

Supplementary Information

Small RNAs derived from avocado sunblotch viroid and their association with bleaching symptoms: implications for pathogenesis in avocado sunblotch disease

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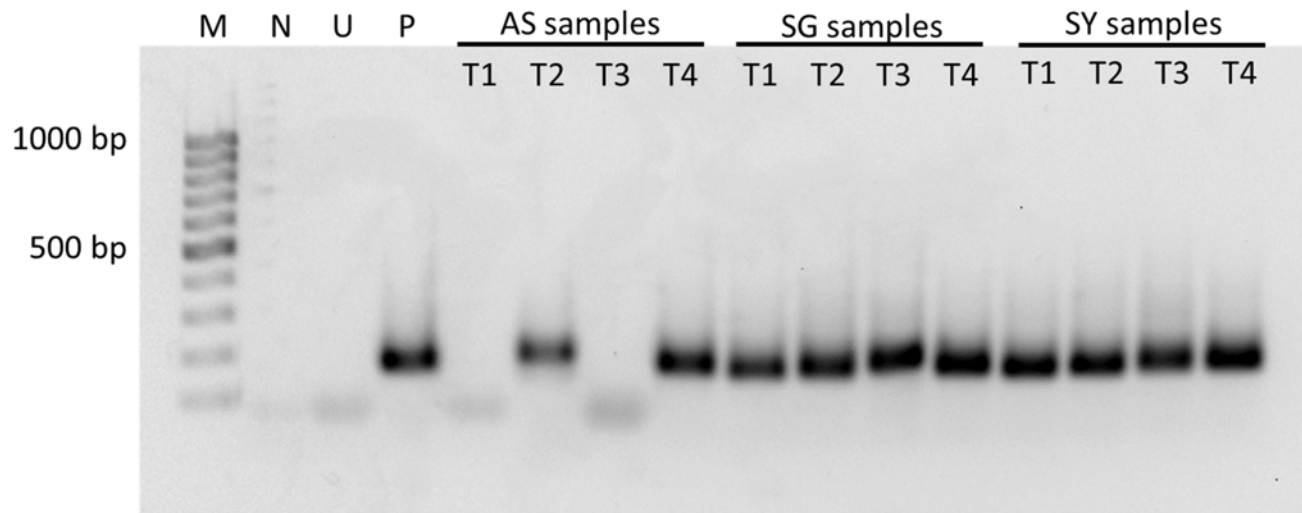


Fig. S1. Confirmation of real-time PCR amplification of avocado sunblotch viroid (ASBVd) in 12 samples. Products of TaqMan™ amplification of ASBVd in pooled leaf samples harvested from four sunblotch-affected trees (T1-T4), shown on a 2% agarose gel and compared to a subset of controls used in the real-time PCR. The infected control (P) and ten samples that produced fluorescence in the diagnostic assay had single bands with the expected size of ~155 bp. The non-template control (N), uninfected control (U), and two samples that did not fluoresce in the real-time assay had no amplification products. M: 100 bp DNA marker; AS samples: Asymptomatic (fully green) leaves; SG samples: Green sectors of bleached leaves (SG); SY samples: Yellow sectors of bleached leaves.

	80	100	120	140
SB-1_J02020.1	ACUCUGAGUU	UCGACUUGUG	AGAGAAGGAG	GAGUCGUGGU
ScHassKZN-A6e
ScHassKZN-A1a
ScHassKZN-A1b
ScHassKZN-A1c
ScHassKZN-A1d
ScHassKZN-A1e
ScHassKZN-A2a
ScHassKZN-A2b
ScHassKZN-A2c
ScHassKZN-A2d
ScHassKZN-A2e
ScHassKZN-A3a
ScHassKZN-A3b
ScHassKZN-A3c
ScHassKZN-A3d
ScHassKZN-A3e
ScHassKZN-A4a
ScHassKZN-A4c
ScHassKZN-A4d
ScHassKZN-A5a
ScHassKZN-A5b
ScHassKZN-A5c
ScHassKZN-A5d
ScHassKZN-A5e
ScHassKZN-A6a
ScHassKZN-A6b
ScHassKZN-A6c
ScHassKZN-A6d
ScHassKZN-A4e
ScHassKZN-A4b
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T2_SG_Clone3
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T2_SG_Clone5
T2_SG_Clone2
T2_SG_Clone1
T2_AS_Clone4
T2_AS_Clone2
T2_AS_Clone5
T4_AS_Clone1
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T1_SG_Clone1
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T3_SY_Clone5
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T4_SY_Clone2
T4_SY_Clone3
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T4_SY_Clone4
T2_AS_Clone1
T2_AS_Clone3
T4_AS_Clone5
T1_SG_Clone2
T1_SG_Clone4
B-variant_1994_S74687.1
Consensus	ACUCUGAGUU	UCGACUUGUG	AGAGAAGGAG	GAGUCGUGGU
Conservation				
Sequence logo	ACUCUGAGUU	UCGACUUGUG	AGAGAAGGAG	GAGUCGUGGU

