

# Biotechnological studies on rootknot nematode resistance in tomato

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Six tomato cultivars i.e. Edkawi, Super Marmand, Renita, PI 376072, Nemared, and Anahu were used in making non reciprocal diallel pattern of crosses to obtain F1 seeds in order to study the genetics of resistance to the root knot nematode. Cultivar Edkawi has not been evaluated for resistance to root knot nematode before. The tomato cultivar Super Marmand is known for susceptibility to root knot nematode. However, the other cultivars line Ronita, PI 376072, Nemared and Anahu are considered resistant. Seeds of cultivars Edkawi and Super Marmand were obtained from Germplasm Preservation Laboratory, Faculty of Agric. Moshtohor, Zagazig University, Department of Horticulture, Egypt. The seeds of the resistant cultivars were obtained from the National Germplasm Resources Laboratory, Beltsville, U.S.A. The seeds of the different parental germplasm and their F1 seeds were used in the greenhouse, field, and bioassay experiments. *Meloidogyne incognita* Race 1 was used in testing the different tomato genotypes for resistance to root knot nematode, since it was found to be the most common race in most of the tomato growing areas in Egypt. Greenhouse Experiment: Seeds of the different tomato parental germplasm in addition to their F1 seeds were planted in clay pots, 25 cm in diameter (one plant/pot) containing sterilized sandy clay soil (1: 1, v/v). When these seedlings reached the (4 true leaves stage), each plant was inoculated with 5000 fresh nematode eggs (initial population,  $P_i$ ). Then pots were arranged in a randomized complete block design, with five replicates. The recorded temperature in the greenhouse ranged from 26 to 32°C during the period of conducting this experiment. Sixty days after inoculation, the plants were cut off at the soil surface and the soil was gently washed off from the roots. The roots of the individual plants were examined and the following measurements were recorded: a) Number of galls (Egg masses) b) Number of eggs c) Reproduction (R) factor. The results can be summarized as follows. 1. The parental cultivars Ronita, Nemared, and Anahu were the most resistant genotypes, while Edkawi, Super Marmand, and PI 376072 showed high susceptibility, using number of galls, number of eggs, and reproduction factor as criteria to evaluate the resistance of the different tomato genotypes to the root-knot nematode. 2. The additive type of gene interaction was more important than non-additive type of gene interaction, in the inheritance of tomato resistance to the root-knot nematode. 3- The resistance to the root-knot nematode was found to be complete dominant over susceptibility in the different F1 hybrids. 4- The parental cultivars Ronita, Nemared and Anahu proved to be good combiners to form F1 hybrids with high levels of resistance to the root-knot nematode. 5- An intermediate broad sense and low narrow sense heritability estimates were calculated, indicating the importance of selecting for resistance to the root-knot nematode on a family mean basis instead of selecting on individual plant basis. II. Field Experiment: Seeds of the different parental germplasm and related F1's were planted in 10 cm pots filled with sterilized sand and clay (1:1, v:v) on March 9, 1997. Ten pots were assigned to each genotype. The emerged seedlings were thinned to one plant /pot, Five pots from each genotype were used for the purpose of artificial inoculation using 5000 fresh nematode eggs (initial population,  $P_i$ ) per pot. The other five pots from each genotype were left intact. The inoculated and uninoculated seedlings of each genotype were transplanted in the field on May 1, 1997. Before transplanting, holes with the size of 60 X 50 X 50 cm were dugged in the field and filled with soil contains sterilized sand and clay (1: 1, v: v). Each hole was 1 meter apart from the next hole.

