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The mechanism and durability of intermediate resistance to Plasmodiophora brassicae pathotype X conferred by two resistance genes

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Genetic resistance is important for management of clubroot (Plasmodiophora brassicae Woronin) on canola (Brassica napus). This study assessed canola lines carrying a single (A03 or A08) or double CR genes (A03, A08) with intermediate resistance to P. brassicae pathotype X found recently in Alberta, Canada. These lines also showed differential resistance to pathogen X LG2 and LG3 populations; only the lines carrying two CR genes (A03, A08) were resistant to both LG2 and LG3. Functional annotation of differentially expressed genes (DEGs) identified via RNA sequencing showed the activation of several biological processes, including responses to stress, biosynthesis and signal transduction with the resistance. Enrichment analysis showed that lines with intermediate resistance, either with a single (A08) or double (A03, A08) CR genes, shared many of the DEGs. These DEGs generally were not found in susceptible lines. A total of 286 DEGs may be involved in defense responses, including those linked to pathogen-associated molecular patterns (PAMPs), activation of innate immunity, hormone signaling, transcription factors, and cell wall modification. These results indicate that the intermediate resistance is through the activation of PAMPs- and effector-trigged immunity. Interestingly, transcription levels for most of the defense-related DEGs were much higher in the line carrying double CR genes (A03, A08) than in the line carrying a single CR gene (A08), implying the two CR genes together trigger stronger defense responses. Resistance durability was also studied by inoculating two double CR-gene lines (intermediately resistant) with a field pathotype X-LG3 population under controlled environment. Galls from each generation were let mature in damp soil for 3 wks before being incorporated into recycled growth media. Resting spores in the media were quantified using qPCR before each planting and the results validated with droplet-digital PCR. After being exposed to the same pathotype X population for five generational cycles (6 wks /cycle), lines with two CR genes maintained the level of resistance. The inoculum increased and decreased slightly in the media of susceptible and resistant lines, respectively, over the five cycles, a circumstance related to different amounts of resting spores from diseased plants of varying resistance.