTG	Me-R	TCAGTTCAGGCAGGATCAACC

PCR products were separated on 2% agarose gels and stained with Goldview (EB substation; Toyobo). A DS2000 DNA ladder (Dongsheng Biotech, Shanghai, China) was used to determine the molecular sizes of the bands. Band patterns were photographed under UV light using the Alphalmager (Alpha Innotech).

Inoculation of cucumber and rootstock seedlings with the J2 of *M. incognita*

The J2s were then re-suspended in sterile water, and the concentration was adjusted to 2000 J2/mL. The cucumber seedlings were inoculated with the J2 of *M. incognita* by pouring 1 mL of the nematode suspension into holes with a 2-cm depth around the bases of the plants when the first two true leaves of cucumber seedlings were spread completely. Cucumber seedlings inoculated with 1 mL water were used as controls. Ten seedlings for each germplasm were included in each experiment, and each experiment was repeated at least three times.

Measurement of growth and resistance indexes

Twenty-five days after inoculation with J2 or water, the roots of all seedlings for all germplasms were gently washed and the following growth indexes were measured: plant height (PH), stem diameter (SD), aerial part fresh weight (APFW), root fresh weight (RFW), and total fresh weight (TFW). The growth indexes showed as the relative growth rate using the followed formulae:

$$\text{Relative growth rate} = \frac{\text{the value of treatment}}{\text{the value of control}} \times 100\%$$

We also measured the following resistance indexes: gall number (GN), root infection percent (RIP; Mao, 2007), gall index (GI; Boiteux and Charchar, 1996), and disease index (DI; Li and Zhu, 2005).

RIP, GI, and DI were calculated using the following formulae:

$$RIP = \frac{Ni}{N} \times 100\%$$

Where, *Ni* is the number of roots infected by root-knot nematodes per seedling, and *N* is the total number of roots per seedling.

$$GI = \frac{N}{W}$$

Where, *N* is the number of galls per seedling, and *W* is the RFW per seedling.

For DI, we first evaluated gall severity per seedling using the levels 0-5, where 0 = no gall, 1 = 1-2 galls, 2 = 3-10 galls, 3 = 11-30 galls, 4 = 31-100 galls, and 5 ³100 galls. We then used the following formula to calculate DI:

$$DI = \frac{\sum Si \times Ni}{N}$$

Where, *Si* is the level of gall severity, *Ni* is the number of seedlings belonging to the same level, *i* pertains to the different levels, and *N* is the number of seedlings. For DI, the resistance of germplasms to root-knot nematodes was classified using five levels, where 0 = immune, 0-1.0 = highly resistant, 1.0-2.0 = resistant, 2.0-3.0 = moderately resistant, 3.0-4.0 = susceptible, and >4.0 = highly susceptible.

Analysis of data for growth and resistance indexes

A similarity matrix was generated based on simple matching coefficients. The similarity coefficient and genetic distance were analyzed according to the method described by Nei (1972). NTSYS-PC 2.2 software (Rohlf, 1998) was used to perform cluster analysis

on resistance indexes in the similarity matrix, using the unweighted pair group method with arithmetic mean (UPGMA). PCA (IBM SPSS Inc., Chicago, IL, USA) was used to detect clustering and to summarize the characteristics of different cultivars inoculated with *M. incognita*. To eliminate the influence of dimension, data for agro-morphological traits were classified into 10 grades for analysis; grade 1<X-2δ and grade 10>X+2δ, where the interval of every grade was 0.5δ, and δ was the standard deviation.

RESULTS

Identification of nematode species

Based on morphology, the J2s used in this experiment were identified as *M. incognita* by Professor Zhang. Sequence-characterized amplified-region technology has been used widely to identify root-knot nematode species (Williamson et al., 1997; Zijlstra et al., 2000; Wu et al., 2005). This method is accurate and sensitive, and overcomes the shortcomings of traditional morphological identification (Wu et al., 2005). In this study, the sequence-characterized amplified-region method was used to identify which species of root-knot nematode were initially collected. A 500-bp fragment of *Meloidogyne* 28s rDNA was produced following amplification with *Meloidogyne*-universal MF/MR primers, and a 1000-bp fragment from *M. incognita* was produced following amplification with the Mi-F/Mi-R primers (Figure 1). This indicated that the root-knot nematodes collected were indeed *Meloidogyne* spp. To check whether these were species other than *M. incognita*, the Me-F/Me-R and Fjav/Rjav primers for *M. enterolobii* and *M. javanica,* respectively, were used to amplify the DNA. No band was observed on the resulting agarose gel; therefore, we could conclude that the root-knot nematodes collected did not include *M. enterolobii* or *M. javanica*, and were from a *M. incognita* population.

