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Controlling the southern root-knot nematode (*Meloidogyne incognita* Chitwood) with grafted and resistant pepper varieties

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Summary: Newly bred resistant bell pepper varieties and those grafted onto resistant rootstocks were tested in soil severely infested with southern root-knot nematode [*Meloidogyne incognita* (Kofoid and White) Chitwood] in unheated plastic house and compared to varieties on their own roots, in order to evaluate the efficiency of this environmentally friendly control method. 'Cinema F1' carrying the N gene yielded significantly more than the two susceptible varieties. Varieties grafted onto resistant rootstocks outyielded those on their own roots although to different extent, which was not always significant. At the end of the vegetation period the roots of the rootstocks were undamaged and the roots of some resistant varieties were slightly infected, whereas the roots of susceptible varieties were severely damaged. According to our results, both the use of resistant varieties and grafted plants offer an effective and environmentally safe way of controlling *M. incognita*.

Key words: pepper, grafting, resistance, *Meloidogyne incognita*

Introduction

Bell pepper (*Capsicum annuum* L.) is the most important forced vegetable in Hungary, spreading over 1800 ha (*Anonym,* 2008). In Hungarian plastic and greenhouses, *Meloidogyne incognita* is the most common pest, causing severe damage to most forced vegetables, including bell pepper (*Dabaj* et al., 1994; *Amin,* 1994). After the mild winters of the past few years, the overwintering of the species outdoors was observed in Hungary (*Budai* et al., 2005). On the basis of our observations, it overwinters in unheated greenhouses along with heated ones, and year by year it causes severe damage. Soil fumigation has not been an option after the 1st January 2005, and other control methods with pesticides are rather costly and harmful to the environment and appliers. Moreover, their efficiency is not perfect. The use of resistant varieties as well as grafting onto resistant rootstocks offer alternative, environmentally friendly ways of control (*Thies* et al., 2003; *Oka* et al., 2004; *Pénzes* et al, 2008). Although trials have been carried out with grafted bell pepper plants, no bell pepper variety resistant to the southern root-knot nematode has been in commercial use in Hungary. Although grafting onto resistant rootstocks is costly, it can provide a solution until resistant varieties are available in commercial use (*Pénzes* et al., 2008).

Research on the resistance against root-knot nematodes in pepper (*Capsicum spp.*) started about 60 years ago. *Martin* (1948) selected Cayenne-type peppers resistant to root-knot nematode (the species is unknown) (red, pointed chili), the result of which was 'Carolina Hot' available for commercial use from 1958. *Hare* (1956) tested 162 *C. frutescens* varieties and lines against *M. incognita acrita*. In his tests, 4 red pepper varieties proved to be resistant. In *C. frutescens* 'Santanka xS', he found a dominant gene (*N*) responsible for resistance to 3 species (*M. incognita, M. javanica* and *M. arenaria*) (*Hare,* 1957). Based on this, he obtained the pimiento-type 'Mississippi Nemaheart', which was launched in the market in 1966. *Hendy* et al. (1983) tested *C. annuum* lines, out of which PM217 and PM687 were found to be resistant against the populations of different root-knot nematode species (*M. incognita, M. javanica* and *M. arenaria*). Later the inheritance of resistance was examined in these two lines, during which *Me1* and *Me2* nematode resistance genes were found in PM217, and *Me3* abd *Me4* genes in PM687. *Me1* is responsible for resistance against *M. incognita, M. javanica* and *M. arenaria*, while *Me2* gives resistance against *M. javanica*. *Me3* is responsible for resistance against *M. arenaria, M. incognita* and *M. javanica*, while *Me4* against the Ain Toujdate isolate of *M. arenaria* (*Hendy* et al., 1985).

