Genetic and biotechnological studies on resistance to root knot nematode in pepper

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Seven pepper parental germplasm (Capsicum annuum L) i.e. California Wonder, Yellow Wax, Aswany, Murch, Domalik Buber, Mirch and No.4791 were used in making non-reciprocal diallel pattern of crosses to study the genetics and nature of resistance to the root knot nematode. Seeds of the parental genotypes Murch, Domalik Buber, Mirth, No.4791, California Wonder, and Yellow Wax were kindly provided by University of Georgia, Plant Genetic Resources Conservation Unit, U.S.A., while seeds of the line Aswany was attained from the germplasm preservation laboratory ; Faculty of Agriculture Moshtohor, Zagazig University, Benha Branch.The pepper cultivar California Wonder is known to be susceptible to root knot nematode. However, the other cultivars, Murch, Domalik Buber, Mirch and No.4791 are known to be resistant, to the root known nematode. While the parental line Aswany and parental cultivars Yellow Wax have not beenevaluated for resistances to the root knot nematode.Meloidogyne incognita race 1, kindly provided by Prof. Dr. Ashraf Afia, Faculty of Agriculture, Department of Nematology, Cairo University, was used in evaluating the different pepper genotypes for resistance to root knot nematode.1- Greenhouse Experiment :Seeds of the different parental pepper germplasm in addition to their F, seeds were planted on April 5, 2001 in claySummary 98pots, 30 cm in diameter (one plant/pot) containing sterilized sand and clay (1:1 , v/v). For each genotype five pots were assigned to be inoculated with the root knot nematode, while another five pots were assigned to be the control, i.e. uninoculated. The pots were arranged in a randomized complete block design with five replicates. When the seedlings reached the 4-5 true leaves stage, each plant in the pots was inoculated with 5000 fresh nematode eggs and juveniles (initial-population PO. The recorded temperature in the greenhouse ranged from 26 to 31 C° during the period of conducting this experiment. Forty live days after inoculation, the plants were cut off at the soil surface and the roots was gently washed off from the soil. The following measurements were recorded for the individual plants :A)Root fresh weight.B)Number of galls (Eggmasses).C)Number of eggs.D)Reproduction Factor (R).The result can be summarized as follows :1-The results indicated that number of galls and number of eggs counted on plant roots after the artificial inoculation with root knot nematode were efficient criteria to evaluate degree of resistance or susceptibility of pepper genotypes to thisnematode.2-The inheritance of plant reaction to root knot nematode in pepper, measured by number of galls or number of eggs, involved both additive and non-additive type of gene action.The GCA/SCA ratio indicated that the additive type of gene action was more important in the inheritance of this character.3-The parental pepper germplasm Murch, Domalik Buber, Mirch No.4791 had relatively high desirable combining ability which indicated the suitability of these parents as combiners in forming pepper hybrids with resistance to the root knot nematode.4-The results indicated that dominance acted in the direction of the parent with higher expression of resistance to root knot nematode measured by number of galls.5-The degree of dominance measured by (H1/D)1/2, averaged over all loci, were 0.95 and 0.92 which indicated that the dominance was close to be complete dominance toward high degree of resistance to root knot nematode measured by number of galls and number of eggs, respectively.6-The relative values of the Vr and Wr showed that the parental cultivars Murch and Mirch had the lowest values which indicated that these parental cultivars contained the most dominant genes. On the other hand, the parental cultivars California Wonder and Yellow Wax had

the highest Vr-Wr values and, hence, contained the most recessive genes.7-The seven parents used in the present study had more dominant than recessive alleles.8-Very high broad sense and above-intermediate narrow sense heritability estimates were recorded which indicated the important role for both the additive and non-additive gene action in the inheritance of resistance to root knot nematodemeasured by number of galls or number of eggs. However the addition type represented the greatest portion.2- Field experiment :Seeds of different parental germplasm and related F,'s were planted in 10 cm pots filled with sterilized sand and clay (1:1, V:V) on April 5, 2000. 