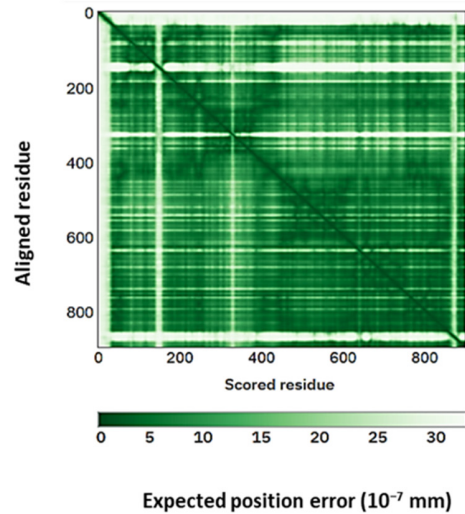


Figure S1. (A) Assessment of inter-domain accuracy for PpAGO6, highlighting expected position errors at residues when aligned with true structures, indicating high prediction reliability (AlphaFold). (B) Direct physical PPI fingerprint curves of Dicer protein homologs, distinguishing dimeric (blue) and tetrameric (green) quaternary structures critical for understanding molecular interactions and regulation, with differently colored boxes indicating motifs and their positions. (C) Assessment of inter-domain accuracy for PpRDR2, showing expected position errors at residues when aligned, indicative of robust prediction of domain positions (AlphaFold).

A.



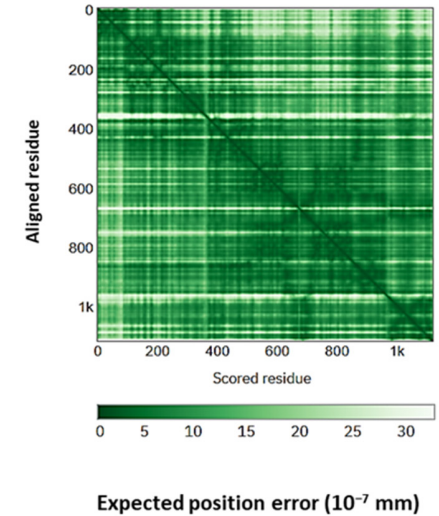
PpAGO6

B.



PpDCL4

C.



PpRDR2

Figure S2 *Cis*-elements in the 2kb upstream region of the RNAi-related genes. A. *Cis*-elemets for *PpAGO* B. *Cis*-elemets for *PpDCL* C. *Cis*-elemets for *PpRDR*. Different colors in the left lines indicate different types of *cis*-elements. The color intensity and number in the cells indicated the number of *cis*-element in these genes.

