Inheritance of Resistance to Powdery Mildew Race 2 in Citrullus lanatus var. lanatus

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Abstract. Information on the mode of inheritance of powdery mildew resistance in watermelon is important for designing a breeding strategy for the development of new cultivars. Resistance in the watermelon accession PI 270545 was investigated by generation means analysis by crossing it with susceptible PI 267677. The analyses showed involvement of two genes, a recessive resistance gene, pmr-1, and a dominant gene for moderate resistance, Pmr-2. Resistance to powdery mildew in the leaf had a large dominance effect and a heritability of 71%. The additive-dominance model was inadequate in explaining variation in leaf resistance as revealed by the joint scaling test. However, nonallelic interactions could not be detected by the nonweighted six-parameter scaling test. For stem resistance, the additive-dominance model was adequate, and inheritance was controlled mainly by additive effects. A high narrow-sense heritability of 79% suggested that selection for stem resistance in early generations would be effective.

Powdery mildew in watermelon [Citrullus lanatus (Thum.) Matsum. and Nakai] caused by the fungus Podosphaera xanthii race 2W has in recent years become a concern among growers as well as plant breeders in United States (Davis et al., 2001; McGrath, 2001), China (Feng, 1996; Zhang et al., 2011), and other parts of the world (McGrath, 2001; Tomason and Gibson, 2006). The disease causes significant yield loss as well as decreased fruit quality (McGrath and Thomas, 1996) through mycelial coverage of the leaves, leaf necrosis, and premature death of the plant (Davis et al., 2001). Powdery mildew of watermelon occurs throughout the southeastern United States, extending north to New York as well as into western states (Davis et al., 2005).

Development of genetic resistance is an important objective in watermelon breeding programs. Screening of the U.S. watermelon germplasm collection identified high resistance in several wild accessions of C. lanatus var. citroides (Davis et al., 2007; Tetteh et al., 2010), but none of the accessions in the primary gene pool of watermelon, a generation means analysis was carried out to determine inheritance, gene action, and heritability of resistance in PI 270545. Information on these parameters would be useful for designing an efficient breeding strategy for watermelon powdery mildew resistance.

Materials and Methods

Plant material. A single population of watermelon segregating for resistance to powdery mildew race 2W-U.S. was derived from a cross between the susceptible P1 (PI 269677) and resistant P2 (PI 270545). The parents were inbred for two generations before crossing to produce plants uniform for powdery mildew resistance. From this, crosses were made to create a total of six generations, F1, F2, BC1P1, the first backcross to P1, and BC1P2 (the first backcross to P2) for a study of inheritance of powdery mildew resistance in watermelon was investigated by generation means analysis by Tetteh et al. (2013). Studies have demonstrated a close correspondence between generation mean analysis and QTL mapping (Jung et al., 1994; Perchepied et al., 2005). Estimation of genetic effects in different crosses should inform the breeding strategy for development of resistant cultivars. Although additive and dominance models can be determined with the scaling tests in generation means analysis, the identification of nonallelic interactions requires more powerful tests such as the joint scaling test (Mather, 1949) or QTL analysis (Perchepied et al., 2005). In most cases, the variation unaccounted for by a major gene is provided by digenic epistasis. A major deficiency of generation means is in nondetection of additive effects resulting from dispersion of alleles with similar effects between parents and internal cancellation of dominance effects exhibited in opposite directions at different loci (Crow and Kimura, 1970). Saudhu and Nittal (1988) studied two Gossypium arboreum crosses and reported the absence of nonallelic interaction in the six-parameter model, whereas the joint scaling test predicted the presence of epistasis for yield of seed cotton per plant (Iqbal and Nadeem, 2003).

Given the lack of genetic information on powdery mildew resistance in the primary gene pool of watermelon, a generation means analysis was carried out to determine inheritance, gene action, and heritability of resistance in PI 270545. Information on these parameters would be useful for designing an efficient breeding strategy for watermelon powdery mildew resistance.

Experimental design. Seeds of inbred powdery mildew-resistant PI 270545 and -susceptible PI 269677, together with their F1, reciprocal F1, F2 (generated by self-pollination of the F1), and backcross generations were produced in 2007 to 2008 in greenhouses at the Department of Horticultural Science, North Carolina State University, Raleigh, NC. Seeds were planted in two sets, each consisting of 10 plants of each parent, 10 plants of F1, 10 plants of F1 reciprocal, 100 plants of F2, and 30 plants of each BC1P2. Pooled over sets, a total of 20 plants for each parent, 17 plants of F1, 19 of F1 reciprocal, 190 plants of the F2, and 59 plants each of BC1P2 and BC1P2 were evaluated for powdery

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mildew leaf and stem resistance, account-
ing for missing plants.

