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VARIABLE RATIOS OF TWO ENDOGENOUS PARA RETROVIRUSES IN THE GENOMES OF TARAXACUM

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Abstract:

Endogenous viral elements of the Caulimoviridae family have been registered in different plant families as integrants. As one of the Asteraceae family, Taraxacum has been studied here to find endogenous pararetroviruses (EPRVs), including its three different agamospecies genomes. Three different agamospecies genomes of Taraxacum O978, A978, and S3, have been sequenced using next-generation sequencing, and then analyzed by bioinformatics techniques to search endogenous pararetroviral sequences within each examined genome. In the three genomic sequences, two caulimovirus-like sequences were found, one belonging to the Caulimovirus genus and is named as Caulimovirus-TOf, while the second was from the Florendovirus genus and was named ToffiV. The length of Caulimovirus-TOf was 6976 bp with three open reading frames encoding protein domains of cauli-D, peptidase-A3, RT-LTR, RVT, and RNaseH. Cauliflower mosaic virus and Caulimovirus-TOf shared peptidase, RT, and RH domains in 62% pairwise identity based on phylogeny analyses. The Florendovirus sequence has been reversely extracted with a length of 8131 bp and three open reading frames that encode movement protein, RT-LTR, RVT, and RNaseH. The O978 genome showed a high rate of EPRVs than the other two, indicating a very accessible genome and possible DNA marker based on widely fluctuating ratios of both integrants.

Keywords: Taraxacum genomes, Endogenous pararetroviruses, Genomics, Caulimovirus-TOf, ToffiV, Next-generation sequencing, Bioinformatics techniques

1. INTRODUCTION

Taraxacum F. H. Wiggers (Asteraceae subfamily Cichorioideae) commonly known as dandelion is a large genus, over 2800 species grouped into 60 sections (Kirschner et al., 2015), a worldwide distributed perennial grassland herbs growing from the subtropics to subarctic regions across the world (Ge et al., 2011; Kirschner et al., 2015). Taraxacum considers one of the taxonomically complex plant genera in the world, it has complex reticular evolution, including different reproduction strategies (sexual and apomixis-meiotic diplospory- clonal reproduction by seeds) (Richards 1973; Asker and Jerling 1992; Kirschner and Štěpánek 1994; Majeský et al., 2015), and polyploidy events (only a few species are sexual diploids). Dandelion basic chromosome number X = 8, and diploid Taraxacum is quite rare and reproduces sexually, however, the majority of its species are triploid and apomictically produced (Richards 1973). Estimation of Taraxacum genome size (Plant genome C-value)

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revealed that Taraxacum has an estimated value of 2C = 1.74 pg in diploid T. linearisquameum Soest, up to an estimated value of 2C = 2.86 pg in triploid T. perdubium Trávníček (Záveský et al., 2005; Vidic et al., 2009; Temsch et al., 2010; Bainard et al., 2011; Iaffaldano et al., 2017; Macháčková et al., 2018). Studying genotypic diversity in the chloroplast genome showed its importance in analyzing the nature of evolutionary processes in nuclear and cytoplasmic genomes, population structures, and breeding systems (Salih et al., 2017). However, an investigation of genotypic diversity in pure apomictic and mixed sexual-apomictic populations showed variation arises from both mutation (accumulation of somatic mutations/allele divergence) and recombination (gene flow between sexual-apomictic individuals) (Mes et al., 2002; Majeský et al., 2012; Majeský et al., 2015; Salih 2017). Dandelion has been registered earlier in Great Britain and Germany to be infected beside lettuce (Lactuca sativa) with a Dandelion yellow mosaic virus that causes chlorotic rings and spots (Kassanis, 1944, 1947; Bos et al., 1983). Mountain et al. (1983) reported that Tomato ring spot virus infects common dandelion and transmits through seeds as a natural reservoir for this virus. In Amsterdam, Dijkstra et al. (1985) characterized a carlavirus that was found restricted to dandelion plants in the experimental garden and suggested to be called dandelion carlavirus. Further, Groves et al. (2002) pointed out that Tomato spotted wilt virus and its vector the tobacco thrips found in dandelion with a comparatively high rate of infection. Rojas et al. (2007) reported that Tomato yellow leaf curl virus could infect tomato and dandelion for the first time in California using the PCR technique. Recently, in rubber dandelion (Taraxacum kok-saghyz), two new viruses belonging to the Amalgaviridae family have been characterized using next-generation sequencing techniques (Debat et al., 2019). It is noteworthy, however, that endogenous pararetroviruses (EPRVs) settle into host genomes from earlier infections and are passed down through generations to act as donors or infectious components, and previously no studies or characterizations have been conducted on dandelion genomes. In this paper, we identified two novel endogenous pararetroviruses belonging to two different genera of the Caulimoviridae family for the first time in the genomes of dandelion.

