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Genetic Characterisation of Resistance Genes against Black Spot (*Diplocarpon rosae* Wolf) in Rose Populations

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Abstract

Developing resistant cultivars against black spot (*Diplocarpone rosae* Wolf) has been a challenge in breeding garden roses. Genetic characterisation is an important step to identify and utilise new sources of such resistant traits in germplasm collections. This study was initiated to characterise black spot resistance gene(s) in a wild rose species *Rosa majalis*; to determine whether it is identical to the Rdr1, number and inheritance of gene(s) involved, and develop molecular markers and map the position in the genome. Forty-six F1 crosses of *R. majalis* and *R. pisocarpa*, 90 F1 crosses of *R. majalis* and König Stanislaus, 16 control genotypes, and 4 black spot single- spore isolates were used to carry out phenotypic (inoculation) assay, microsatellite marker, ploidy level and sequence analyses. Seven of the 46 segregating genotypes were found susceptible to F004, S009, DortE4 and D002 isolates. However, infection severity with D002 and DortE4 was not high as with the other isolates. In both flow cytometry and microsatellite marker analyses, the resistant parent (*majalis*) and segregating genotypes were found to be tetraploids, while *pisocarpa* is a diploid. Microsatellite marker loci, developed for Rdr1; 69E24Mica_F1, 29Mica_F5, 155SSR and Rdr1_gener[3]-pp co-segregated with the resistance against F004 and S009. Hence, the resistance gene in *Rosa majalis* could be identical to Rdr1 or a different gene within the Rdr1 cluster. All alleles which were specific to pisocarpa were absent in any of the segregating progenies in the *majalis* × *pisocarpa* cross. It suggests all progenies in this cross could be derived from selfing within the seed parent majalis. Gene prediction using a 1.7kb region cDNA sequence of resistant segregating genotypes resulted in NBS-LRR type disease resistance gene. Further nucleotide and protein-protein BLAST analyses confirmed that the sequence is part of a putative disease resistance gene in roses. *A phylogenetic* tree using 25 sequence data of different rose species indicated a closer similarity between the sequence and RGA8 of *R. multiflora*. Chi-square test results of 3:1 in *R. majalis* selfing and 1:1 in *majalis*–König Stanislaus cross segregations suggest a single dominant gene found in simplex (Rrrr) configuration in the parent *R. majalis*.

Keywords: Microsatellite marker, *Rosa majalis*, Rdr1, RGA, spot

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