Identification of a novel source of resistance to the root-knot nematode *Meloidogyne incognita* in *Cucumis*

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Summary. *Cucumis* species germplasm was evaluated for resistance to the root-knot nematode, *Melodogyne incognita*. The disease index (DI) score for *Cucumis hystrix* was smaller than for the susceptiple control *C. sativus* cv. Beijingjietou. Additionally, numerous large galls were found on cv. Beijingjietou, while only a few small galls were found on *C. hystrix*. *Cucumis hystrix* infected with *M. incognita* also had more lateral roots compared to the susceptible control. Furthermore, interspecific progenies derived from the cross between *C. hystrix* and cv. Beijingjietou had a high level of resistance to *M. incognita*. Thus, *C. hystrix* could serve as a valuable *M. incognita*-resistant source for cucumber improvement.

Key words: Cucurbitaceae, gall size, germplasm, interspecific progenies.

Cucumber (*Cucumis sativus* L., 2n = 2x = 14) is an important vegetable crop that is highly susceptible to the root-knot nematode (*Meloidogyne* spp.), which causes significant yield losses to cucumber throughout the world (Walters *et al*., 1993). There are four *Meloidogyne* species that primarily cause the root-knot disease on a worldwide basis: *Meloidogyne incognita*, *M. arenaria*, *M. javanica* and *M. hapla* (Fassuliotis, 1982). The southern root-knot nematode, *M. incognita*, is a major limiting factor in commercial production of cucumber worldwide (Walters & Wehner, 1996). Significant progress has been made in selecting and breeding for resistance to *M. arenaria*, *M. javanica* and *M. hapla*, but no such progress has been made for resistance to *M. incognita* in cucumber (Walters & Wehner, 1997). Historically, several investigations have indicated that little or no resistance exists in current cultivars and breeding lines of cucumber (Bharali & Phukan, 1996). By contrast, resistance has been discovered in some related *Cucumis* species, e.g., *C. metuliferus*, *C. anguria*, *C. ficifolius* and *C. longipes* (Walters *et al*., 1993). Successful exchange of genes between *C. sativus* and related wild species is difficult using conventional hybridisation techniques, since the chromosome number of *C. sativus* is different (n = 7) from most other species of *Cucumis* (n = 12) (Dane, 1991). Several attempts have been made to introduce economically important characteristics from wild into cultivated *Cucumis* species with little success (Chen & Adelberg, 2000). So far, these resistances have not been utilised for cucumber improvement due to the cross incompatibility that exists between the species (Walters & Wehner, 2002).

Cucumis hystrix Chakr. (2n = 2x = 24), is a wild *Cucumis* species originating in Asia (Kirkbride, 1993; Chen *et al*., 1995). A successful cross has been made and confirmed between *C. hystrix* and *C. sativus* and interspecific F_1 hybrids have been obtained between these two species (Chen *et al*., 1997). This interspecific hybridisation is the first repeatable cross between a cultivated *Cucumis* species and a wild relative. The fact that there is cross-compatibility between *C. hystrix* and *C. sativus* opens a new avenue for cucumber improvement through interspecific hybridisation. The economically important characters of *C. hystrix* are of great interest to cucumber researchers worldwide.

Genetic resistance is the preferred control strategy in nematode management because it has no harmful effects on human health and the environment (Roberts & May, 1986). Resistant cultivars would suppress or reduce the threat of *M. incognita* in cucumber production. The objectives of this study are: *i*) to identify *C. hystrix* as a potential source of root-knot

nematode resistance; and *ii*) to evaluate whether or not the resistance from *C. hystrix* can be transferred to its interspecific progeny with *C. sativus*.

