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Original article

Identification of new miRNAs in rapeseed (*Brassica napus* L.) and their role in suppressing target genes

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Extended abstract

Introduction

MicroRNAs(miRNAs) are a group of small non-coding RNAs of approximately 18 - 24 nucleotides that play a negative role in post-transcriptional changes in eukaryotes. The miRNAs are then loaded onto the argonate family proteins (AGO) to form a protein complex (RISC). The primary activity of the RISC complex is to direct the mature miRNA to the target RNA and to stop protein production (Megah et al., 2018). miRNAs are involved in response to abiotic stresses in plants such as drought; several miRNA families have been reported in response to drought stress in rice, tomato, Arabidopsis, Medicago truncatula, peach, barley and wheat (Akdogan et al., 2015). The purpose of this study was to identify new microRNAs and their role in suppressing and preventing expression of some of their target genes in rapeseed.

Materials and Methods

A total of 38589 known mature miRAN sequences were downloaded from the miRBase database. The miRNA sequences were used as known sequences to find conserved miRNAs based on homology search for miRNAs with rapeseed GSS sequences.103369 GSS for rapeseed was downloaded from NCBI database. Mature miRNA sequences were uploaded to the BLASTn algorithm to search for homology with rapeseed GSSs in Linux. The miRNA sequences as known sequences and the GSS sequences as sequences were compared with each other for homology search. GSSs with mature miRNA sequences up to four mismatches were selected as candidates (Zhang, 2005). GSS sequences were used instead of EST sequences because miRNAs can generate GSS sequences in addition to EST sequences. Consequently, GSSs were sequenced between the BLASTx miRNA sequences and the GSS coding sequences were deleted and only non-coding GSS sequences remained (Karimi et al., 2017). Mfold software was used to predict the secondary structure of candidate miRNAs (Vivek, 2018). ath-miR5021 and ath-miR8175 from Arabidopsis, bol-miR9410 and bol-miR9411 from wild cabbage and cas-miR11592 from Camelina sativa were selected from the psRNATarget website (Dai and Zhao, 2011.

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Results

For miR5021: The NST1 target gene encodes a protein called Stress response protein NST1, which plays a role in regulating secondary wall thickness in plants and preventing its destruction against a variety of stresses (Mitsuda et al., 2005). For miR9410: The HST gene is one of the target genes that encodes an enzyme called Shikimate O-hydroxycinnamoyltransferase in plants, which participates in the phenylpropanoid biosynthesis and heat stress control in plants, propanoids as secondary metabolites during developmental stages. The plant is synthesized in response to stress conditions (Lukasik et al., 2013).

Discussion

Types of microRNAs and their role in suppressing target genes during live and abiotic stresses in barley, wheat, soybean, cucumber, alfalfa, olive, rice have been reported (Ozhuner et al., 2013). In this study, we tried to identify new microRNAs and their role in suppressing target genes for the first time in rapeseed. The results of this study showed that among the newly identified microRNAs, miR5021 and miR9410 families play an important role in suppressing NST1 and HST target genes, respectively, especially during stress in canola.. Therefore, identifying the molecular mechanism of these microRNAs and their target genes can help us in selecting drought and heat resistant varieties for rapeseed. A study of microRNAs for boron stress tolerance in leaves and roots of barley showed that of the four new microRNAs identified, miR408 was more involved in regulating cell signaling in leaves than the other three microRNAs (Ozhuner et al., 2013). Also, no response has been reported in hybrid and maize inbred lines for miR172 under drought and salt stress conditions (Kong et al., 2010). Therefore, understanding the cellular regulation mechanism for new microRNAs, including how to regulate the activity pathway of antioxidant enzymes such as superoxide dismutase in organs such as leaves, roots and shoots of canola, requires further investigation.

Conclusions

MicroRNAs can be used as a new molecular tool alongside existing classical breeding methods to improve the genetic status of plants to promote tolerance to a variety of biotic and abiotic stresses in plant breeding.

***Keywords*:** Bioinformatics, Eukaryote, GSS sequence, Rapeseed, Target Gene, Target mRNA

Table 1. New miRNAs based on GSS sequences in *Brassica napus*

miRNA	Accession	Identity (%)	L-miRNA (nt)	mismatch	Gap
miR5021	DU105547	100	18	0	0
miR11592	DU099375	100	19	0	0
miR8175	DU099594	100	18	0	0
miR9410	DU103199	100	19	0	0
miR9411	DU107859	95.45	22	0	0

Table 1. Continued

Start-miRNA(nt)	End-miRNA(nt)	Start-GSS Seq	End-GSS Seq	E-value	bite score
1	18	367	384	8×10^{-4}	36
1	19	745	763	2×10^{-4}	38
3	20	547	564	8×10^{-4}	36
2	20	143	161	3×10^{-4}	38
1	22	175	199	1×10^{-4}	36