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 and juveniles (initial population P1) 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 2, 2001. Before transplanting, holes with the size of 50 x 50 x 50 cm were digged in the field and filled with soil containing sterilized sand and clay (1:1, v:v). Each hole was one meter apart from the next hole. The inoculated and uninoculated seedlings of the different genotypes, i.e., parents and F, populations were transplanted into these holes according to the randomized complete block design with 5 replicates. The individual plants of the different genotypes were evaluated for the following characters :-1)Number of fruits.2)Average fruit weight.3)Plant total yield.4)Average fruit length.Summary 1015)Average fruit diameter :-6)Detached leaves fresh weight.7)Plant height.8)Roots fresh weight.9)Root phenols content.10)Leaves chlorophyll content (SPAD Reading).11)Leaves dry weight.12)Shoots dry weight. The results can be summarized as follows :1-Root phenols content in the inoculated plants of the parents and F1 hybrids were higher than that of the uninoculatedplants.2-The increase percentage of root phenols content due to inoculation were higher in the parents which showed different degrees of resistance, i.e., Murch "highly resistant" (34.96%), Domalik Buber "resistant" (39.35%), Mirch "highly resistant" (29.69%), and No.4791 "highly resistant" (50.51%), comparing to parents which showed the highest degree of susceptibility, i.e., Yellow Wax (8.60%) and California Wonder (19.78%). These results indicated that the high increase in root phenols content of resistant parents after inoculation whith root knot nematode act as a defensemechanism.3-The results indicated the presence of both the additive and non-additive gene action in the inheritance of all studiedSummary 102characters, i.e., percentages of root phenols increase, leaf chlorophyll decease and plant total yield decrease due to inoculation with root knot nematode, as well as leaves fresh weight and roots fresh weight measured under the conditions of infection with the nematode. However, with all studied characters, the additive type of gene action was moreimportant.4- The parents which had the highest desirable general combining ability effects, concerning carrying the defense mechanism of root phenols content, were No.4791 (9.14), Murch (5.06) and Mirch (1.27). The previously mentioned parents were classified in the present study as highly resistant to root knot nematode. These results indicated that the parents which possess the defense mechanism of increasing the root phenols content upon infection with the root knot nematode and classified as highly resistant can be considered as good combiners to form pepper hybrids which are resistant to theroot knot nematode.5- The F1 hybrids which showed the highest desirable better-parent heterosis under the conditions of artificial inoculation with root knot nematode were Aswany X Murch, Aswany X Mirch and California Wonder X Murch for root phenols content; Yellow Wax X Mirch, Aswqany X Domalik Buber, Aswany Mirch, Aswany X No. 4791, California Wonder X Murch, California Wonder X Domalik Buber, California Wonder X No. 4791, and Domalik Buber X Mirch for leaf chlorophyll content (SPAD Reading), and Yellow Wax X Murch, Yellow Wax X Mirch, Aswany X Domalik Buber,Aswany X Mirch, California Wonder X Murch, Murch X Mirch and Murch X No. 4791, for total plant yield.6-The results indicated overdominance for high percentage of root phenols content increase and resisting the chlorophyll leaf content deterioration, after exposing pepper plants to nematode infection.7-The broad sense heritability estimates were 93.4% and 91.2%, and narrow sense heritability estimates were 19.8% and 27.9, for percentages of root phenols content and leaf chlorophyll content (SPAD Reading) decrease, respectively. The relatively low value of the narrow sense heritability estimates indicated the involvement of the non-additive and environmental effects on the expression of these characters. According to these results, selection of lines or hybrids carrying the defense mechanisms of plant

ability to increase root phenols content and to resist leaf chlorophyll deterioration upon infection with root knot nematode, should be performed in replicated experiments to eliminate as Much as possible the environmental effects on the expression ofthese characters.8-Plant yield of the different parents and F i hybrids under the condition of artificial inoculation was less than plant yield recorded under nematode free condition.9-Plant yield loss percentage of parents which had been evaluated in the present study as highly susceptible to the root knot nematode, i.e., Yellow Wax and California Wonder (i.e., 37.51% and 29.39%, respectively) were higher thanpercentages recorded for plants of parents which had been classified as resistant i.e., Domalik Buber (2.02%), or as highly resistant, i.e., Murch (4.22%), Mirth (4.78%) and No. 4791 (13.69%). The low percentage of yield loss under infection condition indicated the resistance or tolerance of the pepper germplasm associated with these low percentages.10-The broad and narrow sense heritability estimates for percentage of yield loss under infection condition were 54.27% and 25.73%, respectively. These results indicated that percentage of plant yield decrease due to infection with root knot nematode