Seeds were planted in 100-mm pots con-
taining 4P Fafard soillsoil mix (Conrad Fafard
Incorporated, Agawam, MA) and arranged on
greenhouse benches. Conditions of growth in
the greenhouse were 16-h photoperiod, light
intensity of 200 μmol·m⁻²·s⁻¹, and 20 to 26 °C
day and 13 to 19 °C night air temperature.

Inoculum production and seedling
inoculation. Seedlings were inoculated three
times at weekly intervals, starting at the
first true leaf stage. A spore suspension of
_Podosphaera xanthii_ race 2W-U.S. (4 × 10⁴
conidia/ml) was sprayed over the plants un-
til runoff. The suspension was prepared from
inoculum isolated from infected commercial
watermelon fields in South Carolina over the
surface. After each inoculation, seedlings
were maintained under plastic shading at
100% humidity for 7 d and subsequently
at normal greenhouse conditions of 37% to
70% relative humidity and temperature of 24
to 38 °C (night to day).

Disease assessment. Individual plants
were rated for disease severity on a 0 to 9
scale on leaves and stems at 21 and 30 d after
first inoculation. For leaf resistance rating, 0 =
no symptoms; 1 = faint yellow speck on
leaves; 2 = chlorotic lesions on leaves; 3 =
chlorotic lesions covering 20% of leaves; 4 =
yellow chlorotic lesions on leaves turned to
brown necrotic areas; 5 = two to three healthy
colonies of mycelium on leaves; 6 = less than
20% mycelium coverage on leaves; 7 = 20% to
50% mycelium coverage on leaves; 8 =
50% to 70% mycelium coverage with large
necrotic areas; 9 = all leaves fully covered
with powdery mycelium or plant dead. For stem
resistance rating, 0 = no symptoms; 1 = first
appearance of necrotic spots on stem; 2 = two
to three necrotic spots on the stem; 3 = necrotic
spots covering less than 10% of stem; 4 = first
sign of active mycelium sporulation on stem;
5 = two to three healthy colonies of mycelium
on stem; 6 = less than 20% mycelium cover-
age on leaves; 7 = 20% to 50% mycelium
coverage on stem; 8 = 50% to 70% mycelium
coverage with large necrotic areas; 9 = entire
stem fully covered with powdery mycelium or
plant dead. On the basis of resistance levels of
parental genotypes, plants were classified into
two groups. In Group 1, plants having leaf
and stem resistance ratings of 0 to 2 were
classified as resistant, whereas ratings of 3 to 9
were classified as susceptible. Classification
in Group 2 was based on ratings of 0 to 2 as
resistant, 3 to 5 as moderately resistant, and
6 to 9 as susceptible.

Statistical analysis. Generation means
and variances were calculated. Variances for
the two parents and reciprocal F₁ were ex-
mained for correlation (data not shown).
Corre-
lation of variance with mean indicated a need
for transformation of the data. A log₁₀ trans-
formation was applied but this did not reduce
the correlation. A nonsignificant test of homo-
genesis of F₁ and F₂, variance demonstrated
no maternal effect, hence, the two genera-
tions were pooled. Chi square was used to test
the goodness of fit of the observed ratio of
segregation to expected ratio in the F₂ and
backcross progenies. Analysis of variance was
performed with generations as fixed
effects and blocks (sets) as random effects.
Phenotypic correlation between leaf and stem
resistance was calculated. Data for each gen-
eration were pooled over replicates within
each block. Generation means analysis was
conducted on plot means by the ABC scaling
test and the joint scaling test based on additive-
dominance model (Cavalli, 1952; Mather and
Jinks, 1971) in which the generations were
subjected to a weighted least squares regres-
sion based on the equation:

\[
Y = m + a_i + a_j + a_i j + a_i j d + \text{error}
\]

and a nonweighted scaling test based on the
six-parameter model (Mather and Jinks,
1971). In this equation, \(Y\) is the mean of
a given generation, \(m\) is the midpoint, \(d\) is
the pooled additive effect, \(h\) is the pooled
dominance effect, \(i\) is the additive × additive
effect, \(j\) is the additive × dominance effect,
\(l\) is the dominance × dominance effect, and
\(a_i\) to \(a_{ij}\) are the coefficients of the genetic
effects in the equations (Carson and Hooker,
1981; Mather and Jinks, 1971). The signifi-
cance of the joint scaling test, as tested by \(\chi^2\),
provided evidence of non-allelic interactions.