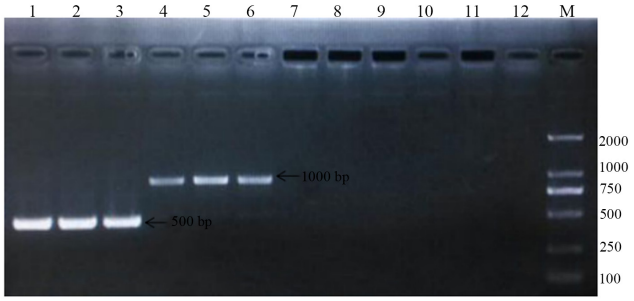

Figure 1. PCR products obtained using specific primers and DNA extracted from the collected root-knot nematodes. *Lane M*: DS2000 DNA marker; *lanes 1-3*: MF/MR; *lanes 4-6*: Mi-F/Mi-R; *lanes 7-9*: Me-F/Me-R; *lanes 10-12*: Fjav/Rjav.

Identification of the resistance levels of 28 germplasms against *M. incognita*

The growth indexes of PH, SD, AFPW, RFW, and TFW of the 28 seedlings inoculated by *M. incognita* differed. The growth indexes of all seedlings decreased following inoculation with *M. incognita* for 25 days, including Jinyan No.4, Jinchun No.4, and Commum CAT. The results showed that the *M. incognita* infestation had a significant effect on seedling growth; however, the decrease in the different growth indexes was not consistent, which indicated that

infection with *M. incognita* on different organs of cucumber and rootstock seedlings was inhibited to varying degrees. The coefficients of variation for the growth indexes were: PH = 3.39%; SD = 3.86%; AFPW = 4.93%; RFW = 9.20%; and TFW = 5.85%, which indicated that inoculation with *M. incognita* had a greater effect on the growth of roots than on the growth of aerial parts (Table 3).

Table 3. Effects of *Meloidogyne incognita* inoculation on the growth of 28 germplasm seedlings.

