EARLY STEPS OF TOMATO BREEDING RESIST TO ROOT-KNOT NEMATODE

Rudi Hari Murti\(^1\); Fardatun Muamiroh, Tata Rina Wahyu Pujjati and Siwi Indarti

Faculty of Agriculture, University of Gadjah Mada
Jl Sosisojustisia, Bulaksumur, Yogyakarta, 55281, Indonesia.

\(^{*}\) Corresponding author: Phone: +62-274-551228 E-mail: rhmurti@ugm.ac.id

Received: January 12, 2011/ Accepted: June 29, 2012

ABSTRACT

The inheritance pattern of resistant tomato to root-knot nematode was studied. GM2 accession and Gondol Putih (GP) cultivars were used as resistant and susceptible parent, respectively. Parental plants, F1 generation, and the F2 generation were grown individually in the sterile soil. One thousands of larvae stage 2 (L2) nematode of \textit{M. incognita} was infested in each plant. Data of root gall intensity, the number of egg mass, population of L2 nematodes in root and soil were analyzed with the Kormogorov-Smirnov’s test, Chi-square test, and potence ratio. The F2 selected resistant plantsthen were grown and self fertilized to identify of homozygote plants (F2) related to nematode resistant and good fruit characters. The result showed that the resistant to root-knot nematode was controlled by a dominant gene. Positive correlation between damage levels of roots, eggs mass number and L2 larvae population in the root was significant. Eleven selected plants, conferred the homozygous resistant gene, was prospective lines to be used pedigree or single seed descent selection in producing variety with resistant to nematode, high yield and quality of fruit.

Keywords: tomato, root-knot nematode, resistant gene, dominant, homozygous, correlation

INTRODUCTION

Soil-borne root-knot nematodes (\textit{Meloidogyne} spp.) are the root systems parasite organism of a wide variety of crops, including cultivated tomato \textit{Lycopersicon lycopersicum} (L.). They could spread in the open air cultivation and controlled environment production systems, in warm temperate to tropical regions such as Indonesia. Nematode infect and penetrate the root at the infective juveniles (L2 stage) and initiate specialized feeding sites by modifying host cells in the vascular cylinder, from which they withdraw nutrients (Williamson and Hussey 1996).

Root galls, stunted growth, and increased susceptibility to drought stress and pathogen attack (Williamson, 1998), wilting, poor fruit yield (Johnson 1998) were as the effect of nematode infection. Susceptible cultivars loosened until 50% of yield due to nematode infection of \textit{M. incognita} and the resistant cultivars was unaffected (Roberts and May, 1986). Heavy root-knot nematode infestations affected profoundly in yield reduction, that making it among the most damaging agricultural pests worldwide (Williamson and Hussey, 1996), especially in tropical countries (Gunawan et al., 1997).

In Indonesia, these losses are predicted to be much higher from where many highly damaging populations of plant parasitic nematodes exist and growers do not have enough resources to invest in nematicide based nematode control. Control of nematodes used nematicides and soil fumigants such as methyl bromide have become increasingly difficult due to the withdrawal from the market (Oka and Cohen, 2001) and have negative effect to environment. As a result, there is an increasing need for new nematode management techniques. Potentially, resistant plant could be utilized to develop alternative control strategies for root-knot nematodes. Estimated losses due to loss of tomato plant nematode \textit{Meloidogyne} spp attacks was around 24-38% (Luc, 1995). In Barcelona, the 3-year average tomato yield increased by 2.6 kg m\(^{-2}\) in the rotations including at least one

http://dx.doi.org/10.17503/agrivita-2012-34-3-p270-277
resistant tomato crop, and by 6.1 kg m\(^{-2}\) when the resistant cultivar was cultivated for two consecutive years (Talavera et al., 2009). There are no cheap practical nematode management options available to the tomato farmers (Trudgill and Blok, 2001). Study on cultivars resistant to nematode and identification of nematode race was barely carried out in Indonesia, so conventional approach with nematode inoculation was reliable to be done. The host plant resistance could be identified based on either the number of females or the egg mass score and gall index recorded on infected roots (Cousins and Walker, 2002). Furthermore genetic analyses have revealed a monogenic or polygenic determinism of the resistances identified in wild plants. Sometime, the same gene has different proportion on phenotype expression depend on the variable observed. Monogenic resistance is desirable for breeding purpose because of their simplicity in introgression into genotype that was good quality and high production but susceptible to nematode. However, these resistances are pathotype or species specific and their effectiveness can be rapidly lost due to the evolution of the pathogen populations. In contrast, quantitative resistances are, in general, non-pathotype specific and they are more interesting in term of management because they may be relatively durable in field (Hammond-Kosack and Jones, 1997). Growing nematode-resistant crops offers an environmentally friendly alternative for controlling the parasites as part of sustainable agriculture system.