Narrow-sense heritability was calculated as

\[
h^2 = \frac{2V_{F_2} - (V_{BC1P1} + V_{BC2})}{V_{F_2}}
\]

where \(V_{F_2}\) = variance among F₂ plants of
the single-cross population; and \(V_{BC1P1}\) and
\(V_{BC1P2}\) are variances among plants from the
backcrosses of F₁ × P₁ and F₁ × P₂ (Warner,
1952). A SE of heritability \(h^2\) was derived as
the square root of

\[
\hat{h}^2 = \sqrt{\frac{2V_{BC1P1} + V_{BC1P2}}{\text{df}_{F_2}}} + \sqrt{\frac{V_{BC1P1}^2}{\text{df}_{BC1P1}}} + \sqrt{\frac{V_{BC1P2}^2}{\text{df}_{BC1P2}}} \times \sqrt{\frac{V_{F_2}^2}{\text{df}_{F_2}}}.
\]

Results and Discussion

Faint specks of mycelium were observed
14 d after inoculation. On Day 21, disease
development was poor with few patches of
mycelium on few plants of the susceptible
genotypes. On Day 30, major differences in
disease scores for resistant, intermediate re-
sistant, and susceptible plants were evident.
Hence, disease ratings on Day 30 were used
for all analyses. All plants of PI 270545
demonstrated resistance to moderate resistance
with none showing absence of disease. The
reaction of PI 269677 to _P. xanthii_ was highly
susceptible.

Table 1 shows the reactions of water-
melon accessions to powdery mildew. For
leaf rating, all plants of the resistant genotype
were consistently rated between 1 and 3.
None of the plants showed absence of disease.
Of 20 plants, 25% scored 1, 55% scored 2,
and 20% had a rating of 3, corresponding to 16
resistant and four moderately resistant plants.
For stem resistance, 40% scored 0, 35% scored
1, 20% scored 2, and 5% scored 3, correspond-
ing to 19 resistant and one moderately resistant
plant. Thus, PI 270545 was between resis-
tant and moderately resistant. All F₁ plants
were susceptible (Table 1) and F₁ mean was
greater than the midparent indicating domi-
nance of the allele for susceptibility. Robinson
et al. (1975) had earlier reported that high
susceptibility to _P. xanthii_ in PI 269677 was
controlled by the single recessive gene, _pm_.
The new and more virulent _P. xanthii_ races on
watermelon recently appear to exhibit differ-
ent mode of inheritance.

For leaf rating in the F₂ progenies, there
were 29 highly resistant, 40 moderately
resistant, and 121 susceptible plants. A \(\chi^2\)
goodness-of-fit test on all three-category
expected segregation ratios gave probability
values < 0.01. However, the combination of
moderately resistant with susceptible individ-
uals fitted a 3:13 segregation ratio (Table 1)
that was supported by a corresponding back-
cross segregation ratio of 1:3. The apparent
model for this ratio was two genes with one
recessive for high resistance and the other
dominant for moderate resistance. The cumu-
labative effect of the two genes in the F₂ produced
some highly resistant and some moderately
resistant plants. Similar inheritance was ob-
served for stem rating, in which the F₂ gave
a good fit for 3:13 model (\(\chi^2\) of 3.89, \(P =
0.53\)) supported by a backcross to the resistant
parent of 1:3 (\(\chi^2\) of 4.75; \(P = 0.03\)). This
provided further evidence that two independ-
ent genes, one recessive and one dominant,

<table>
<thead>
<tr>
<th>Population</th>
<th>Number of plants with diseased reaction</th>
<th>Probability of calculated (\chi^2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PI 269677 × PI 270545</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Leaf resistance</td>
<td>Total</td>
<td>0–1 (R)</td>
</tr>
<tr>
<td>P₁ (PI 269677)</td>
<td>20</td>
<td>0</td>
</tr>
<tr>
<td>P₂ (PI 270545)</td>
<td>20</td>
<td>5</td>
</tr>
<tr>
<td>F₁</td>
<td>25</td>
<td>0</td>
</tr>
<tr>
<td>F₂</td>
<td>190</td>
<td>29</td>
</tr>
<tr>
<td>BC₁P₁</td>
<td>54</td>
<td>0</td>
</tr>
<tr>
<td>BC₁P₂</td>
<td>59</td>
<td>20</td>
</tr>
<tr>
<td>Stem resistance</td>
<td>Total</td>
<td>0–1 (R)</td>
</tr>
<tr>
<td>P₁ (PI 269677)</td>
<td>20</td>
<td>0</td>
</tr>
<tr>
<td>P₂ (PI 270545)</td>
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</tr>
<tr>
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<td>0</td>
</tr>
<tr>
<td>BC₁P₂</td>
<td>59</td>
<td>22</td>
</tr>
</tbody>
</table>

Table 1. Reactions of watermelon plants inoculated with powdery mildew race 2W-U.S isolate.
control powdery mildew resistance in PI 270545. Occurrence of epistasis suggests that the dominant allele at one locus could mask the expression of alleles at the second, whereas the recessive allele at the second locus could mask the expression of the alleles at the first. The resistance genes found in PI 270545 were designated pmr-1 for the recessive gene for high resistance and pmr-2 for the dominant gene for moderate resistance.

