

**Supplementary Table 1.** GenBank accession numbers, hosts, and countries of origin of isolates used in this study

Isolate	Accession no.			Host (specific name)	Country	Genomic region
	RNA1	RNA2	RNA3			
<b>IR-VM</b>	<b>MW014929</b>	<b>MW014930</b>	<b>MW014931</b>	<b>Lesser periwinkle (<i>Vinca minor</i>)</b>	<b>Iran</b>	<b>Complete</b>
<b>IR-WS</b>	<b>MW014932</b>	<b>MW014933</b>	<b>MW014934</b>	<b>Wisteria (<i>Wisteria sinensis</i>)</b>	<b>Iran</b>	<b>Complete</b>
<b>IR-WS2</b>	-	-	<b>MW014935</b>	<b>Wisteria (<i>Wisteria sinensis</i>)</b>	<b>Iran</b>	<b>Complete</b>
FERA160224	KY810767	KY810768	KY810769	Lab host: <i>Nicotiana tabacum</i>	UK	Complete
Manfredi	KC881008	KC881009	KC881010	Alfalfa ( <i>Medicago sativa</i> )	Argentina	Complete
HZ	HQ316635	HQ316636	HQ316637	Lab host: <i>Nicotiana glutinosa</i>	China	Complete
295	LC485018	LC485016	LC485017	Pea ( <i>Pisum sativum</i> )	Australia	Complete
AU-SA80	MK648424	MK648425	MK648426	Alfalfa ( <i>Medicago sativa</i> )	Australia	Complete
Gyn	MH332897	MH332898	MH332899	Jiaogulan ( <i>Gynostemma pentaphyllum</i> )	China	Complete
Tec1	FR715040	FR715041	FR715042	Cape honeysuckle ( <i>Tecomaria capensis</i> )	Spain	Complete
Lst	FN667965	FN667966	FN667967	Lavender ( <i>Lavandula stoechas</i> )	Italy	Complete
CaM	MK607973	MK607975	MK607977	Potato ( <i>Solanum tuberosum</i> )	Canada	Complete
Ca175-1	MK607974	MK607976	MK607978	Potato ( <i>Solanum tuberosum</i> )	Canada	Complete
175	MF990284	MF990285	MF990286	Potato ( <i>Solanum tuberosum</i> )	Canada	Complete
Mint	MK883819	MK883820	MK883821	Mint ( <i>Mentha canadensis</i> )	China	Complete
OH-2-2017	MT669386	MT669387	MT669388	Soybean ( <i>Glycine max</i> )	USA	Complete
425L			L00162	Clover ( <i>Trifolium repens</i> )	USA	CP
425L			K03542	Clover ( <i>Trifolium repens</i> )	USA	CP
425M			K02703	Clover ( <i>Trifolium repens</i> )	USA	CP
TR			V00048	Clover ( <i>Trifolium repens</i> )	USA	CP
Joe Davis			HQ185569	Soybean ( <i>Glycine max</i> )	USA	CP
TN			HQ185568	Soybean ( <i>Glycine max</i> )	USA	CP
NY-A 2002			AY340070	Alfalfa ( <i>Medicago sativa</i> )	USA	CP
NY-B 2002			AY340071	Bean ( <i>Phaseolus vulgaris</i> )	USA	CP
YSMV			M59241	Alfalfa ( <i>Medicago sativa</i> )	USA	CP
SturdyII			JN256023	Soybean ( <i>Glycine max</i> )	USA	CP
S01-18			JN256020	Soybean ( <i>Glycine max</i> )	USA	CP
K1			JN256025	Soybean ( <i>Glycine max</i> )	USA	CP
C			JN256024	Soybean ( <i>Glycine max</i> )	USA	CP
Ar15			JN256022	Soybean ( <i>Glycine max</i> )	USA	CP
SE12			JN256026	Soybean ( <i>Glycine max</i> )	USA	CP
LN			AY957607	<i>Leonotis nepetifolia</i>	Mexico	CP
Ca175			DQ314750	Potato ( <i>Solanum tuberosum</i> )	Canada	CP
Ca401			DQ314753	Potato ( <i>Solanum tuberosum</i> )	Canada	CP
Ca518			DQ314755	Potato ( <i>Solanum tuberosum</i> )	Canada	CP
Ca400			DQ314752	Potato ( <i>Solanum tuberosum</i> )	Canada	CP
Br			FJ858265	Alfalfa ( <i>Medicago sativa</i> )	Brazil	CP
V.tinus			JN040542	Laurustinus ( <i>Viburnum tinus</i> )	Chile	CP
Monofia			HQ288892	Potato ( <i>Solanum tuberosum</i> ) cv. Spunta	Egypt	CP
Mans			LN846979	Tomato ( <i>Solanum lycopersicum</i> )	Egypt	CP
Shark			LN846978	Tomato ( <i>Solanum lycopersicum</i> )	Egypt	CP
Kr			AB126032	Unknown	South Korea	CP
AZ			AB126031	Unknown	South Korea	CP
KR1			AF294432	Potato ( <i>Solanum tuberosum</i> )	South Korea	CP
KR2			AF294433	Potato ( <i>Solanum tuberosum</i> )	South Korea	CP
ZN-1			LK937168	<i>Nicotiana tabacum</i>	China	CP