was influenced greatly by environmental and non-additive gene action. Based on these findings, selection for resistance to root knot nematode in pepper, using low percentage of plant yield loss under the condition of artificial inoculation with the root knot nematode, should be performed in replicated experiments on family mean basis.3. Bioassay Experiment : Root Exudates Preparation :Seeds of two resistant pepper parental genotypes, i.e., Mirch and Murch and a susceptible one, i.e., Yellow Wax, and their F1 hybrids, i.e., Yellow Wax X Mirch and Yellow Wax X Murch, were planted separately in plastic cups with 10 cm diameter, containing sterilized sand. One week after, the germinated seedlings were thinned to one plant/cup. Then, plants were allowed to grow for six weeks. Small amount of water were used frequently in irrigation to avoid accumulation of water atbottom of the cups. Then, each plant was gently removed from sand, and placed in 50 ml-beaker containing distilled water for one day, to obtain root exudates in that water. In addition, the sand which became free of root system in the cup of each plant was soaked in 50 ml distilled water for two hours. Then, the soaking water was received to obtain root exudates in the sandy soil culture. Then, root exudates of each plant from both sources, were mixed and stored in plastic bottles in freezer until required.Nematode Culture :Meloidgyne incognita egg masses were picked up from root galls of infected tomato plants cv. Marmande, and placed on nematode extraction filter, and the hatched juveniles were collected at 72 hour intervals for the juveniles motility test. Someother egg masses were dissolved with 0.5 % sodium hypoclorite and the eggs were collected for hatching test.Juveniles motility (activity) Test :About 100 Juveniles of M incognita were transferred to root exudates of each plant of the previously mentioned pepper genotypes, in sterilized Petri-dishes. Motility of nematodes was examined using a microscope at intervals over seven days (i.e., after 1,2, and 7 days). Distilled water served as a control, and each treatment was replicated 3 times using randomizedcomplete block design.Hatching Test :About 1000 eggs of M incognita were transferred to root exudates of each plant of the previously mentioned genotypes, in sterilized Petri-dishes. Hatching in distilled water served asSummag 106control. Each treatment was replicated three times using randomized complete block design. Petri-dishes were incubatedat 28 ± 2 C°. Number of hatched juveniles was counted after four days.The results can be summarized as followsThe results showed that root exudates of parental cultivarsMirch, Murch which have been previously categorized as highlyresistant and Yellow Wax, which has been categorized as highly susceptible, had no effect on the motility (activity) of the second stage juveniles of root knot nematode. Also, no effects were observed in this respect for root exudates of the F1 hybrids Yellow Wax X Mirch (resistant) and Yellow Wax X Murch (highly resistant). Furthermore, root exudates of the different parental cultivars, whether it is highly resistant, i.e., Murch and Mirch or highly susceptible, i.e., Yellow Wax, as well as that of its F1 hybrids, whether it is resistant, i.e., Yellow Wax X Murch or highly resistant, i.e., Yellow Wax X Mirch, had no significant inhibitory effects on hatching of eggs of root knot nematode. These results indicate that factors of the chemical basis of rootresistance to the root knot nematode only exist inside the cells of root tissue.4. Acid phosphatase Isozyme Analysis Expriment :Seeds of the susceptible parent California Wonder and the three resistant parents, i.e, Murch, Domalik Buber, and Mirch were used to run Acid phosphatase isozyme analysis through gel electrophoresis. The resulted gel was scanned using Scan Pack version 3.0 (Biometer).Summary 107The results can be

summarized as follows :The acid phosphates patterns for the susceptible parent California Wonder and resistant parents, i.e., Mirch, Domalik Buber and Murch as well as F1 hybrid California Wonder X Domalik Buber were investigated. A total of six bands with different densities and Rf were observed and scanned. All detected bands were present in all examined genotypes, except for band number 3 which was present in the resistant parents, i.e., Mirch, Domalik Buber and Murch and the resistant F1 hybrid California Wonder x Domalik Buber, while this band was absent in the susceptible parent California Wonder. These resultsindicated that band number 3 can be used in distinguishingbetween pepper genotypes concerning resistance to root knotnematode. Based on the obtained results it can be mentioned thatacid phosphatase isozyme analysis can be successfully used bypepper breeders to select for resistance to root knot nematodewithout any need to make artificial inoculation with nematode,which well save time and effort as will as avoiding hazardscaused to soil and environment from artificial inoculation.