

Development of PCR-based Markers Associated with Powdery Mildew Resistance using Bulked Segregant Analysis (BSA-seq) in Melon

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Powdery mildew (PM) is a fungus that causes disease in both the feld and the greenhouse. Utilizing resistant cultivars is the most effective approach of disease management. To develop insertion-deletion (InDel) marker associated to this trait, whole genome of PM resistant line M17050 (P1) and PM-susceptible line 28-1-1 (P2) were sequenced. A total of 1,200 InDels, with an average of 100 markers per chromosome, were arbitrarily chosen from the sequencing data for experimental validation. One hundred InDel markers were ultimately selected due to their informative genetic bands. Further, an F2 segregating population of melons generated from these two parents was inoculated by PM pathogen. Based on bulk segregant analysis (BSA) using these 100 InDel markers, the powdery mildew resistance was associated with the genomic region *LVpm12.1* on melon chromosome12. This region overlapped the previously described QTL-hotspot area carrying multiple PM-resistance QTLs. Moreover, conventional QTL mapping analysis InDel markers MInDel89, MInDel92, and MInDel93 were detected. Therefore these markers could be used to track this resistance locus in melon while the lines carrying this locus could be employed in PM melon resistance breeding programs after validation test.



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Fruits morphology and PM disease evaluation. Fruit appearance and fruit longitudinal section of P1 line (a-b) and P2 line (c-d) showing skin and fesh characteristics; Powdery mildew pathogen single spore isolation (e) followed by conidiophore and conidia observation under light microscope (f), Maintenance of PM disease on melon leaves using artificial inoculation in growth chamber (g); Leaves from P1 and resistant F2 plant (h-i) and that from P2 and susceptibility F2 plant (j-k); X and 40X, represents high magnification observation.

