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Full Length Research Paper

Management of *Meloidogyne incognita* in nematode-susceptible watermelon cultivars using nematode-resistant *Cucumis africanus* and *Cucumis myriocarpus* rootstocks

K. M. Pofu, P. W. Mashela* and M. S. Mphosi

Department of Soil Science, Plant Production and Agricultural Engineering, School of Agricultural and Environmental Sciences, University of Limpopo, Private Bag X1106, Sovenga 0727, Republic of South Africa.

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Watermelon (*Citrullus lanatus*) cultivars are highly susceptible to the southern root-knot nematode (*Meloidogyne incognita*), with considerable yield losses when this nematode is not managed. Empirical evidence suggested that wild watermelon (*Cucumis africanus*) and wild cucumber (*Cucumis myriocarpus*) were highly resistant to *M. incognita* race 2. The objective of this study was two-fold; viz. to determine whether (1) *C. africanus* and *C. myriocarpus* seedling rootstocks would be compatible with watermelon cv. 'Congo' and 'Charleston Gray' and (2) the two *Cucumis* spp. rootstocks would retain their nematode-resistance capabilities when grafted with the two highly nematode-susceptible watermelon cultivars. The eight treatment combinations were arranged in a randomised complete block design, with six replications. At harvest, 56 days after transplanting the grafted seedlings, with highly susceptible watermelon cultivars had no effect on nematode-resistance capabilities of *C. africanus* and *C. myriocarpus.* Also, the two *Cucumis* spp. were compatible with the two watermelon cultivars. Consequently, *C. africanus* and *C. myriocarpus* rootstocks have the potential for use as resistant rootstocks in the management of *M. incognita* race 2 in watermelon production.

Key words: *Cucumis* spp., root galls, resistant rootstocks, reproductive factor, watermelon cultivars.

INTRODUCTION

Watermelon (*Citrullus lanatus*) cultivars suffer considerable yield losses due to infection by the southern root-knot nematode (*Meloidogyne incognita)* in tropical areas with sand (Davis, 2007; Sumner and Johnson, 1973; Thies, 1996). Following empirical demonstration that the Cucurbitaceae family had no resistance to *Meloidogyne* spp. (Thomanson and McKiney, 1959), management of *M. incognita* in watermelon husbandry depended wholly on fumigant nematicides. However, the suspension of methyl bromide due to its environment-unfriendliness resulted in the management focus shifting towards alternatives that included the use of nematode-resistant rootstocks.

In Asia, Europe and the Middle East, bottle gourd (*Lagenaria siceraria*) and hybrid squash (*Cucurbita moschata* x *C. maxima*) were widely used as rootstocks in watermelon production due to their resistance to fusarium wilt (Cohen et al., 2007). However, the fusarium-wilt resistant rootstocks were highly susceptible to *M. incognita* (Thies et al., 2008). Widespread screening in wild watermelons (*Citrullus lanatus* var. *citroides*) for nematode resistance resulted in the identification of moderate resistance to *M. incognita* (Thies and Levi, 2007). The availability of nematode resistant germplasm from wild watermelons would provide an alternative to methyl bromide for managing the root-knot nematodes in watermelon production.

In watermelon-producing regions of Limpopo Province, South Africa, following the suspension of methyl bromide, research on nematode management shifted towards using crude extracts from selected plant organs (Mashela, 2002; Mashela et al., 2008) and the screening of various genera for resistance against *M. incognita* race 2 within the Cucurbitaceae family (Pofu et al., 2010a, b,

*Corresponding author. E-mail: phatu.mashela@ul.ac.za.

c). Greenhouse studies suggested that the tested *C. lanatus* cv. 'Congo' and 'Charleston Gray' were highly sensitive to *M. incognita* race 2 (unpublished data), whereas wild watermelon (*Cucumis africanus)* and wild cucumber (*Cucumis myriocarpus*) were highly resistant to this nematode race (Pofu et al., 2010a, b, c). The objective of this study was two-fold; viz. to determine whether (1) *C. africanus* and *C. myriocarpus* seedling rootstocks would be compatible with watermelon cv. 'Congo' and 'Charleston Gray' and (2) the two *Cucumis* spp. rootstocks would retain their nematode-resistance capabilities when grafted with the two highly nematode-susceptible watermelon cultivars.