M.sativa	JQ281522	Alfalfa ( <i>Medicago sativa</i> )	China	CP
XJA-1	KU573958	Alfalfa ( <i>Medicago sativa</i> )	China	CP
XJA-2	KU573961	Alfalfa ( <i>Medicago sativa</i> )	China	CP
178	KC767662	Actinidia ( <i>Actinidia fortunatii</i> )	New Zealand	CP
176	KC767661	Actinidia ( <i>Actinidia fortunatii</i> )	New Zealand	CP
175	KC767660	Actinidia ( <i>Actinidia fortunatii</i> )	New Zealand	CP
NZ1	U12509	Alfalfa ( <i>Medicago sativa</i> )	New Zealand	CP
NZ2	U12510	Alfalfa ( <i>Medicago sativa</i> )	New Zealand	CP
NZ34	AF215664	Alfalfa ( <i>Medicago sativa</i> )	New Zealand	CP
NZ1 Lincoln	AF215663	Alfalfa ( <i>Medicago sativa</i> )	New Zealand	CP
CV1	HE591387	Chinese hibiscus ( <i>Hibiscus rosa-sinensis</i> )	Spain	CP
TM2	HE591386	Chinese hibiscus ( <i>Hibiscus rosa-sinensis</i> )	Spain	CP
Lye80	AJ130703	Tomato ( <i>Solanum lycopersicum</i> )	France	CP
Caa1	AJ130707	Pepper ( <i>Capsicum annuum</i> )	France	CP
Dac16	AJ130708	Carrot ( <i>Daucus carota</i> )	France	CP
S	X00819	Alfalfa ( <i>Medicago sativa</i> )	England	CP
VRU	AF015716	Lupinus polyphyllus	England	CP
15/64	AF015717	Lupinus polyphyllus	England	CP
195-AN	AJ130705	Tomato ( <i>Solanum lycopersicum</i> )	Italy	CP
F-430	AJ130706	Bean ( <i>Phaseolus vulgaris</i> )	Italy	CP
VIBtin	KP233749	<i>Viburnum tinus</i>	Italy	CP
Danza	Y09110	Tomato ( <i>Solanum lycopersicum</i> )	Italy	CP
Tef-1	FR854391	Teucrium fruticans	Italy	CP
PL-2-10	KC182569	Pepper ( <i>Capsicum annuum</i> )	Serbia	CP
196-08	FJ527749	Tobacco ( <i>Nicotiana tabacum</i> )	Serbia	CP
292Saff	KP034961	Safflower ( <i>Carthamus tinctorius</i> )	Serbia	CP
100-08	EU925642	Lilac ( <i>Syringa vulgaris</i> )	Serbia	CP
258-11	KF147805	Tomato ( <i>Solanum lycopersicum</i> )	Serbia	CP
95-08	FJ527748	Alfalfa ( <i>Medicago sativa</i> )	Serbia	CP
373-12	KC288155	<i>Robinia pseudoacacia</i>	Serbia	CP
P-27-09	KC182568	Pepper ( <i>Capsicum annuum</i> )	Serbia	CP
P-10-10	KC182567	Pepper ( <i>Capsicum annuum</i> )	Serbia	CP
70-12	JX996119	Lavandula x intermedia	Croatia	CP
371-13	KJ504107	Lavandula x intermedia	Croatia	CP
R236	KX710198	Pepper ( <i>Capsicum annuum</i> )	Bosnia & Herzegovina	CP
PV1	MG600289	Clover ( <i>Trifolium repens</i> )	Czech Republic	CP
W1	HM807307	Alfalfa ( <i>Medicago sativa</i> )	Australia	CP
Hu	JX112759	Alfalfa ( <i>Medicago sativa</i> )	Australia	CP
EW	JX112757	Medicago littoralis x Medicago truncatula	Australia	CP
Aq	JX112758	Alfalfa ( <i>Medicago sativa</i> )	Australia	CP
N20	AF332998	Lab host: <i>Nicotiana clevelandii</i>	Australia	CP
WC3	JN209847	Clover ( <i>Trifolium repens</i> )	Australia	CP
S30	HM807305	Alfalfa ( <i>Medicago sativa</i> )	Australia	CP
AP1	JX853610	Alfalfa ( <i>Medicago sativa</i> )	Iran	
ADA4	KM655871	Alfalfa ( <i>Medicago sativa</i> )	Iran	CP
AK2	KM655872	Alfalfa ( <i>Medicago sativa</i> )	Iran	CP
PPF	KM655875	Pepper ( <i>Capsicum annuum</i> )	Iran	CP
SMZ	KM655873	Black nightshade ( <i>Solanum nigrum</i> )	Iran	CP
TBN	KM655878	Cowpea ( <i>Vigna unguiculata</i> )	Iran	CP
PSH	KM655874	Black nightshade ( <i>Solanum nigrum</i> )	Iran	CP