2. PROCEDURE

2.1. Plant material and DNA sequencing

Three agamospecies (2n = 3x = 24) of Taraxacum officinale agg. [Section Taraxacum (formerly Ruderalia), Asteraceae], T. obtusifrons Markl. (O978); T. stridulum TraÂvniček ined. (S3); and T. amplum Markl. (A978) were used in this study. Their geographical records of origin and voucher specimens were deposited in the Herbarium of the Department of Botany, Palacký University, Olomouc, Czech Republic (herbarium abbreviation: OL) (Majeský et al., 2012). Total DNA was extracted from fresh green young leaves using standard cetyl-trimethyl- ammonium bromide (CTAB) methods (Doyle 1991), to obtain high-quality genomic DNA. DNA was sequenced commercially (Interdisciplinary Center for Biotechnology Research, University of Florida, USA). Accessions O978 and A978 were sequenced using Illumina Hiseq500 2x150bp reads

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and obtained 12 Gb sequence data from each of them, while accession S3 was sequenced with Illumina MiSeq 2x300bp paired-end reads and collected 22 Gb sequence data.

2.2. Graph-based read clustering with Repeat-Explorer

RepeatExplorer is a computational pipeline that includes identification, characterization, and graph-based clustering of repetitive DNA sequences in next-generation sequencing data (Novák et al., 2013), it is also used to explore EPRV clusters in the genomic whole raw reads. The pipeline only has the capability to recognize EPRVs at the family level (such as the caulimoviruses family), so clusters of EPRVs need further characterization to identify the species level. Each cluster generated from the Repeat Explorer, consists of one to many contigs, each cluster's contigs were extracted, then the total contigs of each cluster were submitted through Repbase (Jurka et al., 2005), Basic Local Alignment Search Tool (Altschul et al., 1990), and then aligned to known viral sequences from Descriptions of Plant Viruses database (DPVweb) (Adams and Antoniw 2005), and Repbase dataset of representative repeat sequences and endogenous viral elements to identify virus sequences on the genus and species levels using the alignment tool in Geneious prime (Kearse et al., 2012).

2.3. Map to reference

The raw reads of all examined next-generation sequences (NGS) data for EPRV sequences (Petuvirus, Florendovirus, and Caulimovirus) assembled using Geneious prime (Kearse et al., 2012) (available from http://www.geneious.com). The assembly report of the result included a number of reads assembled, the whole used reads, and highly frequent overlapped reads, all incorporated in only one contig and consensus sequence. This report data is used to calculate genome proportions and copy numbers as below: 1- Genome proportion: number of assembled reads/numbers of total NGS reads x 100. 2- Copy number: number of assembled reads x read length/reference sequence length (Mustafa 2018).

2.4. Phylogenetic analysis

In order to choose a robust model for phylogeny, MEGA11 (Tamura et al., 2013) was conducted using the maximum likelihood (ML) method. Firstly, the Geneious prime (Kearse et al., 2012) was applied using ClustalW alignment for 16 endogenous virus sequences including an out-group member with default parameters and optimized manually. After that, a phylogenetic tree was reconstructed using General Time Reversible (GTR) as the best substitution model of evolution. Bayesian phylogeny inference was used for analysis with Bayesian inference of phylogeny (MrBayes 3.2.6) (Huelsenbeck and Ronquist 2001).