MATERIALS AND METHODS

Location and sowing

The experiment was conducted in the greenhouse at the University of Limpopo, South Africa (23° 53'10"S, 29° 44'15"E) in December 2009 and repeated in late February 2010. Fruits of *C. africanus* and *C. myriocarpus* were collected from the local field, cut into pieces and seeds were separated from carpal materials. After oven-drying at 52°C for 72 h (Makkar, 1999), seeds were separately wrapped in hand-sewn cotton handkerchief bags and submerged in running tap-water for 8 h to leach out auto-allelochemicals (Mafeo and Mashela, 2009) prior to sowing each species at one seed/cone in separate seedling trays containing Hygromix (Hygrotech, Pretoria North, South Africa). Seven days after sowing the rootstock seeds, primed seeds of watermelon cv. 'Congo' and 'Charleston Gray' were sown at one seed/cone in separate seedling trays containing Hygromix and irrigated to field capacity daily.

Grafting, transplanting and cultural practices

Grafting was performed 14 days after emergence of watermelon cultivars using razor blades which were intermittently sterilised in 5% NaOCl solution. Both rootstock and scion seedlings were taken out of their respective seedling trays. Rootstocks were cut at 45° upward underneath the axis, whereas the respective scions were cut at 45° downward at the same height; the two clefts pushed into each other and joined using plastic grafting clips. The two companions were set in two adjacent cones, with Hygromix being added to firm the seedlings. After 14 days, parts of the watermelon and those of *Cucumis* spp. below and above the graft union, respectively, were severed. Seven days after cutting, plants were transplanted into 20-cm-diameter plastic pots containing a mixture of pasteurised sand and Hygromix at 3:1 (v/v).

At transplanting, seedlings were fertilised with 5 g 2:3:2 (22)/pot. Four moisture meters (Hadeco Magic^{R}, RSA) were inserted at 7- and 14-cm depths in two randomly selected pots of each rootstock and pots were irrigated with 300 ml tap water as soon as 50% of the moisture meters were read below 2 units. When necessary, aphids were controlled using aphicide (a.i. dimethoate 400 g/L) at 0.75 ml/L water.

Preparation of inoculum and inoculation

When required, *M. incognita* inoculum was prepared by extracting eggs from roots of greenhouse-grown nematode-susceptible kenaf (*Hibiscus cannabinus*) plants in 1% NaOCl (Hussey and Barker, 1973). A day after transplanting, pots were each infested by dispensing ca. 1 000 *M. incognita* juveniles using a 20-ml plastic syringe by placing into 5-cm-deep holes on the cardinal points of the base of the stems and then covered with growing mixture.

Experimental design

The eight treatments, viz. cv. 'Congo' alone, 'Charleston Gray' alone, *C. africanus* alone, *C. myriocarpus* alone, cv. 'Congo' grafted on *C. africanus,* cv. 'Congo' grafted on *C. myriocarpus*, cv. 'Charleston Gray' grafted on *C. africanus,* cv. 'Charleston Gray' grafted on *C. myriocarpus*, were arranged in a randomised complete block design, with 10 replicates.

Data collection

Survival of grafts was recorded from transplanting to harvest. Whenever a graft withers within the replication, the entire replication was removed. Due to mortalities, data were collected from six and seven replicates in Experiments 1 and 2, respectively. At harvest, 56 days after inoculation, stolon length was measured and cut at the graft union. Stem diameter of scion 5 cm above the severed end and rootstock diameter 5 cm below the severed ends were measured using a digital vernier caliper. In ungrafted treatments, stem and rootstock diameters were measured at heights similar to those of grafted plants. Shoots were oven-dried at 70°C for 72 h and weighed.