GM-2 accession was the selected plant that resist to nematode. Root gall intensity (gall index) recorded on infected roots of GM-2 was very low (some free of gall), while other accessions showed high root gall intensity (Riyandari, 2002). The weakness of GM-2 fruit is color and soft fruit (Murti dan Sri-Trisnowati, 2001). Breeding strategy to develop new resistance varieties are determined by mode of inheritance of target genes.

In this paper elaborated the resistance gene against the root-knot nematode *Meloidogyne* spp on GM-2. Our objectives were to analyze the inheritance pattern of the resistance through its segregation in their progeny and select homozygote resistant plant of F2 progenies and to identify the F2 progenies that confer homozygote resistance gene to nematode.

**MATERIALS AND METHODS**

**Nematode Inoculum Preparation**

Inoculums preparation of root knot nematode as follows: The egg mass of root knot nematode (*Meloidogyne* spp.) were collected from infected root that discovered from tomato production center. Eggs were extracted from infected tomato roots using 1% NaOCl. Extracted eggs were gently washed with tap water to remove NaOCl before incubated to get L-2 inoculums of root knot nematodes. This procedure was followed filtration and incubation method according to Southey (1985). The eggs were kept in water suspension for 3-4 days until the egg hatched and developed to be larvae stage 2 (L2) then used as inoculums.

**The Population of F1 And F2 Generation and The Resistance Evaluation**

The experiment was carried out in a plastic house in the Horticultural Seed Production Center (BBI) Ngipiksari, Yogyakarta and Nematology Laboratory, Faculty of Agriculture Gadjah Mada University. GM2 accession and Gondol Putih (GP) cultivars were used as resistant and susceptible parents, respectively. Both of parents were crossed reciprocally to produce F1 progenies. About twenty F1 seeds were grown and self-pollinated to produce F2 seeds.

Both of parents and F1 plants consisted of 30 plants and 200 F2 plants were grown in sterilized medium. Approximately 2 to 3 weeks, the seedlings were transplanted individually to polibag 7x10 cm in size. A week later, each plant was infested the root knot nematode by injecting 1000 eggs into the root zone. Plants were maintained in the greenhouse with ambient temperature at 27±5°C under adequate moisture condition. Approximately 3 weeks after infestation, roots were carefully washed until free of soil. The data of root gall intensity (Zeck's method with score 0-10), egg mass number, L2 larva in the root and population of larva in the soil were collected. F2 resistant plant (score of root gall intensity: 0-1) then replanted in the field to produce F3.
Evaluation of F3 Progenies

Selected resistant plant of F2 population, based on root gall intensity, was transplanted in the opened field of Horticultural Seed Production Center. The cultivation procedures were carried out such as tomato seed production with spacing 30 cm in raw and 75 cm between raw in soil bench covered by silver mulching. Fertilizer with dosage Urea 250 kg/ha, SP36 250 kg/ha, KCl 150 kg/ha dan ZA 150 kg/ha mixed in the soil before set up the bench. Maximum three clusters of fruit kept in each plant and each cluster was maintained about three fruit. Mature fruits were harvested and processed to extract the seeds. Seeds from each plant were collected in paper bag separately and marked.

F3 seeds were sown and infested with nematode L2 larva with the procedure described previously. When all progenies of certain plant were resistant to nematode and t test also confirmed no segregation of gene, it concludes that the parent (F2 selected) was homozygote. The homozygote plants progenies (F3) then were replanted to the field to produce fruit for fruit characterization. The cultivation procedure as standard was described before.

The F2 data were analyzed with Kormogorov-Smirnov Goodness-of-Fit test to normal distribution. If the data distributed normally, then concluded that trait was controlled by polygenic. In contrary result, \( \chi^2 \) test was required for identifying the most appropriate of gene segregation pattern. The dominance estimates were computed using "potence ratio" method (Petr and Frey, 1966):

\[
hp = \frac{F1 - MP}{BP - MP}
\]

Where \( hp \): dominance estimate, \( F1 \), MP and BP: values of F1, mid parent and better parent respectively. F3 data were analyzed with t test and when \( H0: \mu = 0 \) accepted, it confirmed that F2 selected plant was homozygote caused of no segregation of given trait observed.