No.	Cultivar	Relative growth rate (%)				
		PH	SD	APFW	RFW	TFW
1	No.14	98.85 ± 1.04	97.33 ± 2.26	91.56 ± 1.92	99.86 ± 0.20	95.18 ± 1.26
2	No.26	93.34 ± 8.08	98.18 ± 0.59	98.77 ± 0.40	94.26 ± 2.29	96.94 ± 0.73
3	Q24	97.28 ± 1.12	99.32 ± 0.19	98.08 ± 1.36	96.91 ± 1.46	97.62 ± 0.25
4	Q8	91.86 ± 6.35	95.75 ± 4.84	99.64 ± 0.17	98.75 ± 0.20	99.61 ± 0.10
5	Q16	97.69 ± 1.13	97.48 ± 0.40	94.89 ± 4.18	95.77 ± 1.76	98.04 ± 2.01
6	Commum CAT	93.34 ± 6.16	88.69 ± 10.24	83.90 ± 0.48	67.30 ± 4.20	74.62 ± 2.14
7	Jinyou No.1	97.75 ± 0.26	94.00 ± 3.23	94.68 ± 3.01	96.98 ± 2.37	95.98 ± 2.74
8	Jinchun No.4	98.76 ± 0.26	96.31 ± 1.12	97.74 ± 2.56	90.40 ± 2.14	95.77 ± 2.44
9	Jinchun No.5	98.56 ± 0.89	90.93 ± 2.36	93.60 ± 6.72	91.88 ± 7.86	94.24 ± 7.75
10	Jinyan No.4	89.73 ± 2.34	95.69 ± 2.15	90.45 ± 2.01	94.48 ± 6.02	92.14 ± 3.59
11	Luyangxinsi	98.08 ± 1.32	98.16 ± 2.60	89.26 ± 1.27	87.28 ± 4.34	94.44 ± 5.99
12	Baisite	95.57 ± 4.80	97.98 ± 0.10	97.26 ± 0.43	98.83 ± 0.33	98.61 ± 0.13
13	Changchunmici	99.75 ± 0.10	98.88 ± 0.23	97.96 ± 1.73	92.72 ± 2.32	95.96 ± 1.82
14	Laolaishao	96.73 ± 3.65	96.90 ± 1.59	96.26 ± 3.30	92.57 ± 4.90	94.30 ± 0.76
15	Lisha	89.05 ± 10.55	90.46 ± 3.42	92.56 ± 6.43	72.59 ± 7.10	82.14 ± 0.71
16	Zhongnong No.16	96.89 ± 3.68	94.95 ± 3.76	94.44 ± 0.87	89.37 ± 5.01	92.70 ± 2.29
17	Zhaibuwan	92.71 ± 9.47	95.93 ± 2.77	80.63 ± 11.45	86.95 ± 4.26	83.33 ± 7.95
18	GaochanwangYapajia	88.65 ± 7.52	96.66 ± 1.58	94.69 ± 3.15	93.59 ± 4.83	94.02 ± 3.89
19	Yanziru	91.27 ± 8.49	93.62 ± 0.92	96.51 ± 3.52	82.17 ± 12.22	89.70 ± 3.95
20	Cuixiang No.6	94.87 ± 3.87	96.89 ± 2.09	93.72 ± 2.22	86.65 ± 6.59	94.17 ± 0.10
21	Liuyangbai	94.95 ± 1.00	92.70 ± 2.24	89.95 ± 1.65	93.00 ± 2.91	91.68 ± 0.93
22	Qianglishi F1	95.44 ± 3.76	82.72 ± 10.73	95.06 ± 0.90	78.90 ± 10.56	90.53 ± 2.32
23	Ganfeng No.1	94.80 ± 0.24	92.86 ± 0.40	95.65 ± 1.76	79.35 ± 3.07	91.23 ± 2.23
24	Guozhen No.2	97.28 ± 2.43	96.64 ± 1.32	94.78 ± 2.27	79.31 ± 4.88	91.86 ± 6.90
25	Banzhen No.3	97.27 ± 0.27	99.14 ± 0.87	84.97 ± 10.75	95.41 ± 2.59	90.20 ± 8.53
26	Liangba	90.38 ± 1.86	99.07 ± 0.44	91.67 ± 0.39	87.01 ± 11.94	90.10 ± 3.84
27	Huangzhen No.1	95.46 ± 3.03	96.67 ± 1.12	91.50 ± 7.44	81.58 ± 12.41	88.37 ± 9.01
28	Xiuli	98.20 ± 1.17	96.41 ± 1.39	98.17 ± 0.65	93.04 ± 6.15	96.40 ± 2.55
Mean		95.16	95.37	93.51	89.18	92.50
Standard		3.22	3.68	4.61	8.20	5.41
Coefficient of Variation (%)		3.39	3.86	4.93	9.20	5.85

Data are mean ± standard deviation of 10 individual seedlings for each germplasm in each experiment. The whole infection experiment was repeated at least three times; PH: plant height; SD: stem diameter; APFW: aerial part fresh weight; RFW: root fresh weight; TFW: total fresh weight.

A significant difference was found among the resistance indexes of the 28 germplasms (Table 4). GN, RIP, DI, and GI for the seven rootstocks were significantly lower than those for the 21 cucumber germplasms. Specifically, the 21 cucumber germplasms were classed as susceptible or highly susceptible to *M. incognita*, and most of the rootstocks had moderate resistance to *M. incognita*. Of the 21 germplasms, 11 were classed as highly susceptible, including Luyangxinsi, Q_{24}, Laolaishao, Baisite, No.26, Q_{16}, Changchunmici, Lisha, Zhaibuwan, Yapajia, and Yanziru, with an average DI of 4.79. Luyangxinsi and Q_{24} had the maximum DI of 5, as well as a much higher GN compared with the 11 highly susceptible germplasms (Figure 2A and B). The other 10 cucumber germplasms, and two of the rootstocks, were classed as susceptible to *M. incognita*, including Cuixiang No.6 and Q_8 (Figure 2C and D). The average DI and GN of these groups were 3.67 and 53, respectively. The remaining five rootstocks, including Huangzhen No.1, Xiuli, Guozhen No.2, Banzhen No.3, and Ganfeng No.1, were classed as moderately resistance to *M. incognita*, in which Huangzhen No.1 and Xiulihad a lower GN than the other rootstocks (Figure 2E and F).

Table 4. Effects of *Meloidogyne incognita* inoculation on resistance indexes of 28 germplasm seedlings.

