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Assessment of the viral protein VPg intrinsic disorder contribution to the *Potato virus Y* adaptation to eIF4E-mediated resistance of pepper (*Capsicum annuum*)

Justine Charon[1], Amandine Barra[1], Jocelyne Walter[1], Vincent Simon[1], Benoît Moury[2], Pauline Millot[2], Eugénie Hébrard[3], **Thierry Michon[1]**

[1] *Biologie du Fruit et Pathologie, UMR 1332 Inra, Université Bordeaux 2, 71 avenue E. Bourlaux, CS 20032, 33882 Villenave d'Ornon Cedex, France*
[2] *I.N.R.A., Unité de Pathologie Végétale, BP94, 84143 Montfavet Cedex, France*
[3] *Institut de Recherche pour le Développement (IRD), UMR RPB (IRD, Cirad, Université Montpellier 2), Montpellier, France*

In the proteins world, the structure-function paradigm was recently questioned with the emergence of the concept of intrinsic disorder. This new notion defines the transitory or permanent absence of 3D-structure in some proteins or regions as directly related to their functionality (1). In RNA viruses, the structural properties of disordered proteins may be linked to the ability of these microorganisms to hijack the cellular machinery of the host by interacting with multiple partners and to rapidly adapt to the many constraints they must face. It was suggested that, because of their low topological requirements, disordered regions could be more permissive to mutations and associated with weaker evolutionary constraints than ordered regions (2, 3). Accordingly, mutations introduced during replication could have less impact on the protein stability and consequently on the virus fitness, thereby enabling it to explore a larger choice of adaptive mutation pathways. To experimentally test these assumptions, we chose the plant virus *Potato virus Y* (PVY)-pepper pathosystem. In this system, the Viral genome-linked (VPg) protein is a key determinant of potyvirus adaptation (4) because of its ability to recruit the host translation initiation factor eIF4E. The VPg was previously characterized as intrinsically disordered (5, 6). We first designed a set of 3 mutants of PVY displaying either an enrichment or a depletion of intrinsic disorder in their VPg.
The assessment of intrinsic disorder contribution to VPg-based PVY adaptation process consisted in monitoring resistance breakdown apparition (i.e adaptation) in a resistant host pepper genotype (pvr23), inoculated either by wild-type PVY or by our three VPg disorder-affected mutants. Interestingly, a PVY mutant carrying a disorder depletion within the VPg was unable to overcome pvr23 resistance. Conversely, mutants displaying higher levels of intrinsic disorder in theVPg were able to better adapt to pvr23 resistance than WT PVY. A comparative analysis of the VPg mutations associated to the resistance overcoming led us to the conclusion that: (i) for one of these mutants, the disorder increment was associated with a tighter binding between VPg and the resistant pepper susceptibility factor eIF4E. (ii) for the other, increasing intrinsic disorder had been beneficial to the adaptive process by facilitating adaptative mutations to occur. To our knowledge, this study provides the first experimental evidence of protein intrinsic disorder involvement in a plant RNA virus adaptation process.

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Mots-clés : VPg, potyvirus, PVY, eIF4E, evolution, adaptation, intrinsically disordered protein.

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