Abstract

Bacterial wilt disease caused by *Rasltonia solanacearum* which is the second most important and destructive bacterial disease on tomato after *Pseudomonas syringae* pv. *tomato* in the world. In this study, the role of *eds1* and *npr1* in the resistance of tomato to bacterial disease was investigated by using genetic engineering and molecular techniques. For this reason, *Tobacco rattle virus* (TRV) was used as a viral carrier in virus-induced gene-silencing (VIGS) technique. With insertion of virus to Agrobacterium and tomato plant agro-infection, gene silencing efficiency of gene silencing and principal role of these genes in plant resistance was determined. To confirm the results of gene silencing, plants were infected with recombinant virus. Twenty hors post infection (hpi), detection of virus in infected young leaves was started and 72 hpi the rate of virus reached to maximum amount. These results showed TRV vector is able to be significantly amplified and spread systemically in young tissues. The results demonstrated that the TRV virus is an efficient tool with high performance to using gene silencing technique of targeted genes. Also, the effect of different biotic and abiotic factors such as plant stage growth, two bacterial concentration and two temperatures at time of plant infection with Agrobacterium on gene silencing efficiency was assayed. Disease severity and bacterial concentration in infected plants as morphological indicators and semi-quantitative PCR reaction at the molecular level was used to determine of the rate of gene silencing of each target gene. Our results showed a significant increase in the severity of disease and bacterial concentration in silenced genes plants when compared with none silenced plants. Also, the results of the semi-quantitative PCR reaction indicated that each of these treatments had a considerable effect on the efficiency of VIGS. The highest efficiency of VIGS was observed in fully-opened cotyledon plants, the inoculation temperature of 20ºC and the Agrobacterium concentration of 1×10^6 CFU. Our results showed that gene silencing was occurred after 24 hours after inoculation and then was continuously increased 48 and 72 hpi. Maximum gene silencing evidence was confirmed 72 hpi. According to obtained data, it can conclude that these genes play a significant role in increase of resistance responses and induce resistance against bacterial wilt disease. As regards to involving of different genes in signaling pathway to plant resistance to pathogen attacks development of VIGS technique could be accurately used for determining of key genes that play important role in plant resistance mechanisms.

Key words: Agrobacterium, Bacterial disease, Gene silencing, Semi-quantitative PCR, Plant resistance genes.

University of Zabol

Faculty of Agriculture

Department of Plant Protection

Thesis for the degree of Ph.D. in Plant Pathology

Efficiency of induced gene silencing on infected tomato to bacterial wilt disease caused by *Ralstonia solanacearum* by recombinant *Tobacco rattle virus*

Supervisors

S. K. Sabbbagh (Ph.D.)

M. Mazaheri (Ph.D.)

Advisors

S. M. Salari **(**Ph.D.)

S. M. Moshtaghioun **(**Ph.D.)

By

H. R. Bolok Yazdi

Summer 2018