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A N I N T E R N A T I O N A L J O U R N A L

In Silico Identification of MicroRNAs with B/CYDV Gene Silencing Potential

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ABSTRACT

Computational investigation of a set of publicly available plant microRNAs revealed 19 barley- and other plants-encoded miRNAs and their near-complement reverse sequences (miRNA) that have potential to bind all B/CYDV open reading frames (ORFs) except ORF0 and ORF6. These miRNAs/miRNAs*, their binding positions and targets are discussed in the context of biological protection of cereals against B/CYDV, based on antiviral silencing.*

Key words: Barley/Cereal Yellow Dwarf virus (B/CYDV), microRNA, Gene silencing, Host defense.

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Barley/Cereal Yellow Dwarf Virus (B/CYDV) infects cereal crops worldwide, causing severe leaf symptoms and decreased yield. B/CYDV is a positive sense single-stranded RNA virus, belonging to the *Luteoviridae* family and its subspecies are assigned either to *Polerovirus* genus (CYDV-RPV), or to *Luteovirus* (BYDV-PAV and BYDV-MAV). Several aphid species transmit the B/CYDV viral particles following a persistent and circulative mode [1]. The genome of B/CYDVs contains six Open Reading Frames (ORFs) [2]. ORF1 is involved in RNA replication; ORF2 encodes the RNA-dependent RNA polymerase (RdRp) and is expressed only fused to ORF1 via ribosomal frame-shifting [3], resulting in a high ratio of the ORF1 product (P1) to the ORF1-ORF2 product (P1-P2 fusion); ORF3 encodes the major coat protein (CP) of 22 kDa, which has peptide motifs involved in viral transmission [4]; ORF4, entirely included in ORF3, but in a different reading frame [2], encodes a 17 kDa movement protein (MP) involved in systemic infection [5]; ORF5 is the product of a translational readthrough the ORF3 stop codon and it is involved in aphid transmission and in long distance movement of viral particles [5]; moreover, it exists, in Luteoviruses, a small and variable ORF6 near the 3' end of RNA whose function is unknown [6]. Finally, Poleroviruses have an ORF0 at the 5′ end that induces virus symptoms [7] and is probably a suppressor of RNA silencing [6]. In addition, ORF1 of Poleroviruses encodes a proteinase motif and the viral genome-linked protein (VPg) [8], which is absent in Luteoviruses. As soon as a phytovirus infects the host plant, a host-pathogen arms race is initiated, the outcome of which determines the fate of viral survival. There are several operative defense mechanisms in plants, among them microRNA responses that are an important and decisive factor in conferring resistance to pathogens [9]. MicroRNAs (miRNAs or miRs) are a class of endogenous non-coding small (18-25 nucleotides) RNAs, encoded by so called *MIR* genes. In plants, miRNAs play fundamental roles such as organogenesis, meristem development, leaf and flower morphogenesis, signal transduction and response to environmental stresses [10-12]. MicroRNA-mediated gene silencing is a widespread mechanism of host defense against viral [13] and bacterial infections [9]. Currently, many miRNAs interfering with the cycles of a number of phytopathogenic viruses have been identified in Solanaceae, such as potato [14], and in cereal plants, such as rice [15], barley [16], wheat [17] and sorghum [18]. Moreover, an over-expression of the carrier/passenger strand called miRNA* (miRNA star) [19], in response to viral infection, has been demonstrated in several plant species, such as *Arabidopsis thaliana* [20] and *Solanum lypersicum* [21]. In this latter species, it was demonstrated that miRNA* sequences have a potential to bind to most of the tomato leaf curl virus (ToLCV) open reading frames (ORFs). In order to limit the expanding of B/CYDV over the world, it has become imperative to set up novel strategies, involving miRNAs (and/or miRNAs*) interactions with cereal hosts. The present paper describes the bioinformatic identification of several barley and non-barley miRNA/miRNA* sequences that were shown, through database mining and computational prediction, to have potential to interact with B/CYDV genome.

