Genetics and Plant Physiology – 2013, Volume 3 (3–4), pp. 113–125

Available online at http://www.ifrg-bg.com

COMPARISON OF THE TAS3 GENES CODING FOR TRANS-ACTING SMALL INTERFERING RNA IN ARACEAE

Krasnikova M. S.**, *A. V. Troitsky, A. G. Solovyev, S. Y. Morozov

A.N.Belozersky Institute of Physico-Chemical Biology, Lomonosov Moscow State University, Moscow, 119992, Russia

Received: *21 May 2014* Accepted: *13 October 2014*

Summary: Ta-siRNAs (trans-acting small interfering RNAs) belong to a plant-specific class of endogenous siRNAs (small interfering RNAs). These siRNAs have been found to regulate gene expression in plants including seed plants and mosses. Particularly, non-protein-coding TAS3 genes produce ta-siARF species which target the mRNAs of three Auxin Response Factor (ARF) genes (ARF2, ARF3/ETT and ARF4) for their subsequent degradation. The function of TAS3 precursor RNA is controlled by two miR390 target sites flanking tandem of ta-siARF sequences. Previous studies have shown that one of the conserved ta-siRNAs, TAS3, plays key roles in mosses and flowering plants. In this paper, we studied the organization of TAS3 genes in the specialized aquatic plants of family Araceae. We characterized several TAS3 loci in these plants. The sequenced loci were found to be similar to TAS3 genes of other flowering plants. Moreover, we revealed that some of TAS3 loci were transcribed in a tissue-specific manner.

Citation: Krasnikova M. S., A. V. Troitsky, A. G. Solovyev, S. Y. Morozov, 2013. Comparison of the TAS3 genes coding for trans-acting small interfering RNA in Araceae. *Genetics and Plant Physiology*, 3(3–4): 113–125.

Keywords: ARF genes; micro RNA; silencing; trans-acting RNA; small interfering RNA.

Abbreviations: dsRNA – double-stranded RNA; miRNA – microRNA; siRNA – small interfering RNA; ssRNA – single-stranded RNA; ta-siARF – trans-acting siRNA specific for ARF gene.

INTRODUCTION

Trans-acting siRNAs (ta-siRNAs) are a plant specific class of endogenous siRNAs that function as posttranscriptional negative regulators. Like miRNAs, ta-siRNAs have been found to regulate gene expression in plants including the seed plants and moss lineages (reviewed in Baulcombe, 2004; Allen and Howell, 2010; Axtell, 2013; Martínez de Alba et al., 2013; Bologna and Voinnet, 2014). After transcription of the TAS gene, specific miRNAs bind to certain members of the Argonaute (AGO1 or AGO7) protein family and annealed to the ssRNA at miRNA recognition sites serving as cleavage points of TAS RNA. TAS3 genes contain two target sites for miR390. It was demonstrated that after the appearance

*Corresponding author: marialaur@mail.ru

of the miR390 family; 450 million years ago, duplication, divergence, and neofunctionalization gave rise to at least seven families of miRNAs in two major superfamilies, while still maintaining miR390 as an important miRNA (reviewed in Rogers and Chen, 2013). Thus, land plants have widely exploited the regulatory functions of miR390 to regulate large gene families.

In plants, most ta-siRNA- and miRNA-encoding loci comprise independent, non-protein-coding transcription units. These genes are transcribed by RNA polymerase II (pol II). The primary RNA transcripts contain cap structures as well as poly(A) tails. In some cases these genes contain introns. Like protein-coding genes, promoters of ta-siRNA and miRNA loci contain canonical *cis*-promoter elements, such as TATA box and transcription initiator, and various transcription factor responsive elements (reviewed in Allen and Howell, 2010; Axtell, 2013; Fei et al., 2013; Yoshikawa, 2013; Rogers and Chen, 2013; Bologna and Voinnet, 2014).

A. thaliana TAS3a and related plant TAS3 species are cleaved at the 3′ miR390 target site but not the 5′ miR390 target site, and ta-siRNAs are derived from the 5′ cleavage fragments. This process specifies formation of long dsRNA by RDR6 polymerase (plant RNA-dependent RNA polymerase) and SGS3 protein (RNA binding protein). Double-stranded RNA of TAS3 gene is then subjected to gradual and phased cleavage with DCL4 (specific endonuclease) assisted by DRB4 protein (dsRNA binding protein). Processing may be limited either by the length of the dsRNA region or the processing of DCL4. The resulting 21 bp ta-siRNAs are methylated by HEN1 protein (specific RNA methylase) (reviewed in Allen and Howell, 2010; Axtell, 2013; Fei et al., 2013; Yoshikawa, 2013; Bologna and Voinnet, 2014). The processing of the dsRNAs by DCL4/DRB4 begins from the miRNA cleavage site in a stepwise manner (Xie et al., 2005). The effective ta-siRNAs are phased in 21-nt increments, and the phasing register of TAS3 is set by the 3'-proximal miR390 binding site to define the region for ta-siRNAs production (Allen et al., 2005; Yoshikawa et al., 2005). In *Arabidopsis*, TAS3a with two near-identical 21-nt ta-siARFs, that co-aligned with the phasing registers D7 and D8 (starting from miR390-dependent cleavage site), was first identified by Allen et al. (2005). These ta-siARFs are bound by AGO1 and cause post-transcriptional cleavage of transcripts coding for ARF3 and ARF4, which are auxin response transcription factors and regulate juvenile to adult phase transition, and the establishment of auxin-mediated polarity of leaf, flower and lateral root growth (Adenot et al., 2006; Fahlgren et al., 2006; Garcia et al., 2006; Marin et al., 2010; Yoon et al., 2010; Yang et al., 2013). Two additional TAS3 family members with a single ta-siARF at D8 or D4 positions were subsequently found in *Arabidopsis* TAS3b and TAS3c, respectively (Howell et al., 2007). In general, the phasing registers may vary considerably between different TAS3 genes because of rather low sequence similarity except for ta-siARF sites and miR390 targeting sites (reviewed in Allen and Howell, 2010).