MATERIALS AND METHODS

Two glasshouse experiments were conducted. The objective of experiment 1 was to identify susceptibility of *C. sativus* and resistance of *C. hystrix* to *M. incognita*. The eight *Cucumis* genotypes used in this experiment were from the collection of J. F. Chen (Chen *et al*., 1994). The germplasm evaluated comprised six cucumber cultivars: the Northern Chinese cucumber cv. Beijingjietou, which is well-known for being highly susceptible to *M. incognita* (Walters & Wehner, 1997); the Southern Chinese cucumber cv. Erzaozi; four *C. sativus* var. *xishuangbannesis* cultivars (SWCC8, SWCC9, SWCC10 and SWCC12); plus two wild species of *Cucumis* (African horned cucumber (2n = 2x = 24), a *C. metuliferus* line well-known for its high resistance to *M. incognita* but cross-incompatibility with *C. sativus* (Deakin *et al*., 1971) and *C. hystrix*). This initial experiment was followed by another to evaluate the transmission of resistance from *C. hystrix* to its interspecific progeny with *C. sativus* cv. Beijingjietou. Interspecific hybridisation between *C. hystrix* and *C. sativus*, and subsequent embryo rescue were performed as described by Chen *et al*. (1997). The resulting sterile F_1 progeny (2n = 19, with genome HC, where H is the genome of *C. hystrix*, and C is for *C. sativus*) went through chromosome doubling as previously described by Chen *et al*. (1998) and dF_1 (*C. hytivus*, 4n = 38, HHCC) was obtained. The BCF_1 (3n = 26, HCC/CCH) was obtained by crossing dF_1 with the original diploid cucumber parent cv. Beijingjietou.

The original nematode population used for inoculations came from a severely infested plot at Nanjing, Jiangsu province, China. A pure population was established using a single egg mass. Accurate identification of this nematode species was based on female perineal patterns (Hartman & Sasser, 1985), malate dehydrogenase (Mdh) and esterase (Est) phenotypes (Esbenshade & Triantaphyllou, 1985a). The identified species was multiplied on tomato, *Solanum lycopersicon* Mill. cv. Rutgers, grown in a glasshouse at 20-28°C. Nematode inoculum was prepared in accordance with the technique described by Hussey & Barker (1973). Nematode inoculum concentration was determined from a 1 ml aliquot containing 2000 eggs or second-stage juveniles (J2).

The experiment was conducted in a glasshouse located at Nanjing Agricultural University, Nanjing, China. Seeds were pre-germinated on moist filter paper in a Petri dish at 28°C. Germinated seeds were sown in 32-cell seeding tray containing sterilised substrate. Seedlings at the two-leaf-stage were transplanted into 30 cm diam. pots (1 seedling/pot) filled with a 2:1 mixture of disinfected sand and soil. Plantlets from *in vitro* culture were also transplanted into that medium at the same time. Once the seedlings reached the three to four leaf stages, the inoculum was applied at the base of each seedling using a micropipette. The treatments were replicated three times with five plants or plantlets of each genotype per replication and arranged in a completely randomised design. Plants were maintained in the glasshouse at 26±4°C and were fertilised weekly with a commercial nutrient formulation (N:P:K = 20:20:20) and watered daily. The procedures and cultural practices were identical for each experiment conducted in this study.

At 8 weeks after inoculation, plants were removed from the pots, roots were gently washed free of soil before traits evaluation: number of galls and egg masses per root system, average gall size, gall index (GI), egg mass index (EI) and disease index (DI). The number of galls for each root system was counted under a stereoscopic microscope at 10× magnification, after which egg mass counts were made after immersion into Phloxine B solution (0.15 g l^{-1}) for 3 to 5 min (Daykin & Hussey, 1985). Average gall size was assessed on a scale of 1 to 3 with 1 = less than 1 mm, 2 = 1-3 mm and 3 = more than 3 mm, based on average gall diameter. Root galling and egg mass production were each assessed on a 0 to 5 scale with 0 = none, 1 = 1-2, 2 = 3-10, 3 = 11-30, 4 = 31-100, and 5 = more than 100 galls (or egg masses) (Hartman & Sasser, 1985). The gall index (GI) and egg mass index (EI) was then counted for each genotype using the formula: GI (EI) = [(no. plants in scale 1×1) + (no. plants in scale 2×2) ... (no. plants in scale 5×5) ×100] / [total no. plants examined×5] (Powell *et al*., 1971). A disease index (DI) was generated to combine both resistance parameters (GI and EI) into a single value $DI=\sqrt{GI^2+EI^2}$ (Kouame *et al*., 1997). Based on DI values, host reactions were classified as immune (IM), DI = 0.0-1.0; highly resistant (HR), DI = 1.0-2.0; resistant (R), DI = 2.0-3.0; moderately resistant (MR), DI = 3.0-4.0; moderately susceptible (MS), DI = 4.0-5.0; susceptible (S), DI = 5.0-6.0; and highly susceptible (HS), DI>6.

