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**Poster Abstract - D.29**

# DEVELOPMENT OF A "FILTER ARRAY" FOR THE DETECTION OF VIRAL RECOMBINANTS IN PLANTS

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Viral recombination between different virus and viral recombination in virus resistant transgenic plants play an important role in their variability and evolution, this is particular for RNA virus. These phenomena happen whit low frequency and it is necessary to use very sensitive methods to detect them. Among different molecular techniques currently used, we preferred macroarray methods for different reasons:

a) simple genome virus necessities a low density of spotted genes compare to microarray;
b) detection is based on $^{33}P$;
c) it is possible to repeat hybridisation on the same array at several time;
d) low cost.

The aim of this research was to optimize an array and to study the limit of sensitivity in detecting viral recombinants. For this study two important tomato phytoviruses, one cucumber mosaic virus (CMV) and one tomato spotted wilt virus (TSWV), were chosen. The viral genome organization was checked to determine possible similarities and homologies between CMV and TSWV sequences by NCBI and EMBL bank. One hundred primers pair were designed and used to amplify whole viral genome. Polymerase chain reaction (PCR) products were spotted on filter by multifunctional station GeneTAC G3 (Perkin Elmer). Artificial recombinants (in vitro syntetized), made by parts of CMV and TSWV genome, were used as $^{33}P$ probe to check array sensitivity. In order to mimic a natural scenario, artificial recombinant transcript (used in decrescent concentration) was mixed to total RNA extracted from tomato leaves. Different concentration of transcript (100, 10, and 1 ng) were mixed by maintaining total RNA quantities. Filter array sensitivity, observed by filter hybridisation, was lower than 1 ng of transcript per 100 ng of total RNA. The signal was so intense to reach lower sensitivity than 1 ng. The lower limit of sensitivity is currently being elaborated.