It is known that ta-siARFs promote adaxial identity through repression of ARF3 and ARF4, although the extent to which ta-siARFs contribute to adaxial identity specification varies in different

organisms (Adenot et al. 2006, Fahlgren et al. 2006, Garcia et al. 2006, Hunter et al. 2006; Husbands et al., 2009; Yamaguchi et al., 2010). In *Arabidopsis*, mutants in the ta-siRNA biogenesis pathway genes do not exhibit obvious lateral organ polarity defects, probably owing to the existence of parallel mechanisms to control adaxial identity (Garcia et al. 2006). In maize, mutations in a gene required for ta-siRNA biogenesis result in abaxialization of leaves (Nogueira et al. 2007). Ta-siARFs are localized on the adaxial side of leaf primordia and probably promote adaxial fate by restricting the expression of micro-RNAs miR165/166, perhaps indirectly through the ARFs. Importantly, two small RNAs, ta-si-ARF and miR165/166, show opposite polar distribution in leaf primordia and establish the adaxial-abaxial axis in leaf development (Nogueira et al., 2009). Further investigations have shown that the TAS3 ta-siRNA pathway also regulates both leaf margin development and lateral organ separation in *Medicago truncatula* (Zhou et al., 2013).

TAS3 and miR390 are well conserved in the moss *Physcomitrella patens* and vascular plants (reviewed in Rogers and Chen, 2013; Axtell, 2013; Zhang et al., 2014; Montes et al., 2014). Previously, we described the new method for identification of plant ta-siRNA precursors based on PCR with oligodeoxyribonucleotide primers mimicking miR390. The method was found to be efficient for dicotyledonous plants, cycads, conifers, and mosses (Krasnikova et al., 2009). Using this PCR-based approach as a phylogenetic profiling tool allowed us to reveal relatives of all four *P. patens* TAS3-like loci in several classes of moss species (Krasnikova et al., 2011; 2013). Interestingly, although moss TAS3-like loci code for ta-siARF species controlling ARF transcription factors homologous to those in flowering plants, other species of ta-siRNAs derived from all moss loci additionally target mRNAs of AP2 transcription factors (Allen and Howell, 2010; Krasnikova et al., 2013). We proposed an evolutionary scheme reflecting the change of TAS3 functional organization during the early plant evolution where significant evolutionary changes of TAS3 structure occurred during transition to vascular plants (Krasnikova et al., 2013). These changes resulted in the loss of ta-siRNA sequences specific for AP2 mRNAs. However, recent studies have revealed a remarkable evolutionary event that lead to substitution of the TAS3-coded AP2-specific ta-siRNAs by siRNAs of the new origin. This important observation has revealed that phased, secondary siRNAs are generated not only from non-coding TAS loci, but also from protein-coding transcripts (Zhai et al., 2011; Fei et al., 2013). These siRNAs, called phasiRNAs, derive from *PHAS* protein-coding genes that are primarily targeted by miRNAs.

Among *PHAS* loci which contained miRNA binding sites, the authors found an *APETALA2* (AP2) gene, which possessed one cleavage site for miR172. It seems the acquisition of the miRNA binding site in *AP2* genes has happened relatively recently in evolution of flowering plants (Zhai et al., 2011).

In this paper, we studied the presence of TAS3 genes in representatives of aquatic angiosperms belonging to order Alismatales. Aqueous habitats are associated with development of a number of specific physiological and morphological adaptations that are

distinctive from terrestrial angiosperms. For instance, underwater marine seagrass species (for example, *Zostera marina*) share a secondarily simplified morphology which includes horizontal rhizomes and strap-like leaves originating from a basal meristem. Additionally, seagrasses have reduced stamen and corolla (Kuo and den Hartog, 2006). On the other hand, freshwater free-floating duckweed displays an extreme morphological reduction and consists of minute fronds or thalli that bear only a few roots (Cabrera et al., 2008).

MATERIALS AND METODS

Plant material

Plant material, total plant DNAs from *Monstera deliciosa*, *Wolffia arrhiza* and *Lemna minor* belonging to family Araceae were taken from the collections of the Biological Faculty of M. V. Lomonosov Moscow State University and Centre "Bioengineering" of the Russian Academy of Sciences, Moscow, Russia.

Analysis of nucleic acids

Genomic plant DNA of *Spirodela polyrhiza* was isolated from 200 mg of fresh or dried plant material by DNA extraction kit (Macherey-Nagel) according to the protocol of the manufacturer. TAS3 genes were amplified and sequenced as described in our previous papers (Krasnikova et al., 2009, 2011).