3. RESULTS

The whole raw reads produced from Illumina platforms were about 59258642, 58713854 and 69056774 raw reads for S3, A978 and O978 genomes respectively. The

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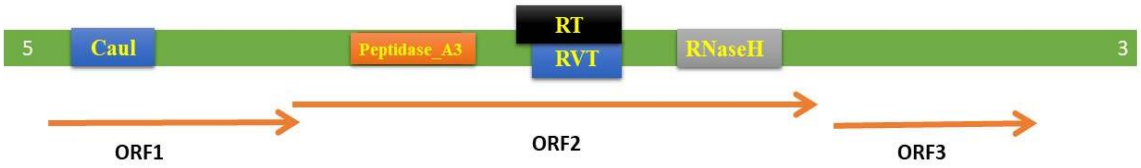
results from the RepeatExplorer pipeline have revealed two clusters of caulimovirus-like sequences found in the raw reads of three Taraxacum genomes under study. Further analyses clarified the taxonomy of both groups that referred to Caulimovirus and Florendovirus genera. Caulimovirus and florendovirus-like sequences are named as Caulimovirus-TOf (https://www.girinst.org/2017/vol17/issue8/Caulimovirus-TOf.html) and ToffiV (https://www.girinst.org/2017/vol17/issue8/ToffiV.html) based on Repbase dataset and Geering et al. (2014) instructions respectively. The length of the Caulimovirus-TOf sequence was 6876 bp with three open reading frames encoding protein domains of cauli-D, peptidase-A3, RT-LTR, RVT, and RNaseH (Fig. 1).

Fig 1: The whole sequence of Caulimovirus-TOf showing three open reading frames encode protein domains of cauli-D, peptidase-A3, RT-LTR, RVT and RnaseH



The Florendo virus sequence has been reversely extracted with a length of 8141 bp and three open reading frames encode movement protein (MP), RT-LTR, RVT, and RNaseH (Fig. 2).

Fig 2: The whole sequence of ToffiV showing three open reading frames encode protein domains of movement protein, RT-LTR, RVT and RNaseH that organized in reverse direction

Cauliflower mosaic virus (CaMV) and Caulimovirus-TOf shared peptidase, RT, and RH domains with 56% pairwise identity, and this has been approved in the phylogeny tree (Fig. 3).

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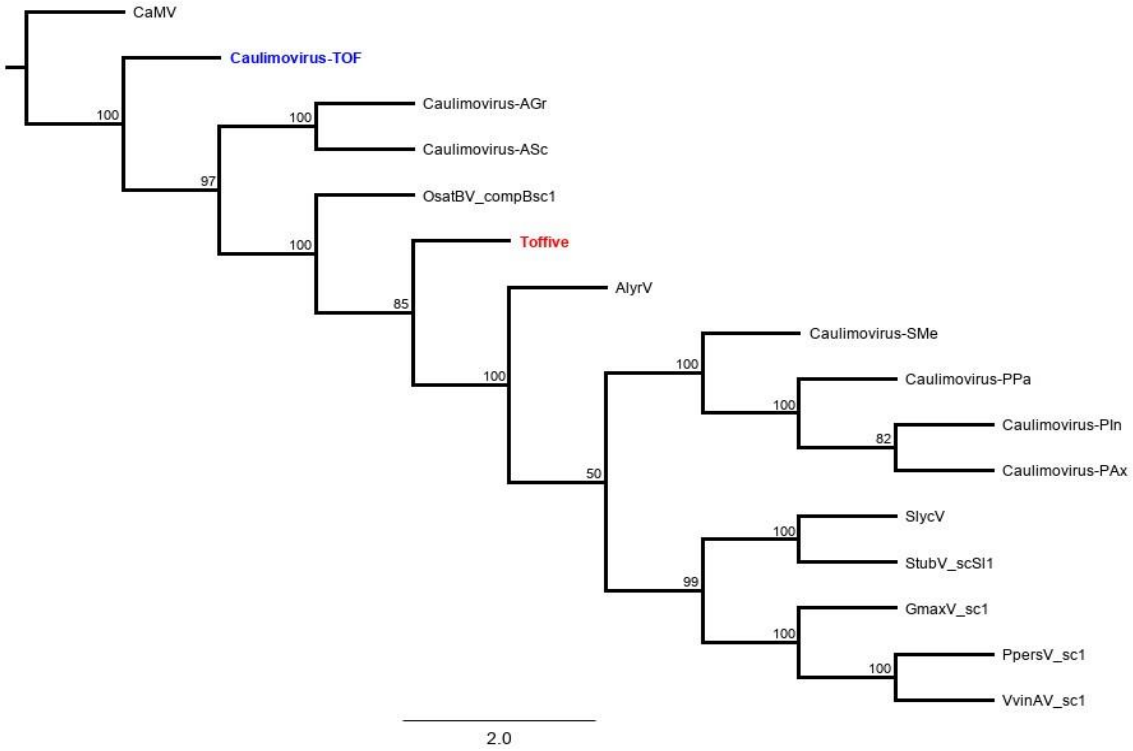
Fig 3: Bayesian phylogenetic tree of a set of caulimoviruses and florendoviruses in comparison with Caulimovirus-TOf, ToffiV and CaMV using 11 entire sequences (about 6500 bp each), showing high similarity between Caulimovirus-TOf and ToffiV to CaMV and other florendoviruses respectively