A total of 5,000 mature miRNA sequences were used in the study. These miRNAs belong to three groups: (a) Group I, consisting of 71 mature barley miRNAs (hvu-miRs), was collected from miRBase [22] (http://www.mirbase.org/cgi-bin/query.pl?terms=hvu&submit=Search); (b) Group II was initially made of 2,441 mature miRNAs from 15 *MIR* families, conserved among 67 plant species, among which barley. From this preliminary set, twelve hvu-miRs already contained in group I were excluded, keeping 2,429 mature miRNAs in group II; and (c) Group III was built from 2,500 complement reverse miRNAs (miRNAs*) belonging to groups I and II.

Using RNA hybrid software version 2.2 (http://bibiserv.techfak.uni-bielefeld.de/rnahybrid/submission.html) [23], a total of 19 sequences were shown to target B/CYDV viral ORFs. These miRNAs/miRNAs*, their binding positions and targets are described in Table 1. Thirteen out of 19 sequences belonged to barley (hvu-miRNAs/miRNAs*), three to *A. thaliana*, two to rice, and a unique miRNA* sequence to *Lotus japonicus*. Among this identified set, eight miRNAs were barley-specific miRNAs (group I), eight were miRNAs conserved across several plant genomes (group II) and three were miRNAs* (group III). The targeted regions were associated with replication (ORF1), RNA-dependent RNA polymerase (ORF2), coat protein (ORF3), viral transmission (ORF3-ORF5) and movement protein (ORF4-ORF5) [6]. In strain BYDV-PAV genome, eleven miRNAs and two

Table 1. miRNAs/miRNAs* and their candidate targets in the B/CYDV genome and the barley transcriptome (DFCI barley contigs, Release 12).

No.	miRNA/miRNA*		B/CYDV target		Barley transcriptome best expectation target			
	miRBase ID (accession no.)	**5'-3' sequence**	**Isolate/genomic region**	**Alignment start position**	**Barley DFCI accession**	**Target annotation**	**Expect-ation**	**Inhibition mode**
1†	osa-miR166a-5p (MIMAT0022855)	GGAAUGUUGUCUGGUUCAAGG	RPV/ORF1	1077	-	-	-	-
2†	hvu-miR168-5p (MIMAT0018215)	UCGCUUGGUGCAGAUCGGGAC	PAV-III/ORF3,4	3096	NP315934	Flame chlorosis virus-like agent [*Hordeum vulgare*]	3.0	Translation
3†	hvu-miR169 (MIMAT0018218)	AAGCCAAGGAUGAGUUGCCUG	PAV-I/ORF3,4	3072	TC242386	Similar to UniRef100_Q0DX39; *Oryza sativa* Japonica Group (Rice)	0.0	Cleavage
4†	hvu-miR171-5p (MIMAT0022971)	UGUUGGCUCGACUCACUCAGA	PAV-I/ORF1	343	-	-	-	-
5†	ath-miR172a (MIMAT0000203)	AGAAUCUUGAUGAUGCUGCAU	PAV-I/ORF5 PAV-II/ORF5 PAV-III/ORF5	3664 3664 3647	TC278536	Similar to UniRef 100_Q0DL60 Cluster: Os05g0121600 protein; *Oryza sativa* Japonica Group	0.5	Cleavage
6†	ath-miR390b-5p (MIMAT0000932)	AAGCUCAGGAGGGAUAGCGCC	PAV-I/ORF3,4 PAV-II/ORF3,4	3217 3217	-	-	-	-
7†	osa-miR393a (MIMAT0000957)	UCCAAAGGGAUCGCAUUGAUC	MAV/ORF5	4190	-	-	-	-
8†	ath-miR394a (MIMAT0000936)	UUGGCAUUCUGUCCACCUCC	PAV-III/ORF1 MAV/NC	367 5252	TC240366	Similar to UniRef100_Q0JGH1; *Oryza sativa* Japonica Group (Rice)	0.0	Cleavage
9‡	hvu-miR5048a	UAUUUGCAGGUUUUAGGUCUAA	MAV/ORF5	4744	TC250387	Similar to	0.0	Cleavage