Total RNA was isolated from green parts of plants with the Trizol reagent according to the manufacturer's instructions (Invitrogen). Digestion of any contaminating DNA was achieved by treatment of samples with RQI RNase-free DNase (Promega). Reverse transcription was performed with 1 μg of total RNA and oligo (dT)-primer t20-xho (attctcgaggccgaggcggccgacatgttttttttttttttttttttttttv) using the RT system (Invitrogen) according to the protocol of the manufacturer. Primers for PCR analysis were: forward primer TAS-P (5'-GGTGCTATCCTATCTGAGCTT-3') and one of two reverse primers - TAS-Mcaa (5'-AGCTCAGGAGGGATAGCAA-3') and TAS-Maca (5'-AGCTCAGGAGGG ATAGACA-3'). For PCR, 25–35 cycles were used for amplification with a melting temperature of 95°C, an annealing temperature of 58°C and an extending temperature of 72°C, each for 30 sec, followed by a final extension at 72°C for 3 min. PCR products were separated by electrophoresis of samples in 1.5% agarose gel and purified using the GFXTM PCR DNA and Gel Band Purification Kit (Amersham Biosciences). For cloning, the PCR-amplified DNA bands isolated from the gel were ligated into pGEM-T (Promega). Cloned products were used as templates in sequencing reactions with the ABI Prism BigDye Terminator Cycle Sequencing Ready Reaction Kit (Applied Biosystems).

Computer analysis and programs

TAS3-like sequences identified in this paper and found in the NCBI database were compared using multiple alignment tool at MAFFT (version 5.8; http://mafft.cbrc.jp/alignment/server). Nucleotide sequences were additionally analyzed at http://blast.ncbi.nlm.nih.gov/BlastAlign.cgi and http://bioinfo.jit.edu.cn/tasiRNADatabase/. The data on the abundance of the TAS3-related siRNAs were taken from *SoMART (*http://somart.ist.berkeley.edu). Search for the seagrass (*Zostera marina*) TAS3

transcripts was performed at Dr. Zompo server (http://drzompo.uni-muenster.de). DNA sequences obtained in this study were deposited at the NCBI data bank (*Monstera deliciosa* - KM396218; *Lemna minor* - KM396219 and KM396220; *Wolffia arrhiza* - KM396221, KM396222 and KM396223; *Spirodela polyrhiza* - KM396224, KM396225 and KM396226); the accession numbers of our previous entries are as follows: FJ804743 (*Nicotiana tabacum*), FJ804745 (*Solanum demissum*), FJ804751 (*Nicotiana tabacum*), JN692262 (*Curio repens*), JN692261 *(Senecio talinoides*), JN692260 (*Senecio talinoides*) and JN692259 (*Curio articulatus*). The accession numbers obtained by other authors are indicated in the text below.

RESULTS AND DISCUSSION

TAS3 genes in the genomes of duckweeds

PCR amplification of total DNA from *S. polyrhiza, W. arrhiza* и *L. minor* resulted in the synthesis of major bands of 150–250 bp (Fig. 1). Nevertheless, some minor size differences were revealed between the PCR products obtained with different M primers (Mcaa and Maca) (Fig. 1). Cloning and sequencing of the obtained DNA bands of *W. arrhiza* revealed that only 7 from the total 18 cloned amplified sequences contained well-conserved TAS3 region composed of ARF-specific sequence and located between miR390 target sites corresponding to PCR primers. To exclude the possibility of the PCR and cloning artifacts, we used multiple sequence alignment tool MAFFT and BLAST search (data not shown). As a result we found three different TAS3 species in *W. arrhiza* (Fig. 2). From two to six TAS3 species were also found in cereal monocots (Shen et al., 2009) and many other plant species (Krasnikova et al., 2013; http://bioinfo.jit.edu.cn/tasiRNADatabase/). In the case of *L. minor*, 5 from the total 14 cloned sequences contained a well-conserved TAS3 region, and two different TAS3 species were revealed (Fig. 2). In *S. polyrhiza*, among 22 sequenced clones only 12 contained TAS3 sequences representing three different structural

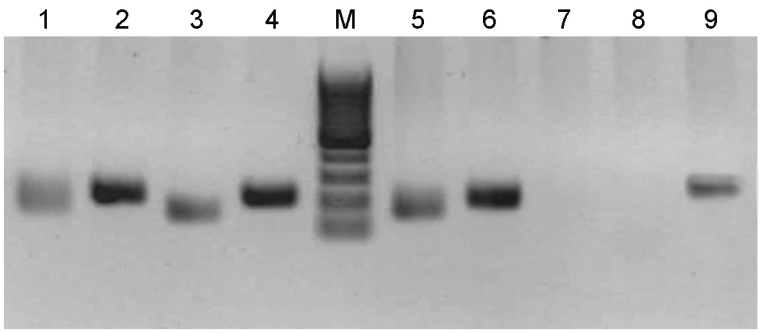

Figure 1. Analysis of PCR products in 1.5% agarose gel. PCR products were obtained on total plant DNAs with miR390-mimicking specific primers. *Spirodela polyrhiza* (1, 2), *Wolffia arrhiza* (3, 4), *Lemna minor* (control) (5, 6), *Selaginella kraussiana* (control) (7), *Arabidopsis thaliana* (control) (9), and primers with no template DNA (control) (8). (M), DNA size markers including bands ranging from 100 bp to 1000 bp with 100 bp step (Sibenzyme).