Di Vito and Saccardo (1986) found the P.I. 159237 and P.I. 159256 lines of *C. annuum* resistant, but the F1 hybrids of these lines crossed with 'Corno di Toro' were susceptible to *M. incognita* race 1. *C. chinense* 'Surrinam-8' and *C. frutescens* 'Tabasco' exhibited high resistance. Later 4 *C. chacoense*, 17 *C. chinense*, and 6 *C. frutescens* lines derived from Hungarian samples proved to be resistant against *M. incognita* (Di Vito et al.1989).

In Hungary, *Amin* (1994) tested 44 *C. annuum* varieties and lines, out of which 8 were resistant against *M. incognita*.

Budai et al. (1997) examined the resistance of 10 red pepper and 5 bell pepper varieties against *M. incognita*, and found resistance in 'Kalocsai merevszárú' and 'Szegedi 80'.

Fery et al. (1986) selected the *M. incognita*-resistant 'Carolina Cayenne' and 'Charleston Hot' from a 'Carolina Hot' population heterogeneous for resistance to *M. incognita*. In 1997, the dominant N gene was transferred from 'Mississippi Nemaheart' to 'Yolo Wonder B' and 'Keystone Resistant Giant' varieties through traditional backcrosses, thus 'Carolina Wonder' and 'Charleston Belle', the first *M. incognita*-resistant bell pepper (blocky type) varieties were marketed (*Fery* et al. 1998). By comparing the near-isogenically resistant 'Charleston Belle' and the susceptible 'Keystone Resistant Giant' varieties, *Thies* et al. (2004) found that the resistant variety yielded more by 35% and 50% in two different plots infected with *M. incognita*.

There are few data available regarding the use of tolerant rootstocks and grafted pepper against *M. incognita*. In South-Korea and Japan, then most common way of control against soil-born pests is grafting in cucumber, egg plant, melon, watermelon, and tomato, however, there is no mentioning of pepper (*Lee*, 2003). *Lee & Oda* (2003) reports about growing grafted pepper as a method recently introduced in South-Korea. According to their data, only 5% of the plants are grafted.

Oka et al. (2004) tested the resistance of several pepper rootstocks and varieties in growth chamber, inoculating plants with 1500 larvae. *C. annuum* 'AR 96023' and the rootstock variety 'Snooker' as well as *C. frutescens* and *C. chacoense* lines showed resistance. The variety 'Celica' grafted onto the rootstock 'AR 96023' yielded twice as much in soil infested with *M. incognita*, than on its own roots. In comparison, the roots of 'Snooker' were damaged under the same conditions, and did not yield significantly more than the non-grafted pepper plants. The forming of root-knots on 'Snooker' might have been due to the high temperature. From this it was concluded that the resistance of 'AR 96023' remains stable at high temperatures. According to these results, in some rootstock-scion combinations yield can decrease compared to varieties on their own roots.

In the test carried out by *Morra & Bilotto* (2006) the variety grafted onto 'Graffito' developed well despite the severe root-knot nematode infection and yielded significantly more than plants grafted onto 'Gc1002' or on its own roots. Also, the number of galls was lower. On the basis of this, the use of 'Graffito' variety as rootstock in soil infested with *M. incognita* is recommended. *Colla* et al. (2008) tested the combination of 5 rootstock varieties with 2 scions, measuring plant growth, and the quantity and quality of yield. Each rootstock variety ('RX600', '97.9001', 'DRO8801', 'Snooker', 'Tresor') significantly increased plant growth, and the quantity of marketable yield in both scions.

In our experiments our aim was to evaluate *M. incognita*-resistant pepper rootstocks, by comparing the quantity and quality of yield of grafted plants to susceptible and newly bred, *M. incognita*-resistant varieties on their own roots in soil severely infested with *M. incognita* in unheated plastic house.