No.	Cultivar	GN(N)	RIP (%)	GI	DI	Resistance
1	No.14	73.50 ± 1.73^{jk}	53	20.47	4.00	Susceptible
2	No.26	108.17 ± 1.32^{fg}	81	33.25	4.89	Highly susceptible
3	Q_{24}	137.72 ± 10.42^{c}	94	41.55	5.00	Highly susceptible
4	Q_{8}	81.67 ± 4.59^{ij}	57	22.92	4.00	Susceptible
5	Q_{16}	99.45 ± 1.49^{gh}	59	51.06	4.33	Highly susceptible
6	Commum CAT	89.75 ± 7.15^{hi}	73	46.91	3.71	Susceptible
7	Jinyou No.1	19.40 ± 2.48^{op}	20	12.94	3.00	Susceptible
8	Jinchun No.4	59.16 ± 2.13^{lm}	35	65.74	3.94	Susceptible
9	Jinchun No.5	52.47 ± 19.89^{m}	34	48.80	3.81	Susceptible
10	Jinyan No.4	59.89 ± 1.67^{lm}	32	19.77	4.00	Susceptible
11	Luyangxinsi	122.67 ± 5.69^{d}	90	40.48	5.00	Highly susceptible
12	Baisite	109.00 ± 1.02^{fg}	90	51.92	4.89	Highly susceptible
13	Changchunmici	110.78 ± 4.01^{ef}	84	54.82	4.89	Highly susceptible
14	Laolaishao	120.33 ± 8.85^{de}	97	31.50	4.94	Highly susceptible
15	Lisha	166.40 ± 2.51^{a}	96	81.64	4.93	Highly susceptible
16	Zhongnong No.16	61.61 ± 7.90^{lm}	32	52.13	4.00	Susceptible
17	Zhaibuwan	171.67 ± 3.17^{a}	90	66.08	4.60	Highly susceptible
18	Gaochanwang Yapajia	90.86 ± 3.88^{hi}	74	39.07	4.33	Highly susceptible
19	Yanziru	154.48 ± 6.55^{b}	94	63.85	4.87	Highly susceptible
20	Cuixiang No.6	64.53 ± 11.00^{kl}	39	47.54	3.93	Susceptible
21	Liuyangbai	29.94 ± 2.99^{n}	26	12.37	3.47	Susceptible
22	Qianglishi F1	26.15 ± 1.45^{no}	23	18.91	3.07	Susceptible
23	Ganfeng No.1	13.35 ± 1.86^{pq}	18	8.52	2.54	Moderate resistance
24	Guozhen No.2	18.22 ± 1.29^{op}	22	12.46	2.94	Moderate resistance
25	Banzhen No.3	14.07 ± 0.46^{pq}	17	13.74	2.60	Moderate resistance
26	Liangba	19.29 ± 1.84^{op}	22	16.05	3.13	Susceptible
27	Huangzhen No.1	5.51 ± 0.32^{q}	8	5.80	2.00	Moderate resistance
28	Xiuli	9.61 ± 1.27^{pq}	13	8.37	2.26	Moderate resistance

Data are reported as means ± standard deviation of 10 individual seedlings for each germplasm in each experiment. The whole infection experiment was repeated at least three times. Means within a column followed by the same letter are not significantly different according to Duncan's New Multiple Range Test at P = 0.05; DI: 0-scale; where 0 = no gall, 1 = 1-2 galls, 2 = 3-10 galls, 3 = 11-30 galls, 4 = 31-100 galls, and 5 ≥100 galls. Resistance of germplasms to root-knot nematodes was based on five levels, where 0 = immune, 0-1.0 = highly resistant, 1.0-2.0 = resistant, 2.0-3.0 = moderately resistant, 3.0-4.0 = susceptible, and >4.0 = highly susceptible (Li and Zhu, 2005). GN: galls number; RIP: root infected percent; GI: gall index.

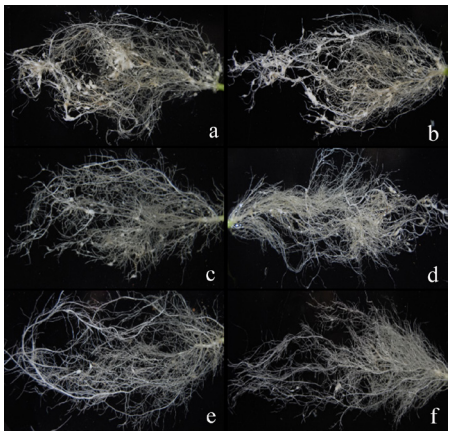

Figure 2. Root symptoms of six germplasms following inoculation with *Meloidogyne incognita*. **a.** Luyangxinsi; **b.** Q_{24}; **c.** Cuixiang No.6; **d.** Q_{8}; **e.** Huangzhen No.1; **f.** Xiuli.

