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Survival, Flowering and Productivity of Watermelon (*Citrullus lanatus*) Cultivars in Inter-generic Grafting on Nematode-resistant *Cucumis* Seedling Rootstocks in *Meloidogyne*-Infested Fields

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ABSTRACT

A new procedure for optimising stem diameters of watermelon (*Citrullus lanatus*) and nematode-resistant wild indigenous *Cucumis* species suggested that the two genera were highly compatible, with rootstock seedlings retaining their ability to suppress *Meloidogyne* species. A field study was initiated using the new procedure to determine the influence of inter-generic grafting of *Citrullus* cultivars onto *Cucumis* species on survival of grafts, flowering, fruit yield and accumulation of essential nutrient elements under field infested with *Meloidogyne* species. Four weeks after transplanting, *Cucumis africanus* and *C. myriocarpus* rootstocks increased flowering by 70-81% and 96-77% in two watermelon cultivars. At harvest, 66 days after transplanting, rootstocks reduced nematode reproductive factor values by 92-93% and 94-96%, but increased fresh fruit yield by 46-60% and 48-115% and dry shoot weight by 50-66% and 30-104% in watermelon cultivars. Rootstocks had no effect on accumulation abilities of essential nutrient elements in leaves except for Mn and Zn. Inter-generic grafting of *Citrullus* cultivars onto *Cucumis* species improved the productivity of the former in areas heavily infested with *Meloidogyne* species. Thus, inter-generic technology with optimised stem diameters needs to be expanded to other genera where incompatibility challenges had been limiting the use of this technology.

Key Words: *Citrullus lanatus; Cucumis species;* Inter-generic grafting; Nematode management; Watermelon

INTRODUCTION

In tropical and subtropical regions of Limpopo Province, South Africa (Pofu *et al.,* 2010a,b; Pofu *et al.*, 2011c), as in other similar regions (Khan & Ahmad, 2000; Khan *et al*., 2000; Jada *et al*., 2007), the root-knot nematodes (*Meloidogyne* spp.) are the most damaging soil pathogens due to suitable climate for nematode reproduction all-year round. Inter-specific (species-to-species) grafting technology had been widely used in plant production for managing soil-borne diseases (Lee, 1994). However, in most cases, all species within the same genus do not have genotypes that are resistant to pathogens and/or nematodes. For instance, all species within the *Citrullus* genus do not have genotypes that are resistant to *Verticillum oxysporum* or the root-knot nematodes, which are both highly injurious to watermelon (*Citrullus lanatus*) cultivars (Oda, 1993). Genera within the same family have conferred some promises in widening the gene pool for resistance to the soil-borne pathogens (Thurau *et al*., 2010). However, inter-generic (genus-to-genus) grafting technology is replete with contradictions on the influence of the seedling rootstocks on survival, flowering, fruit production, bio-mass, fruit quality of scions and accumulation abilities of essential nutrient elements (Davis *et al*., 2008; Flores *et al*., 2010; Rouphael *et al*., 2010; Traka-Mavrona *et al*., 2010; Pofu *et al*., 2011c). The latter, for instance, may result in deficiency and/or toxicity of ions for certain essential nutrient elements to the scion.

Under various conditions, wild watermelon (*Cucumis africanus*) and wild cucumber (*C. myriocarpus*) seedlings were highly resistant to *M. incognita* races 2 and 4 and *M. javanica* (Mofokeng *et al*., 2005; Pofu *et al.,* 2010a,b; Pofu *et al*., 2011b), which are predominant in South Africa (Kleyhans *et al*., 1996). Both *Cucumis* species and *Citrullus* species are within the Cucurbitaceae family (Pitrat *et al*., 1999), with the former being indigenous to South Africa (Kristkova *et al*., 2003), whereas the latter is indigenous to the Kalahari Desert (Jarret *et al*., 1996). The two *Cucumis* species when used as seedling rootstocks in inter-generic

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grafting with highly nematode-susceptible watermelon cv. 'Congo' and cv. 'Charleston Gray' under greenhouse conditions retained their nematode resistant capabilities (Pofu *et al*., 2011c). Also, when *Citrullus* seeds were sown in a 200-hole seedling tray seven days after sowing *Cucumis* seeds in a 160-hole seedling tray, there was 100% survival of the inter-generic grafts as a result of equating the stem diameters of the two genera at the graft union (Pofu & Mashela, 2011a).

In addition to suppressing *Meloidogyne* species in watermelon production, the two *Cucumis* seedling rootstocks would be more attractive to watermelon growers if they were to improve fruit yield and had no deleterious effect on accumulation of essential nutrient elements. The objective of this study was to determine the influence of inter-generic grafting of *Citrullus* cultivars onto *Cucumis* species on survival of grafts, flowering, fruit yield and accumulation of essential nutrient elements under field infested with the root-knot nematodes.