Root systems were removed from pots, immersed in water to remove soil particles, blotted dry and weighed to facilitate the calculation of nematode density/total roots/plant. Root galling was based on the scale of 0 to 5, in which 0 = no galls, 1 = 1 to 2 galls, 2 = 3 to 10 galls, 3 = 11 to 30 galls, 4 = 31 to 100 galls and 5 = >100 galls/root system (Taylor and Sasser, 1978). Nematodes were extracted from total root system/plant by maceration and blending for 30 s in 1% NaOCl (Hussey and Barker, 1973). The material was passed through nested 61- and 38-µm mesh sieves. The contents of the 38-µm mesh sieve were collected for further separation of nematodes from debris using the sugar-floatation and centrifugation method (Jenkins, 1964).

Soil per pot was thoroughly mixed and a 250-ml soil sample was collected, with nematodes extracted using the sugar-floatation and centrifugation method (Coolen and D'Herde, 1972). Eggs and juveniles were counted from a 10-ml aliquot with the use of a stereomicroscope. Nematode numbers from roots were converted to nematodes/total root system/plant, whereas soil nematode numbers were converted to 2700 ml soil/pot. Reproductive factors (RF = Pf/Pi) were computed.

Data analysis

Nematode data were transformed through $\log_2(x+1)$ to homogenise the variances (Gomez and Gomez, 1984). Data were subjected to analysis of variance with SAS software (SAS Institute, Cary, NC). Mean separation was achieved with Waller-Duncan multiple range test when the treatment effect was significant at 5% level of probability. Since the season x season interactions for the two experiments for variables measured were not significant at the probability level of 5%, data were pooled (n = 12) and subjected to analysis of variance. Only the treatment effects where the F-tests were significant at the probability level of 5% are discussed, unless otherwise indicated.

RESULTS

Reproductive factors

Roots of watermelon cv. 'Congo' and ' Charleston Gray'

Table 1. Reproductive factors (P*f*/P*i*) of *Meloidogyne incognita* race 2 on eight scion-rootstock combinations of watermelon cultivars 'Congo' and '*Charleston Gray'* with and without *C. africanus* and *C. myriocarpus* seedlings under greenhouse conditions (n = 12).

Scion-rootstock combination	Root gall/ total root	Final nematode number (P*f*)/total root[y]	P*f*/P*i*[z]
Watermelon cultivar 'Congo'	4.8	7582 (8.81[a])[x]	7.58[a]
Watermelon cultivar 'Charleston Gray'	4.6	7067 (8.80[a])	7.07[a]
C. africanus	0.1	265 (5.47[bc])	0.26[b]
C. myriocarpus	0.0	230 (5.32[bc])	0.23[b]
Congo on *C. africanus*	0.2	617 (6.14[b])	0.62[b]
Congo on *C. myriocarpus*	0.3	543 (6.09[b])	0.54[b]
Charleston Gray on *C. africanus*	0.1	446 (5.93[b])	0.45[b]
Charleston Gray on *C. myriocarpus*	0.4	293 (4.77[c])	0.29[b]

[x]Column means followed by the same letter are not different ($P \leq 0.05$) according to Duncan's multiple-range test; [y]Final nematode numbers (P*f*) in parenthesis represent means of $\log_2(x+1)$-transformed data; [z]Reproductive factor = P*f*/P*i*, where P*i* = 1000.

had fully developed galls, whereas those on the test rootstocks were, when present, small and undeveloped (Table 1). Also, the watermelon cultivars had the highest RF values when compared to those of grafted and ungrafted *Cucumis* seedlings. However, the RF values of *M. incognita* race 2 on grafted and ungrafted *Cucumis* rootstocks did not differ.

Survival of grafts and yield components

In total, eight of twenty replications were removed due to mortalities of inter-generic grafts, which accounted for 40% loss. Dry shoot weight and stolon length of grafted and ungrafted watermelons within a given cultivar did not differ (Table 2). However, stem diameter quotients of cv. 'Charleston Gray' on both *Cucumis* rootstocks were significantly bigger than those of others, whereas that of cv. 'Charleston Gray' onto *C. africanus* did not differ from that of cv. 'Congo' onto *C. myriocarpus* (Table 3).