Cluster analysis

The similarity coefficients of the 28 germplasms ranged from 0.02 to 1.00. The dendrogram of the 28 germplasms constructed from the resistance indexes indicated that they were clustered into three main groups (Figure 3). The first group consisted of 10 cucumber germplasms, eight of which were classed as susceptible, including No.14, Jinchun No.4, Jinyan No.4, and Jinchun No.5, and two of which were classed as highly susceptible, including Q16 and Gaochanwang yapajia. The second group contained nine germplasms, including No.26, Q_{24}, Luyangxinsi, and Laolaishao, which were all classed as highly susceptible. The third group included two cucumber germplasms and seven rootstocks, most of which were classed as moderately resistant, including Ganfeng No.1, Guozhen No.3, Banzhen No.3, and Xiuli, with the exception of Jinyou No.1, Liuyangbai, Qianglishi F1, and Liaba, which were classed as susceptible to *M. incognita*. The results of the cluster analysis were somewhat consistent with the classification of resistance indexes.

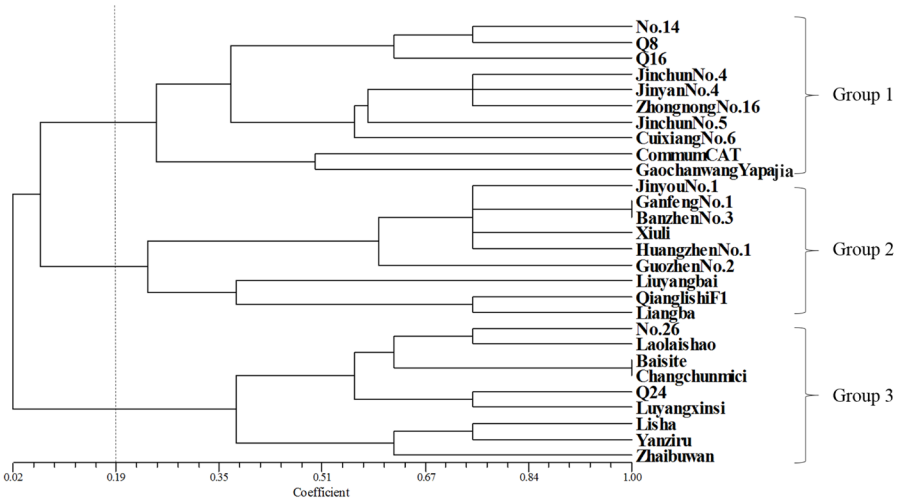

Figure 3. Unweighted pair group method with arithmetic mean dendrogram among the 28 germplasms based on Nei's coefficients. Three groups were defined based on combined markers: 1, 2, and 3. Group 1 includes 10 germplasms, Group 2 includes nine germplasms, and Group 3 includes nine germplasms.

Principal component analysis (PCA)

Grouping of different germplasms using PCA was based mainly on the first three principal coordinates, and the contribution of the first three principal coordinates were 39.93, 31.48, and 10.69%, respectively. PC1 was positively correlated with the relative growth rate of GI, RIP, DI, and RKN, while PC2 was positively correlated with PH, SD, AFPW, RFW, and TFW.

The scatter plot showing the distribution of 28 germplasms determined by PC1 and PC2, showed that the resistance indexes for the germplasms tended to increase with increasing values of PC1. The mean growth indexes of seedlings generally increased with increasing values of PC2. These data indicated that the 28 germplasms examined were partitioned into

three distinct groups, and were consistent with the clustering results. The first group, including all rootstocks, Liuyangbai, and Jinyou No.1, was generally located on the left part of the PC1 axis. In addition to Xiuliand Jinyou No.1, this group had a low relative growth rate and low resistance indexes such as DI, GI, and GN. The second group was mostly positioned in the middle of the PC1 axis, and in the higher part of the PC2 axis. Jinchun No.4, Jinchun No.5, Cuixiang No.6, Zhongnong No.16, and four additional cucumber germplasms belonged to this group, which was characterized by relatively lower resistance indexes than the third group, and a higher relative growth rate than the first group. The third group was situated in the right part of the PC1 axis, which was composed of cucumber germplasm with high susceptibility to *M. incognita*. The relative growth rate of some germplasms of this group was high, and the other germplasms was low (Figure 4).