MATERIALS AND METHODS

Field experiments were conducted during summer (November – January) in 2010/2011 at two locations, *viz*. the Plant Protection Skills Centre, University of Limpopo (UL), South Africa (23°53'10"S, 29°44'15"E) and the Agricultural Research Council – Institute for Industrial Crops (ARC – IIC), Rustenburg, North West Province, South Africa (25°43'40"S, 27°17'30"E). The UL location had Hutton sandy loam (65% sand, 30% clay, 5% silt) containing 1.6% organic C, with EC = 0.148 dS m^{-1}, pH (H_2O) = 6.5 and hot dry summers (November – January), with day maximum temperature (DMT) ranging of 28-38°C. Soil at the ARC - IIC location comprised 81% sand, 13% clay and 6% silt, containing 1.1% organic C, with EC=0.152 dS m^{-1}, pH(H_2O) = 6.7 and hot dry summers (November – January), with DMT range of 26 - 35°C. Average rainfall during the growing period at the UL and the ARC – IIC locations averaged 500 mm and 580 mm, respectively.

Fruits of *C. africanus* and *C. myriocarpus* were collected from the local field, auto-allelochemicals leached out (Mafeo & Mashela, 2009) and sown in a 160-hole seedling trays containing Hygromix (Hygrotech, Pretoria North, South Africa) and irrigated daily to field capacity. Seven days after sowing rootstock seeds, primed seeds of watermelon cv. 'Congo' and cv. 'Charleston Gray' were sown at one seed/cone in separate 200-hole seedling trays containing Hygromix and irrigated daily to field capacity. Grafting was performed as described previously (Pofu & Mashela, 2011a). Each location was initially cropped with nematode-susceptible tomato (*Solanum lycorpersicum*) cv. 'Floradade', which was inoculated after transplanting by dispensing 1000 *M. incognita* race 2 eggs and second-stage juveniles (J2s)/plant. Eggs and J2s were prepared by extracting from roots of greenhouse-grown nematode-susceptible Kenaf (*Hibiscus cannabinus*) plants in 1% NaOCl (Hussey & Barker, 1973). A 20-mL plastic syringe was used to place eggs and J2s into 5-cm-deep holes on the cardinal points of the stem of each plant. Tomato plants were uprooted at 56 days after inoculation and primary land preparation done manually using forks.

Each plot of 22.5 m^2 comprised 10 seedlings at inter- and intra-row spacing of 1.5 m × 1.5 m. The first and the last seedlings in a row were used as buffer, with the middle six plants within a plot being used for harvesting and measurements. The site was irrigated to field capacity using overhead sprinkler and three days later it was sampled for initial nematode population densities (Pi). Six treatments, *viz*. cv. 'Congo' alone, cv. 'Charleston Gray' *alone,* cv. 'Congo' onto *C. africanus,* cv. 'Congo' onto *C. myriocarpus*, cv. 'Charleston Gray' onto *C. africanus* and *cv.* 'Charleston Gray' onto *C. myriocarpus*, were arranged in a randomised complete block design, with 10 replicates. Irrigation was scheduled using four Hadeco moisture meters (Hadeco Magic^R, RSA) with plants being irrigated when the average reading was less than two units. Two days after transplanting, soil samples were collected for the determination of Pi. Each soil sample was collected in a systematic pattern between the plants within a row and comprised five composited cores of 2.5-cm diameter at 20-cm deep per plot. Nematodes were extracted from a 250 mL subsample soil using the modified sieving and centrifugation procedure (Jenkins, 1964). In the UL and ARC – IIC trials the mean Pi comprised 230 and 375 *Meloidogyne* species, respectively. Fertilisation and pest management were executed as prescribed for commercial watermelon production in South Africa. Weeds were removed using hand-held hoes when necessary.

Flowering and fruit set were scored at 4, 6 and 8 weeks after inoculation. At harvest, 66 days after transplanting, soil samples for the determination of the final nematode population densities (Pf) were collected per plant as described earlier. Vine length was measured and the graft union was marked with a grafting clip for measuring stem diameter from the clip to 5 cm above (D_2) and 5 cm below (D_1) using a digital vernier caliber. Shoots were cut from the crown, oven-dried for 72 h at 70°C, weighed and leaves ground in a Wiley mill to pass 1-cm opening sieve. Nematodes were extracted from 10 g roots/plant by maceration in a blender for 30 s in 1% NaOCl solution and then passed through a nested series of 150, 63 and 45-µm-pore sieves onto a 25-µm-pore sieve (Hussey & Barker, 1973). Eggs and juveniles from the 25-µm sieve were collected for counting under a light microscope.