	160	180	200					
SB-1_J02020.1	CACUCGUCUU	CAAUCUCUUG	AUCACUUCGU	CUCUUCAGGG	AAAGAUGGGA	AGAACACUGA	UGAGUCUCGC	204
ScHassKZN-A6e	204
ScHassKZN-A1a	204
ScHassKZN-A1b	204
ScHassKZN-A1c	204
ScHassKZN-A1d	204
ScHassKZN-A1e	204
ScHassKZN-A2a	204
ScHassKZN-A2b	204
ScHassKZN-A2c	204
ScHassKZN-A2d	204
ScHassKZN-A2e	207
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ScHassKZN-A3d	204
ScHassKZN-A3e	204
ScHassKZN-A4a	204
ScHassKZN-A4c	205
ScHassKZN-A4d	204
ScHassKZN-A5a	204
ScHassKZN-A5b	205
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ScHassKZN-A5e	206
ScHassKZN-A6a	204
ScHassKZN-A6b	206
ScHassKZN-A6c	207
ScHassKZN-A6d	205
ScHassKZN-A4e	205
ScHassKZN-A4b	204
SC-variant_1994_S73860.1	204
T2_SG_Clone3	204
T2_SG_Clone4	.	G	205
T2_SG_Clone5	.	G	205
T2_SG_Clone2	203
T2_SG_Clone1	203
T2_AS_Clone4	210
T2_AS_Clone2	206
T2_AS_Clone5	206
T4_AS_Clone1	.	G	206
T4_AS_Clone2	.	G	206
T4_AS_Clone3	.	G	206
T4_AS_Clone4	.	G	206
T1_SG_Clone1	206
T1_SG_Clone3	206
T1_SG_Clone5	.	.	C	206
T3_SG_Clone1	.	G	205
T3_SG_Clone2	.	G	206
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T3_SG_Clone4	.	G	206
T3_SG_Clone5	.	G	205
T4_SG_Clone1	.	G	207
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T3_SY_Clone3	206
T3_SY_Clone4	206
T3_SY_Clone5	206
T4_SY_Clone1	.	G	206
T4_SY_Clone2	207
T4_SY_Clone3	207
T4_SY_Clone5	.	G	206
T4_SY_Clone4	.	G	206
T2_AS_Clone1	207
T2_AS_Clone3	206
T4_AS_Clone5	.	G	206
T1_SG_Clone2	206
T1_SG_Clone4	202
B-variant_1994_S74687.1	.	G	207
Consensus	CACUCGUCUU	CAAUCUCUUG	AUCACUUCGU	CUCUUCAGGG	AAAGAUGGGA	AGAACACUGA	UGAGUCUCGC	
Conservation								
Sequence logo								

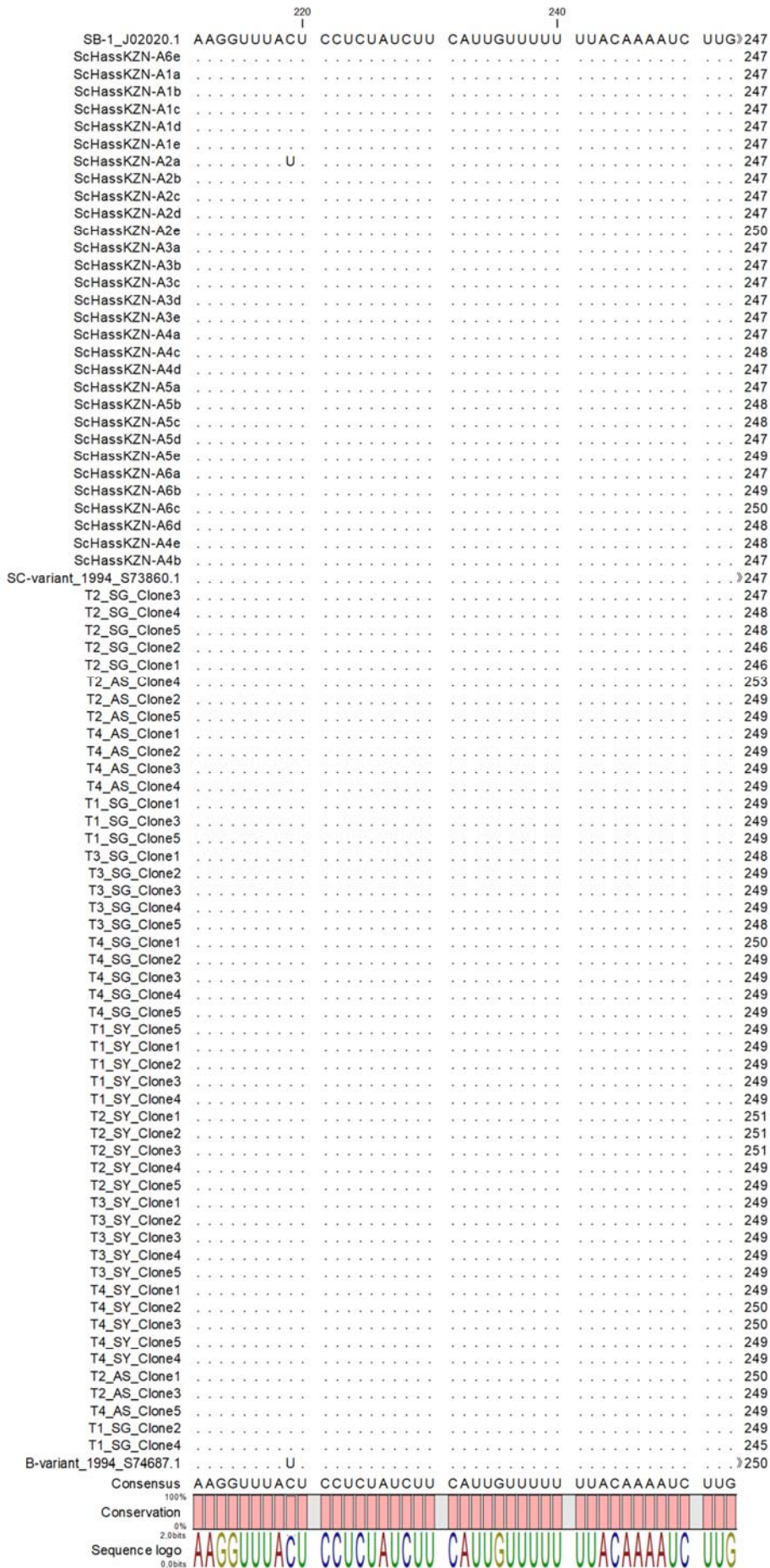


Fig. S2. Alignment of avocado sunblotch viroid (ASBVd) clones sequenced in this study with previously characterised variants. Variant sequences were obtained from ASBVd isolates cloned from yellow sectors (SY samples) and green sectors (SG samples) of bleached leaves for four biological replicates: Tree 1 (T1), Tree 2 (T2), Tree 3 (T3) and Tree 4 (T4), and from asymptomatic (AS) samples from T2 and T4. The 50 clones sequenced in this study were aligned to the ASBVd type sequence SB-1, the previously characterised bleached (B) and symptomless carrier (SC) variants, and 30 clones sequenced from six asymptomatic ASBVd-infected nursery trees (ScHassKZN A1-A6) in our previous study. The most significant sequence variation was observed at positions 115-128 relative to SB-1 (highlighted by a black border), representing a portion of the right terminal loop (RTL) of the viroid secondary structure. The insertion of at least one uracil residue at positions 115-118 was observed in green and yellow tissues of trees with sunblotch symptoms, but not in asymptomatic (fully green) leaf tissue from symptomless carrier trees.