Materials and methods

Tests were carried out in 2008. All the tested varieties *(Table 1.)* belonged to the sweet bell pepper variety-group: two varieties available in commercial use and susceptible to the southern-root-knot nematode ('Cibere F1' and 'Citera F1'), two *M. incognita*-resistant rootstock varieties ('Snooker F1' and 'RT 17') onto which susceptible varieties had been grafted, as well as two new, *M. incognita*-resistant test varieties (in the meantime one of them has become available in commercial use as 'Cinema F1') *(Table 1.)*. According to the information gained from breeders, the rootstock variety 'Snooker' carries the *Me7* resistance gene, whereas 'Cinema F1' carries the *N* gene. In order to obtain seedlings of the same developmental stage, the seeds of the rootstocks and varieties to be grafted were sown on 13.3.2008, whereas those of the other varieties were sown on 4.4.2008., into trays of 4x4 cm holes, containing peated seeding medium. The seedlings were planted into soil heavily infested with *M. incognita* in an unheated plastic tunnel in Soroksár on the 17th of May. The species had been morphologically identified as *M. incognita* from the roots of tomato grown in the plastic house in the previous year. The soil and air temperature was measured every 15 min with TinyTag measurer. Seedlings were placed in double rows, where spacing between rows was 100+50 cm, and 30 cm within rows, in such a way that the same varieties grafted onto different rootstocks were next to each other in the rows *(Fig.1.)*. There were 17 replica in each variety and rootstock-scion combination. Plants were grown with two stems, and continuously pruned during the vegetation period, and 1 fruit was left on every main stem and side-branch. Fertiliser comprising Plantaaktiv NK Spezial Plus (12:0:41+te) + Yara Liva Calcinit (15,6:0:0+25,6CaO), as well as Scotts Universol Orange (16:5:25+te) was applied regularly with micro sprinkle irrigation. Fruits were picked from the end of June until frost about every two weeks. During fruit picking, fruits were collected from and separated by each stem, and the fruits were measured and recorded one by one. Fruits were not categorised according to their size, but statistical analysis was done with the number of fruits weighing more than 90 grams, which account for a fruit size of 6 cm width and more than 10 cm length. The data obtained was analysed with SPSS programme by variance analysis, and significant differences were determined with Tukey-Kramer test.

Table 1. Tested varieties and combinations (Soroksár, 2008)

Number	Variety and combination
1	Cibere F1
2	Citera F1
3	Cibere F1 grafted onto 'Snooker'
4	Cibere F1 grafted onto 'RT 17'
5	Citera F1 grafted onto 'Snooker'
6	Citera F1 grafted onto 'RT 17'
7	'Cinema F1'
8	resistant trial variety 2

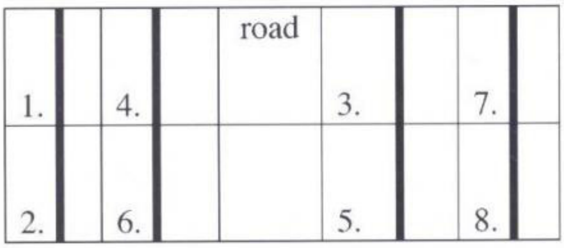

Figure 1. Plot design (Soroksár, 2008)

Results

The developmental stage of the seedlings before replanting was unanimous. It was found that the seeds of grafted plants have to be sown approx. 3 weeks before the others, in order to obtain unanimous seedlings before replanting.

At the end of the vegetation period, the roots were divided from the soil, and although galls were not counted, serious damage was observed on the roots of all plants belonging to the susceptible varieties (the whole root system was covered with galls). On the roots of rootstock varieties no galls were observed. Out of 17 plants, slight damage (15–20% of roots covered with galls) was found on the roots of 5 'Cinema F1' plants, and no damage was observed on the roots of the other 12 plants *(Fig.2. and 3.)*.