PKH	KM655876	Pepper ( <i>Capsicum frutescens</i> )	Iran	CP
Ke.Or.Am	KX535523	<i>Ammi majus</i>	Iran	CP
Si.Ze.A	KX535500	Alfalfa ( <i>Medicago sativa</i> )	Iran	CP
Es.Es.A	KX535478	Alfalfa ( <i>Medicago sativa</i> )	Iran	CP
Ch.Sh.A	KX535475	Alfalfa ( <i>Medicago sativa</i> )	Iran	CP
Ke.Si.Ru	KX535524	<i>Rumex</i> sp.	Iran	CP
Ke.Sa.Pe	KX535514	Pepper ( <i>Capsicum annuum</i> )	Iran	CP
Ke.Ke.Pe	KX535513	Pepper ( <i>Capsicum annuum</i> )	Iran	CP
Ke.Ma.Po	KX535510	Potato ( <i>Solanum tuberosum</i> )	Iran	CP
Kh.Bj.A	KX535495	Alfalfa ( <i>Medicago sativa</i> )	Iran	CP
Fa.Ma.A	KX535479	Alfalfa ( <i>Medicago sativa</i> )	Iran	CP

The Alfalfa mosaic virus isolates, which have been sequenced in this study, are bolded.  
CP, coat protein.

**Supplementary Table 2.** Recombination/reassortment putative events identified in AMV isolates by using seven programs packaged in the RDP4 software