RESULTS AND DISCUSSION

RESULTS

Pattern of Inheritance The Resistant Gene To Root-Knot Nematode (Meloidogyne Spp)

The result of normality test for all the parameters showed that the root gall intensity, the number of egg mass (egg mass), the L2 larva number in the root and the population L2 larva in the soil were not normally distribute at the level of significance 99%, as shown in Table 1. It is indicated that all variable were not controlled by polygenic, otherwise was controlled by major gene. The number of gene was identified with \( \chi^2 \) test for fit-goodness segregation and potence ratio for identifying of dominance action.

Table 2 showed the result of fit-goodness test of pattern of inheritance. Root gall intensity of the cross GM2 and GP showed 9:7 ratios, while its reciprocal cross was 12:3:1. Both of segregation ratio indicated that the resistance traits of GM2 was controlled by dominant major gene (two loci, two allele per locus). While ratio segregation of egg mass was fit goodness with 3:1 ratio, pointed out that resistant traits with egg mass as variable was controlled by one gene, in both crossing GM2 x GP and its reciprocal. This showed that the resistance of tomato plants is controlled by a single dominant gene.

Table 1. Normality test of Kormogorov-Smirnov* of F2 plants

<table>
<thead>
<tr>
<th>F2 GM2xGP</th>
<th>Root gall intensity</th>
<th>Egg mass number</th>
<th>L2 larva number in the root</th>
<th>L2 larva number in the soil</th>
</tr>
</thead>
<tbody>
<tr>
<td>Statistics value</td>
<td>0.160</td>
<td>0.251</td>
<td>0.385</td>
<td>0.250</td>
</tr>
<tr>
<td>(Pr &gt; D)</td>
<td>0.000</td>
<td>0.000</td>
<td>0.000</td>
<td>0.000</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>F2 GPxGM2</th>
<th>Root gall intensity</th>
<th>Egg mass number</th>
<th>L2 larva number in the root</th>
<th>L2 larva number in the soil</th>
</tr>
</thead>
<tbody>
<tr>
<td>Statistics value</td>
<td>0.232</td>
<td>0.345</td>
<td>0.435</td>
<td>0.266</td>
</tr>
<tr>
<td>(Pr &gt; D)</td>
<td>0.000</td>
<td>0.000</td>
<td>0.000</td>
<td>0.000</td>
</tr>
</tbody>
</table>

Remarks: *: Lilliefors significance correction
Distribution of L2 larva number in the root and in the soil in GM2 x GP had fit goodness with 9:7 ratios, while in its reciprocal had similar ratio except for L2 larva number in the root. It could be conclude that resistance to nematode, which was expressed through L2 larva number in the root and in the soil, controlled by two dominant genes.

Based on the results, F2 plants crossing GM2 x GP with segregating 9:7 pattern is controlled by two genes with double recessive epitasis. While crossing GP X GM2 with 12:3:1 ratio is controlled by two genes with dominant epitasis effect. This result gave significant useful information for resistance breeding to root-knot nematode.

**Potence Ratio**

The average degree of dominance as indicated by the potence ratio revealed all of crossing and its reciprocal was over dominance for all variable, except root gall intensity that indicated positive incomplete dominance towards the parent with low root gall intensity. It showed the consistency of dominance effect to all variable of resistant plant to nematode, although differed in magnitude. Lower egg mass and root gall intensity in F1 plants than the better parent (low root gall intensity or egg mass number) indicated that production of F1 nematode resistant plant is visible with involving just one resistant parent.

Data in Table 4 suggest that there is positive correlation between root gall intensity, the number of egg masses, and L2 larva population in the root, while no significant negative correlation between L2 larva populations in the root with others was exist, except with root gall intensity. Root gall intensity correlated significantly with number of egg, in which the correlation coefficient was the highest ($r=0.52^*\)).

The t-test result of F2 progenies resistant plant showed in Table 5. Among thirty eight selected plant of 200 F2 plants, eleven plants (No 1, 10, 14, 15, 16, 18, 20, 22, 23, 26, 27) had homozygous resistant gene. This resistant progenies plants could be used as population to be selected with single seed descent or pedigree selection method for developing pure line variety with resistant to nematode.