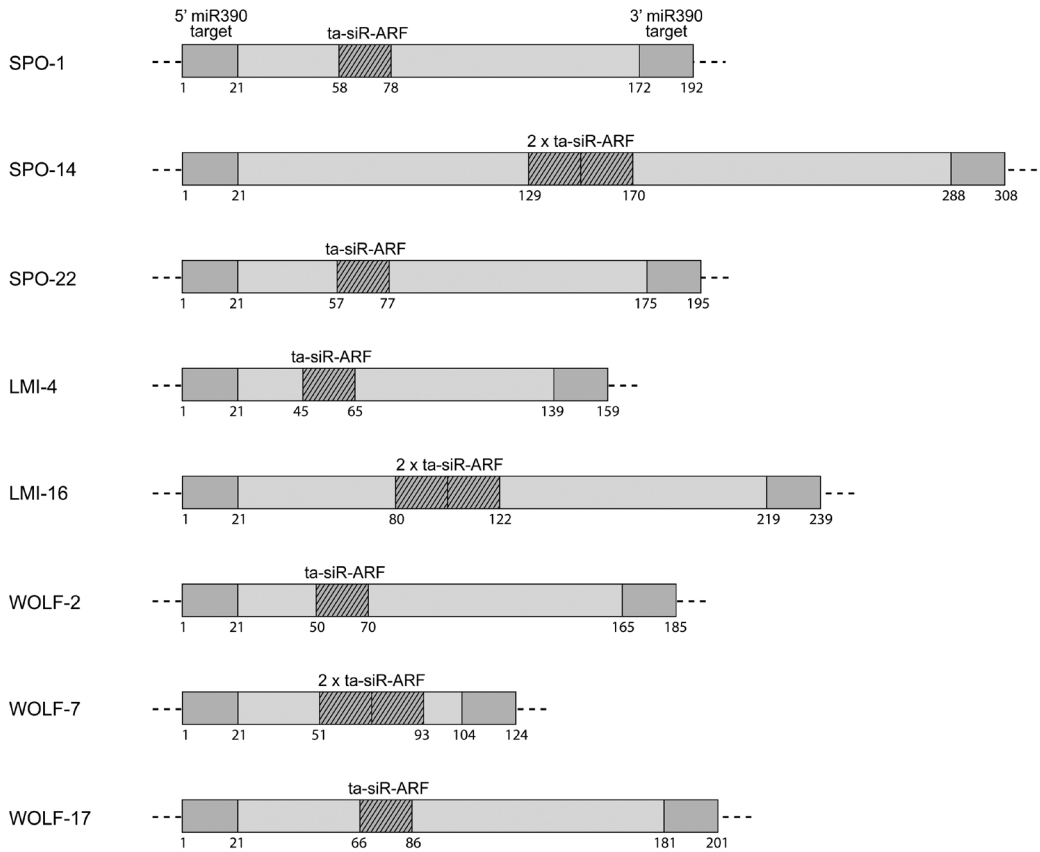

Figure 2. Comparison of TAS3 internal organization in plants (*Spirodela polyrhiza* - *SPO, Wolffia arrhiza* - WOLF and *Lemna minor* - LMI) belonging to family Araceae. Boxes represent the functional regions of TAS3 genes including miR390-targeting sites and ta-siARF sites. Numbers below boxes show relative nucleotide positions of miR390 target sites and ta-siRNAs.

classes (Fig. 2).

In December 2013, NCBI released the sequence data on the genome of *S. polyrhiza* obtained in the frame of the project "The *Spirodela polyrhiza* whole genome shotgun" (Waksman Institute, USA). We found that all three TAS3 sequences revealed in this paper had obvious homologs (99.5% identity) among genomic sequences determined in the frame of the above project (see GeneBank accession numbers SRR497624.214636.2, ATDW01013065.1,SRR072268.818059.2, ATDW01004610.1) (data not shown). Minor differences may represent the results of differences between plant isolates or/and sequence errors.

"Canonical" and "non-canonical" TAS3 structure types in duckweeds

We have shown that only part of the amplified TAS3 sequences in the plant from family Solanaceae contain "canonical" ta-siARF site composed of two tandem copies of ARF-specific sequence blocks typical for *A. thaliana* TAS3a species (Krasnikova et al., 2009; Fei et al., 2013). The other Solanaceae clones revealed the

novel TAS3-like sequences possessing ta-siARF site composed of the only copy of ARF-specific sequence ("non-canonical" site) (Krasnikova et al., 2009). Occurrence of two TAS3 structural types were then confirmed for other dicots (Xia et al., 2012; Krasnikova et al., 2013; Yang et al., 2013; Fei et al., 2013), as well as for some monocots (Shen et al., 2009; Liu et al., 2013) (see also http://bioinfo.jit.edu.cn/tasiRNADatabase/). Usually, the length of "canonical" TAS3 sequences is obviously larger than that of "non-canonical" loci (Krasnikova et al., 2009) (Fig. 2). Strikingly, in *W. arrhiza*, the length of the "canonical" TAS3 (WOLF-7) was considerably smaller than that of "non-canonical" species (WOLF-2 и WOLF-17) (Fig. 2 and 3). However, consensus sequence of ta-siARF block was well