	(MIMAT0020544)					UniRef100_Q0ITC3 ; *Oryza sativa* Japonica Group (Rice)		
10‡	hvu-miR5049b (MIMAT0024797)	AGUAUUUAGGUACAGAGGGAG	PAV-II/NC	5501	-	-	-	-
11‡	hvu-miR6177 (MIMAT0024800)	UACCAUGGACAGAAGGCACUUA	PAV-II/ORF2 MAV/ORF2	1751 1715	-	-	-	-
12‡	hvu-miR6182 (MIMAT0024806)	UGAGUGUGUGAUGGAUGGCUUU	MAV/ORF2	923	-	-	-	-
13‡	hvu-miR6196 (MIMAT0024822)	AGGACGAGGAGAUGGAGAGGA	PAV-II/ORF2	2703	-	-	-	-
14‡	hvu-miR6199 (MIMAT0024825)	CCACAGAAUUCUCACAGUGAUGG	RPV/ORF2	3210	-	-	-	-
15‡	hvu-miR6211 (MIMAT0024839)	CAGAUCAAGACGCUCCGGCA	PAV-III/ORF1	379	-	-	-	-
16‡	hvu-miR6214 (MIMAT0024842)	CGACGACGACGAGCACGACA	PAV-I/ORF2	2373	-	-	-	-
17*	hvu-miR166a* (MIMAT0018213)	UCGGACCAGGCUUCAUUCCCC	MAV/ORF1	391	CV063912	Weakly similar to UniRef100_A7R0J5 ; *Vitis vinifera* (Grape)	3.0	Cleavage
18*	lja-miR171d-3p* (MIMAT0029317)	GCGAUGUUGGUGAGGUUCAAUC	PAV-II/ORF5 PAV-III/ORF5	4365 4342	-	-	-	-
19*	hvu-miR6177* (MIMAT0024800)	GCAAGUGCUUUCAUGUCCAUGGGU	PAV-I/ORF5	4497	-	-	-	-

ORF: Open Reading Frame; NC: Non coding genome; † : conserved miRNAs (group II), ‡ : barley-specific miRNAs (group I) ; * : microRNA* sequences (group III).

miRNAs* were predicted to target all ORFs except ORF6. In strain BYDV-MAV genome, only three ORFs (ORF1, ORF2 and ORF5) had putative miRNAs/miRNAs* counterparts. Finally, in strain CYDV-RPV genome, only two miRNAs, osa-miR166a-5p and hvu-miR6199, were able to target ORF1 and ORF 2 of this strain, respectively. The identification of three miRNA* species with potential to silence ORFs of B/CYDV, provides a valuable support to the hypothesized role of miRNAs* in host defense [14,20,24]. In animals, it has been demonstrated that certain miRNAs* can be functionally active [25,26]. If such a mechanism operates in plants, we expect that the present study will broaden our understanding of miRNAs* as potential contributors to host-pathogen interactions.

Using psRNATarget software (http://plantgrn.noble.org/psRNATarget/), predicted targets of miRNAs/miRNAs* were obtained from *H. vulgare* Expressed Sequence Tags (ESTs, DFCI gene index). Results showed that among 19 miRNA/miRNA* sequences identified by RNA hybrid analysis, six, namely hvu-miR168-5p, hvu-miR169, ath-miR172a, ath-miR394a, hvu-miR5048a, and hvu-miR166a*, had also potential targets among barley ESTs (Table 1). Among these, hvu-miR168-5p showed complementarities with NP315934, a barley EST corresponding to flame chlorosis virus-like agent. Flame chlorosis (FC) is a soil-borne virus-like disease of cereals, associated with a double-stranded linear RNA, containing at least one ORF [27]. Based on this, we speculate that miR168 plays an important role through its hybridization potential to viral/virus-like genomes (e.g. B/CYDV and FC).

In conclusion, results of our study suggest that at least 16 miRNAs and three miRNAs*, here reported, would play a regulatory role in conferring barley/cereals resistance to B/CYDV infection. Future research focuses will encompass the expression profiling and mechanistic investigation of this role, as well as the establishment of a balance between barley yield and antiviral defense.

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