The F3 selected plant then grown in field for evaluating fruit characters. Genetically, the geno-
Type resistant to nematode wouldn’t segregate for thus character, so selection next generation just focused on fruit characters. Tomato consumers in Indonesia interested to round or oval but not flat fruit form. Some fruit characters of F3 selected plants showed Figure 1. Fruit characters segregated and resulted variation of fruit weight and long/diameter ratio in F3. First plant of T18 (T18-1) produced weight/fruit about 213 g/fruit with long/diameter about 1. This was a potential line to produce new cultivar. Another potential line was T10 and T20-2 that conferred weight fruit about 169 and 127 g/fruit respectively, with round shape.

Table 3. Ratio potency of root gall intensity, egg mass number, L2 larva number in the root, L2 larva number in the soil

<table>
<thead>
<tr>
<th>Parameter</th>
<th>GM2/GP</th>
<th>F1</th>
<th>Mid parent</th>
<th>Better parent (BP)</th>
<th>Dominance</th>
<th>Category</th>
</tr>
</thead>
<tbody>
<tr>
<td>Root gall intensity</td>
<td>2.30</td>
<td>2.35</td>
<td>1.43</td>
<td>0.0</td>
<td>ID</td>
<td></td>
</tr>
<tr>
<td>Egg mass number</td>
<td>1.46</td>
<td>12.52</td>
<td>5.50</td>
<td>1.57</td>
<td>OD</td>
<td></td>
</tr>
<tr>
<td>L2 larva number in the root</td>
<td>55.59</td>
<td>40.08</td>
<td>52.88</td>
<td>1.21</td>
<td>OD</td>
<td></td>
</tr>
<tr>
<td>L2 larva number in the soil</td>
<td>18.02</td>
<td>47.36</td>
<td>59.88</td>
<td>-2.54</td>
<td>OD</td>
<td></td>
</tr>
</tbody>
</table>

B. GP/GM2

<table>
<thead>
<tr>
<th>Parameter</th>
<th>GP/GM2</th>
<th>F1</th>
<th>Mid parent</th>
<th>Better parent (BP)</th>
<th>Dominance</th>
<th>Category</th>
</tr>
</thead>
<tbody>
<tr>
<td>Root gall intensity</td>
<td>2.03</td>
<td>2.35</td>
<td>1.43</td>
<td>0.34</td>
<td>ID</td>
<td></td>
</tr>
<tr>
<td>Egg mass number</td>
<td>2.53</td>
<td>12.52</td>
<td>5.50</td>
<td>1.42</td>
<td>OD</td>
<td></td>
</tr>
<tr>
<td>L2 larva number in the root</td>
<td>55.77</td>
<td>40.08</td>
<td>52.88</td>
<td>1.22</td>
<td>OD</td>
<td></td>
</tr>
<tr>
<td>L2 larva number in the soil</td>
<td>34.71</td>
<td>47.36</td>
<td>59.88</td>
<td>-1.09</td>
<td>OD</td>
<td></td>
</tr>
</tbody>
</table>

Remarks: ID = incomplete dominance, OD = over dominance

Table 4. The correlation between the root gall intensity (RGI), the number of egg masses (NEM), L2 larva population in the root (LPR) and in the soil (LPS) of the F2 plants (GM2 x GP) population

<table>
<thead>
<tr>
<th>Parameter</th>
<th>RGI</th>
<th>NEM</th>
<th>LPR</th>
<th>LPS</th>
</tr>
</thead>
<tbody>
<tr>
<td>RGI</td>
<td>1.00</td>
<td>0.52</td>
<td>0.19</td>
<td>-0.20</td>
</tr>
<tr>
<td></td>
<td>0.00</td>
<td>0.001</td>
<td>0.006</td>
<td>0.003</td>
</tr>
<tr>
<td>NEM</td>
<td>1.00</td>
<td>0.21</td>
<td>0.003</td>
<td>0.141</td>
</tr>
<tr>
<td></td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.25</td>
</tr>
<tr>
<td>LPR</td>
<td>1.00</td>
<td>-0.08</td>
<td>0.00</td>
<td></td>
</tr>
<tr>
<td>LPS</td>
<td>1.00</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Remarks: Italic number is $\alpha$

Table 5. t-test of F3 progenies of selected resistant plant in F2

<table>
<thead>
<tr>
<th>F2 selected plants no-</th>
<th>1</th>
<th>10</th>
<th>14</th>
<th>15</th>
<th>16</th>
<th>18</th>
<th>20</th>
<th>22</th>
<th>23</th>
<th>26</th>
<th>27</th>
<th>17</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0.24</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0.52</td>
<td>0.77</td>
</tr>
<tr>
<td>Sd</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0.66</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0.77</td>
</tr>
<tr>
<td>$t_{\text{test}}$</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1.81</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>3.38*</td>
<td></td>
</tr>
</tbody>
</table>