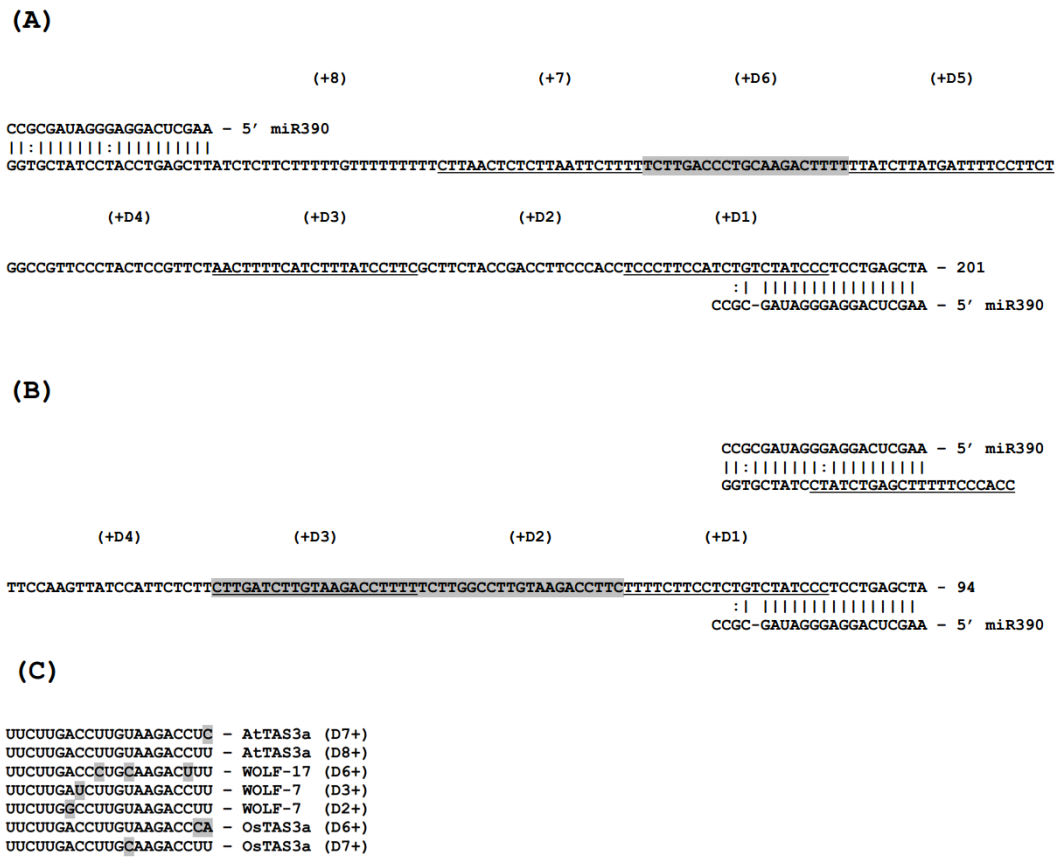

Figure 3. (A) Nucleotide sequence of WOLF-17 TAS3 locus. The 5′ and 3′ miR390 target sites are shown as alignments. Predicted Dicer-generated siRNA registers between processing sites are shown by underlining. TAS3 siRNA which is complementary to the auxin response factors are indicated by shading (register - +D6). **(B)** Nucleotide sequence of WOLF-7 TAS3 locus. The 5′ and 3′ miR390 target sites are shown as alignments. Predicted Dicer processing sites are shown by underlining. TAS3-siRNAs that are complementary to the auxin response factors are indicated by shading (+D3) and (+D2). **(C)** Sequence alignment of ta-siARF RNAs from the TAS3 species of *W. arrhiza* and selected TAS3 species from *A. thaliana* and rice (*Oryza sativa*). Minor nucleotide deviations in the alignment are shaded.

conserved in WOLF-7 in comparison with "canonical" TASs in rice and *A. thaliana* (Fig. 3C).

Identification of TAS3 RNA precursors in the representatives of order Alismatales

To study the possible differences in expression of "canonical" and "non-canonical" TAS3 genes, we first isolated total RNA from whole plants of *L. minor* and expanded leaves of *Monstera deliciosa* which is a terrestrial plant from family Araceae. The RNA preparation was used for reverse transcription with oligo(dT)-primer. Then, cDNA was used as template for PCR with primers TAS-P and t20-Xho (see Materials and Methods). These experiments resulted in identification of the "canonical" TAS3 transcripts only (MON-14 and LMI-16 for *M. deliciosa* and *L. minor*, respectively) (Fig. 4). Bioinformatic analysis of EST libraries at NCBI Genebank revealed only "canonical" TAS3 transcript in one more representative of Araceae, *Zantedeschia aethiopica* (Zae-6754, GenBank accession GAOT01006754) (Fig. 4). A similar result was obtained in our previous analysis of TAS3 expression of fully developed leaves of tobacco and succulent plants from tribe Senecioneae (Krasnikova et al., 2009; Ozerova et al., 2013). However, we unexpectedly revealed only one "non-canonical" TAS3 in EST library of duckweed *Wolffia australiana* (WOA-JZ078031, GenBank accession JZ078031) (Fig. 4). On the other hand, the database in