Prior to analysis, data for numbers of nematode, flower, fruits and micronutrient were transformed with $\log_{10}(x + 1)$ in order to homogenise the variances (Gomez & Gomez, 1984), but untransformed data are reported. All data were subjected to analysis of variance with SAS software (SAS Institute, Cary, NC), followed by Duncan's multiple-range test or Fisher's least significant different test. The interaction between locations for each *Cucumis* species was not significantly different and data were pooled (n = 120) and subjected to statistical analysis. Only data where the F-test was significant ($P \leq 0.01$) are discussed, unless otherwise indicated.

RESULTS AND DISCUSSION

From grafting to harvest, all grafts survived (data not shown). Using a 250 mL subsample soil as a unit of nematode population density, the soil RF values for *Meloidogyne* species at both locations were above one for ungrafted plants and below one for the grafted plants (Table I). Relative to ungrafted cv. 'Congo', *C. africanus* and *C. myriocarpus* rootstocks grafted with cv. 'Congo' reduced the RF values by 92% and 93%, respectively whereas in the cv. 'Charleston Gray' the two rootstocks reduced the RF values by 94% and 96%, respectively.

Four weeks after transplanting, relative to cv. 'Congo' alone, *C. africanus* and *C. myriocarpus* rootstocks increased flowering by 70% and 81%, respectively (Table II). In cv. 'Charleston Gray' the rootstocks increased the variable by 96% and 77% respectively. Sixty-six days after transplanting, relative to cv. 'Congo' alone, *C. africanus* and *C. myriocarpus* increased fresh fruit yield of cv. 'Congo' by 46% and 60%, respectively whereas the two rootstocks each increased fresh fruit yield of cv. 'Charleston Gray' by 48% and 115%, respectively (Table III). Relative to ungrafted watermelon cultivar, the two rootstocks increased dry shoot weight in cv. 'Congo' by 50% and 66%, respectively, whereas in cv. 'Charleston Gray" by 30% and 104%, respectively.

Rootstock had no effect on vine length and stem diameter (Table IV). In all treatments, the stem diameter quotients were approximately equal to unity. The treatment had no effect on accumulation of essential nutrient elements in leaves, except for Mn and Zn (Table V). Rootstock *C. africanus* increased leaf Mn in cv. 'Congo', but the variable was not different in cv. 'Congo' alone and cv. 'Congo' grafted onto *C. myriocarpus*. However, leaf Mn of cv. 'Congo' grafted onto *C. myriocarpus* was higher than that in cv. 'Charleston Gray' alone and all its scion-rootstock combinations. In contrast, *C. myriocarpus* rootstock increased the accumulative ability of leaf Zn in cv. 'Charleston Gray', which was different from that of cv. 'Congo' grafted onto *C. myriocarpus* and cv. 'Charleston Gray' alone.

The 100% survival of the grafts of *Citrullus* onto *Cucumis* supported observations under greenhouse conditions, which suggested that the recently developed procedure for inter-generic grafting of the two genera would provide a consistent result in survival, which would be an important consideration in mass production (Pofu & Mashela, 2011a). Generally, inter-generic grafting is characterised by high percentages of graft mortalities due to the inherent high incompatibilities of the genera as opposed to species in inter-specific grafting (Tiedermann, 1989; Thies *et al.,* 2010; Traka-Mavrona *et al.,* 2010). The high survival rates of the grafts in this and the previous study could possibly be ascribed to the new procedure for raising seedlings of the two genera, as shown by the stem diameter quotients, which were approximately equal to one.

Control watermelon cultivars had high RF values and root gall numbers, which confirmed observations on inter-generic grafts in the greenhouse study (Pofu *et al.*, 2011c) and other watermelon studies (Thies *et al.,* 2010). The RF values lower than one on *Cucumis* rootstocks and the absence of noticeable root galls suggested that the two plant species retained their nematode resistance under field studies as was previously observed on inter-generic grafting of the two genera under the greenhouse conditions (Pofu *et al*., 2011c).

