Tagging SSR Markers Associated with Genomic Regions Controlling Anthracnose Resistance in Chilli (Capsicum baccatum L.)

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Abstract

Anthracnose, caused by Colletotrichum spp. is a serious pre- and post-harvest disease in chilli (Capsicum annuum L.) which is a remunerative spice-cum-cash crop of the India. An attempt was made to tag genomic regions controlling anthracnose resistance using reported microsatellite markers. Out of 60 polymorphic SSR markers screened, only four differentiated the individual constituents of resistant and susceptible bulks. Of these four, only one (HmpsE 081) was found associated with genomic regions controlling anthracnose resistance. However, the association was weak as suggested by low contribution of the marker towards the variance of response to anthracnose disease in terms of lesion size.

Keywords

Chilli; SSR markers; Anthracnose resistance; Tagging

Introduction

Chilli (Capsicum annuum L.) is a remunerative vegetable and spice-cum-cash crop of the Indian subcontinent. India is the largest producer accounting for 26 per cent of the global production followed by China. Andhra Pradesh and Karnataka together account for more than fifty per cent production in India. However, chilli productivity in India (1.60 t ha⁻¹) is lower than that in the developed countries such as USA and South Korea (3.4 t ha⁻¹) [1].

Among the biotic stresses that constrain the chilli production, anthracnose, caused by Colletotrichum spp. is a serious pre- and post-harvest disease. C. capsici (Syd.) Butler and Bisby, C. gloeosporioides (Penz.) Penz. and Sacc., C. acutatum (Simmonds) and C. coccodes (Wallr.) Hughes [2], cause anthracnose of chilli; the former two are predominant in India. Yield losses due to anthracnose in India range from 50 per cent [3] to 66-84 per cent [4] and loss in fruit quality attributes such as oleoresin, capsaicin and phenol content due to anthracnose could be 50 per cent [5] resulting in reduced market price.

Material and Methods

Plant material

The material for the study consisted of anthracnose resistant PBC 80 and susceptible SB1 both belonging to Capsicum baccatum. The genotypes were cross at the experimental plots of Department of Genetics and Plant Breeding (GPR), University of Agricultural Sciences (UAS), Gandhi Krishi Vignyana Kendra (GKVK), Bengaluru.

Methods

Resistance response of PBC 80 was confirmed by screening against seven C. capsici and four C. gloeosporioides isolates (data not shown). Seeds from the crossed 'PBC 80 × SB 1′ fruits were sown to raise F₁ plants in an insect proof net house. True F₂’s were selfed individually to obtain F₃ seeds. F₃ seeds of the cross were sown in nursery to raise the F₃ mapping population and 40 days old seedlings were transplanted in insect proof net house, along with their parents and F₂ by maintaining a spacing of 0.45 m between plants within a row and 0.9 m between rows. All the recommended package of practices was followed to raise a good crop.

Phenotyping F₂ population for reaction to anthracnose disease

A total of 240 F₂ plants were raised from the selfed F₃ seeds and phenotyped for reaction to anthracnose. Twenty random fruits from each F₂ plant were picked at red ripe stage and brought to the laboratory. The fruits were surface sterilised, rinsed in sterile water and inoculated with virulent strain of Colletotrichum capsici i.e., ‘Cc 38’ in two replications. Thereafter, the fruits were inoculated with homogenized spore suspension containing 5×10⁴ spores/ml at two spots on the fruit (one µl/spot) using Hamilton micro syringe [6]. The inoculated fruits were incubated in plastic boxes with moist filter papers placed at the bottom and on top of the fruits to maintain relative humidity of over 90 per cent and then incubated at 27 ± 1°C for eight days (Figure 1). Disease reaction was recorded in terms of lesion size and was expressed as overall lesion diameter (OLD) across

Figure 1: Microinjection method of screening and experimental set up for screening against anthracnose.
all inoculated points on the fruits and true lesion diameter (TLD) using the following formulae

\[
OLD = \frac{\sum \text{lesion diameter}}{\text{Total number of inoculated points}}
\]

True lesion diameter (TLD): average of lesion diameter that are truly developed

\[
TLD = \frac{\sum \text{lesion diameter}}{\text{Total number of points that developed true lesions}}
\]

Genotyping

Genomic DNA was extracted from young and healthy leaves of 50 days old seedlings of parents, F₁ and F₂ plants following the extraction protocol given by Prince et al. (1997) with a few modifications.