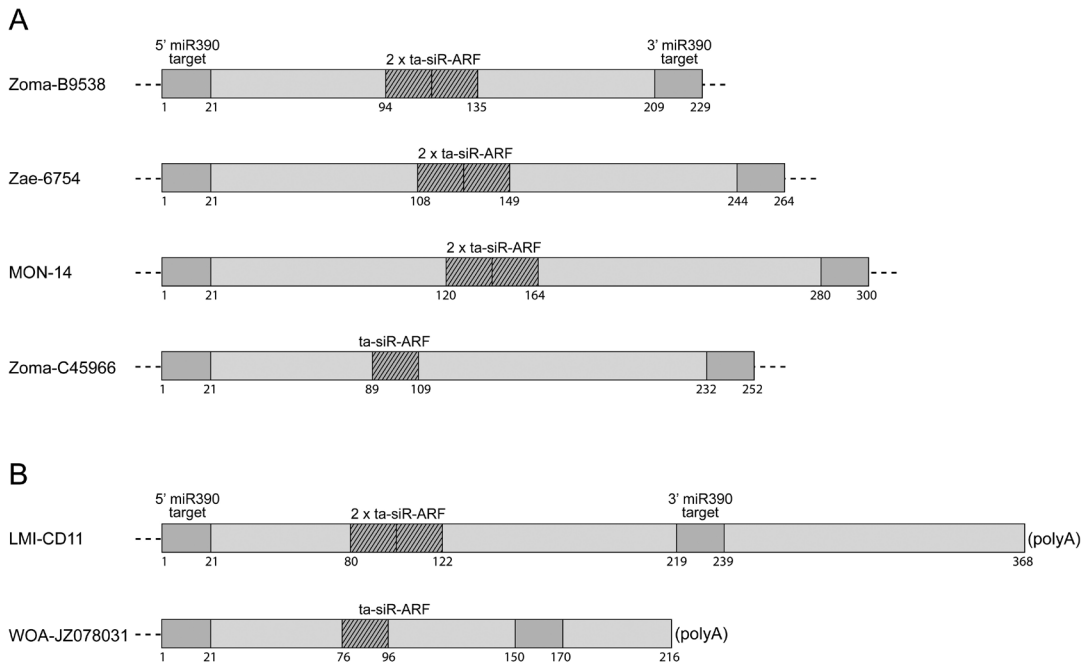

Figure 4. (A) Comparison of TAS3 transcripts in plants (*Zostera marina - Zoma,* and *Monstera deliciosa* - MON) belonging to order Alismatales. Boxes represent the functional regions of TAS3 genes including miR390-targeting sites and ta-siARF sites. Numbers below boxes show relative nucleotide positions of miR390 target sites and ta-siRNAs. **(B)** Comparison of the 3'-terminal area of TAS3 transcripts in duckweed plants (*Lemna minor - LMI,* and *Wolffia australiana* - WOA).

the frame of the project Dr. Zompo (http://drzompo.uni-muenster.de) revealed RNA transcripts for both "canonical" and "non-canonical" TAS3 genes (Zoma-B9538 and Zoma-C45966, respectively) (Fig. 4).

Expression peculiarities of TAS3 RNA precursors in the representatives of order Alismatales

Global transcriptome next-generation sequencing studies in many plant species have shown significant deviations in the pools of the different ta-siRNAs and miRNA classes in various tissues and organs (Egan et al., 2012) (http://smallrna.udel.edu). In general, comprehensive analysis can be compiled on variations in these small RNAs present in a large population of dozens of millions sequences derived from several libraries representing multiple tissues/organs of the plant. Recently, SoMART, a web server for miRNA/ta-siRNA Analysis Resources and Tools, was designed for researchers who are interested in identifying miRNAs or ta-siRNAs (Li et al., 2012). Some of the small RNAs were very abundant in certain tissues. For example, some miRNAs and ta-siRNAs showed preferential expression in either of the flowers, germinating cotyledons, stems, or leaves (Strickler et al., 2012; Zhu et al., 2012; Jagadeeswaran et al., 2012; Xia et al., 2012; Li et al., 2012). Thus, our data on the differences in expression peculiarities of TAS3 RNA precursors in the representatives of order Alismatales are in agreement with the published data and suggest the potential impact of expression deviations rather than the structures of TAS3 genes in the specific physiological and morphological adaptations of these aquatic plants.

Expression peculiarities of TAS3 RNA precursors in the representatives of family Solanaceae

To go deeper insight the expression deviations between TAS3 genes, we also used the data available at SoMART (http://somart.ist.berkeley.edu) for comparative bioinformatic analysis of tissue-specific expression between "canonical" and "non-canonical" TAS3 genes of family Solanaceae (Krasnikova et al., 2009). Mapping of siRNA reads against Solanaceae *TAS3* revealed ta-siRNA generation region between miR390 dual target sites (data not shown). Mostly 21-nt ta-siRNAs were generated in the flower tissue, which correlated with flower-specific expression of miR390 (see Zhu et al., 2012). It was found that abundance of siRNAs originating from "non-canonical" TAS3 tobacco genes in flowers was 9-fold higher than abundance of "canonical" siRNAs. Moreover, these differences were even more for TAS3 genes of *Solanum demissum* (11-fold prevalence for "non-canonical" siRNAs (data not shown). Thus, our bioinformatic analysis confirmed different levels of tissue-specific expression of distinct TAS3 genes.

CONCLUDING REMARKS

In plants, ta-siRNAs and miRNAs are both highly conserved and rapidly evolving, and these features have been characterized in a variety of lineages. While extensive research has been carried out on model plants, less is known about the characterization and functional analysis of miRNAs from plant species of no agricultural and horticultural significance such as aquatic plants belonging to family

Araceae (duckweeds). Our earlier studies reported computational and experimental identification of many TAS3 and miR390 loci from different plant species including mosses and flowering plants (Krasnikova et al., 2009; 2013; Ozerova et al., 2013).