Table 2. Characteristics of newly identified miRNA in Brassica napus

GSS ID	Pre-miRNA	LS (5' – 3')	LP	LM	(A+U) %	(G+C) %	MFE	AMFI
DU105547	miR5021	UGAGAAGAAGAAGA AGAAAA	171	20	63.74	36.26	-47.6	-77.23
DU099375	miR11592	GAACCGAACCGAA CCGAAA	84	19	65.48	34.54	-16.6	-58.12
DU099594	miR8175	GAUCCCCGGCAACGGCGCCA	189	20	49.74	50.26	-127.6	-135.02
DU103199	miR9410	UACUUAAUUAUAAGUCGUCUGG	98	22	60.2	39.8	-45.4	-118.79
DU107859	miR9411	UACUGGACGACUUA CACGGAAG	94	22	62.77	37.23	-49.9	-143.49

MFEs: minimal folding free energies (kcal mol-1). LP: length of pre-miRNA. LM: length of mature miRNAs. MFEIs: minimal folding free energy indices. GSS ID: Genome Survey Sequences. LS: Length of miRNA Sequence

Table 3. Type and Percentage of organic Bases in miRNAs Precursor for *Brassica napus*

GSS Accession	miRNA Precursor	LP-miRNA Precursor	Type and Percentage of Organic Bases					
			A	T/U	G	C	GC%	AU%
DU105547.1	**miR5021**	171	45	64	46	16	36.26	63.74
DU099375.1	**miR11592**	84	25	30	14	15	34.52	65.48
DU099594.1	**miR8175**	189	49	45	44	51	50.26	49.74
DU103199.1	**miR9410**	98	28	31	19	20	39.8	60.2
DU107859.1	**miR5021**	171	29	30	17	18	37.23	62.77

LP: length of miRNA precursor. GSS ID: Genome Survey Sequences.

Table 4. Predicted gene targets for candidate miRNAs in Brassica napus

miRNA	Target protein	Biological function	Target gene	Target accession
ath-miR5021	CCR4-NOT transcription complex subunit 11	Organize the protein-protein interactions platform, total scaffolding, the evolution of sexual organs in plants, RNA degradation and gene expression regulation.	CNOT11	BnaC09g39370D
	Stress response protein NST1	Adjustment of secondary wall thickness and prevent it's destruction against all kinds of living and non-living stresses in plants.	NST1	BnaA09g32160D
ath-miR8175	CBL-interacting serine/threonine-protein kinase 26	Specific signal transmission in cells, Regulating cell cycle progression and transcriptional activation processes of cellular signaling.	CIPK26	BnaC09g36730D
bol-miR9410	Shikimate O-Hydroxycinnamoyltransferase	Participate in the biosynthesis of phenylpropanoids.	HST	BnaC01g42010D
	LOB domain-containing protein 41	Regulation of gene transcription from DNA template strands, Contribute to post-translation and transcription settings.	LBD41	BnaC08g26570D
bol-miR9411	: O-acyltransferase (WSD1-like) family protein	In processes such as glycerol lipid biosynthesis and cuticular wax biosynthesis is involved.	WSD1	BnaA05g02390D
cas-miR11592	Ubiquitin-conjugating enzyme E2 variant 1D	Cell cycle process and its differentiation, DNA repair when transcription errors, Cell survival after DNA damage.	UEV1D	BnaC08g23790D
	Luc7-like protein 3	Binds to cAMP and acts as a regulatory element for DNA sequencing, It is involved in RNA splicing, mRNA and mRNA production processes.	LUC7L3	BnaA10g08020D