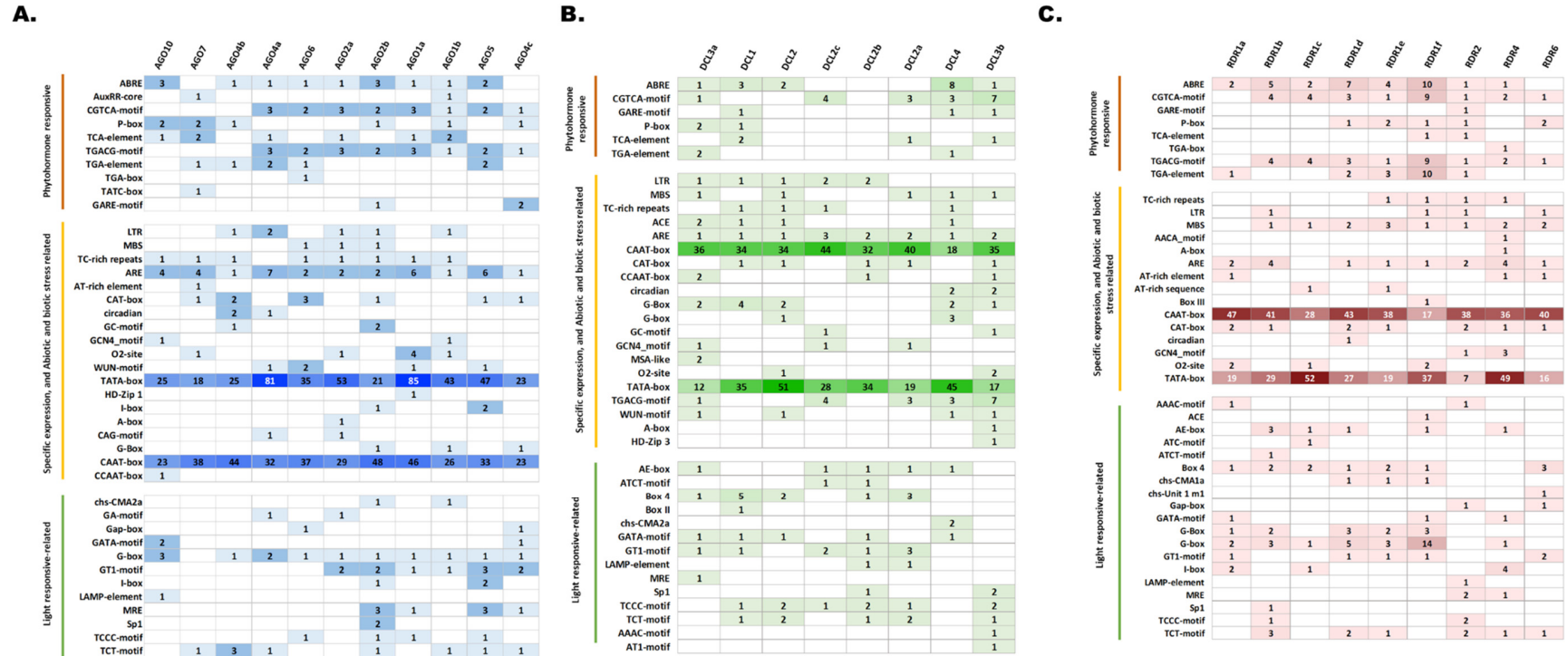


Table S1 Physicochemical characterization of Rosaceae AGO genes

Cluster	Species	Gene ID	Seq. L.	pI	MW
AGO1	<i>P. dulcis</i>	Prudul26A012572	1103	9.7	121.6
	<i>Rubus occidentalis</i>	Ro06_G13161	878	9.9	98.5
	<i>Rubus occidentalis</i>	Ro06_G13162	961	9.7	107.9
AGO2	<i>Fragaria vesca</i>	FvH4_3g11980	1057	9.7	117.11
	<i>Malus x domestica</i>	MDP0000292511	2251	8.5	253.10
	<i>P. armeniaca</i>	PARG11581m02	1101	9.4	121.22
	<i>P. dulcis</i>	Prudul26A015045	1065	9.7	117.22
	<i>P. dulcis</i>	Prudul26A030904	1014	9.4	114.92
	<i>Pyrus communis</i>	pycom05g23520	2348	10	268.21
	<i>Pyrus communis</i>	pycom10g19770	1059	9.6	117.67
	<i>Pyrus communis</i>	pycom10g19780	997	9.3	111.91
	<i>Rosa chinensis</i>	RcHt_S135.34	1053	9.8	117.48
	<i>Rosa chinensis</i>	RcHt_S1438.2	1056	9.7	117.77
AGO2a	<i>Rubus occidentalis</i>	Ro03_G10872	1051	9.7	116.93
AGO4a	<i>Fragaria vesca</i>	FvH4_3g27260	877	8.7	97.61
	<i>P. armeniaca</i>	PARG16951m01	911	9.6	101.75
	<i>P. dulcis</i>	Prudul26A004792	818	8.8	91.41
	<i>P. dulcis</i>	Prudul26A027167	903	9.5	101.52
	<i>P. dulcis</i>	Prudul26A029493	912	9.5	101.64
	<i>Pyrus communis</i>	pycom03g11750	1021	8.8	112.75
	<i>Pyrus communis</i>	pycom07g04080	577	8.8	65.06
	<i>Pyrus communis</i>	pycom11g15700	511	9.8	56.87
