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Screening of T_2 transgenic plants to evaluate the expression of RKN parasitism gene

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Root Knot Nematode (RKN) infection is a major plant pathogenic disease that is of great economic importance. RKN parasitism genes are responsible for the formation of 'giant feeding cells' by up-regulating the plant transcription factors during infection. T_2 transgenic plants which were previously developed to overcome RKN infection were screened in this study for the presence of RKN parasitism gene. The presence of RKN parasitism gene in the transgenic tomato plant genome was tested with qPCR amplification. This was further confirmed with sequencing the positive samples and blast analysis. Expression levels of the transgene under pathogenic stress were analyzed with qPCR amplification. Two different techniques were optimized for the staining of the RKN egg masses. Non transgenic tomato plants under similar conditions were used as the control group. The Presence of transgene were detected in strains $17T_2$ 1.2, $17T_2$ 2.1, $17T_2$ 3.3 and $19T_2$ 2.2. This establishes the presence of the introduced transgene across generations. Relative quantification of transgene cDNA with the housekeeping gene *lat* indicates a 4.72-fold higher expression level of transgene in transgenic plants due to pathogenic stress by RKN infection. This indicates the presence and increased expression of the targeted gene in the transgenic tomato plants. This may be due to activation by the pathogenic stress induced from RKN infection. Thus the plant strain $19T_2$ 2.2 can be used as host plants for RNAi silencing of the parasitic RKN expressing RKN parasitism gene. McCormick Red food dye can be used to effectively stain RKN egg masses.

Keywords: parasitism, root knot nematode (RKN), transgenic and qPCR

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