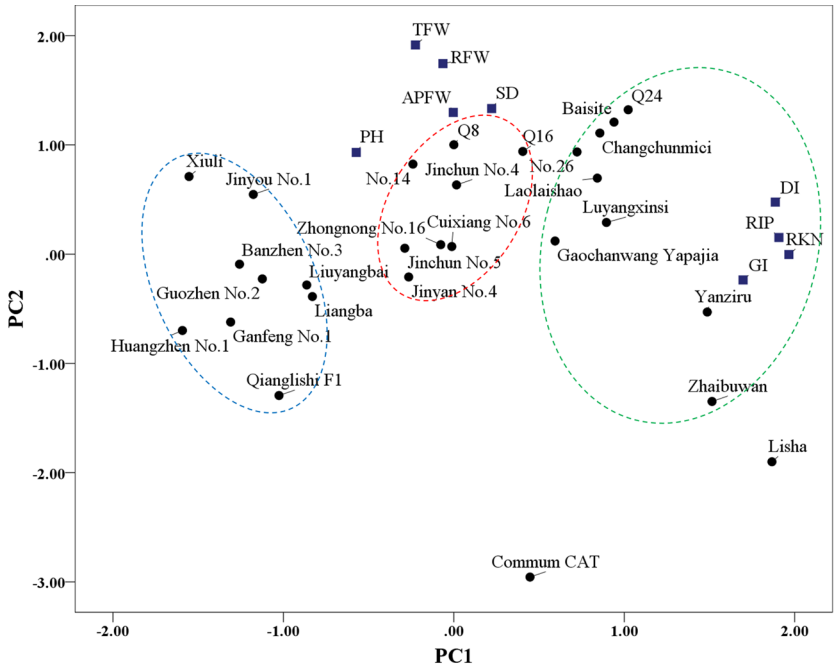

Figure 4. Scatter plot of the first two principal components based on the growth and resistance indexes of 28 germplasm. PH: plant height; SD: stem diameter; APFW: aerial part fresh weight; RFW: root fresh weight; TFW: total fresh weight; GN: galls number; RIP: root infected percent; GI: gall index.

DISCUSSION

Previous studies on the ability of Cucurbitaceae crops to resist root-knot nematodes have been carried out and can divided into different types. Jin et al. (2010) used galls index to evaluate the resistance of cucumber rootstocks and identified two resistance and susceptibility types. Shen et al. (2007) showed that based on disease index and population resistance evaluation standards, the cucumber and pumpkin rootstocks in their experiment could be divided into two types, moderate resistance and susceptibility. Thus, results obtained by evaluating different resistance indexes differ, making them difficult to compare. Because

nematode infestation had different effects on different evaluation indexes, it was difficult to accurately assess plant resistance (Wang et al., 2013). The cluster analysis used in the present experiment was a comprehensive statistical analysis of multiple measurement indexes. It divided the subjects into categories according to the principle of similarity and eliminated the interference caused by artificially inconsistent standards.

In this experiment, all 21 cucumber germplasm seedlings exhibited little resistance to *M. incognita*, including the wild germplasm Commum CAT, which we expected would be more resistant. Most seedlings were classed as susceptible or highly susceptible to *M. incognita*, which was consistent with the findings of previous studies (Roberts, 1992). Aboulipour et al. (2011) reported that 15 cucumber cultivars in their sample were susceptible to *M. javanica,* and only two local cultivars were recognized as tolerant (Aboulipour et al., 2011). Walters et al. (1993) evaluated 884 cucumber (*Cucumis sativus* L.) and 24 horned cucumber (*C. metuliferus* Naud.) germplasms for their resistance to root-knot nematodes (*Meloidogyne* spp). They found that 24 of the *C. metuliferus* cultigens evaluated were resistant to all root-knot nematodes tested, and only 50 of the 884 *C. sativus* cultivars were somewhat resistant to *M. arenaria* and *M. incognita*.

Li et al. (2014) reported that resistance to root-knot nematodes was higher when the cucumber cultivar Cuilv was grafted to Huangzhen 3 and Xindongli rootstocks, with their disease indexes being reduced by 20.7 and 16.9% in spring, and 58.1 and 47.4% in autumn, respectively. Most rootstocks in the present experiment had higher resistance to *M. incognita* than the cucumber germplasms. The resistance indexes of Huangzhen No.1 were lowest among all rootstocks tested. Future experiments will aim to use Huangzhen No.1 as rootstocks for grafting experiments, and to determine the mechanism of grafting to alleviate root-knot nematode disease.

Conflicts of interest

The authors declare no conflicts of interest.

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