The inoculated and uninoculated seedlings of the different genotypes i.e. parents and F<sub>1</sub> populations were transplanted into these holes according to the randomized complete block design. The individual plants of the different genotypes were evaluated for the following characters: 1. Number of days from transplanting to first flower anthesis 2. Fruit set percentage 3. Number fruits/plant 4. Average fruit weight (gm) 5. Plant Yield (gm) 6. Detached Leaves fresh weight (gm) 7. Plant height (cm) 8. Number of branches 9. Roots fresh weight/plant (gm) 10. Root Phenols Content 11. Leaf Chlorophylls Content

The results can be summarized as follows:

- 1- Under the condition of the artificial inoculation with root-knot nematode, number of days from transplanting to first flower anthesis was less than that recorded for the uninoculated plants for the cultivars Edkawi, Super Marmand, and PI 376072.
- 2- The cultivar Ronita showed the highest percentages of fruit set whether the plants were inoculated (78.8%) or not (83.0%).
- 3- Relatively high percentage of fruit set was associated with the inoculated plants of the F<sub>1</sub> hybrids Edkawi x Nemared (83.8%), and Edkawi x Ronita (82.2%), which indicated that such F<sub>1</sub> hybrids could be recommended in the areas which may have possible soil infestation with root-knot nematode.
- 4- The values of the fruit weight of the inoculated and uninoculated plants of the parental genotypes Ronita, PI 376072, Nemared, and Anahu were not significantly different from each other, which could be due to the high level of resistance to the root-knot nematode showed by these tomato germplasm.
- 5- The cultivar Nemared showed the highest plant yield under the condition of artificial infestation with root-knot nematode. On the other hand, the parental genotype PI 376072 had the lowest plant yield under such conditions.
- 6- The fresh weight of the leaves detached from the inoculated plants of the parental cultivars Edkawi, Super Marmand, and PI 376072 were significantly less than the fresh weights of the leaves detached from the uninoculated plants of the same genotypes which can be attributed to the effect of inoculation with root-knot nematode.
- 7- Under the condition of the artificial infestation with root-knot nematode, the plants of the parental cultivar Nemared had the highest number of branches/plant (15.0) followed by Ronita (14.0), PI 376072 (13.0), and Anahu (11.0).
- 8- With all parental genotypes, the healthy plants were higher than the inoculated ones within the same genotype.
- 9- The highest root fresh weight of the parental genotypes was that associated with the cultivar Nemared which had an average of 565 gm for the inoculated and uninoculated plants. The parental genotypes Edkawi, Nemared, Ronita, and Anahu showed no significant difference between the fresh weight of the inoculated plants and that of the uninoculated plants. On the other hand, the susceptible cultivar Super Marmand showed significant difference between the root fresh weight of the inoculated plants (172.6 gm) and that of the uninoculated plants (225 gm).
- 10- Both the healthy and infected plants of the parental cultivars which previously reported as resistant to the root-knot nematode, i.e., Ronita, PI 376072, Nemared and Anahu, had highest root phenols content than that of the other cultivars, i.e. Super Marmand, which was previously reported as susceptible cultivar, and cultivar Edkawi. However, the root phenols content of healthy and inoculated plants of the cultivar Edkawi were higher than that of the cultivar Super Marmand. The obtained results in the present study indicated that root phenols content can be used as a criterion in selection for resistance to the root-knot nematode in tomato breeding programs.
- 11- The leaf chlorophyll content of the uninoculated plants of the different parental genotypes and F<sub>1</sub> hybrids were, either significantly or not significantly, higher than the leaf chlorophyll content of the inoculated plants.
- 12- The mean square values for general (gca) and specific (sca) combining ability were significant for the different studied characters under the conditions of inoculation with root-knot nematode, indicating the involvement of both the additive and non-additive types of gene interaction in the inheritance of these characters.
- 13- The gca/sca ratios exceeded unity for the characters which were evaluated for the plants of the different parents and F<sub>1</sub> hybrids under the condition of artificial with root-knot nematode. These characters were: number of days from transplanting to first flower anthesis, percentage of fruit set, average fruit weight, plant yield, plant height, number of branches, root fresh weight, and root phenols content. These results indicated that the additive type of gene interaction was more important than the dominance type, in the inheritance of the previously mentioned characters.
- 14- The gca/sca ratios were less than 1, for leaf chlorophyll content and number of fruits/plant, under the condition of infection with root-knot nematode, which indicated that the dominance type of gene interaction was more important than the additive type of gene interaction.

in the inheritance of these characters. 15- The additive and dominance type of gene interaction were equally important in the inheritance of detached leaves fresh weight under the condition of artificial infestation with root-knot nematode. 16- The narrow sense heritability for the different studied characters ranged from very low to intermediate which indicated the importance of selecting for these characters in the segregating generations under the condition of artificial inoculation with root-knot nematode, based on family mean basis in replicated experiments to reduce the non-additive effects on the expression of these characters.