The two *Cucumis* seedling rootstocks induced early flowering in watermelon cv. 'Congo' and cv. 'Charleston Gray'. Incidentally, the rootstock-scion combination might have changed the concentration of plant-hormones and therefore influenced sex expression and flowering order in grafts as observed in cucumber grafted on rooted squash rootstocks (Satoh, 1996). Also, early flowering of grafts in this study confirmed observations where watermelon cultivars grafted onto bottle gourd (*Lagenaria siceraria)* flowered earlier than in the control ungrafted plants (Kurata, 1976; Sakata *et al*., 2007). In contrast, Yamasaki *et al.* (1994) observed that when pumpkin (*Cucurbita maxima*), bottle gourd, wax gourd (*Benin-casa hispida*) and watermelon were each serving as seedling rootstocks to watermelon, all delayed flowering.

Grafting of watermelon cv. 'Congo' and cv. 'Charleston Gray' onto *C. africanus* and *C. myriocarpus* increased fresh fruit yield and dry shoot weight of watermelon cultivars. The observation, when combined with the high nematode resistance, makes the *Cucumis* seedling rootstocks highly commendable for use in watermelon production. However, this observation does not confirm that of Thies and Kousik (2009) who observed that among the evaluated five lines of wild watermelon seedling rootstocks, only one line (RKVL 318) increased fruit numbers of seedless watermelon cv. 'Tri-X 313'. Satoh (1996) proposed that since roots of grafts are different from the original roots of the scion, inter-generic grafting might alter the physiology of each of the components, resulting in different flowering dates. In both watermelon cultivars, inter-generic grafting increased dry shoot weight of scions. Due to the incompatibility of grafts in inter-generic grafting, almost always, grafting resulted in reduced shoot weight (Tiedermann, 1989; Traka-Mavrona *et al*., 2010). Generally, watermelons are determinate plants, where flowering curtails shoot growth. Thus, in this, study the experiments were terminated at 66 days after transplanting in order to minimise confounding the effect of flowering and treatments on shoot growth. The stem diameter quotients in all treatments were approximately equal to one under field conditions.

Table I: Rootstock effect on reproductive factor values of *Meloidogyne incognita race 2* on un-grafted watermelon cultivars and grafted onto *Cucumis africanus* and *C. myriocarpus* seedling rootstocks under greenhouse conditions at 56 days after inoculation with 1000 nematodes (n = 120)

Seedling Rootstock	Cultivar 'Congo'		Cultivar 'Charleston Gray'	
	RF	Relative effect (%)	RF	Relative effect (%)
Ungrafted control	7.58a	–	7.07a	–
Cucumis africanus	0.62b	–92	0.45b	–94
Cucumis myriocarpus	0.54b	–93	0.29b	–96

Relative effect = [(Rootstock/Control) – 1) × 100]

Table II: Flower induction and fruit set in watermelon cv. 'Congo' and cv. 'Charleston Gray' grafted on nematode-resistant *Cucumis africanus* and *C. myriocarpus* seedling rootstocks under field conditions at four, six and eight weeks after inoculation (n = 120)

Treatment	Number of flowers			Number of fruits	
	Week 4	Week 6	Week 8	Week 6	Week 8
Congo alone	2.00 (0.67b)	10.50 (2.01a)	3.50 (1.28a)	0.80 (0.41a)	1.30 (0.70a)
Onto *C. africanus*	2.40 (1.14a)	11.60 (2.49a)	5.30 (1.77a)	1.20 (0.69a)	1.80 (0.94a)
Onto *C. myriocarpus*	3.30 (1.21a)	11.10 (2.35a)	7.10 (1.75a)	0.60 (0.36a)	1.30 (0.70a)
Charleston Gray alone	2.20 (0.77b)	13.80 (2.64a)	8.00 (1.76a)	1.20 (0.69a)	1.80 (0.94a)
Onto *C. africanus*	4.10 (1.51a)	10.30 (2.03a)	3.10 (1.14a)	0.70 (0.40a)	1.00 (0.60a)
Onto *C. myriocarpus*	3.70 (1.36a)	11.10 (2.11a)	3.80 (1.30a)	1.50 (0.77a)	0.90 (0.57a)

Column means in brackets with the same letter were not different ($P \leq 0.05$) according to Duncan's multiple-range test.

Table III: Fresh fruit weight and dry shoot weight in two watermelon cultivars grafted on two nematode-resistant *Cucumis* seedling rootstocks under field conditions at 66 days after transplanting (n = 120)

Scion-rootstock combination	Fresh fruit weight (g)	Relative impact (%)^y	Dry shoot weight (g)^z	Relative impact (%)
Congo alone	239.02b	-	62.66b	-
Onto *C. africanus*	347.81a	46	93.73a	50
Onto *C. myriocarpus*	383.41a	60	103.73a	66
Charleston Gray alone	240.86c	-	74.90b	-
Onto *C. africanus*	356.13b	48	97.58b	30
Onto *C. myriocarpus*	517.12a	115	153.13a	104