	<i>P. armeniaca</i>	RcHt_S102.24	892	9.6	100.44
	<i>Rubus occidentalis</i>	Ro03_G14302	891	8.1	98.85
	<i>Rubus occidentalis</i>	Ro03_G26924	944	7.9	104.68
	<i>Rubus occidentalis</i>	Ro07_G13592	914	9.4	102.24
AGO5	<i>Fragaria vesca</i>	FvH4_3g30202	955	9.8	106.35
	<i>Fragaria vesca</i>	FvH4_5g18150	1091	9.5	120.44
	<i>Fragaria vesca</i>	FvH4_5g18151	1127	9.6	125.22
	<i>Malus x domestica</i>	MDP0000161046	1115	9.4	121.53
	<i>Malus x domestica</i>	MDP0000232035	978	9.9	109.13
	<i>Malus x domestica</i>	MDP0000774227	983	9.9	109.50
	<i>P. armeniaca</i>	PARG01102m02	1018	9.9	113.65
	<i>P. dulcis</i>	Prudul26A005261	990	9.9	110.57
	<i>P. dulcis</i>	Prudul26A019891	1070	9.6	118.56
	<i>Pyrus communis</i>	pycom11g12470	1011	10	111.32
	<i>P. armeniaca</i>	RcHt_S1892.2	872	9.6	98.96
	<i>P. armeniaca</i>	RcHt_S1892.3	890	9.7	100.94
	<i>P. armeniaca</i>	RcHt_S3780.4	890	9.7	100.98
AGO6	<i>Fragaria vesca</i>	FvH4_4g08040	885	9.4	98.64
	<i>Fragaria vesca</i>	FvH4_4g08050	891	9.0	100.13
	<i>Malus x domestica</i>	MDP0000285251	2583	9.0	288.50
	<i>P. armeniaca</i>	PARG13812m01	898	8.7	100.13
	<i>P. dulcis</i>	Prudul26A000010	899	8.9	100.07
	<i>P. armeniaca</i>	RcHt_S2853.5	923	9.2	104.24
	<i>Rubus occidentalis</i>	Ro06_G06676	2660	9.3	298.34

Table S1 Physicochemical characterization of Rosaceae AGO genes (Continued)