Analysis of variance (Table 2) revealed significant differences ($P < 0.01$) for both leaf and stem resistance among the generations, indicating the presence of genetic variability for both leaf and stem resistance. Variation was similar for both leaf and stem resistance. This is in contrast to the observation made on watermelon families of the PI 189225 resistant accession in which leaf resistance among the generations was higher than stem resistance (Tetteh et al., 2013). Comparison of means of P1 and P2 showed significant differences for both leaf and stem rating (Table 3). Disease development in F1 and F2 generations were not significantly different. Both had significantly lower ($P < 0.05$) disease development than the susceptible parent and were less resistant ($P < 0.05$) than the resistant parent (Table 3). These values were higher than the backcross to the resistant parent but lower than the backcross to the susceptible parent. Mean performance of the BC1P1 individuals showed more resistance to powdery mildew than the BC1P2. There was a highly significant correlation ($r = 0.99, P < 0.001$) between leaf and stem resistance.

Leaf resistance. In the generation means analysis, although the ABC scaling test (Table 4) was not significant, indicating that a simple additive-dominance model was adequate, the joint scaling test indicated a model that goes beyond additive-dominance for leaf resistance ($\chi^2_{(3)} = 564, P < 0.001$). Therefore, the six-parameter model was required to explain the observed variation. The estimates of mean ($m$) as well as additive ($d$) and dominance ($h$) effects were significant, and the dominance effect was greater than the additive effect (Table 4). The large and negative dominance effect indicated that leaf resistance was predominantly controlled by nonadditive genetic effects with recessive alleles for leaf resistance in PI 270545, hence the lowest positive degree of dominance.

The large dominance effect suggests that selection of resistant plants should be done after self-pollination to identify the resistant recessive genotypes. Recurrent selection can also be useful, because it increases the frequency of resistant alleles for leaf resistance in the population. Failure to detect nonallelic interaction in leaf rating, as inferred from non-significance of the epistatic effects (Table 4), may arise from an unequal dispersion of genes with similar effects between the two parents. Judging from the mean value of F2, it is likely that genes for powdery mildew susceptibility are not only in the susceptible parent, but also present in the moderately resistant parent. The negative dominance effect in leaf rating indicates that, in PI 270545, the high resistance gene is recessive, whereas the moderate resistance gene is dominant. Significant additive ($0.90$) and dominance ($-0.08$) effects explained $71\%$ of the variation in leaf rating, respectively (Table 4). The remaining variation may be contributed by undetected nonallelic effects. This agrees with the high narrow-sense heritability of $71\%$, indicating that improvement in leaf resistance can be achieved through selection. Application of QTL mapping analysis may contribute to the detection of epistatic effects in this population. Zalapa et al. (2007) reported detection of digenic epistatic effects in melon architectural traits using QTL analysis.

**Stem resistance.** In contrast to leaf resistance, nonsignificance of the ABC components was supported by the joint scaling test. The additive-dominance model was adequate in explaining the variation in stem rating ($\chi^2_{(3)} = 1.98, P = 0.6$). Both additive ($d$) and dominance ($h$) effects were significant, but the additive effect was negative and considerably larger than the dominance effect, indicating that stem resistance was predominantly controlled by additive effects. Because larger additive effects were important for stem resistance, breeding progress would be faster than for leaf resistance.

The $i, j$, and $l$ effects were not significant, demonstrating absence of a nonallelic interaction. The contribution of additive and dominance effects was $58\%$ and $15\%$, respectively. A high heritability (79%) indicates that selection for stem resistance in this population would be effective.

**Conclusions**

Powdery mildew resistance in watermelon PI 270545 was controlled by a recessive gene for high resistance and a dominant gene for moderate resistance. Based on generation means analysis, the dominance genetic effect was larger for leaf resistance, whereas the additive genetic effect was larger for stem resistance. The large narrow-sense heritability in PI 269677 × PI 270545 combined with major additive genetic effects suggested that selecting for powdery mildew stem resistance in the segregating population of this cross could be done efficiently using single-plant selection, whereas for leaf resistance, selection in self-pollinated progeny rows would be necessary.

**Literature Cited**