^{y}Relative impact = [(Grafted/ungrafted) – 1] × 100

^{z}Column means with the same letter were not different ($P \leq 0.05$) according to Duncan's multiple-range test

Table IV: Vine length, vine diameter and the quotient in two watermelon cultivars grafted on two nematode-resistant *Cucumis* seedling rootstocks under field conditions at 66 days after transplanting (n = 120)

Scion-rootstock combination	Vine length (cm)	stem diameter (mm)		Quotient (D_2/D_1)
		Rootstock (D_1)	Scion (D_2)	
Congo alone	166.41	9.85	10.18	1.03
Onto *C. africanus*	198.01	9.46	10.25	1.08
Onto *C. myriocarpus*	237.14	9.36	9.75	1.04
$LSD_{0.05}$	76.73	0.94	0.85	0.53
Charleston Gray alone	291.20	10.90	10.19	0.93
Onto *C. africanus*	192.34	10.25	9.53	0.93
Onto *C. myriocarpus*	222.09	9.08	9.05	1.00
$LSD_{0.05}$	36.89	2.28	1.41	0.39

D_1 and D_2 each is 5 cm from the graft union

Table V: Selected macro- and micro-nutrient elements in leaves of watermelon cv. 'Congo' and cv. 'Charleston Gray' grafted on nematode-resistant *Cucumis africanus* and *Cucumis myriocarpus* seedling rootstocks under field conditions at 66 days after transplanting (n = 120)

Scion-rootstock	Macro-nutrient elements (%)					Micro-nutrient elements (ppm)				
Combination	N	P	K	Ca	Mg	B	Cu	Fe	Mn^z	Zn^z
Congo alone	2.58	0.40	2.41	2.24	0.53	16.61	7.44	108.16	51.94a	45.44b
Onto *C. africanus*	2.48	0.40	2.27	2.40	0.54	17.67	7.17	131.79	55.89a	41.72b
Onto *C. myriocarpus*	2.63	0.39	2.36	1.91	0.51	15.72	6.78	107.42	44.56b	34.44c
Charleston Gray alone	2.66	0.39	2.57	2.10	0.47	17.00	7.83	106.74	37.33b	35.44b
Onto *C. africanus*	2.59	0.41	2.40	1.91	0.45	16.28	7.44	107.00	38.24ab	45.28b
Onto *C. myriocarpus*	2.54	0.40	2.49	1.77	0.43	15.72	7.39	107.78	44.44ab	52.50a
$LSD_{0.05}$	2.75	0.42	2.72	2.66	0.61	18.65	8.36	210.21	-	-

^{z}Column means with the same letter across the two cultivars were not different ($P \leq 0.05$) according to Duncan's multiple-range test

This could be attributed to the innovative procedure developed for the raising of inter-generic seedlings, where the negative effects of different stem diameters were eliminated (Pofu & Mashela, 2011a).

In this study, grafting affected the accumulation abilities of Mn and Zn in leaves of watermelon scions. Generally, cv. 'Charleston Gray' alone was a poor accumulator of both Mn and Zn. Rouphael *et al*. (2008) demonstrated that relative to the ungrafted cucumber cv. 'Akito', the accumulation of Cu in leaf and fruit tissues of cv. 'Akito' grafted onto the 'Shintoza'-type rootstock (*C. maxima x C. moschata*) was 15,567% and 31,233%, respectively. Eldenstein *et al.* (2005) demonstrated that, when irrigated with marginal water under field conditions, the concentrations of B, Zn, Mn and Cu in fruit of melons grafted onto pumpkin were lower in grafts than in control plants, suggesting incompatibility of scions and rootstocks in relation to the union of the vascular bundle (Tiedermann, 1989). Mashela (2002) demonstrated that fruit of *C. myriocarpus* from different regions of Limpopo Province, South Africa, had excessively high concentrations of Fe, but this high accumulative ability for Fe was not observed in leaves of the grafts. Root systems of different plant species may have different or similar absorptive capabilities of essential nutrient elements (Mamphiswana *et al*., 2010). Essentially, failure to detect differences in all macro-nutrient and certain micro-nutrient elements could be interpreted to imply that the root systems of both *Cucumis* and *Citrullus* genera within the Cucurbitaceae Family have similar absorptive capabilities.

CONCLUSION

Positive attributes in results of this study were probably due to the successful optimization of stem diameters in the two inter-generic grafts. Therefore, the optimization of stem diameters of various genera could be opening a new era in inter-generic grafting technology for managing *Meloidogyne* species in plant genera where incompatibility due to different stem sizes has previously been a limiting factor.

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