Figure 2. The root of susceptible 'Citera F1'

Figure 3. The root of 'Cinema F1'

At the end of the vegetation period, data regarding all the fruits was analysed. Based on the results of the variance analysis, it has been established that there is significant difference between the varieties and rootstock-scion combinations. The *M. incognita*-resistant 'Cinema F1'variety was statistically proved to exceed the susceptible varieties in both fruit-weight and fruit number ($p<0,001$). Although the other resistant test variety did not lag behind 'Cinema F1' in fruit number, its fruit weight was significantly lower; the fruits in many case became small despite the continuous thinning. Regarding total fruit weight, varieties grafted on rootstocks exceeded the susceptible varieties to various extents in every case, although it was not statistically significant in every case. Neither 'Cibere F1', nor 'Citera F1' variety showed significant difference on 'RT 17' rootstock compared to plants on their own roots ($p=0,111$, and $p=0,108$), although the difference manifested in 30 and 36% more fruit weight in grafted plants. 'Citera F1' grafted on 'Snooker' bore more fruit significantly ($p<0,017$). On the basis of total fruit number, it was found that 'Citera F1' yielded more fruit on both rootstock varieties ($p<0,014$, ill. $p<0,011$), whereas there was no significant difference between grafted and own-rooted 'Cibere F1' plants. Comparing the grafted plants and the resistant 'Cinema F1' variety, it was established that they did not outyield the resistant variety with regard to the total fruit yield, but statistically there was no difference ('Cibere F1' scion and 'RT 17' rootstock; 'Citera F1' scion and 'Snooker' rootstock combination) *(Fig. 4. and 5.)*.

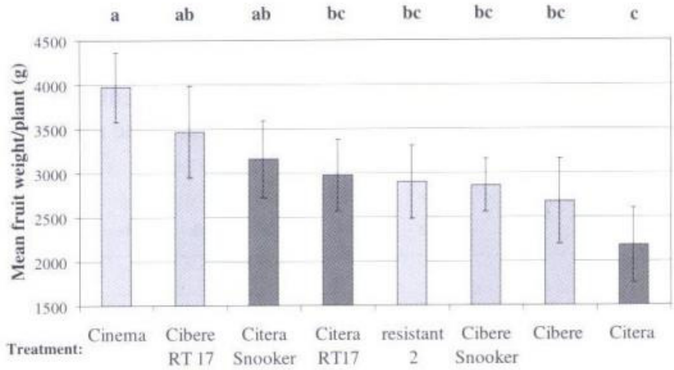

Figure 4. Mean fruit weight per pepper plant (different letters show significant difference at $p<0,05$)

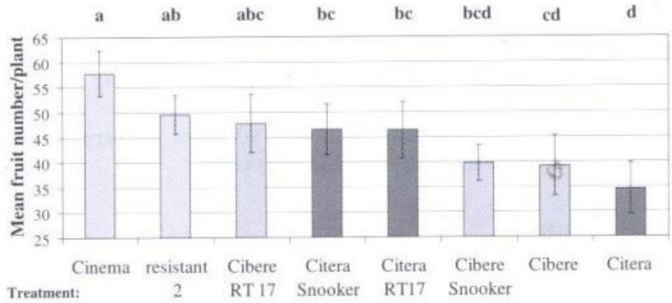

Figure 5. Mean fruit number per pepper plant (different letters show significant difference at $p<0,05$)

Regarding the number of fruits exceeding 90grams, 'Cibere F1' on 'RT 17' rootstock bore fruits of statistically equal weight and number with 'Cinema F1'. On their own roots both species yielded less fruit exceeding 90grams than the grafted plants. 'Cibere F1' grafted onto 'Snooker' yielded 20% more fruit exceeding 90grams, whereas 'Citera F1' yielded 90% more, although this difference is not statistically significant. There was no significant difference between the grafted plants and 'Cinema F1', and only 'Citera F1' on its own roots and the resistant 2 variety differed from them ($p<0,026$ ill. $p<0,018$). This also shows that although the number of fruits of the resistant 2 variety was high, they remained small. *(Fig. 6. and 7.)*.