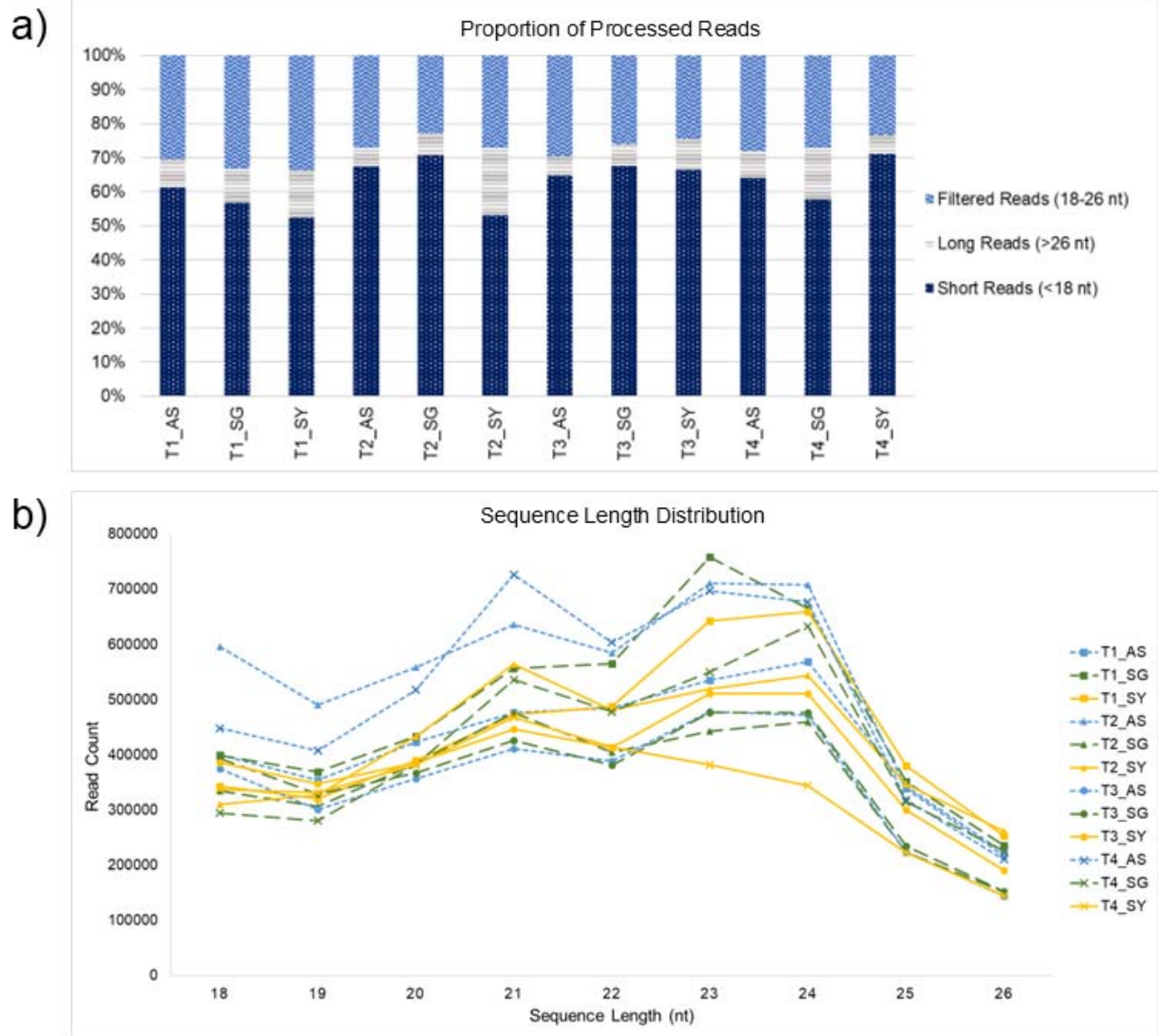


Fig. S3. Size distribution of small RNA (sRNA)-sequencing reads in different samples. **(a)** The proportion of reads for each sample that were maintained for further analysis (average 27.8%), reads discarded for being too short (average 62.7%) and discarded reads that were too long (average 9.5%). **(b)** The sequence length distribution for filtered reads maintained for further analysis (18-26 nt). Data is shown for three sample types (asymptomatic (AS), green sectors of bleached leaves (SG), and yellow sectors of bleached leaves (SY)) for four biological replicates (Tree 1-4 (T1-T4)).