Event	Recombinant isolate	Reass/ Rec	<i>P</i> -value for the seven detection methods							Position <sup>a</sup>	Segment	Parental sequences	
			RDP	Gene-Conv	Boot-scan	Max-Chi	Chi-maera	SiScan	3SEQ			Major	Minor
1	HZ	Reass	2.474 ×10 <sup>-20</sup>	5.569 ×10 <sup>-19</sup>	5.537 ×10 <sup>-18</sup>	2.874 ×10 <sup>-17</sup>	1.320 ×10 <sup>-07</sup>	4.672 ×10 <sup>-20</sup>	8.762 ×10 <sup>-20</sup>	3696-6408	RNA2	Unknown <sup>b</sup> (Manfredi)	175
2	FERA160224	Reass	1.039 ×10 <sup>-17</sup>	1.474 ×10 <sup>-11</sup>	2.991 ×10 <sup>-17</sup>	3.486 ×10 <sup>-11</sup>	1.392 ×10 <sup>-05</sup>	2.415 ×10 <sup>-18</sup>	8.487 ×10 <sup>-08</sup>	3542-6408	RNA2	Unknown <sup>b</sup> (295)	Lst
3	Lst	Reass	8.716 ×10 <sup>-05</sup>	2.802 ×10 <sup>-07</sup>	8.572 ×10 <sup>-03</sup>	1.008 ×10 <sup>-11</sup>	4.323 ×10 <sup>-08</sup>	3.051 ×10 <sup>-03</sup>	1.489 ×10 <sup>-03</sup>	1-3696	RNA1	Mint	295
4	295	Reass	3.821 ×10 <sup>-11</sup>	3.858 ×10 <sup>-09</sup>	2.781 ×10 <sup>-11</sup>	1.306 ×10 <sup>-13</sup>	2.061 ×10 <sup>-06</sup>	1.455 ×10 <sup>-11</sup>	8.189 ×10 <sup>-01</sup>	3696-6408	RNA2	Gyn	Unknown <sup>b</sup> (Tec1)
5	AU-SA80	Reass	6.539 ×10 <sup>-11</sup>	4.439 ×10 <sup>-04</sup>	3.777 ×10 <sup>-10</sup>	7.454 ×10 <sup>-14</sup>	1.496 ×10 <sup>-05</sup>	4.454 ×10 <sup>-11</sup>	4.041 ×10 <sup>-14</sup>	3696-6408	RNA2	Gyn	Unknown <sup>b</sup> (Tec1)
6	175	Reass	4.166 ×10 <sup>-02</sup>	14.01 ×10 <sup>-04</sup>	1.772 ×10 <sup>-05</sup>	9.247 ×10 <sup>-10</sup>	8.571 ×10 <sup>-04</sup>	2.450 ×10 <sup>-22</sup>	5.127 ×10 <sup>-02</sup>	3696-6408	RNA2	Unknown <sup>b</sup> (OH-2-2017)	CaM
7	Ca175-1	Rec	4.355 ×10 <sup>-02</sup>	18.45 ×10 <sup>-04</sup>	3.795 ×10 <sup>-06</sup>	5.545 ×10 <sup>-09</sup>	8.571 ×10 <sup>-04</sup>	8.482 ×10 <sup>-20</sup>	5.127 ×10 <sup>-02</sup>	3759-5147	RNA2	Unknown <sup>b</sup> (OH-2-2017)	CaM

For each putative event is reported type of event (reassortment/recombination), parental (major and minor), position and segment involved.

<sup>a</sup>Position in alignment.

<sup>b</sup>Sequence used to infer unknown parent.

**Supplementary Table 3.** Codon positions of the coding regions of AMV isolates affected by positive selection

Genomic region	SLAC	FEL	IFEL	FUBAR	MEME
<b>P1</b>	-	-	-	<b>228</b>	10, 75, <b>228</b> , 724, 986
<b>P2</b>	-	<b>46, 168, 193</b>	<b>142, 193, 498</b>	<b>27, 46, 168, 193, 229</b>	<b>46, 72, 168, 193, 652</b>
<b>MP</b>	-	-	-	-	150
<b>CP</b>	-	-	-	-	15

Bold numbers are the positively selected codon sites detected by two methods or more.