III. Penetration bioassay experiment: Root tips of the different parental cultivars and related  $F_1$ 's were used in conducting a bioassay to determine the penetration rate of nematode larvae. Seeds of each genotype were soaked for three min. in 95% ethanol followed by 10 min. soaking in 0.5% sodium hypochloride. Then seeds were washed with sterilized distilled water and then transferred to sterile 0.8-9A water agar plates where they were maintained for four days at 28°C. Then a 5 mm-long root tip was excised from each new developing seedling and transferred to a sterile petri plate containing Gamborg's B-5 medium. Three days later each root tip was inoculated with one egg mass taken from the susceptible cultivar (Marmand) inoculated with *M. incognita* race 1. Five replications were used for each genotype and another five root tips were left uninoculated as control. All petri plates were incubated at 28°C for ten days after inoculation. All root tips were then washed and stained, using the acid-fuchsin lactophenol technique. The penetrations of the tested genotypes by the nematode was judged by the number of penetrated larvae. The results can be summarized as follows:

- 1- Number of larvae penetrated the root tips within 10 days after inoculation were 90.00, 81.67 and 53.33 for the parental genotypes Edkawi, Super Marmand, and PI 376072, respectively. On the other hand, larvae of the root-knot nematode did not penetrate the root tips of the parental cultivars Ronita, Nemared, and Anahu.
- 2- The root tips of the  $F_1$  hybrids Edkawi x Super Marmand, Super Marmand x PI 376072, and Edkawi x PI 376072, were penetrated by 77.00, 41.67, and 1.67 larvae, respectively within 10 days after inoculation, while the root tips of the other  $F_1$  hybrids had not been penetrated.
- 3- The penetration bioassay was efficient and dependable in detecting the resistance and the degree of the susceptibility of the different tomato germplasm to the root-knot nematode.
- 4- Both the additive and dominance type of the gene interaction were found to be involved in the inheritance of resistance to the penetration of root-knot nematode larvae. However, the role of the additive type of gene interaction was more important than the role of the dominance type.
- 5- An intermediate value of broad sense heritability (63.72%) was calculated for resistance to penetration of root-knot nematode larvae, while the narrow sense heritability estimate was below intermediate (35.65%).

Based on these results, selection for resistance to larvae penetration in the segregating generations should be done on family mean basis.

V. Resistance bioassay experiment. Seeds of the different parental genotypes and related  $F_1$ 's were planted in sterilized sandy clay soil (1:1, v:v). One seed was sown in each pot, and five pots were used for each genotype. Twenty five days after emergence of seedlings each seedling received 5000 *M. incognita* eggs. Then the pots were arranged in a randomized complete block design at the greenhouse. The recorded greenhouse temperature ranged from 28°C to 31°C. After 15 days from inoculation, the seedlings were removed and its roots were washed with running water. The number of galls formed at that time was counted under a dissecting microscope. The reactions of the tested genotypes to nematode were judged by the number of galls produced on the roots of the tested seedlings. The gall rating was made as in the greenhouse experiment. The results can be summarized as follows:

- 1- The bioassay was efficient and accurate in detecting the differences among the parental genotypes and  $F_1$  hybrids concerning resistance to the root-knot nematode.
- 2- The additive type of gene interactions was more important in the inheritance of resistance to the root-knot nematode, comparing to the dominance type of gene interaction.
- 3- The broad and narrow sense heritability estimates calculated for resistance to root-knot nematode, evaluated by resistance bioassay were (69.33%), and (30.67%), respectively.