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## PROJECT DESCRIPTION

Viruses are important pathogens of plants, significantly contributing to almost half of the agricultural losses deriving from pathogen attack. Climate change is predicted to induce considerable losses in agricultural production for major crops, in both temperate and tropical regions. As global and climate changes are expected to modify pest and pathogen landscapes, crop production will undergo further threatening(Challinor et al., 2014). Understanding how plants cope with global and climate changes and pathogen offensive is not just a major scientific challenge but has relevant implications from the agricultural and societal point of view, as increase in crop productivity is mandatory to feed a growing world population. Maintaining the crop production using sustainable strategies is therefore a challenging task in the face of changing climate.

**Viruses** are of particular concern, as they are rapidly vectored by few polyphagous insect species to large populations of plants, limiting the effectiveness of traditional chemical and biological disease management. Sources of resistance/tolerance to viruses are not always available and, if present, require long and expensive processes of introgressions into cultivated varieties. Conversely, efficient **pathogen-derived resistance** may be reached in transgenic plants expressing virus-derived nucleic acid sequences inducing RNA silencing-like or protein interference mechanisms (Sanford and Johnston, 1985; Baulcombe, 1996; Prins et al., 2008). However, producing transgenic plants is expensive, time consuming, requires the characterization of the viral genome and efficient protocols of plant transformation, and entails social and ethical acceptance issues. Therefore, **new strategies to generate virus resistance are sought, exploiting natural exchanges of genetic material between viruses and hosts.**

Actually, viruses can occasionally **naturally exchange genetic material with their hosts**, as supported by several examples of viral derived sequenced integrated into plant genomes, or of viruses expressing protein coding genes of eukaryotic origin, suggesting that **horizontal gene transfer between viruses and hosts can occur in nature** (Gilbert and Cordaux, 2017).

Best candidates for such gene exchanges are **viruses with circular small circular single stranded DNA (ssDNA) genomes**, including geminiviruses (GVs), plant-infecting agents of worldwide importance. This statement is supported by several reasons. First, GV replicate in the nuclei through double-stranded DNA (dsDNA) intermediates (Jeske, 2009), binding histones and closely mimicking the structure of minichromosomes (Pilartz and Jeske, 1992). Second, GV evolve through recombination/re-assortment (Lefeuvre and Moriones, 2015), due to a mix of rolling-circle and recombination-dependent replication (RDR) mechanisms (Jeske, 2009). RDR allows recovery of GV DNA fragments resulting from incomplete synthesis or nucleolytic attack, favoring recombination with even distantly related GVs, which infect the same cell (Martin et al., 2011), producing intra- and inter-specific or even inter-generic recombinations. For example, the genus *Becurtovirus* originated from

two GV genera, mastreviruses and curtoviruses (Varsani et al, 2014). Interestingly, GV replication strongly resembles that of Helitrons, transposable elements common in plants (Grabundzija et al., 2016), so that it has been proposed that *Helitrons* and GVs have common ancestors (Feschotte and Wessler, 2001; Murad et al., 2004; Kapitonov and Jurka, 2001). Finally, many GV-related sequences (GRDs) integrated in the genome of different plant species (Murad et al., 2004; Bejarano et al., 1996; Ashby et al., 1997; Filloux et al., 2015), further supporting the GV attitude to exchange genetic material with the host. Again, such findings are associated to historical events identified through phylogenetic and metagenomics studies, suggesting that virus-host horizontal gene transfer is rare in nature, possibly related to evolutionary adaptations with no relevance to short-term acquisition of resistance or increased fitness and resilience, as obtained with transgenic approaches.