In the present work, we provided a comprehensive analysis of ta-siRNA genes (TAS3) in family Araceae. Like the model plant systems (*Arabidopsis* etc), duckweeds have two or even more conserved TAS3 genes with similar structural features responsible for *trans*-acting siRNA formation. Some of the identified TAS3 RNAs were found to be tissue-specific, as previously observed for other plant species (see references above). We hypothesize that known ta-siRNA targets, mostly ARF transcription factors, may control specific physiological processes and genetic programs associated with aquatic habitats. Thus distinct TAS3 RNAs produced in different tissues and characterized by their specific expression patterns may also be involved in morphogenesis. Since auxin signaling and modulation is essential for diverse biological processes in all flowering plants, miR390-*TAS3*-derived ta-siARFs in specific tissues could orchestrate auxin signaling that could be directly relevant to growth and development of aquatic plants. We hope that the research directions presented here stimulate further studies focused on better understanding of the TAS3 evolution in the highly morphologically specialized groups of flowering plants.

ACKNOWLEDGEMENTS

The authors acknowledge Dr. N.V. Ravin and Dr. E.Z. Kochieva (Centre "Bioengineering' of RAS) for providing total plant DNAs.

REFERENCES

Adenot X, T Elmayan, D Lauressergues, S Boutet, N Bouche, V Gasciolli, H Vaucheret , 2006. DRB4-dependent TAS3 trans-acting siRNAs control leaf morphology through AGO7. Curr Biol, 16: 927–932.

Allen E, Z Xie, A Gustafson, J Carrington, 2005. MicroRNA-directed phasing during trans-acting siRNA biogenesis in plants. Cell 121, 207–221.

Allen E, M Howell 2010, miRNAs in the biogenesis of trans-acting siRNAs in higher plants. Seminars in Cell and Developmental Biology, 21: 798–804.

Axtell M, 2013. Classification and comparison of small RNAs from plants. Annu Rev Plant Biol, 64: 137–159.

Baulcombe D, 2004. RNA silencing in plants. Nature, 431: 356–363.

Bologna N, O Voinnet, 2014. The Diversity, Biogenesis, and Activities of Endogenous Silencing Small RNAs in Arabidopsis. Annu Rev Plant Biol, 65: 473–503.

Cabrera L, G Salazar, M Chase, S Mayo, J Bogner, P Dávila, 2008. Phylogenetic relationships of aroids and duckweeds (Araceae) inferred from coding and noncoding plastid DNA. Am J Bot, 95:1153–1165.

Egan A, J Schlueter, D Spooner, 2012. Applications of next-generation sequencing in plant biology. Am J Bot, 99: 175–185.

Fahlgren N, T Montgomery, M Howell, E Allen, S Dvorak, A Alexander, J Carrington, 2006. Regulation of AUXIN RESPONSE FACTOR3 by TAS3 ta-siRNA affects developmental timing and patterning in Arabidopsis.

Curr Biol, 16: 939–944.

Fei Q, R Xia, B Meyers, 2013. Phased, secondary, small interfering RNAs in posttranscriptional regulatory networks. Plant Cell, 25: 2400–2415.

Garcia D, S Collier, M Byrne, R Martienssen, 2006. Specification of leaf polarity in Arabidopsis via the trans-acting siRNA pathway. Curr Biol, 16: 933–938.

Howell M, N Fahlgren, E Chapman, J Cumbie, C Sullivan, S Givan, K Kasschau, J Carrington, 2007. Genome-wide analysis of the RNA-DEPENDENT RNA POLYMERASE6/DICER-LIKE4 pathway in Arabidopsis reveals dependency on miRNA- and tasiRNA-directed targeting. Plant Cell, 19: 926–942.

Hunter C, M Willmann, G Wu, M Yoshikawa, M de la Luz Gutierrez-Nava, S Poethig, 2006. Transacting siRNA-mediated repression of *ETTIN* and *ARF4* regulates heteroblasty in *Arabidopsis*. Development, 133: 2973–2981.

Husbands A, D Chitwood, Y Plavskin, M Timmermans, 2009. Signals and prepatterns: new insights into organ polarity in plants. Genes Dev, 23: 1986–1997.

Jagadeeswaran G, P Nimmakayala, Y Zheng, K Gowdu, U Reddy, R Sunkar, 2012. Characterization of the small RNA component of leaves and fruits from four different cucurbit species. BMC Genomics, 13: 329.

Krasnikova M, I Milyutina, V Bobrova, A Troitsky, A Solovyev, S Morozov, 2009. Novel miR390-dependent transacting siRNA precursors in plants revealed by a PCR-based experimental approach and database analysis. Journal of Biomedicine and Biotechnology, Article ID 952304. doi: 10.1155/2009/952304.

Krasnikova M, I Milyutina, V Bobrova, L Ozerova, A Troitsky, A Solovyev, S Morozov, 2011. Molecular diversity of mir390-guided trans-acting siRNA precursor genes in lower land plants: experimental approach and bioinformatics analysis. Sequencing, Article ID 703683. doi: 10.1155/2011/703683.

Krasnikova M, D Goryunov, A Troitsky, A Solovyev, L Ozerova, S Morozov, 2013. Peculiar evolutionary history of miR390-guided TAS3-like genes in land plants. Scientific World Journal, Article ID 924153. doi: 10.1155/2013/924153.

Kuo J, C den Hartog, 2006. Seagrass Morphology, Anatomy, and Ultrastructure. In the *Seagrasses: Biology, Ecology and Conservation.* (eds. A.W.D. Larkum, R.J. Orth, C.M. Duarte), Springer, Dordrecht, Netherlands, 51–87.