Primers of 282 publically available microsatellite markers [7,8,9] were custom synthesized from Sigma genosys, Bengaluru. Reaction mixture for amplification consisted of Template DNA (12.5 ng/µl) 2 µl, Forward primer (10 pmol/µl) 2 µl, Reverse primer (10 pmol/µl) 2 µl, 1mM each dNTP 2 µl, 10 X Taq buffer 1 µl, 1 U Taq polymerase (SU/µl) 0.2 µl.

The PCR amplified products were initially visualized on 3% agarose and where clear resolution was not observed the products were denatured and separated on 6% Polyacrylamide Gel Electrophoresis (PAGE) gel and products were visualized by silver staining.

Depending on the lesion size (mm diameter) caused by infection with virulent 'Cc 38' isolate, the F₁ plants were categorised as resistant and susceptible following the scale modified from Hartman and Wang [10]. DNA from 10 resistant plants and ten susceptible plants were bulked. The bulks were constituted by combining equal quantity of the two bulks. Hence these four SSR markers were selected for further confirmation of their association with anthracnose resistance through single marker analysis (Figure 3). These results suggested putative association of the four SSR markers with genomic regions controlling anthracnose resistance.

Single marker analysis

Of the four SSR markers which consistently differentiated the resistant and susceptible bulks and their constituents, only one (HmpsE 081) was found associated with genomic regions controlling anthracnose resistance as indicated by significance of mean squares due to “between marker classes” (Table 2). Lower magnitudes of variance of response to anthracnose disease in terms of overall and true lesion size (explained by linked SSR marker) was amply reflected through low heritability (Table 3) suggesting weak association between the marker and the genetic determinants controlling anthracnose resistance. Voorrips et al. [16] have identified one major quantitative trait locus (QTL) with larger effects in the F₂ population (derived from C. annuum × C. chinense cross).

In single marker analysis, the distance between the linked SSR marker locus and per cent trait variation explained by the linked marker are confounded [17]. Further as F₂ individuals are not replicable, the SSR marker-trait (anthracnose resistance) association need to be confirmed in a replicable mapping population such as recombinant inbred lines (RILs) for effective use in marker assisted selection.

Inheritance pattern of anthracnose resistance

Mean lesion diameter of fruits sampled from individual F₂ plants were used to estimate skewness, the third degree statistics and kurtosis, the fourth degree statistics [13] to understand the nature of distribution and hence inheritance pattern using ‘STATISTICA’ software program. Genetic expectations of skewness (-3/2 d²h) reveal the nature of genetic control of the traits [14] and Kurtosis indicates the relative number of genes controlling the traits [15].

Results and Discussion

Out of 282 SSR markers screened only 60 (Table 1) differentiated the resistant (PBC 80) and susceptible (SB 1) parents indicating low level of parental polymorphism at SSR loci (21.3%), though the parents were diverse for several morphological traits. Kwon et al. [7] also reported low level of polymorphism at the SSR loci among commercial chilli varieties tested.

Bulk segregant analysis

Out of seven primers, which could differentiate the resistant and susceptible bulks, only four viz., HmpsE 081, HmpsE 097, HmpsE 116 and HmpsE 139 consistently differentiated the individual constituents of the two bulks. Hence these four SSR markers were used to genotype all the 125 F₂ individuals for further confirmation of their association with anthracnose resistance through single marker analysis (Figure 3). These results suggested putative association of the four SSR markers with genomic regions controlling anthracnose resistance.