The ToffiV shows a close relationship to florendovirus-like sequence members in the phylogeny analysis (Fig. 3). The Caulimovirus member has colonized the O978 genome with a proportion of 0.077 and 1161 copy numbers more than the other two genomes were 0.0046 genome proportion and 600 copies for S3, and 0.049 genome proportion and 628 copies for A987 (Fig. 4). The ToffiV like Caulimovirus-TOf was found with an extremely high rate of genome proportion of 1.4 and 18755 copy numbers in the O978 genome, while the A978 genome has 0.049 genome proportion and 7570 copies, and 0.060 genome proportion and 658 copies for S3 genome (Fig. 4).

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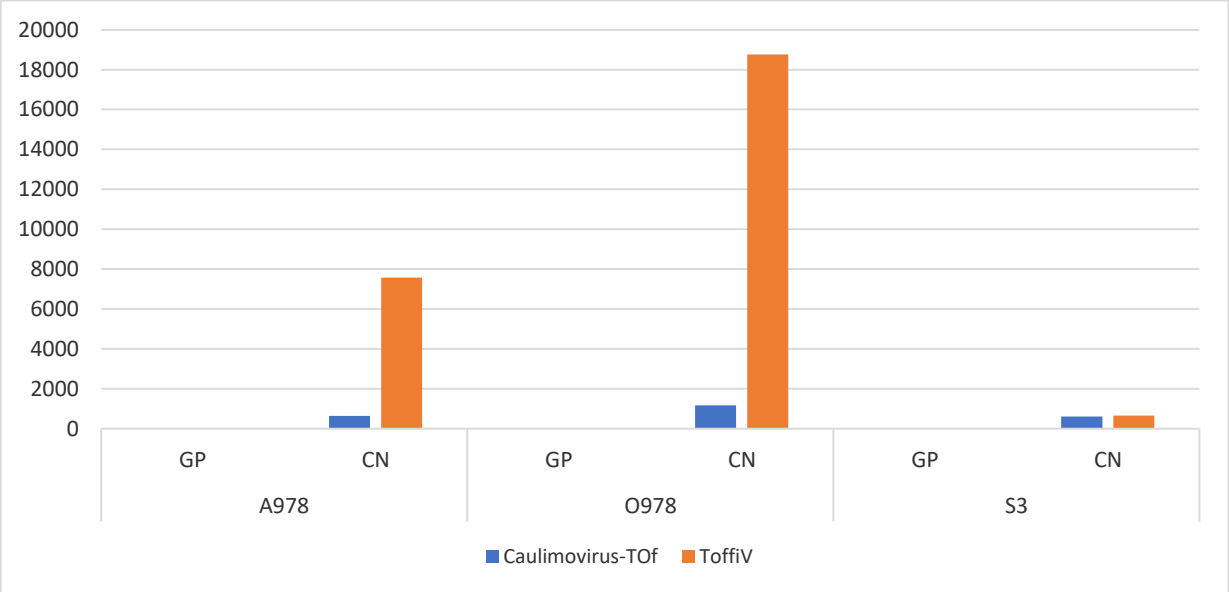
Fig 4: Genome proportion (GP) and copy numbers (CN) of Caulimovirus-TOf and ToffiV over three Taraxacum genomes showed higher rates of both in O978 genome