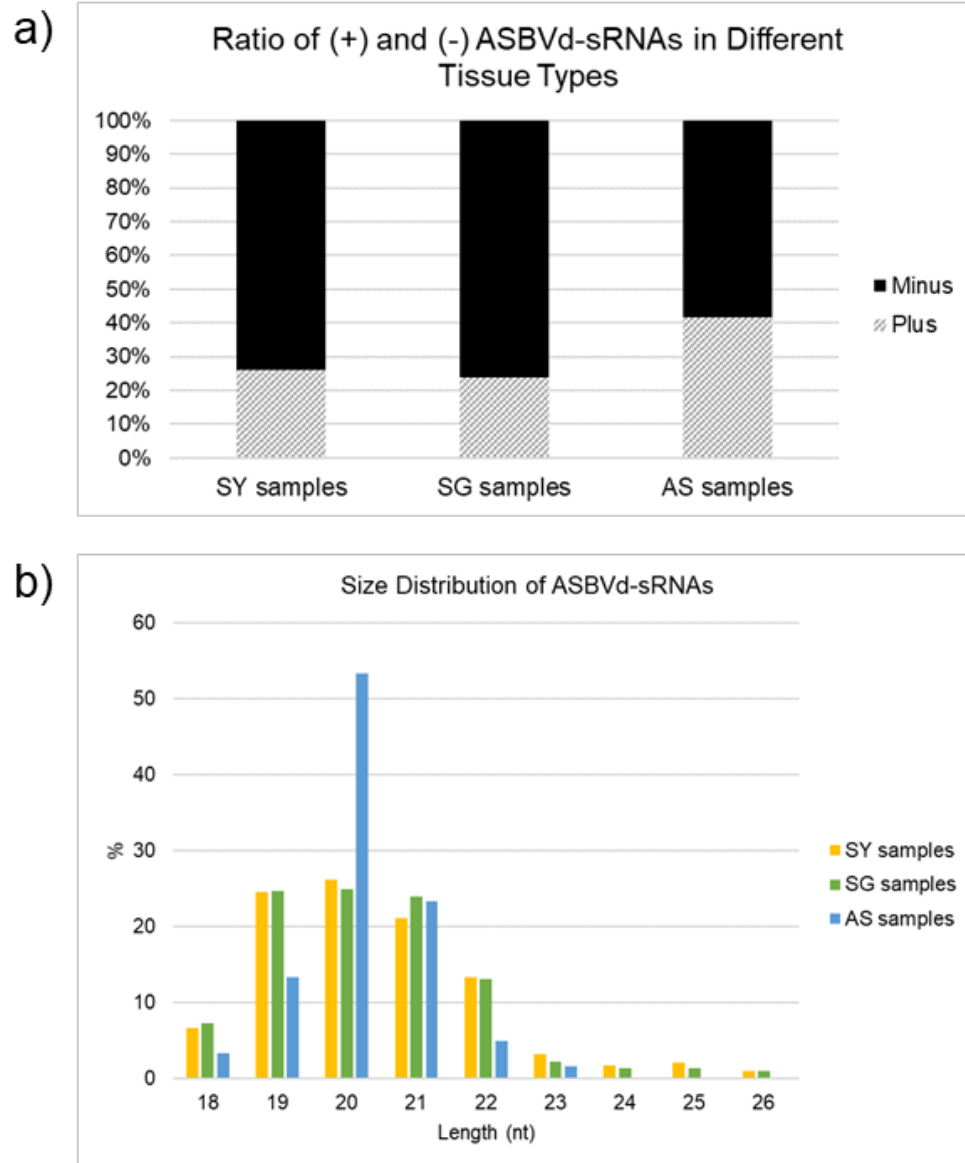


Fig. S4. Polarity and size distribution of avocado sunblotch viroid-derived small RNAs (ASBVd-sRNAs) in different sample types. **(a)** The proportion of ASBVd-sRNAs derived from the sense (plus; +) and antisense (minus; -) strands of the ASBVd genome according to tissue type. **(b)** The percentage of ASBVd-sRNAs corresponding to specific sizes within assorted sample types. AS samples were obtained from asymptomatic (fully green) leaves from symptomatic trees, while SG and SY samples consist of material from the green and yellow sectors of bleached leaves, respectively.

Target genes downregulated in yellow tissues

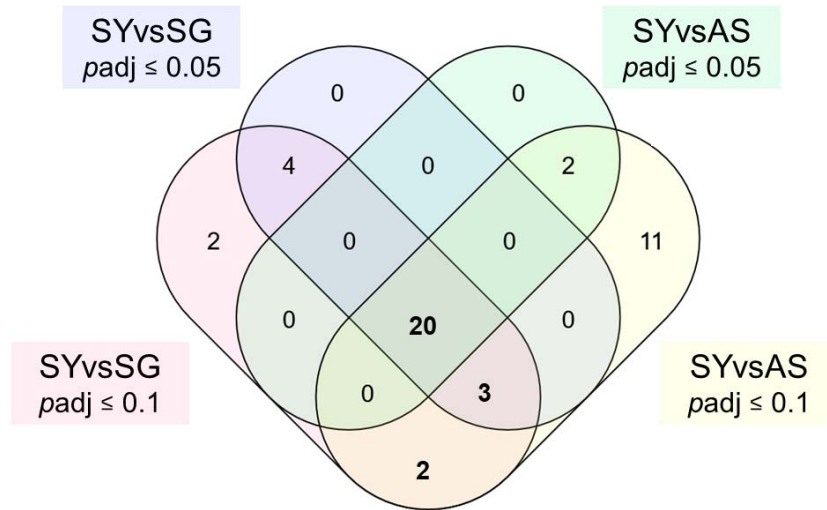


Fig. S5. Venn diagram of 44 target genes downregulated in yellow leaf tissues in ASBVd-infected trees displaying bleaching symptoms. The genes were predicted as targets of avocado sunblotch viroid-derived small RNAs (ASBVd-sRNAs) for post-transcriptional gene silencing by *in silico* analysis. Putative targets were all significantly downregulated ($p_{adj} \leq 0.1$) in yellow sectors of bleached leaves (SY) relative to either green sectors of bleached leaves (SG) or asymptomatic leaves from symptomatic trees (AS). Genes downregulated in both SY vs. SG and SY vs. AS are indicated in bold. No targets were significantly downregulated in SG vs. AS.

Table S1. The source of leaf material used in this study.

Biological replicate	Cultivar ^a	Bleached leaves present	Variiegated leaves present	Chlorotic fruit present ^b	Number of bleached leaves pooled for SY and SG samples ^c	Number of asymptomatic (fully green) leaves pooled for AS samples ^c
Tree 1	A	Yes	Yes	No	30	5
Tree 2	B	Yes	Yes	Yes	22	5
Tree 3	B	Yes	Yes	No	14	5
Tree 4	C	Yes	Yes	Yes	19	5

^aSamples were collected under a proprietary agreement with South African avocado growers. Individual cultivars are therefore indicated by different letters.

^bAll trees sampled were in fruit at the time of collection, though some trees did not have visible fruit symptoms.

^cAll leaves collected were fully developed at the time of sampling.

AS: material obtained from asymptomatic (fully green) leaves; SG: material obtained from the green sectors of bleached leaves; SY: material obtained from the yellow sectors of bleached leaves

Table S2. Average fluorescence values for real-time PCR performed to detect ASBVd in individual samples.