Inheritance pattern of anthracnose resistance

Broad sense heritability of the response to anthracnose infection was estimated following the method suggested by Hanson et al. [12]. The additive and dominance genetic effects of the linked SSR marker was tested following two-sample 't' test with unequal variances [11].
while positively skewed, platykurtic distribution of $F_2$ was observed for average TLD caused due to infection by ‘Cc 38’ (Figure 4) isolate. Positively skewed distribution of individuals of $F_2$ for overall and true lesions produced in response to inoculation by ‘Cc 38’ (Figure 5) is on the expected lines as all $C. baccatum$ lines have been reported to have some level of resistance to anthracnose. Mild selection is expected to maximize the genetic gain. However, lepto and platy kurtic distribution of $F_2$ individuals with respect to overall and true lesion produced upon inoculation with ‘Cc 38’ (Figures 4 and 5) indicates that fewer to large numbers of genes, respectively are involved in

Table 1: List of SSR markers polymorphic to F2 mapping population parents (PBC 80 and SB 1) in Capsicum baccatum

<table>
<thead>
<tr>
<th>Sl.no</th>
<th>Primer</th>
<th>Sl.no</th>
<th>Primer</th>
<th>Sl.no</th>
<th>Primer</th>
<th>Sl.no</th>
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<th>Sl.no</th>
<th>Primer</th>
<th>Sl.no</th>
<th>Primer</th>
</tr>
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<td>1</td>
<td>Hpms E 001</td>
<td>11</td>
<td>Hpms E 035</td>
<td>21</td>
<td>Hpms E 070</td>
<td>31</td>
<td>Hpms E 097</td>
<td>41</td>
<td>Hpms E 146</td>
<td>51</td>
<td>AA840 689</td>
</tr>
<tr>
<td>2</td>
<td>Hpms E 003</td>
<td>12</td>
<td>Hpms E 036</td>
<td>22</td>
<td>Hpms E 072</td>
<td>32</td>
<td>Hpms E 100</td>
<td>42</td>
<td>Hpms E 147</td>
<td>52</td>
<td>CAN 09</td>
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<td>3</td>
<td>Hpms E 005</td>
<td>13</td>
<td>Hpms E 051</td>
<td>23</td>
<td>Hpms E 074</td>
<td>33</td>
<td>Hpms E 101</td>
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<td>CM 008</td>
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<td>4</td>
<td>Hpms E 012</td>
<td>14</td>
<td>Hpms E 058</td>
<td>24</td>
<td>Hpms E 075</td>
<td>34</td>
<td>Hpms E 104</td>
<td>44</td>
<td>Hpms 24</td>
<td>54</td>
<td>Gpms 3</td>
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<tr>
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<td>15</td>
<td>Hpms E 059</td>
<td>25</td>
<td>Hpms E 078</td>
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<td>Hpms E 116</td>
<td>45</td>
<td>Hpms 13</td>
<td>55</td>
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<td>6</td>
<td>Hpms E 019</td>
<td>16</td>
<td>Hpms E 063</td>
<td>26</td>
<td>Hpms E 081</td>
<td>36</td>
<td>Hpms E 122</td>
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<td>Hpms 04</td>
<td>56</td>
<td>Gpms 4</td>
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<td>7</td>
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<td>17</td>
<td>Hpms E 064</td>
<td>27</td>
<td>Hpms E 083</td>
<td>37</td>
<td>Hpms E 125</td>
<td>47</td>
<td>Hpms 1-106</td>
<td>57</td>
<td>Gpms 93</td>
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<td>8</td>
<td>Hpms E 027</td>
<td>18</td>
<td>Hpms E 065</td>
<td>28</td>
<td>Hpms E 084</td>
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<td>Hpms E 139</td>
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<td>Hpms E 029</td>
<td>19</td>
<td>Hpms E 066</td>
<td>29</td>
<td>Hpms E 090</td>
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<td>Hpms E 141</td>
<td>49</td>
<td>Hpms 1-155</td>
<td>59</td>
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<td>30</td>
<td>Hpms E 096</td>
<td>40</td>
<td>Hpms E 145</td>
<td>50</td>
<td>Hpms 1-216</td>
<td>60</td>
<td>Gpms 140</td>
</tr>
</tbody>
</table>

