Datos suplementarios

Exploración de la regulación postranscripcional por miR-528 en la resistencia del arroz al virus de la hoja blanca

Tabla S1. Lista de patosistemas, microRNAs asociados y sus targets.

Patógeno viral *	**Precursor**	**Locus del precursor**	**mRNA targets (MSU ID)**	**Fuente**	**Gene Target**
Rice Stripe Virus (RSV)	miR528	Chr3:1667328-1667415 [+]	LOC_Os06g37150 LOC_Os01g24880.2 LOC_Os06g06050 LOC_Os08g42640	[1] [2] [6] [6] [6] [11]	OsAAO2 OsVIP2 OsDWARF3 OsRFI2
Rice Stripe Virus (RSV)	miR528	Chr3:1667328-1667415 [+]	LOC_Os11g01869	[3] [10]	OsDRB1
Rice Stripe Virus (RSV)	miR528	Chr3:1667328-1667415 [+]	LOC_Os02g45070	[2]	OsAGO1a
Rice Stripe Virus (RSV)	miR166c-3p	Chr3:3487788-3487912 [+]	LOC_Os07g06130	[4]	OsEIN2
Rice Stripe Virus (RSV)	miR160a	Chr2:3511585-3511672 [-]	LOC_Os06g47150.3	[5]	HD-ZIP III
Rice Stripe Virus (RSV)	miR167a	Chr12:25476808-25476948 [+]	LOC_Os04g57610	[6]	ARF8
Rice Stripe Virus (RSV)	miR156b	Chr1:4666341-4666516 [+]	LOC_Os04g46580	[6]	SCARECROW
Rice Stripe Virus (RSV)	miR1425	Chr5:8862149-8862250 [+]	LOC_Os11g15060	[6]	SAM-dependent_MTases
Rice Dwarf Virus (RSV)	miR172d-3p	Chr1:42923736-42923973 [-]	LOC_Os06g43220.1	[6]	OsAP2
Rice Stripe Virus (RSV)	miR168a-5p	Chr2:1553154-1553240 [-]	LOC_Os02g45070	[7]	OsAGO1a
Rice Ragged Stunt Virus (RRSV)	miR319a-5p	Chr1:26823282-26823472 [-]	LOC_Os07g05720	[8]	OsTCP21
Rice Stripe Virus (RSV)	miR171b	Chr8:5369153-5369252 [+]	LOC_Os02g44370 LOC_Os05g33730 LOC_Os03g49990	[9] [13] [6] [12] [13]	OsSCL6-IIa OsGID1 OsGAI

*****Todos los experimentos y literatura consultada usaron como modelo *Oryza sativa* subsp. *japonic*a cv. Nipponbare

Tabla S2. miRNAs escogidos para este estudio

microRNA	Locus del precursor	mRNA targets	Gene target
osa-miR-528	Chr3:1667328-1667415 [+]	LOC_Os06g37150 LOC_Os01g24880.2 LOC_Os06g06050 LOC_Os08g42640	*L*-Ascorbate Oxidase OsVIP2 OsDWARF3 OsRFI2
osa-miR-171b	Chr8:5369153-5369252 [+]	LOC_Os02g44370 LOC_Os05g33730 LOC_Os03g49990	OsSCL6-IIa OsGID1 OsGAI

Secuencias codificantes de genes candidatos

Secuencia precursora de osa-miR-528

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>AP014959.1:1667329-1667415 Oryza sativa Japonica, chromosome 3, cultivar:
Nipponbare, complete sequence
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TCTCTCTCCTGTGCTTGCCTCTTCCATT
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Secuencia precursora de osa-miR-171b

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>ENA| AY551247.1: 5369153-5369252 Oryza sativa precursor microRNA 171b
gene, complete sequence
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GAGTGCATGAATCTGATGCAGTCTCAGTGTAGTATGCTCCATGCTGGAACTTCTGATTGA
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CDS *L*-Ascorbate Oxidase (Os06g37150)

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CDS OsSCL6-IIa (LOC_Os02g44370)

>LOC_Os02g44370.1
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Tabla S3. Primers usados para amplificar ADNc de cada gen