Cluster	Species	Gene ID	Seq. L.	pI	MW
AGO7	<i>Fragaria vesca</i>	FvH4_4g30730	1020	9.3	115.71
	<i>Malus x domestica</i>	MDP0000118779	1016	9.5	114.86
	<i>Malus x domestica</i>	MDP0000159246	1024	9.6	116.22
	<i>P. armeniaca</i>	PARG06502m02	1012	9.5	114.80
	<i>Pyrus communis</i>	pycom13g06000	535	9.4	60.39
	<i>Pyrus communis</i>	pycom16g05840	1020	9.8	115.29
	<i>P. armeniaca</i>	RcHt_S3636.8	1018	9.6	115.82
	<i>Rubus occidentalis</i>	Ro04_G00017	1017	9.3	115.63
AGO8	<i>P. armeniaca</i>	RcHt_S917.14	892	9.8	100.50
AGO9	<i>Fragaria vesca</i>	FvH4_7g04840	916	9.2	102.07
	<i>Malus x domestica</i>	MDP0000176861	866	8.0	96.08
	<i>Malus x domestica</i>	MDP0000209079	888	9.7	99.85
	<i>Malus x domestica</i>	MDP0000215105	845	9.7	94.78
	<i>Malus x domestica</i>	MDP0000272708	974	8.8	108.08
	<i>P. armeniaca</i>	PARG01636m01	942	8.7	104.38
	<i>P. armeniaca</i>	RcHt_S664.14	985	9.1	109.79
	<i>P. armeniaca</i>	RcHt_S1308.7	1097	9.4	123.14
	<i>P. armeniaca</i>	RcHt_S3236.5	1008	8.8	112.23
	<i>P. armeniaca</i>	RcHt_S3474.2	935	9.3	104.48
AGO10	<i>Fragaria vesca</i>	FvH4_4g01630	993	9.4	111.18
	<i>Malus x domestica</i>	MDP0000069525	1093	9.5	121.50
	<i>Malus x domestica</i>	MDP0000071268	988	9.4	110.56
	<i>Malus x domestica</i>	MDP0000191579	956	9.2	106.88
	<i>Malus x domestica</i>	MDP0000199819	974	9.4	109.45
	<i>Malus x domestica</i>	MDP0000305971	2043	8.1	227.87
	<i>P. armeniaca</i>	PARG03504m01	990	9.4	111.20
	<i>P. armeniaca</i>	PARG25313m01	1092	9.7	120.56
	<i>P. dulcis</i>	Prudul26A007562	1016	9.4	114.18
	<i>Pyrus communis</i>	pycom06g21250	1114	9.6	121.95
	<i>Pyrus communis</i>	pycom06g21260	1080	9.6	120.01
	<i>Pyrus communis</i>	pycom13g17460	991	9.4	110.78
	<i>Pyrus communis</i>	pycom14g20500	1081	9.6	120.36
	<i>Pyrus communis</i>	pycom16g16970	914	8.6	102.23
	<i>P. armeniaca</i>	RcHt_S751.29	993	9.3	111.33
	<i>P. armeniaca</i>	RcHt_S844.20	993	9.3	111.34
	<i>P. armeniaca</i>	RcHt_S1713.17	1098	9.6	121.29
	<i>P. armeniaca</i>	RcHt_S1713.18	1081	9.6	119.34
	<i>P. armeniaca</i>	RcHt_S3446.14	1009	9.5	112.17
	<i>Rubus occidentalis</i>	Ro04_G13543	1015	9.4	113.76
	<i>Rubus occidentalis</i>	Ro05_G03676	2833	9.2	314.02

Table S2 The detailed information of *DCL* genes among five Rosaceae genomes

Protein	Species	Gene identifier	Chr	5' End	3' End
DCL1	<i>Rubus occidentalis</i>	Ro07_G20422	7	673857	683803
	<i>Rosa chinensis</i>	Rcg0361361	1	53342837	53352195
	<i>Fragaria vesca</i>	Frv7g17920	7	15080110	15089980
	<i>P. armeniaca</i>	PARG18582m01	5	19491995	19501399
	<i>P. persica</i>	Prupe.2G200900	2	23762166	23772469
	<i>P. dulcis</i>	Prudul26A022109	2	19442051	19451461
	<i>Pyrus communis</i>	pycom01g11560	1	12559735	12567397
	<i>Malus x domestica</i>	MDP0000767313	1	20653300	20662517
	<i>Pyrus communis</i>	pycom01g11560	1	12559735	12567397
	<i>Malus x domestica</i>	MDP0000164658	1	20653300	20662517
	<i>Malus x domestica</i>	MDP0000268554	1	21052478	21061418
	<i>Malus x domestica</i>	MDP0000237286	1	21052478	21061418
DCL2	<i>Pyrus communis</i>	pycom14g07170	14	7180140	7183892
	<i>Malus x domestica</i>	MDP0000316580	14	9863509	9877023
	<i>Pyrus communis</i>	pycom12g07520	12	7946455	7950076
	<i>P. dulcis</i>	Prudul26A006390	7	7145719	7151040
	<i>P. persica</i>	Prupe.7G047700	7	8457811	8464704
	<i>P. persica</i>	Prupe.7G048000	7	8471883	8476896
	<i>P. armeniaca</i>	PARG26002m01	8	6007756	6024585
	<i>P. dulcis</i>	Prudul26A013521	7	7151919	7162920
	<i>P. persica</i>	Prupe.7G047900	7	8465187	8471404
	<i>Rubus occidentalis</i>	Ro05G07364	5	12471049	12480637
	<i>Rubus occidentalis</i>	Ro06_G14366	6	18536064	18547083
	<i>Rubus occidentalis</i>	Ro06_G28732	6	24490467	24501374
	<i>Rosa chinensis</i>	Rcg0447571	3	232685	241890
	<i>Fragaria vesca</i>	Frv6g00200	6	152041	161520
	<i>Pyrus communis</i>	pycom04g21800	4	22794339	22799537
	<i>Malus x domestica</i>	MDP0000194327	4	30664164	30679873
	<i>P. armeniaca</i>	PARG03276m01	1	24105028	24116477
	<i>P. persica</i>	Prupe.6G363600	6	30547664	30558666
	<i>P. dulcis</i>	Prudul26A002319	6	29350412	29360408

