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Selection of Bacterial Wilt-resistant Lines from Regenerated Tobacco Plants

Kazuyuki CHATANI**, Hideyoshi TOYODA*, Yoshinori MATSUDA* and Seiji OUCHI*

One of the most promising applications of biotechnological strategies to plant disease control is to establish an efficient system for rapidly and effectively producing disease resistant lines in major crop plants. Genetic somaclonal variations induced in plant tissue cultures have been utilized for this purpose[1-3]. In our laboratory, plants resistant to viral[4], bacterial[5], and fungal pathogens[6] have been successfully selected through *in vitro* culture and plant regeneration. To further verify the feasibility of this strategy, the present study was designed to select bacterial wilt resistant tobacco plants by the use of somaclonal variation. In our previous study[5], we selected tomato lines resistant to *Pseudomonas solanacearum* through direct inoculation of regenerated plants with the pathogen rather than *in vitro* selection of resistant callus tissues under the pressure of toxic substances produced by the pathogen. In the present study, therefore, the selection was conducted on the basis of post-inoculation survival of regenerated tobacco plants derived from axillary bud-derived callus tissues.

Callus induction and plant regeneration were carried out according to the method reported previously[7]. Axillary buds were harvested from one-month-old seedlings of tobacco (*Nicotiana tabacum* cv. Bright Yellow), surface-sterilized with 70% ethanol and 2% sodium hypochlorite, and cultured on agar-solidified Murashige-Skoog[8] (MS) medium supplemented with 0.5 μg/m*l* 2, 4-D and 0.01 μg/m*l* kinetin for callus induction. Induced callus tissues were transferred to MS medium containing 0.05 μg/m*l* IAA and 1 μg/m*l* kinetin for plantlet regeneration. The regenerated plants were transplanted to soil in plastic pots and acclimated in a moist growth chamber for one week and then placed in a shaded greenhouse for two weeks. Regenerated plants with 4-5 leaves and non-cultured parental tobacco plants (as a control) at the same growth stage were inoculated with a virulent strain U-10 of *P. solanacearum* which was kindly given by Dr. H. Tanaka, Japan Tobacco Inc. To secure bacterial inoculation, roots of these plants were cut by plunging a razor blade down into the soil (5 cm downward) at positions 2 cm distant from plants in order to help bacterial cell penetration into the vascular system[9]. The U-10 was shake-cultured to an exponential phase in PCG medium (10 g pepton, 10 g casamino acids, and 1 g glucose in 1 *l* water), collected by centrifugation, and suspended in sterilized water. Ten m*l* of the bactarial suspension (10^6 cells/m*l*) of U-10 was poured into soil at the plunged site.

The inoculated susceptible control plants initially showed typical yellowing of leaves and then severely wilted. **Fig. 1** shows the distribution of wilted plants during incubation periods. All of the control plants (50 plants) exhibited severe wilting within 2 weeks after inoculation (**Fig. 1-A**), indicating that the present injured root inoculation is a reliable method for eliminating infection escape which may lead to erroneous selection. Under the same inoculation conditions, therefore,

* *Faculty of Agriculture, Kinki University*, Nara, 631 Japan
** Present address: *Plant Biotech Division, Daiichi Engei Plantech*, Shizuoka, 410-13 Japan

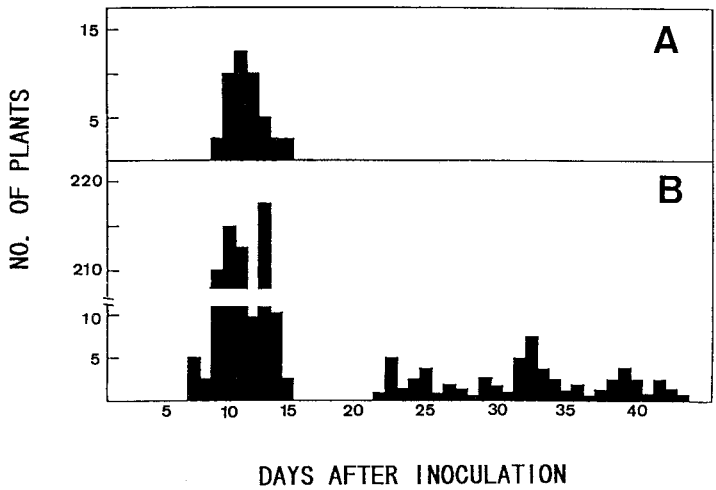

Fig. 1 Time course of wilt appearance in non-cultured parental tobacco cultivar, Bright Yellow (A) and plants regenerated from axillary bud calli (B) after inoculation with a virulent strain U-10 of *P. solanacearum*.