Data were subjected to analysis of variance using SAS (SAS Inst., Cary, N.C.), and germplasm means were compared using Fisher's least significant difference (LSD) at $P = 0.05$.

Table 1. Resistance evaluation of *Cucumis* species, interspecific progeny from cross between *C.hystrix* and *C.sativus* to *Meloidogyne incognita* in greenhouse.

Genotypes or Generations	Gall index (GI)	Egg mass index (EI)	Disease index (DI)	Host reaction (HR)
African horned	1.50a[e]	0.38a	1.55a	HR
C.hystrix	1.38a	0.50a	1.47a	HR
Beijingjietou	4.88b	3.88c	6.23d	HS
Erzaozi	4.75b	3.75c	6.05d	HS
SWCC8	5.00b	2.00b	5.39bc	S
SWCC9	4.60b	1.40b	4.81b	MS
SWCC10	4.40b	1.60b	4.68b	MS
SWCC12	4.80b	1.80b	5.13bc	S
F_1(2n)	1.60a	0.80a	1.78a	HR
BCF_1(3n)	1.80a	0.60a	1.89a	HR
dF_1(4n)	1.60a	0.60a	1.71a	HR

[e] Significant difference at 0.05. The same letter within the column of GI, EI and DI are not significantly different at 0.05 probability level using Fisher's least significant difference (LSD).

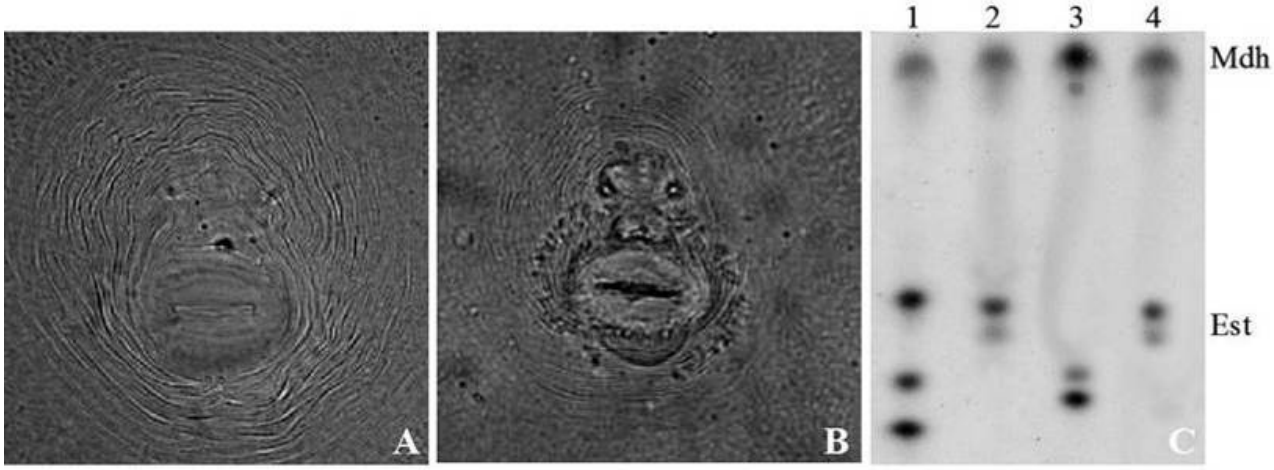

Fig. 1. A. Perineal pattern of the female of the *Meloidogyne* population tested; B. Perineal pattern of the female of *Meloidogyne incognita*; C. Malate dehydrogenase (Mdh) and esterase (Est) phenotypes of *M.javanica* (lane 1), *M.incognita* (lane 2), *M. arenaria* (lane 3) and *Meloidogyne* population tested (lane 4).