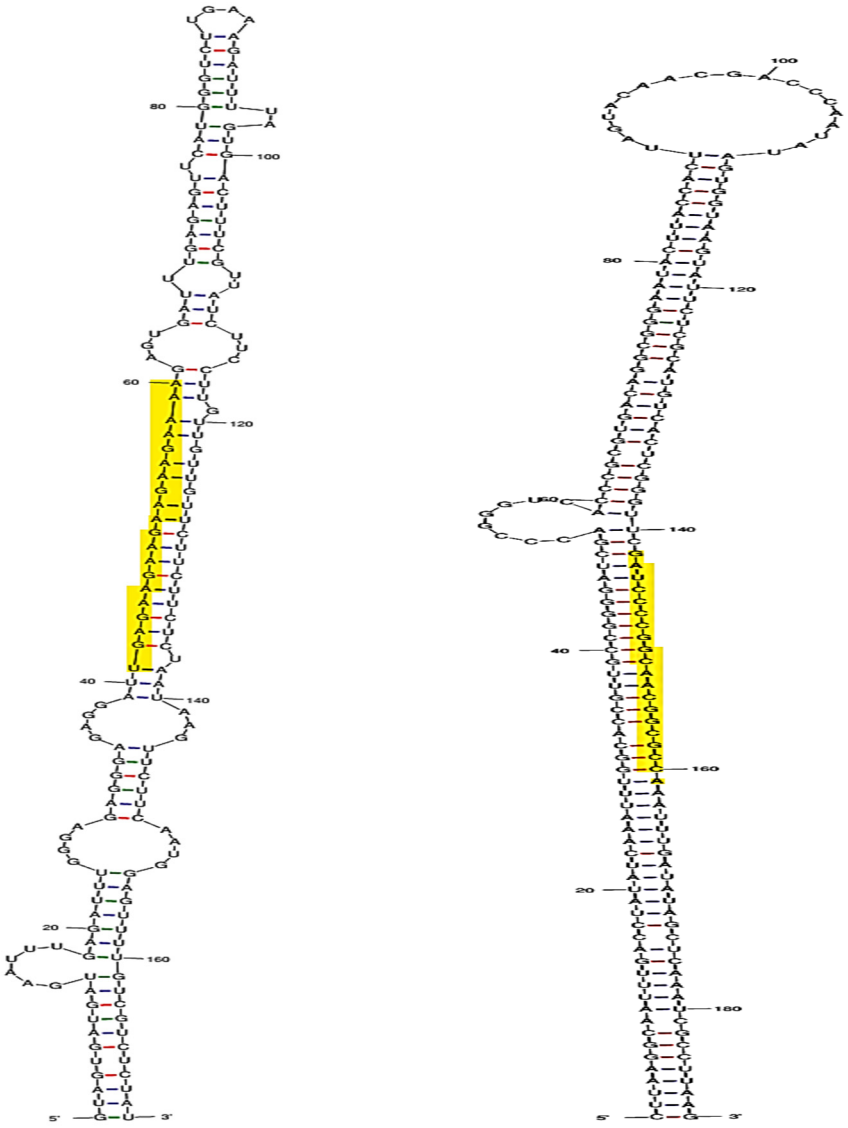
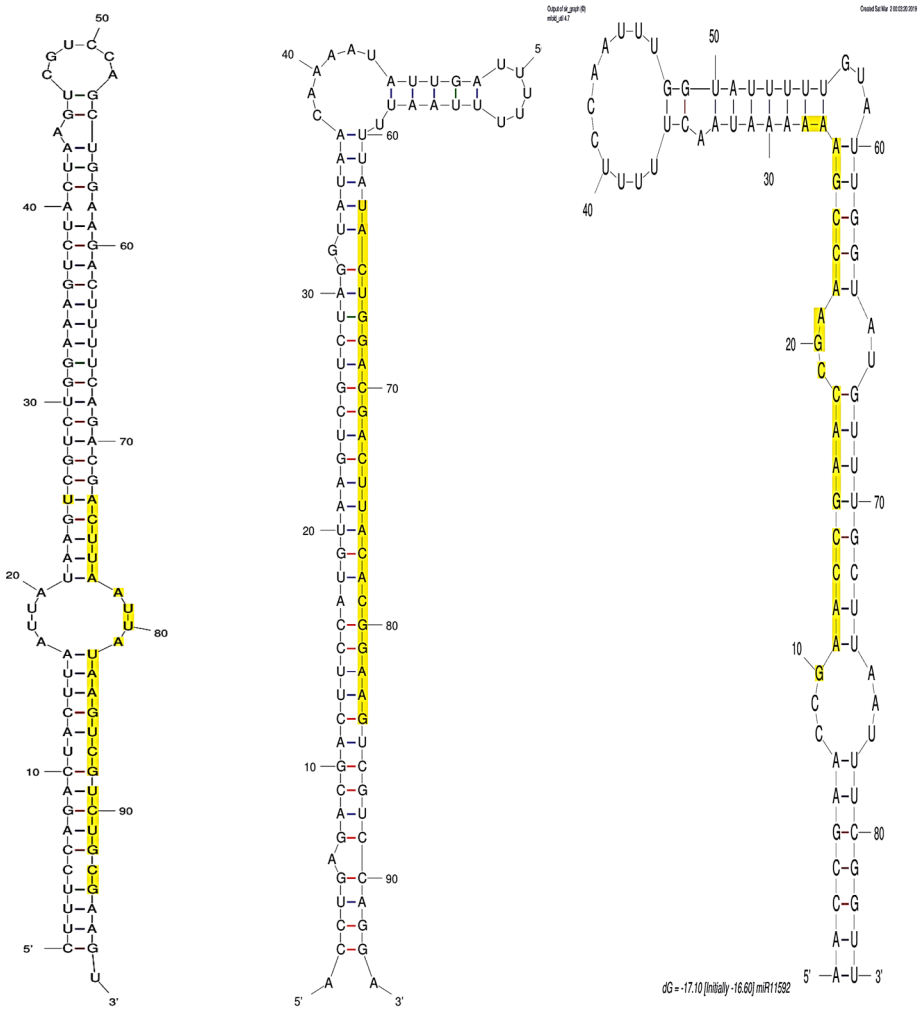


Fig. 1. Secondary stem-loop precursor structures of new miRNAs identified in rapeseed, the mature part of miRNA highlighted in yellow



miR9410 **miR9411** **miR11592**

T Fig. 2. Secondary stem-loop precursor structures of new miRNAs identified in rapeseed, the mature part of miRNA highlighted in yellow