regenerated plants were screened for resistance against the pathogen. In a previous paper[5], we demonstrated that tomato regenerant responses to the inoculated pathogen could be classified into three types; susceptible plants showing a rapid and severe wilting, moderately resistant plants with delayed appearance of wilting, and completely resistant plants without any symptom throughout the entire life cycle. In the present study, 877 out of 950 regenerated plants showed rapid and severe wilting similar to that of parental control plants (**Fig. 1-B**). In 70 regenerants, the appearance of wilting was considerably delayed compared with that of the control plants (**Fig. 1-B**), hence these were considered to be moderately resistant to the pathogen, as pointed out in the previous paper[5].

Three regenerants did not show any wilt symptom throughout the experimental period of 3 months, suggesting that they are truely resistant candidates. For confirming the validity of inoculation method, we attempted to isolate the bacteria from U-10-inoculated regenerants after harvesting self-pollinating seeds. Stems were cut into small pieces, surface-sterilized, and homogenized in phosphate-buffered saline. Homogenates were spread onto PCG medium containing 2, 3, 5-triphenyltetrazolium chloride and the number of colonies formed was counted. On this medium, virulent bacteria formed typical fluidal pinkish colonies[10]. Bacterial population detected in the resistant regenerants was in the renge of $1.5\text{-}6.8\times10^3$ cells/gram fresh weight of tissue. These values were considerably lower than those (10^9-10^{10} cells/g. fr. wt.) in wilted plants. Bacteria isolated from non-wilted resistant regenerants were proved to be virulent by inoculation of susceptible parent plants, suggesting that no wilt symptom in these three regenerated plants was due to the suppression of bacterial growth in the vascular system, but not to a failure of inoculation. Although the mechanism of resistance has not been elucidated, the results strongly support the notion that plant tissue culture is a useful strategy for improving crop plants, especially for production of disease resistant lines.

The resistance trait in these regenerants, however, has to be stably transmitted to their offspring to validate the usefulness of somaclonal variation. The result of self-pollination and backcrossing with the susceptible parent cultivar will be reported elsewhere.

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References

1) Larkin, P. J., W. R. Scowcroft, 1981. Theor. Appl. Genet., **60**: 197-214.
2) Brettell, R. I. S., D. S. Ingram, 1979. Biol. Rev., **54**: 329-345.
3) Toyoda, H., S. Ouchi, 1991. In "Molecular Strategies of Pathogens and Host Plants" (eds. by Patil, S. S. *et al.*), p. 229-239, Springer-Verlag, New York.
4) Toyoda, H., K. Chatani, Y. Matsuda, S. Ouchi, 1989. Plant Cell Rept., 8: 433-436.
5) Toyoda, H., K. Shimizu, K. Chatani, N. Kita, Y. Matsuda, S. Ouchi, 1989. Plant Cell Rept., 8: 317-320.
6) Toyoda, H., K. Horikoshi, Y. Yamamoto, S. Ouchi, 1991. Plant Cell Rept., **10**: 167-170.
7) Toyoda, H., Y. Oishi, Y. Matsuda, K. Chatani, T. Hirai, 1985. Phytopath. Z., **114**: 126-133.
8) Murashige, T., F. Skoog, 1962. Physiol. Plant., **15**: 473-497.
9) Toyoda, H., H. Hashimoto, R. Utsumi, H. Kobayashi, S. Ouchi, 1988. Phytopathology, **78**: 1307-1311.
10) Husain, A., A. Kelman, 1958. Phytopathology, **48**: 155-165.