Surprisingly, we identified **rapid and frequent events of re-assortment during GV infection** between the becurtovirus *Beet curly top Iran virus* (BCTIV) and its natural host plant, *Beta vulgaris*. Circular molecules about half the size of BCTIV (1.2-1-5 knt) were detected in BCTIV-infected beet plants, named minicircles (MC) (Catoni et al., 2018). MCs consist of a portion of the BCTIV genome, including the intergenic region and the origin of replication, with no open reading frames, and of portions of non-coding regions from different chromosomes of beet. A cloned MC, unable of autonomous replication, was trans-replicated by BCTIV not only in beet plants, but also in unrelated species (tobacco and Arabidopsis), implying that the recombined beet element present in the MC can be carried over by BCTIV and propagated in other hosts, acting as natural gene transfer vector. Moreover, MC DNA was detected in BCTIV virions purified from plants experimentally inoculated with BCTIV and the cloned MC (Catoni et al., 2018). As BCTIV virions are transmitted by the polyphagous leafhopper *Circulifer hematoceps*, spread of MCs to other plant species and the environment is highly probable in natural contexts.

The existence of MCs suggests that exchange of genetic material between viruses and plants is more frequent that previously observed. However MC evolutionary and physiological roles (if any), as well as the mechanism for their formation, have still to be elucidated.

MCs accumulation in plants reminds the known proliferation of defective GV molecules, associated to reduced disease severity (Frischmuth and Stanley, 1994; Simon et al., 2004). In addition, MCs could potentially interfere with the host in at least two ways: (i) the captured host DNA could be transcribed by viral promoters present in the intergenic region, or (ii) secondary small RNAs could induce epigenetic changes in corresponding homologous sequences on the host genome.

Thus, MCs are good candidates to alter the host response to viral infection, the progression of the disease and to shape the genetic, epigenetic and transcriptome landscape of a plant. Their characterization may thus help to develop more efficient and cost-effective strategies to manage viral diseases and, additionally, improve the tools available for the use of synthetic biology.

This project has the following objectives:

1. **Characterize the molecular nature of MCs**

   Though found in naturally and experimentally BCTIV-infected *B. vulgaris* plants, it is unclear if MC formation is specific for this virus-host association. Therefore, a panel of other plant hosts (sugarbeet, tomato, watermelon, tobacco, spinach) will be inoculated with BCTIV, or other GVs (e.g. *Beet curly top virus, Tomato yellow leaf curl virus, Chickpea chlorotic virus*) to determine MC formation. To ensure efficient sequence-independent MC detection, the MobilomeSeq strategy will be optimized and applied for a genome-wide detection of chimeric virus/host circular DNA molecules (Lanciano et al., 2017). Newly identified MCs will be characterized and captured host sequences mapped on host reference genomes to reveal any bias in their chromosomal location (position, sequence homology or microhomology, transposon sequences, etc). In addition, genetic/environmental factors affecting virus replication or genome stability, including abiotic/biotic

stresses, will be tested to investigate their impact in the quality and amount of MC formation. This will be accomplished using mutant genotypes of MC forming plant species (e.g. for tomato and arabidopsis).

2. **Analyze the effect of MCs on GV infection and plant symptoms.**
   As MCs can potentially interact with the host and the parental virus, the effect of MC accumulation on the progression of GV infection and disease severity will be evaluated, linking possible phenotypic alterations to epigenetic (bisulfite conversion and methylome analysis), transcriptome or small RNA accumulation changes.
3. **Biotechnological exploitation of MCs**

   The genetic structure of naturally formed MCs will be used as scaffold to generate artificial MCs incorporating host sequences targeting genes relevant for virus infection (either via small RNA regulation or dominant negative mutants). Artificial MCs will therefore serve as viral vectors for VIGS and screened for their effect in limiting virus replication and disease progression, or as vectors allowing heterologous expression of genes of interest in beet or other crops. The latter is expected to improve and optimize the tools available for synthetic biology (e.g antibody production in roots of sugarbeet). Artificial MCs harboring sequences alien to plant genomes will also be engineered to evaluate their potential horizontal transfer from the artificial MC to the plant host genome. For this, artificial MCs will be delivered with their helper virus into plants and alien sequence integration will be tested by next generation sequencing.

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