Table S2 The detailed information of *DCL* genes among five Rosaceae genomes (Continued)

Protein	Species	Gene identifier	Chr	5' End	3' End
DCL3	<i>Rubus occidentalis</i>	Ro02G19685	2	11611804	11621593
	<i>Fragaria vesca</i>	Frv2g18930	2	16120223	16128487
	<i>Rosa chinensis</i>	Rcg0284721	6	48073740	48082027
	<i>Malus x domestica</i>	MDP0000263070	10	26819970	26827854
	<i>P. armeniaca</i>	PARG20479m01	6	3870212	3878692
	<i>P. dulcis</i>	Prudul26A020901	8	16787796	16796450
	<i>P. persica</i>	Prupe.8G202000	8	19052450	19061495
	<i>Rubus occidentalis</i>	Ro02G04571	2	5013330	5023579
	<i>Rosa chinensis</i>	Rcg0312121	6	68828384	68837990
	<i>Fragaria vesca</i>	Frv2g30780	2	23755878	23765432
	<i>P. armeniaca</i>	PARG07485m01	2	30582898	30595667
	<i>P. dulcis</i>	Prudul26A017452	1	32063803	32079394
	<i>P. persica</i>	Prupe.1G401900	1	35456786	35468115
	<i>Pyrus communis</i>	pycom15g04670	15	2767094	2771462
	<i>Malus x domestica</i>	MDP0000308156	15	3281917	3301223
	<i>Pyrus communis</i>	pycom08g03840	8	2924288	2930103
	<i>Malus x domestica</i>	MDP0000155170	8	3185951	3196633
	<i>Pyrus communis</i>	pycom08g03830	8	2919459	2923894
DCL4	<i>Rubus occidentalis</i>	Ro01G05100	1	724883	744279
	<i>Fragaria vesca</i>	Frv1g01480	1	774802	787119
	<i>Rosa chinensis</i>	Rcg0086421	2	1581834	1595215
	<i>P. armeniaca</i>	PARG28161m01	8	21048930	21061307
	<i>P. persica</i>	Prupe.7G252800	7	21319142	21332373
	<i>P. dulcis</i>	Prudul26A023796	7	20244885	20257824
	<i>Malus x domestica</i>	MDP0000120528	15	11742630	11753048
	<i>Pyrus communis</i>	pycom15g14140	15	9625036	9635325
	<i>Malus x domestica</i>	MDP0000411671	15	11742630	11753048
	<i>Malus x domestica</i>	MDP0000619907	15	11742630	11753048