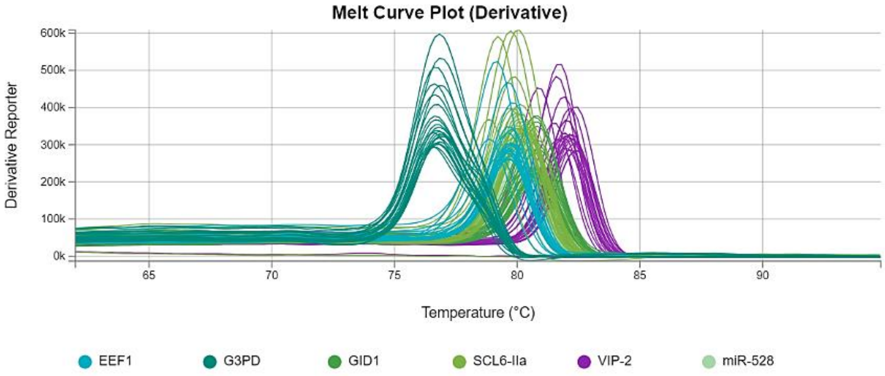
Gen	**Forward (5’-3’)**	**Reverse (5’-3’)**	**Tamaño amplicón (pb)**
OsAAO2	CGAGAACGTGGAGACCTGCGTCGA	CCACCACCGTCATCTTGTGCCCTTG	218
OsVIP	TTGCCTGTATCAGCACCTGG	ACCACAGGGATTGCCATCTG	287
OsDWARF3	GGAGGACTATTATCCAGCCCCAGA	TCCCATTTGTCACCAAGCCAGGAAT	180
OsRFI2	GCCCACACCTCGCAACGGAA	TGGCTCTGCGGCATTCGCTC	453
OsSCL6-IIA	GATATGGTGCTTGGACGGCA	AGGTTGCTGGGTGGAACAG	95
OsGID1	ACCGCAACGAGTGCAAGAC	TTGTACGACAGCTTGAAGTTGGA	73
OsGAI	GACGTCAACGAACGCTCAATT	CGGAGTCCAGTCGTCGATCT	206
osa-miR-528-precursor	CAGAGGAGCAGGAGATTCA	AAACTTCCACAGAACAGCCT	60
osa-miR-171b-precursor	TGATTGAGCCGTGCCAATATC	CAGTGCAGGGTCCGAGGTAT	60

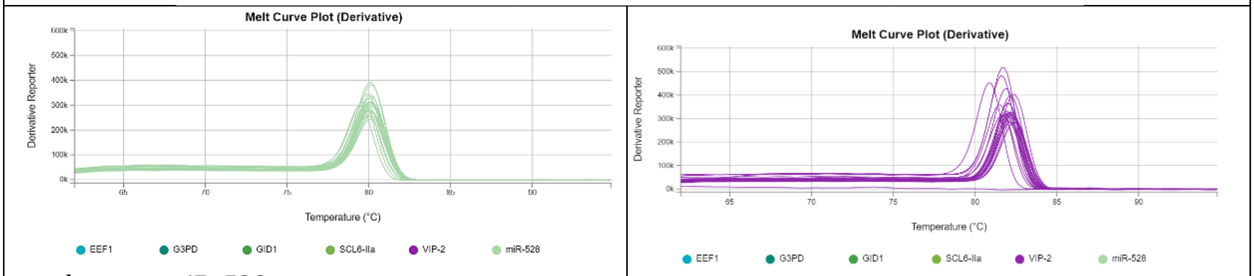
Tabla S4. Datos de cuantificación de RNA de *Oryza sativa* L.