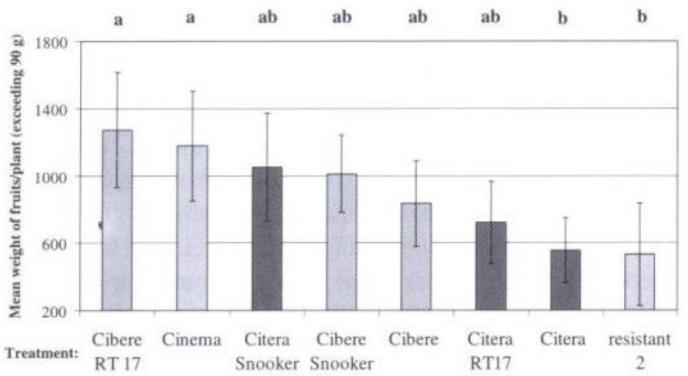

Figure 6. Mean weight of fruits (exceeding 90 grams) per pepper plant (different letters show significant difference at $p<0,05$)

In order to clarify the effect of the southern root-knot nematode on the yield and to obtain successful rootstock-scion combinations, comparing resistant 'Cinema F1' to other varieties with high yield potential and their grafted combinations is justified. It should also be elucidated how such varieties and combinations react in a longer period under heated conditions, and up to what extent are the advantages of rootstocks exhibited compared to the root-knot nematode resistant 'Cinema F1'.

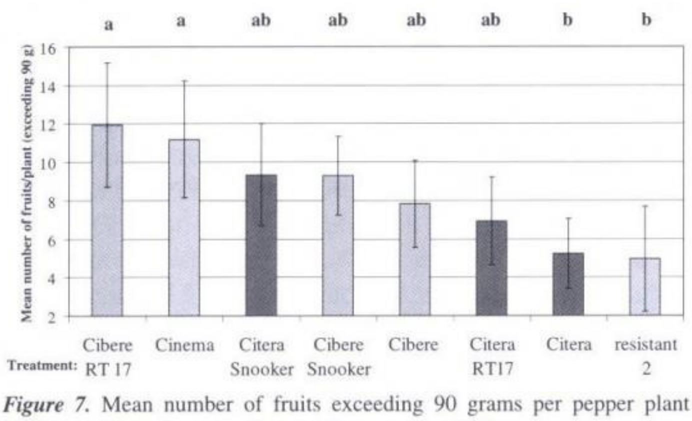

Figure 7. Mean number of fruits exceeding 90 grams per pepper plant (different letters show significant difference at $p<0,05$)

Discussion

No galls were observed on the roots of the tested pepper rootstocks. This contradicts the results of *Oka* et al. (2004), who found in their tests that the roots of 'Snooker' were severely damaged. This can be down to the fact that in our experiment the soil temperature never rose above 30°C during the vegetation period, whereas it rose above 32°C in their test. This justifies that 'Snooker' exhibited resistance in the tests carried out by Oka et al. (2004) in growth chamber. Out of 17, the roots of 5 'Cinema F1' plants were slightly covered (15–20%-os) with galls, whereas no infection was observed on the other 12 plants. Similarly, *Thies and Fery* (2003) found slight damage on 'Carolina Wonder' and 'Charleston Belle' carrying the *N* gene. Although the results of *Oka* et al. (2004) contradict those of *Thies* et al. (2003) (he found the above mentioned two varieties susceptible), it might be due to the high temperature, and the different *M. incognita* races, as the resistant 'Cinema F1' in our test also carries the *N* gene. To specify the spectre of resistance, it is necessary to identify the race of the nematode population.

The *M. incognita*-resistant 'Cinema F1' significantly differed from the susceptible varieties regarding both weight and number of fruits. (*Thies* et al, 2004).

Regarding total yield weight, the grafted plants on resistant rootstocks outyielded the susceptible ones to a different extent. This coincides with the results of *Colla* et al. (2008). The results of *Morra and Bilotto* (2006), who tested grafted pepper plants in soil severely infested with *M. incognita,* confirms our results, as to in case of successful rootstock-scion combination, grafting onto resistant rootstock is an effective way of controlling the southern root-knot nematode.

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