4. DISCUSSION

The family Caulimoviridae has a tremendous impact on a plant's evolution as infectious units or as donors of new genetic material taking into account the high adaptability of the members of this virus family (Diop et al., 2018). The big influence of this family came from two shapes of its components; endogenous pararetroviral and exogenous units. This study highly approved the existence and diversity of endogenous pararetroviral sequences within host plant genomes and highlighted more evidence of the integration between plant viruses and their hosts (Geering et al., 2010; Geering et al., 2014). The results showed that Taraxacum genomes are widely differentiated in EPRV existence, and reported that the O978 genome is very accessible to these components than the other two genomes. The accessibility and endogenization search of EPRVs have been well noticed and explained in petunia genomes that showed a variable existence of these integrants. Additionally, the diversity among host genomes based on the integration of EPRVs has confirmed phylogeny and evolution oncoming over plant species and revealed the relationship of wild and hybrid species based on EPRVs. As well as, EPRVs have cytogenetically confirmed their important role as a DNA marker to recognize host species based on their abundance within each genome (Alisawi 2019). Although Taraxacum has been recorded as a host of multiple plant viruses, this study is the first one to register the colonization of EPRVs inside its genomes. The Caulimovirus and florendovirus-like sequences have been registered in some recent studies as fragmented or full EPRV sequences that are found together within host genomes. The resemblance between Caulimovirus-TOf and Cauliflower

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mosaic virus in protein domains and organization has clearly explained both viruses' relationship. CaMV considers a typical DNA plant virus that infects Brassicaceae (Cruciferae) plant family with a fairly restricted host range (Haas et al., 2002). The hypothesis that suggests both caulimoviruses are ancestrally correlated is strongly proved here to explain how those viruses are related to each other. More importantly, the high similarity of three protein domains (peptidase, RT, and RH domains) within most caulimoviruses especially Dahlia mosaic virus, Strawberry vein banding virus, Cauliflower mosaic virus, and Caulimovirus-TOf probably reflected similar emerging events of those viruses. Florendoviruses have recently been discovered over multiple plant species of monocot and eudicot having abundant copy numbers and 34 distinct viral species. Most of these components have two open reading frames that encode different protein domains that are indispensable for viral replication (Geering et al., 2014). In this work, the dandelion is added as one more host to the florendoviruses plant hosts, this list is extended continuously over time based on research within new plant genomes. Recently, Alisawi (2019) has added four species of petunia to that list recording new four florindoviruses, one in each species with a range of ORFs from two to six. This member of florendoviruses has colonized the Taraxacum genome especially O978 with a big gap from the other two genomes having a huge number of copies similarly to what is found in other listed hosts. This result is very supportive of using ToffiV as well as Caulimovirus-TOf as DNA markers for distinguishing Taraxacum genomes similar to what Alisawi (2019) reported in the case of EPRVs in petunia genomes. Further, Geering et al. (2014) found representative members of florendoviruses in EST (expressed sequence tag) databases according to only three from 27 examined genomes reporting that the components are strongly transcribed. Contrariwise, Alisawi (2019) studied the expression activity of florendoviruses with no significant outcome suggesting that transcription of florendoviruses has dependent activity based on specificity and host impact. Here, as a future plan, we recommend extending this study for expression activity taking into account the two ingterants existence.

5. CONCLUSION

Two endogenous viral elements belong to Caulimovirus and Florendovirus genera exist in the agamospecies genomes of Taraxacum O978, A978, and S3 with highly differentiated ratios. The whole lengths and genome organization of each virus have been characterized, and phylogenetically analyzed. The pararetoviruses found in a higher rate within O978 genome, more than other two genomes, indicating a very accessible genome and possible DNA marker based on widely fluctuating ratios of both integrants within the examined genomes.

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