Table S3 The RDR proteins profiles in seven Rosaceae species

Protein	Species	Gene identifier	Seq. L.	IP	MW
RDR1	<i>Malus x domestica</i>	MDP0000409112	1042	8.2	118.67
	<i>P. armeniaca</i>	PARG29388m02	1363	6.3	154.95
	<i>P. dulcis</i>	Prudul26A002362	1105	6.5	124.55
	<i>P. dulcis</i>	Prudul26A025346	1131	8.0	128.96
	<i>P. armeniaca</i>	pycom16g01590	1117	6.3	127.01
	<i>Rosa chinensis</i>	RcHt_S11920.1	506	7.0	57.56
	<i>Rubus occidentalis</i>	Ro04_G36148	1643	7.4	187.15
	<i>Rubus occidentalis</i>	Ro04_G36603	1189	7.4	135.41
	<i>Rubus occidentalis</i>	Ro04_G37223	1000	6.1	113.80
	<i>Rubus occidentalis</i>	Ro05_G31556	1160	7.7	132.33
RDR2	<i>Fragaria vesca</i>	FvH4_4g33391	1120	7.2	127.26
	<i>Fragaria vesca</i>	FvH4_5g05070	1137	6.4	129.60
	<i>Malus x domestica</i>	MDP0000220527	1119	7.0	126.77
	<i>Malus x domestica</i>	MDP0000228855	1451	6.7	165.48
	<i>Malus x domestica</i>	MDP0000242926	1444	6.6	162.97
	<i>Malus x domestica</i>	MDP0000287328	1030	7.5	115.18
	<i>Malus x domestica</i>	MDP0000939266	1144	6.9	129.95
	<i>P. armeniaca</i>	PARG06882m01	1104	6.6	124.59
	<i>P. armeniaca</i>	PARG11920m01	1131	6.2	129.21
	<i>P. armeniaca</i>	PARG11921m01	1131	7.8	128.61
	<i>P. armeniaca</i>	PARG24674m01	1118	6.2	127.17
	<i>P. dulcis</i>	Prudul26A013912	1345	6.0	152.80
	<i>P. dulcis</i>	Prudul26A018874	1119	6.4	127.40
	<i>P. armeniaca</i>	pycom13g01640	852	6.5	96.88
	<i>P. armeniaca</i>	pycom13g01650	1026	7.3	116.89
	<i>P. armeniaca</i>	pycom13g03550	903	7.2	102.55
	<i>P. armeniaca</i>	pycom14g15310	1161	6.6	131.97
	<i>Rosa chinensis</i>	RcHt_S404.27	1128	6.0	128.34
	<i>Rosa chinensis</i>	RcHt_S1558.23	1110	6.4	126.48
	<i>Rosa chinensis</i>	RcHt_S3209.1	1124	6.2	128.00
	<i>Rosa chinensis</i>	RcHt_S8225.1	1037		
				7.4	118.57
	<i>Rubus occidentalis</i>	Ro05_G12136	1136		
				6.7	128.95

Table S3 The RDR proteins profiles in seven Rosaceae species (Continues)