Variedad	Réplica biológica	ng/µL	A260/A280	A260/230
Fedearroz 2000	Control 1	373,2	2,14	1,74
	T1.1	306,1	2,18	1,82
	T2.1	329,4	2,19	1,85
	Control 2	312,4	2,15	2,26
	T1.2	327,5	2,13	1,94
	T2.2	153,7	2,15	2,23
	Control 3	331,3	2,18	2,02
	T1.3	215,9	2,14	1,76
	T2.3	203,3	2,14	1,80
Bluebonnet 50	Control 1	165,8	2,16	2,26
	T1.1	225,0	2,16	1,95
	T2.1	315,8	2,17	1,71
	Control 2	206,8	2,16	2,21
	T1.2	448,2	2,20	1,87
	T2.2	192,2	2,17	2,16
	Control 3	297,0	2,19	2,23
	T1.3	258,8	2,17	2,25
	T2.3	343,6	2,16	2,21
AGO2P33-1	Control 1	202,8	2,19	1,83
	T1.1	492,3	2,03	1,92
	T2.1	164,2	2,18	1,97
	Control 2	228,8	2,18	1,95
	T1.2	261,2	2,18	1,93
	T2.2	475,0	2,20	2,24
	Control 3	484,1	2,20	2,26
	T1.3	486,6	2,20	2,14
	T2.3	255,9	2,16	2,17

Figura S1. Curvas de denaturación de los productos de qPCR en tiempo real

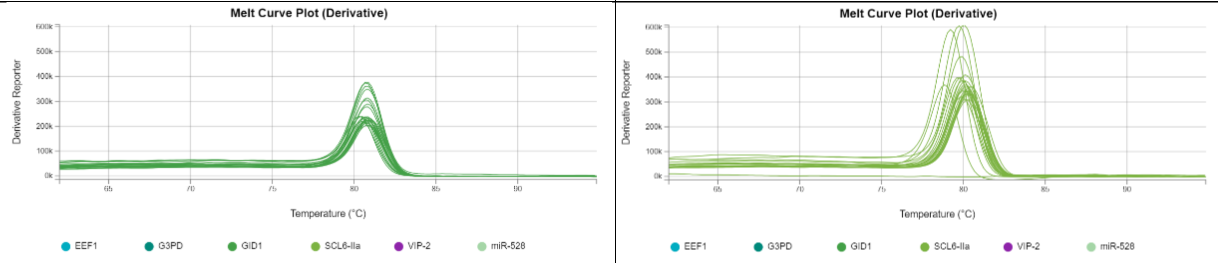
a. Conglomerado





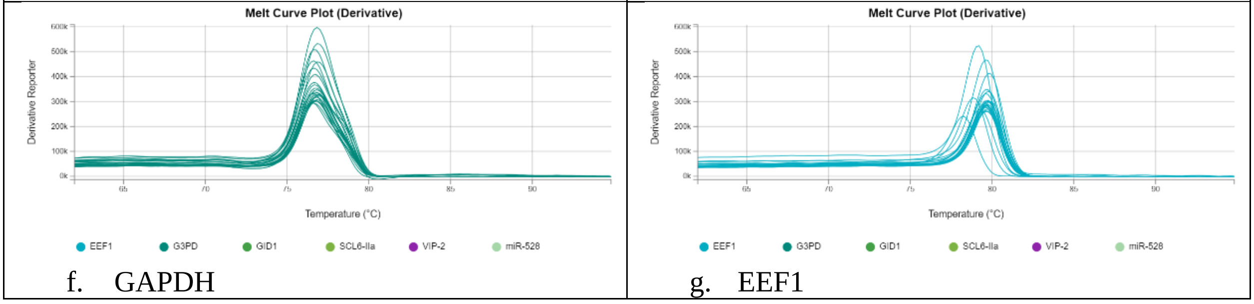
b. osa-miR-528 precursor

c. VIP-2



d. GID1

e. SCL6-IIa



f. GAPDH

g. EEF1

Tabla S5. Tratamiento de datos replicados: gen osa-miR-528-precursor

Muestra Var. Fedearroz-2000	**osa-miR-528 Ct1**	**osa-miR-528 Ct2**	**osa-miR-528 Ct3**	**GPDH Ct1**	**GPDH Ct2**	**GPDH Ct3**	**ΔCt**	**ΔΔCt**	**2-ΔΔCt**
Control 1	30,68	30,48	30,63	27,58	27,10	27,59			
Control 2	28,54	28,36	28,39	25,51	25,06	25,28			
Control 3	29,83	31,61	29,71	26,09	26,97	26,95			
PROMEDIO		29,80			26,46		3,34	0,00	**1,02**
Tratamiento 1	28,28	28,27	28,30	25,84	25,66	25,75			
Tratamiento 2	29,77	29,45	29,53	25,55	25,47	24,82			
Tratamiento 3	27,73	27,87	27,91	25,40	25,73	25,28			
PROMEDIO		28,57			25,50		3,07	-0,28	**1,41**

Tabla S5. Representa el promedio de valores Ct determinados del gen precursor de osa-miR-528 para las muestras de *Oryza sativa* de la variedad Fedearroz 2000. Nivel Relativo en la expresión génica utilizando GPDH como normalizador.