* Significant @ $P = 0.05$; $m_2$, $m_0$ and $m_1$ takes the meaning as described in the material and methods
Figure 4: Distribution of intra-Capsicum baccatum F2 mapping population (PBC 80 × SB 1) individuals for average over all size of the lesion (mm) caused by C. capsici.

Figure 5: Distribution of intra-Capsicum baccatum F2 mapping population (PBC 80 × SB 1) individuals for average over all size of the lesion (mm) caused by C. capsici.

Table 2: Analysis of variance of response to anthracnose disease between and within SSR marker (Hpms E081, Hpms E097, Hpms E116, Hpms E139) classes in an intra Capsicum baccatum (PBC 80 × SB 1) F2 mapping population.

<table>
<thead>
<tr>
<th>Sl. No.</th>
<th>Response to anthracnose disease infection</th>
<th>Total no. of plants</th>
<th>SSR marker class</th>
<th>F' cal</th>
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<td></td>
<td></td>
<td></td>
<td>m₀</td>
<td>m₁</td>
</tr>
<tr>
<td>Hpms E081</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.</td>
<td>Average OLD for Cc (mm)</td>
<td>121</td>
<td>1.56</td>
<td>2.94</td>
</tr>
<tr>
<td>2.</td>
<td>Average TLD for Cc (mm)</td>
<td>121</td>
<td>2.11</td>
<td>3.60</td>
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<tr>
<td>Hpms E097</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>Average OLD for Cc (mm)</td>
<td>117</td>
<td>2.73</td>
<td>2.44</td>
</tr>
<tr>
<td>2</td>
<td>Average TLD for Cc (mm)</td>
<td>117</td>
<td>3.36</td>
<td>3.04</td>
</tr>
<tr>
<td>Hpms E116</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.</td>
<td>Average OLD for Cc (mm)</td>
<td>123</td>
<td>2.89</td>
<td>2.15</td>
</tr>
<tr>
<td>2.</td>
<td>Average TLD for Cc (mm)</td>
<td>123</td>
<td>3.54</td>
<td>2.68</td>
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<tr>
<td>Hpms E139</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>Average OLD for Cc (mm)</td>
<td>113</td>
<td>2.44</td>
<td>3.59</td>
</tr>
<tr>
<td>2</td>
<td>Average TLD for Cc (mm)</td>
<td>112</td>
<td>2.44</td>
<td>3.59</td>
</tr>
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</table>

* Significant @ P = 0.05; m₀, m₁, and m₂ takes the meaning as described in the material and methods.
the response to anthracnose disease infection. Polygenic inheritance of anthracnose resistance was also reported by Voorrips et al. [16]. Several researchers have assessed the resistance to be controlled by a single recessive gene [18,19,20]. The inheritance patterns vary depending on the resistance sources and the Colletotrichum isolates.

Acknowledgments

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References


Table 3: Estimates of variance explained by the linked SSR marker (HpmsE081), broad sense heritability and additive and dominance effect.

<table>
<thead>
<tr>
<th>SSR Marker</th>
<th>Traits</th>
<th>$h^2$</th>
<th>Broad sense $h^2$</th>
<th>Test statistic $t_1$ (additive effect)</th>
<th>Test statistic $t_2$ (dominance effect)</th>
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<tr>
<td>HpmsE 081</td>
<td>Cc OLD</td>
<td>0.41</td>
<td>0.07</td>
<td>0.12</td>
<td>0.02</td>
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<tr>
<td></td>
<td>Average</td>
<td>0.59</td>
<td>0.09</td>
<td>0.14</td>
<td>0.05</td>
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