Protein	Gene identifier	Species	Seq. L.	IP	MW
RDR3	FvH4_1g05980	<i>Fragaria vesca</i>	1226	6.7	138.02
	MDP0000223167	<i>Malus x domestica</i>	1117	7.2	126.41
	MDP0000544932	<i>Malus x domestica</i>	401	5.5	45.21
	PARG27852m01	<i>P. armeniaca</i>	1613	7.2	180.84
	pycom02g05070	<i>P. armeniaca</i>	724	6.2	83.05
	pycom15g17460	<i>P. armeniaca</i>	1003	7.1	113.08
	RcHt_S1387.1	<i>Rosa chinensis</i>	1454	7.1	162.54
	RcHt_S2399.1	<i>Rosa chinensis</i>	1163	6.6	131.94
	Ro01_G29550	<i>Rubus occidentalis</i>	1222	7.3	138.32
	Ro03_G06008	<i>Rubus occidentalis</i>	2331	7.7	262.32
	Ro07_G01985	<i>Rubus occidentalis</i>	408	5.6	46.07
	Ro07_G27199	<i>Rubus occidentalis</i>	897	8.0	100.90
RDR4	Prudul26A018643	<i>P. dulcis</i>	1258	6.7	142.03
RDR5	MDP0000203919	<i>Malus x domestica</i>	256	4.5	29.42
	MDP0000298597	<i>Malus x domestica</i>	1002	7.3	113.63
	PARG27853m01	<i>P. armeniaca</i>	1189	8.0	134.85
	Prudul26A018300	<i>P. dulcis</i>	1118	6.4	127.43
	Prudul26A026199	<i>P. dulcis</i>	1124	7.3	128.47
	Prudul26A032366	<i>P. dulcis</i>	1079	6.9	122.06
	RcHt_S371.18	<i>Rosa chinensis</i>	1118	6.5	126.77
	RcHt_S434.41	<i>Rosa chinensis</i>	1088	6.3	122.58
	RcHt_S1387.2	<i>Rosa chinensis</i>	1065	7.6	121.59
	RcHt_S3330.1	<i>Rosa chinensis</i>	1037	7.1	118.47
RDR6	FvH4_2g29160	<i>Fragaria vesca</i>	1104	8.0	125.88
	FvH4_2g29170	<i>Fragaria vesca</i>	1198	7.0	137.00
	MDP0000475729	<i>Malus x domestica</i>	1115	6.2	127.00
	MDP0000524927	<i>Malus x domestica</i>	1198	7.3	136.96
	MDP0000589856	<i>Malus x domestica</i>	1115	7.2	126.80
	PARG06863m01	<i>P. armeniaca</i>	1257	6.3	142.10
	PARG08233m01	<i>P. armeniaca</i>	1197	6.6	136.83
	Prudul26A015798	<i>P. dulcis</i>	1197	6.7	136.44
	pycom13g03580	<i>P. armeniaca</i>	629	7.1	71.04
	pycom15g11240	<i>P. armeniaca</i>	1198	7.2	136.78
	RcHt_S68.81	<i>Rosa chinensis</i>	1086	7.5	124.04
	RcHt_S220.32	<i>Rosa chinensis</i>	1105	7.8	126.14
	RcHt_S378.24	<i>Rosa chinensis</i>	581	7.3	67.02
	RcHt_S1234.6	<i>Rosa chinensis</i>	1197	7.2	136.93
	RcHt_S4536.1	<i>Rosa chinensis</i>	1247	7.5	142.79
	Ro02_G01597	<i>Rubus occidentalis</i>	2085	7.5	238.48

Table S4 qRT-PCR primers used to validate the expression levels of *PpAGO*, *PpDCL*, *PpRDR* genes