Sample Name	Sample Contents	Average Ct Value (3 technical replicates)
NTC	Non-template control	N/A
cDNA NTC - Y	Non-template control for viroid cDNA synthesis from yellow samples	N/A
PreAmp NTC - Y	Non-template control for pre-amplification reaction for yellow samples	N/A
cDNA NTC PreAmp - Y	Non-template control for viroid cDNA synthesis, pre-amplified with yellow samples	N/A
Neg - Y	Known uninfected – cDNA synthesised and pre-amplified with yellow samples	N/A
Pos - Y	Known infected (symptomatic – bleached leaves) – cDNA synthesised and pre-amplified with yellow samples	29.40
cDNA NTC - G	Non-template control for viroid cDNA synthesis from green samples	N/A
PreAmp NTC - G	Non-template control for pre-amplification reaction for green samples	N/A
cDNA NTC PreAmp - G	Non-template control for viroid cDNA synthesis, pre-amplified with green samples	N/A
Neg - G	Known uninfected – cDNA synthesised and pre-amplified with green samples	N/A
Pos - G	Known infected (asymptomatic) – cDNA synthesised and pre-amplified with green samples	29.71
Tree1_AS	Asymptomatic leaves – biological replicate 1	N/A
Tree2_AS	Asymptomatic leaves – biological replicate 2	37.12
Tree3_AS	Asymptomatic leaves – biological replicate 3	N/A
Tree4_AS	Asymptomatic leaves – biological replicate 4	30.15
Tree1_SG	Green sectors of bleached leaves – biological replicate 1	28.00
Tree2_SG	Green sectors of bleached leaves – biological replicate 2	26.18
Tree3_SG	Green sectors of bleached leaves – biological replicate 3	30.37
Tree4_SG	Green sectors of bleached leaves – biological replicate 4	26.62
Tree1_SY	Yellow sectors of bleached leaves – biological replicate 1	17.39
Tree2_SY	Yellow sectors of bleached leaves – biological replicate 2	17.42
Tree3_SY	Yellow sectors of bleached leaves – biological replicate 3	19.02
Tree4_SY	Yellow sectors of bleached leaves – biological replicate 4	19.86

N/A – No fluorescence detected after 40 amplification cycles

Table S3. Number of small RNA (sRNA) reads derived from avocado sunblotch viroid (ASBVd) in individual samples.

Sample Type	Biological Replicate	Number of raw sRNA reads	Number of reads after filtering	Number of ASBVd-sRNAs in filtered library	ASBVd-sRNAs per million filtered reads
AS	Tree1	12,490,597	3,806,703	0	0.00
	Tree2	17,810,540	4,840,219	3	0.62
	Tree3	10,628,969	3,149,350	0	0.00
	Tree4	16,507,218	4,606,403	57	12.37
SG	Tree1	13,005,385	4,334,959	227	52.36
	Tree2	13,890,995	3,184,772	684	214.77
	Tree3	12,420,530	3,236,852	79	24.41
	Tree4	13,565,594	3,696,011	334	90.37
SY	Tree1	11,682,135	3,949,789	29,914	7,573.57
	Tree2	13,970,063	3,787,878	38,000	10,032.00
	Tree3	14,235,126	3,491,534	29,203	8,363.95
	Tree4	12,958,727	3,026,825	25,725	8,499.00

AS: Asymptomatic (fully green) leaves from symptomatic trees; SG: Green sectors of bleached leaves from symptomatic trees; SY: Yellow sectors of bleached leaves from symptomatic trees

Table S4. ASBVd-sRNAs originating from the pathogenic region of bleaching-associated ASBVd variants, sequenced from yellow leaf tissues.

vd-sRNA name	Length (nt)	ASBVdB-sRNA sequence ^a	sRNA reads ^b	Viroid strand ^c	5' position ^d
ASBVdB-sRNA1	25	AAGGAGGAGUCGUGGUGAAC <u>UUUUU</u>	1	(+)	95
ASBVdB-sRNA2	26	UAAGGAGGAGUCGUGGUGAAC <u>UUUUU</u>	1	(+)	94
ASBVdB-sRNA3	24	AGGAGGAGUCGUGGUGAAC <u>UUUUU</u>	1	(+)	96
ASBVdB-sRNA4	26	AGGAGGAGUCGUGGUGAAC <u>UUUUU</u> AU	2	(+)	96
ASBVdB-sRNA5a	26	GGAGGAGUCGUGGUGAAC <u>UUUUU</u> AUU	9	(+)	97
ASBVdB-sRNA5b	26	GGAGGAGUCGUGGUGAAC <u>UUUUU</u> ACU	1	(+)	97
ASBVdB-sRNA5c	26	GGAGGAGUCGUGGUGAAC <u>UUUUU</u> AUG	2	(+)	97
ASBVdB-sRNA5d	26	GGAGGAGUCGUGGUGAAC <u>UUUUU</u> AUC	5	(+)	97
ASBVdB-sRNA5e	26	GGAGGAGUCGUGGUGAACUUUUAU <u>AUC</u>	1	(+)	97
ASBVdB-sRNA5f	26	GGAGGAGUCGUGGUGAACUUUAU <u>AAU</u>	1	(+)	97
ASBVdB-sRNA6a	25	GGAGGAGUCGUGGUGAAC <u>UUUUU</u> AU	2	(+)	97
ASBVdB-sRNA6b	25	GGAGGAGUCGUGGUGAACUUUAU <u>AUC</u>	1	(+)	97
ASBVdB-sRNA7a	25	GAGGAGUCGUGGUGAAC <u>UUUUU</u> AUU	1	(+)	98
ASBVdB-sRNA7b	25	GAGGAGUCGUGGUGAAC <u>UUUUU</u> AUC	1	(+)	98
ASBVdB-sRNA8	24	GAGGAGUCGUGGUGAAC <u>UUUUU</u> AUC	1	(+)	98
ASBVdB-sRNA9a*	24	AGGAGUCGUGGUGAAC <u>UUUUU</u> AUU	1	(+)	99
ASBVdB-sRNA9b*	24	AGGAGUCGUGGUGAACUUUAU <u>AUC</u>	1	(+)	99
ASBVdB-sRNA10*	23	AGGAGUCGUGGUGAAC <u>UUUUU</u> AUC	1	(+)	99
ASBVdB-sRNA11a*	23	GGAGUCGUGGUGAAC <u>UUUUU</u> AUC	2	(+)	100
ASBVdB-sRNA11b*	23	GGAGUCGUGGUGAAC <u>UUUUU</u> AUU	6	(+)	100
ASBVdB-sRNA11c*	23	CGAGUCGUGGUGAAC <u>UUUUU</u> AUU	1	(+)	100
ASBVdB-sRNA12*	20	GGAGUCGUGGUGAAC <u>UUUUU</u>	1	(+)	100
ASBVdB-sRNA13*	22	GGAGUCGUGGUGAAC <u>UUUUU</u> AU	3	(+)	100
ASBVdB-sRNA14a*	22	GAGUCGUGGUGAAC <u>UUUUU</u> ACU	1	(+)	101
ASBVdB-sRNA14b*	22	GAGUCGUGGUGAAC <u>UUUUU</u> AUU	6	(+)	101
ASBVdB-sRNA15	23	AAC <u>UUUUU</u> AUUAAAAAAAA <u>UUGG</u>	1	(+)	112
ASBVdB-sRNA16a*	21	AGUCGUGGUGAAC <u>UUUUU</u> AUC	1	(+)	102
ASBVdB-sRNA16b*	21	AGUCGUGGUGAAC <u>UUUUU</u> AUU	2	(+)	102
ASBVdB-sRNA16c	21	AGUCGUGGUGAACUUUAU <u>AUU</u>	3	(+)	102
ASBVdB-sRNA17*	25	AGUCGUGGUGAACUUUUUAUU <u>AAAAU</u>	1	(+)	102
ASBVdB-sRNA18*	20	AGUCGUGGUGAAC <u>UUUUU</u> AU	1	(+)	102
ASBVdB-sRNA19a	20	GUCGUGGUGAAC <u>UUUUU</u> AUC	1	(+)	103
ASBVdB-sRNA19b	20	GUCGUGGUGAAC <u>UUUUU</u> AUU	4	(+)	103
ASBVdB-sRNA20a	19	UCGUGGUGAAC <u>UUUUU</u> AUU	2	(+)	104
ASBVdB-sRNA20b*	19	UCGUGGUGAACUUUAU <u>AUU</u>	2	(+)	104
ASBVdB-sRNA21a	18	CGUGGUGAAC <u>UUUUU</u> AUU	6	(+)	105
ASBVdB-sRNA21b	18	CGUGGUGAAC <u>UUUUU</u> AUC	1	(+)	105
ASBVdB-sRNA21c	18	CGUGGUGAAC <u>UUUUU</u> AUG	1	(+)	105
ASBVdB-sRNA21d	18	CGUGGUGAACUUUAU <u>AUU</u>	1	(+)	105
ASBVdB-sRNA22	20	CGUGGUGAAC <u>UUUUU</u> AUUAG	1	(+)	105