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, 50 F1-6, 125 F2 . 0

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| . F1-6, (D). | | 17050 | 28-1-1, |
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|-------|----------|----------|---------------------------|---------------------------|-----|
| chr12 | MInDel23 | 20998229 | GTGATTCGACCTTTAGTGGCAAAA | ATCTAAACTTGGCCAACTCACTTG | 228 |
| chr12 | MInDel24 | 21001671 | TGCTGGCGGAAGAAATTAAATGAG | GTCTAGAGCAGGGCAAAGATTTTG | 204 |
| chr12 | MInDel25 | 21012231 | CCTACAACTTCACCCACTTTTGAG | CGAGAATGTTTTACTAGCTCAACCA | 263 |
| chr12 | MInDel26 | 21012734 | ACGTGTTTACAATGTACCGTGA | TGGGGATAAAGTGAGGAAGAGTTG | 173 |
| chr12 | MInDel27 | 21013801 | ACTGGATTACATGACCACTAAGTG | CTAACAACTTATCACAAGTACTCGC | 288 |
| chr12 | MInDel28 | 21014354 | CGACTTGATAATAAGCACACGTGT | TGTGAACCTATTAGTCCACCAAGT | 87 |
| chr12 | MInDel29 | 21016018 | AAAATCGACCTACCTTCAACGA | TACTGTTGTTGTCCCACATATCGA | 297 |
| chr12 | MInDel30 | 21202574 | GTGGGGATTTGATTACGTGTTCAA | CAACTGGGAAGGATGACATGAAAA | 273 |
| chr12 | MInDel31 | 21229417 | GCGATCATGTTTTCCTCAACCTTC | CTACTTCGAGTGGCCTAAATGACT | 250 |
| chr12 | MInDel32 | 21229663 | AGTCATTTAGGCCACTCGAAGTAG | TTTCTCCACATGTAACTGTACCCA | 174 |
| chr12 | MInDel33 | 21351602 | AGCCATCAAAGCCCAAAGTCTATA | TGAATCCTTGAAAGTTTCCGACAC | 100 |
| chr12 | MInDel34 | 21362591 | CCAGACCCACCATGTAAAATACCA | AAAATCGATTAGCCAAACCTCGTG | 217 |
| chr12 | MInDel35 | 21362703 | CACGATTGTTTAGATTTGGACCCA | AAAATCGATTAGCCAAACCTCGTG | 84 |
| chr12 | MInDel36 | 21363548 | GGCCATTGAACCACACTTACAAAT | AATCCATAGACGACCACAAGAGTC | 246 |
| chr12 | MInDel37 | 21374693 | | TTTTGTGCCGTGGAAAGATAGAGA | 285 |
| chr12 | MinDel38 | 21377989 | GTGATATGTACCCTTTCTGAACACA | | 145 |
| chr12 | MInDel39 | 21378699 | CCCCTTCTCCTTCTCACTTATT | | 129 |
| chr12 | MinDel40 | 21382890 | | GGGCGTGTTTTAACCTCCTTTTAT | 267 |
| chr12 | MinDel40 | 21302030 | | | 180 |
| chr12 | MinDel41 | 21303090 | | | 263 |
| ohr10 | MinDel42 | 21402047 | | | 200 |
| | MinDel43 | 21424600 | | GGAAGGIGIGIIGIIIICIGAGII | 202 |
| | MINDel44 | 21426178 | | | 232 |
| chr12 | MInDel45 | 21426418 | | | 130 |
| chr12 | MInDel46 | 21427266 | AAAATACTCCGTCAACCATGCATC | | 107 |
| chr12 | MInDel47 | 21429242 | GCAATGATCCTTTGAAACTCACATC | ATAAGGTTGTGGGTTTGGGTTTGTC | 270 |
| chr12 | MInDel48 | 21430150 | TGATGGGGAAGAGATCAGAAAGTG | AACAAGAAACAAAATAGGTCGGCC | 281 |
| chr12 | MInDel49 | 21430740 | GAGCATTGAGGTTAACGTAAAGACA | GACTTTGCAAAAGAACGTGTAGAC | 219 |
| chr12 | MInDel50 | 21440365 | TGGTTAGAATAGTTTGTGTTGGAGG | TTGGGGCAAAGATTATCTAGGACT | 270 |
| chr12 | MInDel51 | 21441123 | CACAAAAGCGAAGATGACAACCTT | TTGGCTTGAATGATCTGGTTGTTC | 139 |
| chr12 | MInDel52 | 21443795 | AGAAGAAGGAGAAGAGAGAGAGGGA | CCCTCCTTCTCTTGATCACTTTCA | 113 |
| chr12 | MInDel53 | 21443959 | TGAAAGTGATCAAGAGAAGGAGGG | TATTGGAAGAGGCATTAGAAGGGG | 180 |
| chr12 | MInDel54 | 21445083 | TGTACTTCCTTCTACCTAAAGCGA | CGTGGTTAGATTTGTTGTTGCA | 248 |
| chr12 | MInDel55 | 21445095 | TGTACTTCCTTCTACCTAAAGCGA | CGTGGTTAGATTTGTTGTTGCA | 248 |
| chr12 | MInDel56 | 21445714 | TGTAAAGAGAGGCACATGTGTTAGA | TGTCTATCATCTTGCCATCATAGTC | 86 |
| chr12 | MInDel57 | 21446584 | ATTGGGTTGGGCTCTTTTATTTGG | AAGAGACAGGCCGGAACTTCAATA | 300 |
| chr12 | MInDel58 | 21449903 | CCCCAACCTATAATGATCAACACC | ATATAGGTGGAAAGAAGAGGGCTA | 191 |
| chr12 | MInDel59 | 21450126 | TCTAGCCCTCTTCTTTCCACCTAT | CCAAAAGTGTTGCAATAGGAGTTCA | 296 |
| chr12 | MInDel60 | 21453528 | AGAGATCGCATGTGTGTATGGTTA | TAGACAAAGTTAACAGAACGCCCA | 263 |
| chr12 | MInDel61 | 21453748 | TGTCTATTAGAGCCGTACCACATG | AGGAAACACTAAGACAATCGAGCT | 155 |
| chr12 | MInDel62 | 21459671 | AAAGGCGATAAGTAGTGGTGAAGT | ATGTCCGTCGTCAAAACTTTCTTC | 234 |
| chr12 | MInDel63 | 21459939 | GAAGAAAGTTTTGACGACGGACAT | GGTTTGCCTTGTGAAAATGAGCTT | 177 |
| chr12 | MInDel64 | 21460111 | ACAACTACACTCCAAATTCCCCAT | TGAGAGAGAGAGAGAGAGAGAGAG | 248 |
| chr12 | MInDel65 | 21468921 | ACCCACGAGCATGTAGAAATAAGA | GCAAGGTGATATAAATGGTTGGAGC | 258 |
| chr12 | MInDel66 | 21469302 | ACACACACACACACACACTTTT | TGGCAATCAACAAAAGGGATGA | 294 |
| chr12 | MInDel67 | 2147 | | & 930¾ 2 | |
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