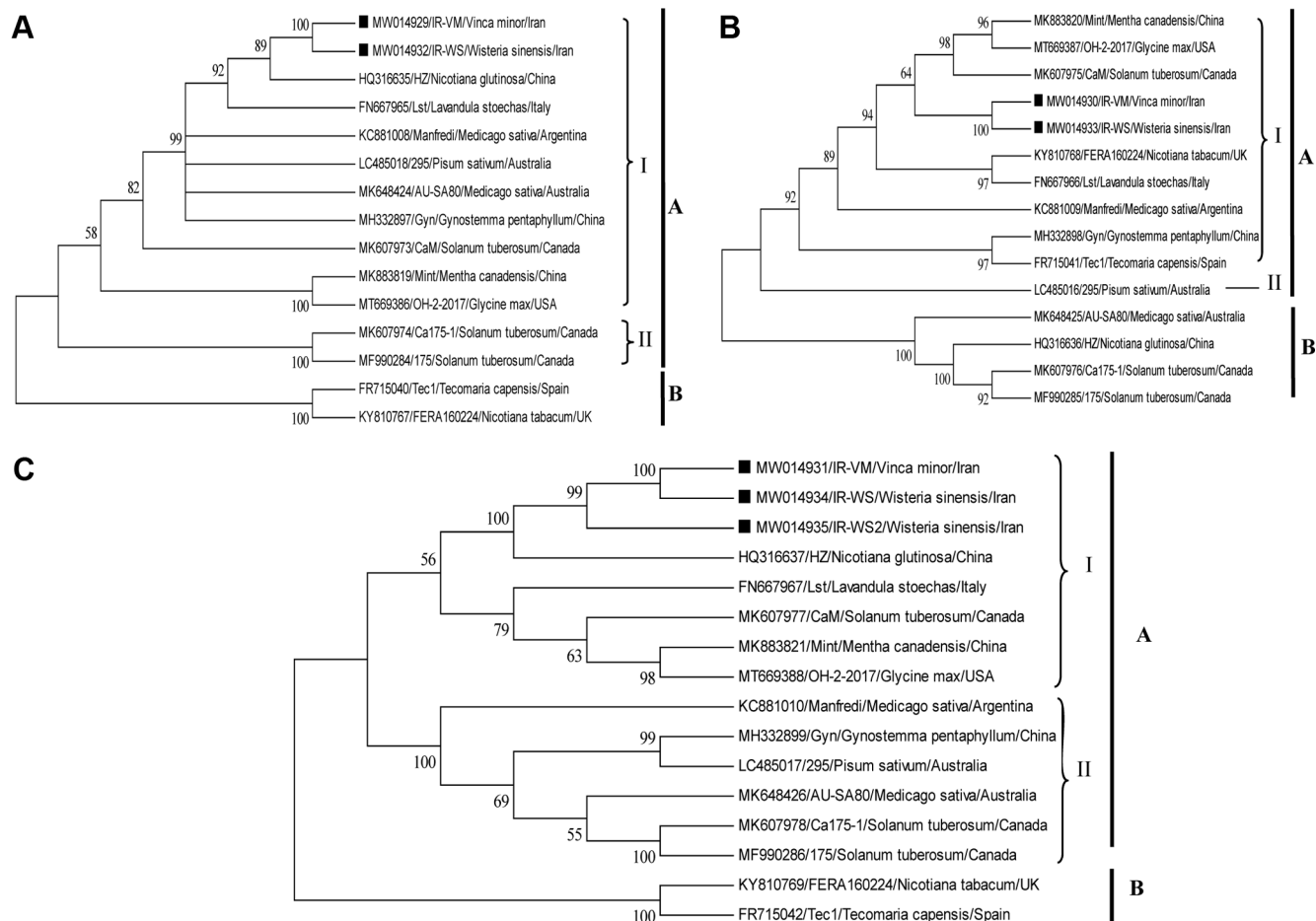
Significance levels set at P = 0.1 for SLAC, FEL, IFEL, and MEME programs, posterior probability of 0.9 for FUBAR.

AMV, Alfalfa mosaic virus; SLAC, single-likelihood ancestor counting; FEL, fixed effects likelihood; IFEL, internal fixed effects likelihood; FUBAR, fast unbiased Bayesian approximation; MEME, mixed effects model of evolution; -, no positively site was found.

**Supplementary Table 4.** Genetic distances within and between phylogroups in clade A

	Groups		
	I	II	III
Group I	0.022 ± 0.003		
Group II	0.051 ± 0.008	0.012 ± 0.004	
Group III	0.052 ± 0.007	0.045 ± 0.007	0.027 ± 0.004

Standard error estimates were obtained by a bootstrap method (1,000 replicates). Analyses were performed using MEGA6, and the codon positions included were first, second, third, and noncoding. All positions containing gaps and missing data were removed from the dataset (complete deletion option).



**Supplementary Fig. 1.** Phylogenetic relationships of complete RNA1 (A), RNA2 (B) and RNA3 (C) sequences of IR-VM and IR-WS with other different Alfalfa mosaic virus (AMV) isolates retrieved from GenBank database, constructed using MEGAX by the neighbor joining method, with 1,000 bootstrap replications and with a 50% bootstrap threshold score. Isolates were indicated in the tree by accession number/isolate name/host/geographical origin of collection. Iranian AMV isolate generated from this study was marked.