ASBVdB-sRNA23*	20	UUUAAU <u>AAAA</u> GUUCACCAC	4	(-)	125
ASBVdB-sRNA24*	25	UAAUAAAGUUCACCACGACUCCUCC	1	(-)	121
ASBVdB-sRNA25	24	<u>UAAAA</u> GUUCACCACGACUCCUCC	1	(-)	120
ASBVdB-sRNA26a	25	AU <u>AAAA</u> AGUUCACCACGACUCCUCC	1	(-)	121
ASBVdB-sRNA26b	25	AUAUAAAGUUCACCACGACUCCUCC	1	(-)	121
ASBVdB-sRNA27	24	AU <u>AAAA</u> AGUUCACCACGACUCCUC	1	(-)	121
ASBVdB-sRNA28	22	UUAAUAAAGUUCACCACGACUCC	1	(-)	121
ASBVdB-sRNA29	23	AUAUAAAGUUCACCACGACUCCC	1	(-)	121
ASBVdB-sRNA30	21	AU <u>AAAA</u> AGUUCACCACGACUC	1	(-)	121
ASBVdB-sRNA31	20	<u>AAAA</u> AGUUCACCACGACUUC	3	(-)	119
ASBVdB-sRNA32*	22	UAAU <u>AAAA</u> AGUUCACCACGACU	1	(-)	123
ASBVdB-sRNA33	20	AU <u>AAAA</u> AGUUCACCACGACC	2	(-)	121
ASBVdB-sRNA34*	21	UAAU <u>AAAA</u> AGUUCACCACGAC	5	(-)	123
ASBVdB-sRNA35*	22	UUAAU <u>AAAA</u> AGUUCACCACGAC	1	(-)	124
ASBVdB-sRNA36	18	AAU <u>AAAA</u> AGUUCACCACG	1	(-)	122
ASBVdB-sRNA37*	19	UAAU <u>AAAA</u> AGUUCACCACG	1	(-)	123
ASBVdB-sRNA38a*	20	UUAAU <u>AAAA</u> AGUUCACCACG	6	(-)	124
ASBVdB-sRNA38b	20	UUAAUAAAGUUCACCACG	1	(-)	124
ASBVdB-sRNA39a*	21	UUUAAU <u>AAAA</u> AGUUCACCACG	5	(-)	125
ASBVdB-sRNA39b*	21	UUUAAU <u>AAAA</u> AGUUCACCACC	1	(-)	125
ASBVdB-sRNA39c*	21	UUUAAU <u>AAAA</u> AGUUCACCAUG	1	(-)	125
ASBVdB-sRNA40	18	UAAU <u>AAAA</u> AGUUCACCAC	3	(-)	123
ASBVdB-sRNA41a*	19	UUAAU <u>AAAA</u> AGUUCACCAC	3	(-)	124
ASBVdB-sRNA41b*	19	UAAU <u>AAAA</u> AGUUCACCAC	1	(-)	124

^aThe sequences of ASBVd-sRNAs originating from the right terminal loop (RTL) of the viroid genome, where the pathogenic region is underlined for ASBVdB-sRNAs containing the pathogenic determinant of bleaching symptoms.

^bNumber of reads found in yellow sectors of bleached leaves (SY samples).

^cThe strand of the viroid genome from which each sRNA originated; where (+) indicates sRNAs derived from the sense strand of the genome, and (-) sRNAs are generated from the antisense ASBVd genome.

^dThe position on the ASBVd genome to which the 5' terminus of each sRNA is mapped.

*ASBVdB-sRNAs predicted to target one or more of the seven avocado mRNA candidates with the strongest evidence for duplex formation.

Table S5. Avocado mRNA targets, predicted to form duplexes with ASBVdB-sRNAs, which were downregulated in yellow tissues.