Gene name	AGO Genes ID	Forward 5'–3'	Reverse 5'–3'	Size (bp)
<i>PpAGO10</i>	Prupe.1G022900	GGGACGGTGTAAAGTGAAGGG	ATAATCGTGTGTGGTGCCGT	144
<i>PpAGO7</i>	Prupe.1G279900	GAAGGGGATGACTGGAAGCC	AGTCCTTGTGGGTTGGTCG	200
<i>PpAGO4b</i>	Prupe.2G056600	GAAAATAGGGCGCTGGGCTA	ACAGAAGCTGTGGTTTGGT	218
<i>PpAGO4a</i>	Prupe.2G056700	ATGGGAAAGGCATTGGCAGA	CATATCCCCGCACTCTCAC	227
<i>PpAGO6</i>	Prupe.3G209300	CAACGCAGAGGGCATCCTTA	AACAAGCCAACGGTCAATGC	281
<i>PpAGO2a</i>	Prupe.4G108000	GGCTAACAAAGCAAATGACCAG	CCAAAGAGGGGAAACCGAT	105
<i>PpAGO2b</i>	Prupe.4G108100	CTGAAGAAGGCTTTGGGAGG	CCCGGTAACACATTGCCTGT	131
<i>PpAGO1a</i>	Prupe.5G241500	AAATCGGCTCCCTGCCTATG	AAATGAACGCCCCACAGGAT	295
<i>PpAGO1b</i>	Prupe.5G241600	AGCTCGCATTTCTCCTCCAC	TCAGAACAGAATCCACGGGC	189
<i>PpAGO5</i>	Prupe.6G115400	CTGGCCGAAAACCAGAGAGA	AGGGTGGCAAATCTTGGTGT	266
<i>PpAGO4c</i>	Prupe.6G154800	GCCAAGGATGGAGAGGATGG	AAGAACAAAGCTCCAGGGGG	154
<i>PpDCL2a</i>	Prupe.7G048000	CATGTGTGCAGAGAGTGAGC	TTCGCTCCTCATGCCAAGTA	181
<i>PpDCL2b</i>	Prupe.7G047900	AATTTCTTGGCGATGCGGTG	TGTGGAGGACACAGTCCGAA	186
<i>PpDCL2c</i>	Prupe.7G047700	ATGCCTTGACTGCCTCTTGC	CCTCAGGCCAAGCACAAACAT	182
<i>PpDCL3b</i>	Prupe.8G202000	GCTTCCCTAACATGGAGCAC	TTTCCAACACC GCAATCGTG	168
<i>PpDCL4</i>	Prupe.7G252800	GCCAAAGAAAAATCTTCCAGGCG	AAGTGGCTTGGTCCTTCTCA	128
<i>PpDCL2</i>	Prupe.6G363600	CGAAGCCTACCAAGCATTTG	TCTCAAATCCGTCAATCCCCTAA	175
<i>PpDCL1</i>	Prupe.2G200900	GCCAGGAATAGCGAGGAGAC	AATGGATGAGCCCAACAGCA	172
<i>PpDCL3a</i>	Prupe.1G401900	ACTGAAGGAACTTGAGAAAAGAGA	GACGTTATAGTTGATGTCTAGGAGA	112
<i>PpRDR4</i>	Prupe.7G221200	AGCAAGGTCGCAAGAGAAGG	TTCACTCCTGTCAATCGCGG	162
<i>PpRDR2</i>	Prupe.5G176700	AGTTTCTGGCTTTCTCGGCT	TTGACCCATCCTAGCTGCAC	152
<i>PpRDR1a</i>	Prupe.4G078900	TGGTCAGTCTTTCAGCTCTTCC	CTCAAAC TTTCTTGCCACCCT	166
<i>PpRDR1b</i>	Prupe.4G078800	ATTATCCCCGGCACGAACT	GAGAGTCAACAGCGAGGGAG	204
<i>PpRDR6</i>	Prupe.1G480300	GTGATTCTGTTGCGGCTGAC	GGCCTTCTTCAACTTCGCAC	207
<i>PpRDR1c</i>	Prupe.1G334600	AAAGGTGTGGTGGCTGTTGA	ACTGGCGGTT CAGGAAACAA	139
<i>PpRDR1d</i>	Prupe.1G334500	GAGAGCGACAGAAGGCTACC	TTTCACCTGTCGGAAGAGCC	203
<i>PpRDR1e</i>	Prupe.1G332600	TGGGTTGACTGCACAAGACA	ACTTGCTTGCCACTCTTCCA	242
<i>PpRDR1f</i>	Prupe.1G132100	CGAGTCAACTGCGAGAGCA	AGCTGAAAGCCTGACCTAACC	144
<i>PpACTIN</i>	Prupe.8G132000	TGCCATTGAAATCCTGAAAC	ACCAATTGGATCATCTCTCT	-

Preparation of Proline Standard Curve

To prepare the L-proline standard curve, different concentration of L-proline (MW; 115.13) was dissolved in ddH₂O as shown in the table below.

Table S5 Preparation of proline standard curves

Tubes	1	2	3	4	5	6	7
ddH ₂ O (mL)	1	1	1	1	1	1	1
L-Proline (mg)	0	5	10	15	20	25	30

Then, 3 mL reaction solution was added to each tube containing acid Ninhydrin and 3% Sulfosalicylic acid, The solution was boiled in a water bath cool on ice and absorbance was measured at 520 nm using a spectrophotometer. The L-proline standard curve was drawn based on the absorbance and concentration.