Remarks: Total selected plant in F2 = 28 and 15 of 28 did not segregate (no. 1, 10,...,27) and displayed in this table, df = degree of freedom, Sd = standard deviation, $\alpha = 0.05$%; * = Significantly different at $\alpha = 5$%
DISCUSSION

Tomato resistant to nematode is the ability of tomato to restrict or prevent nematode multiplication. Resistance against root-knot nematodes does not protect the plants against nematode invasion, but inside the root the induction of the feeding site is either inhibited or initially established feeding structures disintegrate in early stages of nematode development. Giant-cell formation by *Meloidogyne* spp. in tomato plants carrying the single dominant *Mi* gene is arrested by a localized hypersensitive response at or near the induction site (Milligan *et al.*, 1998). Heterozygous parent would produce segregation progenies plants.

In this experiment, all resistance indicators observed in F2 population were not distributing normally, which pointed out that non polygenic gene controlled this trait. The segregation ratio of resistant to susceptible plants indicating by egg mass number suggested that a single dominant gene was a major factor in resistance controlled. The similar result was in Yaghoobi *et al.* (1995) and Ammiraju *et al.* (2003) experiment used the molecular marker method. The gene, which have designated *Mi*-3 and *Mi*-9 respectively, confers resistance against nematode strains that can’t infect plants carrying *Mi*. Similar but not same in cotton, Ynturi et al. (2004) experiment showed segregation ratio of 3:1 for susceptible and resistant in both F2 populations, suggesting a single recessive gene or one dominant and one recessive gene may be involved in controlling the resistance trait. Williamson (1998) said that the gene *Miconfers* resistance to several species of root-knot nematode. Recent cloning of this gene revealed that it encodes a member of the plant resistance protein family characterized by the presence of a putative nucleotide binding site and a leucine-rich repeat. Brown *et al.* (1996) used QTL analysis confirmed supports a genetic model equivalent to monogenic dominant control.

However, when considering root gall intensity and L2 larva in the root as indicator, the resistance to nematode was controlled by two genes. The epitasis interaction in both crossing showed different type, in which if GM2 as mother then the gene action in segregation of F2 was double recessive epitasis. While in the reciprocal, the epitasis dominant and epitasis codominant were exist in gall intensity index and L2 Larva nematode in the root, respectively.

The different type of gene controlled resistance (monogenic and two genes) was similar with Abouet al.(2006) result at *Solanum sparsipilum* resistance to *Meloidogyne* fallax. A monogenic control of the resistance if necrosis used as a variable but when considering the number of nematode females developed in their roots, a continuous distribution was observed for both
“necrotic” and “non-necrotic” hybrid genotypes, indicating a polygenic control of the resistance.

Potency ratio showed the over dominant at all variables except root gall intensity that was incomplete dominant and linearly with Zhang et al. (2007) result in cotton for root-knot nematode (Meloidogyne incognita (Kofoid and White) Chitwood). The over dominant of resistance to nematode is advantage condition for inventing of resistant tomato hybrid.

The significant correlation between root gall intensity and number of egg mass was exist, and negative correlation with larva population in the root. Both of them could be used as resistance indicator interchangeable. In this case, the root gall intensity was used for selection of the F3 progenies resistant plant. Eleven selected plant of F2 produced resistant progenies without segregation of resistance gene. It suggested that thus genotype of selected plants was homozygous. The selected plant was useful for next breeding program, as backcross parent or single seed or pedigree selection program. Concerning the parent that was resistant to nematode and big fruit, the variation of fruit size in the resistant progenies indicated no linkage between resistance gene and fruit characters (size, form). Continuing of selection using pedigree or single seed descent method was expected find out the homozygote plant with good characters and resistant to nematode.

Based on facts were concluded that resistance to nematode in GM2 accession was controlled by a dominant gene when the variable observed was eggs mass number, while two dominant genes if L2 larvae in the roots and root gall intensity variables. Significant positive correlation between root gall intensity, eggs mass number and L2 larvae population in the root was exist, and they had negative correlation with L2 larvae in the soil. Homozygous selected plant of F3 could be used as based population for pedigree or single seed descent selection for producing high yield and quality, also resist to nematode.

ACKNOWLEDGEMENTS

This project was supported by Directorate of Research and Society Services, Department of National Education, Indonesia (Contract no. 034/SPPP/PP/DPPM/IV). We thank Mr. Sumbogo Walijyono for assistance with planting and harvesting.

REFERENCES