Target gene ID ^a	Target annotation ^a	Log2FC - SYvsSG ^b	<i>p</i> adj - SYvsSG ^b	Log2FC - SYvsAS ^c	<i>p</i> adj - SYvsAS ^c	Target localisation ^d	vd-sRNA name	vd-sRNA length (nt)	Duplex score - Default ^e	Duplex score - Strict ^f	Minimum free energy (kcal/mol) ^g	Pathogenic determinant within duplex? ^h
C01g021570	Cytochrome P450 94A1	-1.33	0.05	-1.40	0.05	Endoplasmic reticulum	ASBVdB-sRNA14b	22	2.5	2.5	-28.9	Yes
							ASBVdB-sRNA11b	23	2.5	2.5	-30.5	Yes
							ASBVdB-sRNA13	22	2.5	2.5	-30.5	Yes
							ASBVdB-sRNA14a	22	2.5	2.5	-29.1	Yes
							ASBVdB-sRNA11a	23	2.5	2.5	-30.5	Yes
							ASBVdB-sRNA12	20	2.5	2.5	-29.8	Yes
							ASBVdB-sRNA11c	23	3.0	3.0	-29.2	Yes
							ASBVdB-sRNA16b	21	3.5	3.5	-25.4	Yes
							ASBVdB-sRNA16a	21	3.5	3.5	-25.4	Yes
							ASBVdB-sRNA17	25	3.5	3.5	-25.4	Yes
							ASBVdB-sRNA18	20	3.5	3.5	-25.4	Yes
							ASBVdB-sRNA9a	24	3.5	3.5	-30.8	Yes
							ASBVdB-sRNA9b	24	3.5	3.5	-28.5	Yes
C11g017560	Glycosyltransferase	-1.00	2.748E-04	-1.32	2.857E-08	Cytoplasm	ASBVdB-sRNA10	23	3.5	3.5	-30.8	Yes
							ASBVdB-sRNA38a	20	2.0	2.0	-18.3	Yes
							ASBVdB-sRNA35	22	2.0	2.0	-20.1	Yes
							ASBVdB-sRNA41a	19	2.0	2.0	-16.6	Yes
							ASBVdB-sRNA23	20	2.5	2.5	-17.2	Yes
							ASBVdB-sRNA39a	21	2.5	2.5	-18.9	Yes
							ASBVdB-sRNA39b	21	2.5	2.5	-18.0	Yes
							ASBVdB-sRNA39c	21	2.5	2.5	-17.3	Yes
							ASBVdB-sRNA34	21	3.0	3.5	0.0	Yes
							ASBVdB-sRNA32	22	3.0	3.5	0.0	Yes
							ASBVdB-sRNA37	19	3.0	3.5	0.0	Yes
							ASBVdB-sRNA38b	20	3.5	4.0	0.0	Yes
							ASBVdB-sRNA41b	19	3.5	4.0	0.0	Yes
C03g004440	Ammonium_transp domain-containing protein	-2.19	0.01	-1.50	0.13	Cytoplasm	ASBVdB-sRNA12	20	3.5	4.0	0.0	Yes

C12g016840	protein TRIGALACTOSYLDIAC YLGLYCEROL 2, chloroplastic	-0.57	0.03	-0.47	0.06	Plastid	ASBVdB-sRNA38a	20	3.5	3.5	-22.3	Yes
							ASBVdB-sRNA35	22	3.5	3.5	-23.8	Yes
							ASBVdB-sRNA41a	19	3.5	3.5	-20.6	Yes
							ASBVdB-sRNA34	21	3.5	3.5	-23.6	Yes
							ASBVdB-sRNA32	22	3.5	3.5	-26.1	Yes
							ASBVdB-sRNA37	19	3.5	3.5	-22.1	Yes
							ASBVdB-sRNA26a	25	3.5	4.0	0.0	Yes
							ASBVdB-sRNA27	24	3.5	4.0	0.0	Yes
							ASBVdB-sRNA30	21	3.5	4.0	0.0	Yes
C07g020980	DNA polymerase I A, chloroplastic	-0.60	0.04	-0.55	0.14	Plastid	ASBVdB-sRNA33	20	3.5	4.0	No duplex predicted	Yes
							ASBVdB-sRNA23	20	3.5	4.0	0.0	Yes - with mismatch
							ASBVdB-sRNA39a	21	3.5	4.0	0.0	Yes - with mismatch
							ASBVdB-sRNA39b	21	3.5	4.0	0.0	Yes - with mismatch
							ASBVdB-sRNA39c	21	3.5	4.0	0.0	Yes - with mismatch
C08g027830	O-fucosyltransferase family protein	-0.40	0.04	-0.49	2.435E-03	Golgi apparatus	ASBVdB-sRNA23	20	3.5	4.0	0.0	Yes - with mismatch
							ASBVdB-sRNA39a	21	3.5	4.0	0.0	Yes - with mismatch
							ASBVdB-sRNA39b	21	3.5	4.0	0.0	Yes - with mismatch
							ASBVdB-sRNA39c	21	3.5	4.0	0.0	Yes - with mismatch
C01g008790	ABC transporter-like	-0.90	1.908E-05	-1.13	2.411E-11	Plastid	ASBVdB-sRNA34	21	3.5	4.0	0.0	Yes
							ASBVdB-sRNA32	22	3.5	4.0	0.0	Yes
							ASBVdB-sRNA37	19	3.5	4.0	0.0	Yes
							ASBVdB-sRNA26a	25	3.5	4.0	0.0	Yes
							ASBVdB-sRNA27	24	3.5	4.0	0.0	Yes
							ASBVdB-sRNA30	21	3.5	4.0	0.0	Yes