DISCUSSION

M. incognita race 2, as shown by the RF values, reproduced prolifically in both watermelon cultivars 'Congo' and 'Charleston Gray'. The RF values measure the reproductive potential of a nematode in a host, serving as an indicator for host-status to the test nematode (Ferris and Wilson, 1987). Fully developed root galls on watermelon cv. 'Congo' and 'Charleston Gray' were consistent with those in studies which demonstrated that watermelon cultivars were highly susceptible to *M. incognita* (Montalvo and Esnard, 1994; Tanveer and Saad, 1971). Generally, the presence of root galls is an indication that giant cells developed and that nematode feeding and reproduction occurred (Ferraz and Brown, 2002).

Reproductive factors of less than one in the grafted *Cucumis* spp*.* were consistent with those of ungrafted *Cucumis* spp. in this and other related studies (Pofu et al., 2010a, b, c). The results of this study show that grafting highly nematode-susceptible watermelon scions on highly nematode-resistant *Cucumis* rootstocks had no effect on the resistance capabilities of the *Cucumis* spp. to *M. incognita* race 2. Essentially, results of this study confirmed those of Thies and Levi (2007), who suggested that certain wild watermelon species in the Cucurbitaceae family have some resistance to *M. incognita.* Also, the observation agrees with Fassuliotis (1970) who demonstrated that some resistance to *M. incognita acrita* existed in "fig-leafed" goured (*Cucurbita ficifolia*) and African horned cucumber (*Cucurbita metuliferus*).

In Cucurbita, Cucumis *melo* and *Langeria* rootstocks, grafting of watermelon cultivars for the management of fusarium wilt, resulted in vigorous scion growth and increased yields (Cohen et al., 2007). Generally, within the short-term period of this study, grafting had no effect on yield components, which served as another indicator for compatibility. Results of this study provided additional evidence of inter-generic compatibility within the Cucurbitaceae family, with unacceptable high levels of mortality, which are common in inter-generic grafts (Thies et al., 2008).

In conclusion, grafting nematode-susceptible watermelon cv. 'Congo' and 'Charleston Gray' onto *Cucumis* spp. had no effect on the resistance capabilities of the two *Cucumis* spp. against *M. incognita* race 2. Results of this study suggest that the two *Cucumis* spp. have the potential to serve as alternatives to methyl bromide in the management of *M. incognita* race 2 in watermelon production. However, since rootstocks have influence on yield quantity and quality, these parameters need to be evaluated under field conditions using the two Cucumis

Table 2. Yield components of watermelon cultivars 'Congo' and 'Charleston Gray' with and without *Cucumis africanus* and *C. myriocarpus* nematode-resistant seedling rootstocks (n = 12).

Yield component	Control	*C. africanus*	*C. myriocarpus*	$LSD_{0.05}$
Grafted watermelon cultivar 'Congo'				
Dry shoot weight (g)	14.65	16.05	15.36	1.83
Stolon length (cm)	337.33	369.83	381.33	54.19
Grafted watermelon cultivar 'Charleston Gray'				
Dry shoot weight (g)	14.68	14.52	16.62	3.21
Stolon length (cm)	381.33	403.33	353.67	27.22

Row means are not different (P ≤ 0.05) according to Fisher's least significant difference test.

Table 3. Comparison of the stem and the rootstock diameters at two positions relative to the graft union of watermelon cultivars 'Congo' and 'Charleston Gray' with and without rootstocks of *C. africanus* and *C. myriocar*pus (n = 12).

Scion-rootstock	Position relative to graft union		Quotient
Combination	**5-cm above (D_2)**	**5-cm below (D_1)**	**(D_2)/ (D_1)**
Congo alone	5.45	5.65	0.96^{c}
Congo onto *C. africanus*	4.57	4.20	1.09^{c}
Congo onto *C. myriocarpus*	5.03	4.18	1.20^{bc}
Charleston Gray alone	5.18	5.50	0.94^{c}
Charleston Gray onto *C. africanus*	4.98	3.58	1.39^{ab}
Charleston Gray onto *C. myriocarpus*	5.38	3.52	1.53^{a}

Column means (quotient) followed by the same letter are not different (P ≤ 0.05) according to Duncan's multiple-range test.

rootstocks. Also, procedures need to be further developed to improve survival of the two inter-generic grafts.

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