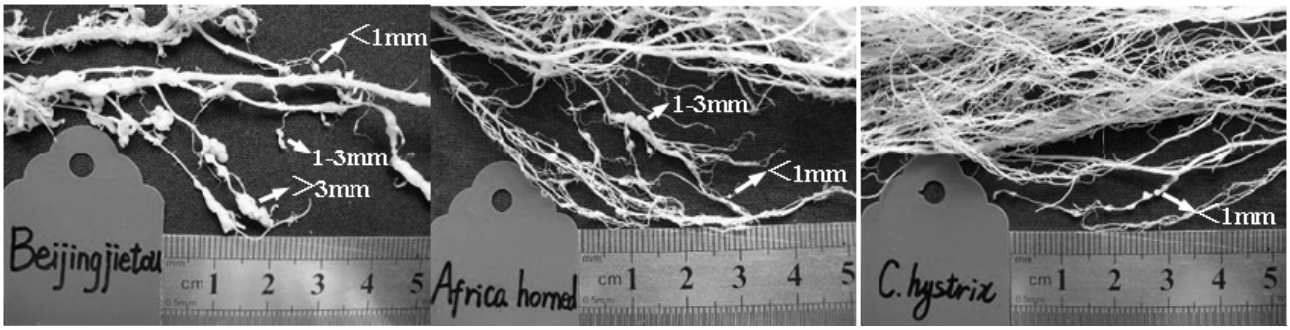

Fig. 2. Morphological aspects of *Cucumis* species roots infected by *M. incognita*. Aspect of the numerous and large sized-galls in the infected roots of the susceptible cv. Beijingjietou (gall diam.>3 mm) (left) and few and small sized-galls in the highly resistant cv. African horned (gall diam.<3 mm) (middle) and in *C. hystrix* (gall diam.<1mm)(right).

RESULTS

The perineal pattern of the *Meloidogyne* population tested match previously published descriptions of *M. incognita* (Jepson, 1987). The dorsal arch is high and squared, the lateral field is indistinct, and the dorsal and ventral striae are not interrupted at this point (Fig. 1A). All of these features for the perineal pattern of the female are similar to *M. incognita* (Fig. 1B). Enzymatic electrophoresis (malate dehydrogenase (Mdh) and esterase (Est)) was also conducted for the population. Based on the established relationship of Mdh-Est profiles and species of *Meloidogyne* (Esbenshade & Triantaphyllou, 1985b), the *Meloidogyne* population used was typical of *M. incognita* (Mdh-Est phenotype N1-I2) distinct from *M. javanica* or *M. arenaria* (Fig. 1C). Both the perineal pattern and enzymatic phenotypes revealed that the *Meloidogyne* population used in this study was *M. incognita*.

The evaluated germplasms were broken out into seven classes based on their DI scores (Table 1). None of the genotypes evaluated were considered immune (DI<1) to *M. incognita*. However, different class reactions were found among the plant species. All *C. sativus* germplasm genotypes evaluated were moderate to highly susceptible (DI>4) to *M. incognita* with DI values varying from 4.68 to 6.23. The DI score of cv. Beijingjietou was 6.23, whereas it was only 1.55 for *C. metuliferus* line Africa horned cucumber. The resistant and susceptible controls behaved as expected. We also discovered *C. hystrix* exhibited a high level of resistance similar to *C. metuliferus* line Africa horned cucumber. Furthermore, *C. hystrix* had smaller DI scores than the susceptible control ($P<0.05$), and the DI score of *C. hystrix* was also smaller than the resistant *C. metuliferus* line Africa horned cucumber ($P>0.05$).

Subsequently, we focused on three species of *Cucumis* germplasm: *C. sativus* cv. Beijingjietou, *C. metuliferus* line Africa horned cucumber and *C. hystrix* for further evaluation. For these three species, we found a large difference in the type of galls produced. Three types of galls were observed in susceptible cv. Beijingjietou, while only two gall types were observed in the resistant *C. metuliferus* line Africa horned cucumber and only one gall type was seen in *C. hystrix* (Fig. 2). For the susceptible species, 58.1% of the galls were classified in the medium sized-gall category (diameter between 1 and 3 mm); as opposed to 28.6% for *C. metuliferus* line Africa horned cucumber which displays high resistance. More importantly 29.1% of the galls in cv. Beijingjietou were large with a diameter > 3 mm, whereas this gall type was never observed in *C. metuliferus* line Africa horned cucumber and *C. hystrix*. For *C. metuliferus* line Africa horned cucumber and *C. hystrix*, small galls with a diameter < 1 mm were most numerous. In the *C. metuliferus* line Africa horned cucumber, 71.4% of the galls were classified in this category while all the galls were less than 1 mm in *C. hystrix* (Fig. 3). The number of lateral roots observed *in situ* revealed that there were large quantitative differences among these three species. The susceptible *C. sativus* cv. Beijingjietou only had a few lateral roots while the resistant *C. metuliferus* line Africa horned cucumber and *C. hystrix* produced many (Fig. 2). Although the number of lateral roots found on *C. metuliferus* line Africa horned cucumber and *C. hystrix* did not differ, both were significantly higher than the susceptible cv. Beijingjietou. There were large differences in the number of egg masses and females among these three species. On *C. sativus* cv. Beijingjietou, 24 egg masses per root were found, compared to 2 to 3 for *C. metuliferus* line Africa horned cucumber and *C. hystrix*. Moreover, egg mass size for the susceptible cv. Beijingjietou was slightly larger than for either *C.metuliferus* line Africa horned cucumber or *C. hystrix*. All large galls (diameter > 3mm) each contained 1 to 2 females for the susceptible cv. Beijingjietou, while females were never observed in *C. metuliferus* line Africa horned cucumber or *C. hystrix*.