Li F, R Orban, B Baker, 2012. SoMART, a web server for plant miRNA, tasiRNA and target gene analysis. Plant J, 70: 891–901.

Liu Y, Y Wang, Q Zhu, L Fan, 2013. Identification of phasiRNAs in wild rice (Oryza rufipogon). Plant Signal Behav, 8: e25079.

Marin E, V Jouannet, A Herz, A Lokerse, D Weijers, H Vaucheret, L Nussaume, M Crespi, A Maizel, 2010. miR390, Arabidopsis TAS3 tasiRNAs, and their AUXIN RESPONSE FACTOR targets define an autoregulatory network quantitatively regulating lateral root growth. Plant Cell, 22:

1104–1117.

Martínez de Alba A, E Elvira-Matelot, H Vaucheret, 2013. Gene silencing in plants: a diversity of pathways. Biochim Biophys Acta, 1829: 1300-1308.

Montes R, F de Fátima Rosas-Cárdenas, E de Paoli, M Accerbi, L Rymarquis, G Mahalingam, N Marsch-Martínez, B Meyers, P Green, S de Folter, 2014. Sample sequencing of vascular plants demonstrates widespread conservation and divergence of microRNAs. Nat Commun, 5: 3722.

Nogueira F, S Madi, D Chitwood, M Juarez, M Timmermans, 2007. Two small regulatory RNAs establish opposing fates of a developmental axis. Genes Dev, 21: 750–755.

Nogueira F, D Chitwood, S Madi, K Ohtsu, P Schnable, 2009. Regulation of Small RNA Accumulation in the Maize Shoot Apex. PLoS Genet, 5, e1000320.

Ozerova L, M Krasnikova, A Troitsky, A Solovyev, S Morozov, 2013. TAS3 genes for small ta-siARF RNAs in plants belonging to subtribe Senecioninae: occurrence of prematurely terminated RNA precursors. Mol Gen Mikrobiol Virusol (Moscow), 28: 79-84.

Rogers K, X Chen, 2013. Biogenesis, turnover, and mode of action of plant microRNAs. Plant Cell, 25: 2383-2399.

Shen D, S Wang, H Chen, Q Zhu, C Helliwell, L Fan, 2009. Molecular phylogeny of miR390-guided trans-acting siRNA genes (TAS3) in the grass family. Plant Syst Evol, 283: 125-132.

Strickler S, A Bombarely, L Mueller, 2012. Designing a transcriptome next-generation sequencing project for a nonmodel plant species. Am J Bot, 99: 257–266

Xia R, H Zhu, Y An, E Beers, Z Liu, 2012. Apple miRNAs and tasiRNAs with novel regulatory networks. Genome Biol, 13: R47.

Xie Z, E Allen, A Wilken, J Carrington, 2005. DICER-LIKE 4 functions in *trans*-acting small interfering RNA biogenesis and vegetative phase change in *Arabidopsis thaliana*. Proc Natl Acad Sci USA, 102: 12984–12989.

Yamaguchi T, S Yano, H Tsukaya, 2010. Genetic framework for flattened leaf blade formation in unifacial leaves of Juncus prismatocarpus. Plant Cell, 22: 2141–2155.

Yang X, L Wang, D Yuan, K Lindsey, X Zhang, 2013. Small RNA and degradome sequencing reveal complex miRNA regulation during cotton somatic embryogenesis.

J Exp Bot, 64: 1521–1536.

Yoon E, J Yang, J Lim, S Kim, S Kim, W Lee, 2010. Auxin regulation of the microRNA390-dependent transacting small interfering RNA pathway in Arabidopsis lateral root development. Nucleic acids research, 38: 1382–1391.

Yoshikawa M, A Peragine, M Park, R Poethig, 2005. A pathway for the biogenesis of trans-acting siRNAs in *Arabidopsis*. Genes Dev, 19: 2164–2175.

Yoshikawa M, 2013. Biogenesis of *trans*-acting siRNAs, endogenous secondary siRNAs in plants. Genes Genet Syst, 88: 77–84.

Zhai J, D Jeong, E De Paoli, S Park, B

Rosen, Y Li, A González, Z Yan, S Kitto, M Grusak, S Jackson, G Stacey, D Cook., P Green, D Sherrier, B Meyers, 2011. MicroRNAs as master regulators of the plant NB-LRR defense gene family via the production of phased, trans-acting siRNAs. Genes Dev, 25: 2540–2553

Zhang C, G Li, S Shinong Zhu, S Zhang, J Fang, 2014. tasiRNAdb: a database of ta-siRNA regulatory pathways. Bioinformatics, 30: 1045–1046.

Zhou C, L Han, C Fu, J Wen, X Cheng, J Nakashima, J Ma, Y Tang, Y Tan, M Tadege, K Mysore, G Xia, Z Wang, 2013. The Trans-Acting Short Interfering RNA3 Pathway and NO APICAL MERISTEM Antagonistically Regulate Leaf Margin Development and Lateral Organ Separation, as Revealed by Analysis of an argonaute7/lobed leaflet1 Mutant in Medicago truncatula. Plant Cell, 25: 4845-4862

Zhu H, R Xia, B Zhao, Y An, C Dardick, A Callahab, Z Liu, 2012. Unique expression, processing regulation, and regulatory network of peach (*Prunus persica*) miRNAs. BMC Plant Biol, 12: 149.