Rootstock genetic engineering for GFLV resistance in grapevine

Valérie Goldschmidt, Emmanuelle Vigne, Aurélie Marmonier, Véronique Komar, Gérard Demangeat, Jean Masson and Olivier Lemaire

Institut National de la Recherche Agronomique, Unité Mixte de Recherche UMR 1131 «Santé de la Vigne et Qualité du Vin », INRA/Université de Strasbourg, 28 rue de Herrlisheim, BP 20501, 68021 Colmar, France.
lemaire@colmar.inra.fr

The grapevine fanleaf disease, the major virus disease for the viticulture worldwide, is caused mainly by nepoviruses transmitted in a semi-persistant manner by longidorid ectoparasite nematodes. The fanleaf disease is causing to the French grapevine industry one billion euros losses per year with a prevalence of 540 000 ha (60 % of the total acreage cultivated with grapevines). *Grapevine fanleaf virus* (GFLV) belonging to the genus *Nepovirus* in the family *Comoviridae* is the main aetiological agent. The control of fanleaf disease is currently based on prophylactic measures, cultural practices (fallows over ten years) and certification programs. Despite soil desinfection and nematicides with the use of environmental unfriendly agrochemicals (some of them being already prohibited in the EU), fanleaf disease remains an expanding pandemy with technical deadlock in control strategies.

To date, no dominant nor recessive resistance genes have been found in grapevine toward GFLV. The implementation of genetic engineering provides new strategies based on rootstock mediated resistance, to develop virus-resistant grapevines generating RNA silencing. Transgenic rootstocks expressing the coat protein (CP) of GFLV genes have been obtained in the early 90ies. Their resistance toward fanleaf disease in vineyard conditions, has been assessed in an open-field trial in Champagne between 1996 and 1999, where 3 lines out of 18 were selected to be included in a following open-field trial starting in 2006 at INRA Colmar. This unique non-confined experiment has been set up through debates and recommendations issued from a local steering committee, including members of the public, of associations, of the local viticulture, wine growers and researchers. Results generated in a next future from this field experiment, will enable us to assess the behavior of these first generation of CP expressing transgenic rootstocks (GFLV resistance). In addition, their environmental impacts on the wild-type GFLV populations, on their longidorid vectors and on the putative horizontal transfer of the NPTII gene to the soil inhabiting bacteria will be analyzed.

Recent knowledge on RNA silencing led us to develop new types of constructs called "stem-loop" in order to optimize the induction, robustness and durability of the RNA mediated resistance. Transgenic *Nicotiana benthamiana* have been produced to assess the effectiveness of these constructs against GFLV infection. In parallel, rootstock transformation with these new constructs is being initiated.