All three interspecific progenies between *C. hystrix* and *C. sativus* tested proved to be highly resistant to *M. incognita* based on their DI scores (Table 1). The DI scores for F_1, BCF_1 and dF_1 were 1.78, 1.89 and 1.71 respectively, and no significant differences were observed among them as well as for the *C. hystrix* parent (DI = 1.47), although differences were detected among the progenies and the parent *C. sativus* cv. Beijingjietou (DI = 6.23). In addition, a large difference existed in the number of roots among these three distinct ploidy interspecific progenies. With the exception of the triploid BCF_1, which had many roots, the diploid F_1 and tetraploid dF_1 only had a few roots, which is similar to the *C. hystrix* parent.

DISCUSSION

Results from this study indicate that no resistance to *M. incognita* was found in *C. sativus*, which is in accordance with the earlier results (Walters *et al*., 1993). However, resistance does exist in *C. hystrix*. The highly resistant phenotype in *C. hystrix* was characterised by much smaller sized galls and reduced quantity of galls than in the susceptible *C. sativus* cv. Beijingjietou. In another experiment, we have confirmed *C. hystrix* is

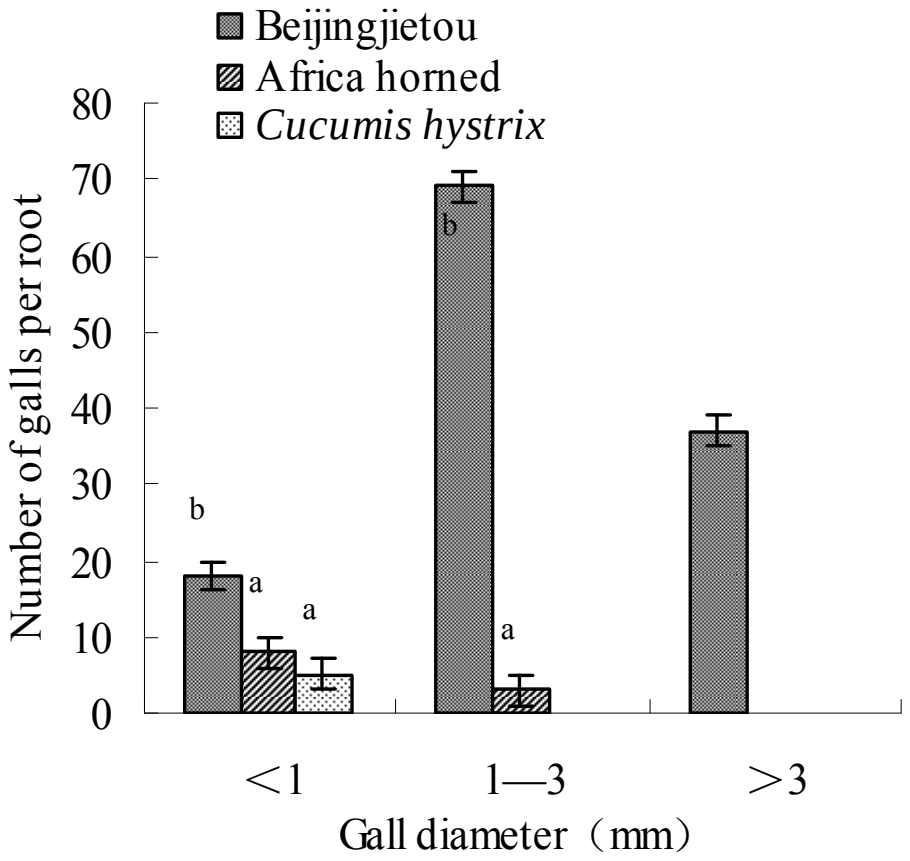

Fig. 3. Distribution of *Meloidogyne incognita* root galls according to their diameter in three *Cucumis* species. The same letter on the top of the column is not significantly different at 0.05 probability level using Fisher's least significant difference (LSD).