C06g011910	Ribokinase	-0.53	0.03	-0.61	7.680E-04	Plastid	ASBVdB-sRNA33	20	3.5	4.0	0.0	Yes
							ASBVdB-sRNA22	20	3.5	4.0	-16.7	Partly - not relevant
C00g040390	Unknown protein	-2.06	0.01	-3.46	1.306E-03	Cytoplasm /Nucleus	ASBVdB-sRNA1	25	3.5	3.5	-27.3	No
C01g034200	Protein SPIRRIG	-1.76	0.05	-1.98	0.02	Lysosome /Vacuole	ASBVdB-sRNA1	25	3.5	3.5	-32.7	Partly - not relevant
C08g004490	Guard cell S-type anion channel SLAC1	-1.95	1.189E-05	-2.14	5.269E-08	Cell membrane /Lysosome /Vacuole	ASBVdB-sRNA24	25	3.5	4.0	0.0	Yes - with mismatch
C05g020570	Thioredoxin domain-containing protein	-1.02	3.477E-03	-1.16	1.471E-04	Plastid	ASBVdB-sRNA20b	19	3.5	3.5	-29.2	Yes
C09g002300	50S ribosomal protein L34, chloroplastic	-1.20	2.022E-03	-1.36	5.758E-05	Plastid	ASBVdB-sRNA23	20	2.0	2.0	-23.0	Yes
							ASBVdB-sRNA39a	21	2.0	2.0	-23.0	Yes
							ASBVdB-sRNA39b	21	2.0	2.0	-23.0	Yes
							ASBVdB-sRNA39c	21	2.0	2.0	-23.1	Yes
							ASBVdB-sRNA38a	20	3.0	3.0	-22.1	Yes
							ASBVdB-sRNA41a	19	3.0	3.0	-22.1	Yes
C11g021230	Pentatricopeptide repeat-containing protein At1g10270	-0.60	0.01	-0.52	0.01	Mitochondrion	ASBVdB-sRNA3	24	3.0	3.0	-33.2	No
							ASBVdB-sRNA7a	25	3.0	3.0	-31.6	No
							ASBVdB-sRNA1	25	3.0	3.0	-33.5	No
							ASBVdB-sRNA8	24	3.0	3.0	-31.6	No
							ASBVdB-sRNA7b	25	3.0	3.0	-31.6	No
C06g015900	E3 ubiquitin-protein ligase arkadia-A, putative	-1.33	0.01	-1.92	1.007E-03	Cytoplasm /Nucleus	ASBVdB-sRNA23	20	3.0	4.0	0.0	Yes
							ASBVdB-sRNA39a	21	3.0	4.0	0.0	Yes
							ASBVdB-sRNA39b	21	3.0	4.0	0.0	Yes
							ASBVdB-sRNA39c	21	3.0	4.0	0.0	Yes
							ASBVdB-sRNA38a	20	3.0	4.0	0.0	Yes
							ASBVdB-sRNA35	22	3.0	4.0	0.0	Yes
							ASBVdB-sRNA41a	19	3.0	4.0	0.0	Yes
							ASBVdB-sRNA34	21	3.5	4.5	0.0	Yes

							ASBVdB-sRNA32	22	3.5	4.5	0.0	Yes
							ASBVdB-sRNA37	19	3.5	4.5	0.0	Yes
C02g035850	40S ribosomal protein S15a	-0.51	0.02	-0.56	0.02	Cytoplasm	ASBVdB-sRNA15	23	3.0	3.5	0.0	Yes - with G:U
C04g000140	Aspartate--tRNA ligase	-0.90	2.418E-03	-0.88	3.014E-03	Cytoplasm	ASBVdB-sRNA15	23	3.0	3.5	0.0	Yes - with G:U
C03g048730	serine/threonine-protein kinase pakF-like	-1.37	0.11	-1.29	0.07	Nucleus	ASBVdB-sRNA24	25	3.0	3.0	-25.4	Yes
							ASBVdB-sRNA29	23	3.5	4.0	0.0	Yes
							ASBVdB-sRNA26b	25	3.5	4.0	0.0	Yes
C02g040700	Unknown protein	-1.04	2.193E-03	-0.90	0.02	Cytoplasm/Plastid	ASBVdB-sRNA7a	25	3.5	3.5	-29.2	No
							ASBVdB-sRNA8	24	3.5	3.5	-29.2	No
							ASBVdB-sRNA7b	25	3.5	3.5	-29.2	No
C01g051500	Peroxidase superfamily protein	-1.64	9.870E-04	-1.80	4.240E-04	Nucleus	ASBVdB-sRNA38a	20	3.5	4.0	0.0	Yes - with mismatch
							ASBVdB-sRNA35	22	3.5	4.0	0.0	Yes - with mismatch
							ASBVdB-sRNA41a	19	3.5	4.0	0.0	Yes - with mismatch
C09g014610	ATP citrate synthase	-0.70	2.340E-03	-1.00	1.156E-04	Cytoplasm	ASBVdB-sRNA25	24	3.5	4.0	0.0	Yes
C10g012530	Pentatricopeptide repeat-containing protein At2g20540	-0.91	6.134E-04	-0.89	1.072E-03	Nucleus	ASBVdB-sRNA15	23	3.5	4.0	0.0	Yes
C12g004290	Signal recognition particle 9 kDa protein	-0.37	0.11	-0.58	0.11	Cytoplasm /Nucleus	ASBVdB-sRNA15	23	3.5	4.0	0.0	Yes
C01g017850	Protein GrpE	-1.08	2.973E-08	-1.55	1.429E-13	Plastid	ASBVdB-sRNA41b	19	3.5	4.0	0.0	Yes
C07g004620	Xyloglucan endotransglucosylase /hydrolase	-1.32	4.95E-03	-1.19	3.52E-03	Extracellular	ASBVdB-sRNA41b	19	3.5	3.5	-18.3	Yes

^aTarget gene identifier and putative annotation in the *Persea americana* West Indian pure accession genome (Peame105).

^bLog₂(Fold Change) (log₂FC) and adjusted *p*-value (*padj*) of target gene expression in yellow sectors (SY) relative to green sectors (SG) of bleached leaves.

^cLog₂FC and *padj* of target gene expression in yellow sectors of bleached leaves (SY) relative to asymptomatic (fully green) leaves (AS).

^dSubcellular localisation of target protein as predicted by DeepLoc 2.1 software (<https://services.healthtech.dtu.dk/services/DeepLoc-2.1/>).

^eE-value score for ASBVd-sRNA:mRNA target duplex from psRNATarget (<https://www.zhaolab.org/psRNATarget/analysis>) prediction at default parameters.

^fE-value score for ASBVd-sRNA:mRNA target duplex from psRNATarget prediction at stricter parameters recommended by Fahlgren and Carrington (2010) (with penalties doubled in seed region).

^gMinimum free energy (mfe) of ASBVd-sRNA:mRNA target duplex as predicted by RNAhybrid (<https://bibiserv.cebitec.uni-bielefeld.de/rnahybrid>).

^hPresence of the full region containing the pathogenic determinant in the duplex formed between ASBVd-sRNAs and mRNA targets. Cases where the pathogenic region was interrupted by mismatches, bulges or G:U base pairing within the duplex are indicated.