Tabla S6. Tratamiento de datos replicados: gen OsGID1

Muestra Var. Fedearroz-2000	**OsGID1 Ct1**	**OsGID1 Ct2**	**OsGID1 Ct3**	**GPDH Ct1**	**GPDH Ct2**	**GPDH Ct3**	**ΔCt**	**ΔΔCt**	**2-ΔΔCt**
Control 1	30,50	29,81	30,26	27,58	27,10	27,59			
Control 2	27,98	27,97	27,32	25,11	25,28	25,06			
Control 3	27,50	27,81	27,83	26,09	26,97	26,95			
PROMEDIO		28,55			26,41		2,14	0,00	**1,17**
Tratamiento 1	27,99	27,93	27,71	25,75	25,66	25,84			
Tratamiento 2	28,18	28,74	28,55	24,82	25,47	25,55			
Tratamiento 3	28,82	28,13	28,63	25,98	25,73	25,28			
PROMEDIO		28,30			25,57		2,73	0,59	**0,70**

Tabla S6. Representa el promedio de valores Ct determinados del gen OsGID1para las muestras de *Oryza sativa* de la variedad Fedearroz 2000. Nivel Relativo en la expresión génica utilizando GPDH como normalizador.

Tabla S7. Tratamiento de datos replicados: gen OsVIP2

Muestra Var. Fedearroz-2000	**OsVIP2 Ct1**	**OsVIP2 Ct2**	**OsVIP2 Ct3**	**GPDH Ct1**	**GPDH Ct2**	**GPDH Ct3**	**ΔCt**	**ΔΔCt**	**2-ΔΔCt**
Control 1	28,58	28,89	28,25	27,58	27,10	27,59			
Control 2	26,73	26,80	26,45	25,11	25,28	25,06			
Control 3	28,43	28,52	27,99	26,09	26,97	26,95			
PROMEDIO		27,85			26,41		1,43	0,00	**1,01**
Tratamiento 1	26,63	26,84	26,28	25,75	25,66	25,84			
Tratamiento 2	26,21	26,61	26,12	24,82	25,47	25,55			
Tratamiento 3	28,21	27,64	28,00	25,98	25,40	25,73			
PROMEDIO		26,95			25,58		1,37	-0,06	**1,14**

Tabla S7. Representa el promedio de valores Ct determinados del gen OsVIP2para las muestras de *Oryza sativa* de la variedad Fedearroz 2000. Nivel Relativo en la expresión génica utilizando GPDH como normalizador.

Tabla S8. Tratamiento de datos replicados: gen OsSCL6-IIa

Muestra Var. Fedearroz-2000	**OsSCL6-IIa Ct1**	**OsSCL6-IIa Ct2**	**OsSCL6-IIa Ct3**	**GPDH Ct1**	**GPDH Ct2**	**GPDH Ct3**	**ΔCt**	**ΔΔCt**	**2-ΔΔCt**
Control 1	28,17	28,16	28,15	27,58	27,10	27,59			
Control 2	26,80	26,87	26,65	25,11	25,28	25,06			
Control 3	27,25	27,65	27,94	26,09	26,97	26,95			
PROMEDIO		27,52			26,41		1,10	0,00	**1,03**
Tratamiento 1	27,22	28,44	26,11	25,75	25,66	25,84			
Tratamiento 2	25,96	25,93	25,47	24,82	25,47	25,55			
Tratamiento 3	27,18	27,12	26,94	25,40	25,73	25,28			
PROMEDIO		26,71			25,50		1,21	0,10	**0,99**

Tabla S8. Representa el promedio de valores Ct determinados del gen OsSCL6-IIa para las muestras de *Oryza sativa* de la variedad Fedearroz 2000. Nivel Relativo en la expresión génica utilizando GPDH como normalizador