also resistant to *M. arenaria*, *M. javanica* and *M. hapla* (data unpublished). The findings of this study indicate that *C. hystrix* may provide a useful source of *M. incognita* resistance for cucumber breeding programmes. The incorporation of this resistance into cultivated cucumber to control root-knot nematodes would be beneficial to growers, and genetic resistance would contribute to reduced nematicide use. The inheritance of the resistance in *C. hystrix*, however, remains to be determined. Further investigations are therefore required to gain a clearer understanding of the underlying mechanisms of resistance involved in *C. hystrix* at both cellular and molecular levels.

Results also revealed that interspecific progenies derived from the cross between *C. hystrix* and *C. sativus* have a high level of resistance to *M. incognita* and the level of resistance was similar to that found in *C. hystrix*, possibly indicating a common genetic basis for resistance. Pederson & Windham (1989) showed that root-knot nematode resistances may be correlated with chromosome numbers and ploidy level in *Trifolium* species. In our study, although chromosome numbers and ploidy level varied among these three interspecific progenies, additional cytogenetics studies are needed to understand whether such a relationship exists. However, large differences for the number in root were observed among the interspecific progenies. This difference in root number may partially explain the different reactions observed due to nematode infection as more roots provided a greater area for root infection (Anzueto *et al*., 2001). The resistance for interspecific F_1 hybrids was introgressed from *C. hystrix*. This resistance was further transferred to the BCF_1 progeny when the dF_1 was backcrossed to *C. sativus* cv. Beijingjietou. The high levels of resistance in the F_1 and BCF_1 progenies from the crosses possibly indicated that the resistance was inherited in a dominant fashion, and thus *C. hystrix* can be used in the cucumber improvement.

Meloidogyne-resistance in tomato was introgressed from *Solanum peruvianum* to *S. lycopersicum* and is present in all resistant commercial tomato cultivars (Verdejo-Lucas *et al*.,

2008). *Cucumis hystrix* showed strong growth and displayed stable resistance to *M. incognita* under infection in three consecutive years, so it could be used as the source for developing *M. incognita*-resistant cucumber varieties. However, numerous backcrosses to cucumber and evaluations for *M. incognita* resistance would be necessary to provide cucumber lines with acceptable horticultural characters and nematode resistance. Currently, we have obtained numerous populations of introgression lines between these two species (Chen *et al*., 2004). The high frequency of resistant interspecific progenies suggests the possibility of developing near-isogenic lines with resistance to *M. incognita*. It should also be possible to detect DNA markers linked with resistance to *M. incognita* and one approach, namely bulk segregate analysis (BSA) (Halden *et al*., 1997), is currently being pursued.

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De-You Ye1, Chun-Tao Qian, C. Kurowski. Выявление нового источника генетической устойчивости к *Meloidogyne incognita* у *Cucumis*.

Резюме. Проведен поиск генов устойчивости к галлообразующим нематодам *Melodogyne incognita* в геноме огурцов. Индекс заболевания DI для *Cucumis hystrix* был существенно ниже, чем для восприимчивого контрольного сорта *C. sativus* cv. Beijingjietou. К тому же, на огурцах cv. Beijingjietou были обнаружены многочисленные крупные галлы, тогда как на *C. hystrix* обнаружено лишь небольшое количество мелких галлов. Пораженные *M. incognita* огурцы *Cucumis hystrix* имели больше боковых корней по сравнению с восприимчивым контролем. Межвидовые гибриды, полученные скрещиванием *C. hystrix* и cv. Beijingjietou, показали высокий уровень устойчивости к *M. incognita*. Таким образом, *C. hystrix* может служить источником ценной устойчивости к *M. incognita* при селекции огурцов.