

Table S1 List of WRKY family members identified genome-wide in *Nelumbo nucifera*.

Gene Name	Annotation/Genbank ID	Chromosome	Location	ORF Length (Nts)	Group	WRKY Domain	Zinc-finger Type	Note
NnWRKY2a	NNU_20595/XM_010254575	1	60331779-60325443	2232	I	WRKYGQK/WRKYGQK	C2H2	
NnWRKY2b	NNU_17962/XR_736581	1	6381110-6371515	2358	I	WRKYGQK/WRKYGQK	C2H2	
NnWRKY4a	NNU_07349/XM_010260422	3	93380654-93385304	1617	I	WRKYGQK/WRKYGQK	C2H2	
NnWRKY4b	NNU_04581/XM_010259913	4	73164730-73182056	2694	I	WRKYGQK/WRKYGQK	C2H2	
NnWRKY6a	NNU_16107/XM_019196196	5	46697578-46699727	1512	IIb	WRKYGQK	C2H2	
NnWRKY6b	NNU_00165/XM_010248058	1	30869961-30867814	1512	IIb	WRKYGQK	C2H2	
NnWRKY7a	NNU_13622/XM_010272500	5	9965414-9966974	1038	IIId	WRKYGQK	C2H2	
NnWRKY7b	NNU_17024/XM_010265142	1	117568068-117566457	1077	IIId	WRKYGQK	C2H2	
NnWRKY9	NNU_16105/XM_010246787	5	46661891-46663927	1581	IIb	WRKYGQK	C2H2	
NnWRKY12a	NNU_14361/XM_010260434	6	35217906-35210883	651	IIc	WRKYGQK	C2H2	
NnWRKY12b	NNU_16551/XM_010247799	1	200462041-200470342	669	IIc	WRKYGQK	C2H2	
NnWRKY13a	NNU_20076/XM_010262725	5	32536749-32539936	672	IIc	WRKYGQK	C2H2	
NnWRKY13b	NNU_06423/XM_010270288	1	91952526-91946637	477	IIc	WRKYGQK	C2H2	
NnWRKY14	NNU_08414/XM_010249539	7	18757624-18759965	1305	IIe	WRKYGQK	C2H2	
NnWRKY17a	NNU_14734/XM_010267113	8	32431912-32433307	972	IIId	WRKYGQK	C2H2	
NnWRKY17b	newGene_16312/XM_010258956	1	158699740-158700138	312	IIId	WRKYGQK	/	No Zinc-finger structure
NnWRKY20a	NNU_16003/XM_010271865	1	148596912-148607860	1482	I	WRKYGQK/WRKYGQK	C2H2	No Zinc-finger structure in the C-terminal WRKY domain
NnWRKY20b	NNU_20475/XM_010254390	1	64157925-64156753	972	I	WRKYGQK/WRKYGQK	C2H2	No Zinc-finger structure in the C-terminal WRKY domain
NnWRKY21a	NNU_19902/XM_010279826	1	162954901-162952926	1083	IIId	WRKYGQK	C2H2	
NnWRKY21b	NNU_14264/XM_010279826	3	60701675-60700616	915	IIId	WRKYAQK	/	No Zinc-finger structure
NnWRKY23a	NNU_05834/XM_010269637	4	21149116-21147855	933	IIc	WRKYGQK	C2H2	
NnWRKY23b	NNU_13849/XM_010260939	3	3896431-3897619	900	IIc	WRKYGQK	C2H2	

NnWRKY27a	NNU_01367/XM_010263082	1	180154804-180153481	1098	IIe	WRKYGQK	C2H2	Only one WRKY domain available
NnWRKY27b	NNU_04335/XM_010280754	6	17631405-17630060	867	IIe	WRKYGQK	C2H2	
NnWRKY28a	NNU_06594/XM_010277346	4	4102930-4101049	966	IIc	WRKYGQK	C2H2	
NnWRKY28b	NNU_19178/XM_010245403	3	22658175-22659903	984	IIc	WRKYGQK	C2H2	
NnWRKY31a	NNU_20314/XM_010254164	1	68179403-68181856	1842	IIb	WRKYGQK	C2H2	
NnWRKY31b	NNU_24624/XM_010246172	1	144761011-144747256	2058	IIb	WRKYGQK	C2H2	
NnWRKY32a	NNU_12096/XM_010267283	2	78178981-78166858	1698	I	WRKYGQK/WRKYGQK	C2H2	
NnWRKY32b	NNU_19344/XM_010277517	8	21729170-21740338	1827	I	WRKYGQK/WRKYGQK	C2H2	
NnWRKY33a	NNU_18828/XM_010258703	2	98850785-98853547	1767	I	WRKYGQK	C2H2	
NnWRKY33b	NNU_13013/XM_010266210	2	63037802-63040904	1977	I	WRKYGQK/WRKYGQK	C2H2	
NnWRKY40a	NNU_12117/XM_010267315	2	78900468-78899025	1050	IIa	WRKYGQK	C2H2	
NnWRKY40b	NNU_19326/XM_010277476	8	21258631-21260028	942	IIa	WRKYGQK	C2H2	
NnWRKY40c	newGene_3441/XM_019199981	8	21285537-21286840	714	IIa	WRKYGQK	C2H2	
NnWRKY41	NNU_01465/XM_010263210	1	177536134-177537992	1089	IIIb	WRKYGQK	C2HC	
NnWRKY43a	NNU_01372/XM_010263088	1	180041789-180035669	597	IIc	WRKYGQK	C2H2	
NnWRKY43b	newGene_98/XM_010248991	6	21869050-21857863	615	IIc	WRKYGQK	C2H2	
NnWRKY44a	NNU_12426/XM_010255152	2	105416670-105419110	1386	I	WRKYGQK/WRKYGQK	C2H2	
NnWRKY44b	NNU_15232/XM_010261834	2	68034522-68036671	1422	I	WRKYGQK/WRKYGQK	C2H2	
NnWRKY47	NNU_08253/XM_010281369	4	30139432-30133355	1494	IIb	WRKYGQK	C2H2	
NnWRKY49a	NNU_18755/XM_010258584	2	96951968-96956010	912	IIc	WRKYGQK	C2H2	
NnWRKY49b	NNU_03597/XM_010264244	2	61257443-61259593	909	IIc	WRKYGQK	C2H2	
NnWRKY50a	NNU_11881/XM_024052442	8	3882291-3886495	318	IIc	WKKYGKK	C2H2	
NnWRKY50b	NNU_05136/XM_030592689	6	63880471-63877082	615	IIc	WKKYGKK	C2H2	
NnWRKY51a	NNU_20102/XM_010262768	5	31909752-31907769	387	IIc	WKKYGKK	C2H2	
NnWRKY51b	NNU_09891/XM_010245010	1	109917294-109916003	321	IIc	WKKYGKK	C2H2	
NnWRKY53a	NNU_02487/XM_010268162	3	68565759-68563758	1092	IIIb	WRKYGQK	C2HC	

NnWRKY53b	NNU_02488/XM_010268164	3	68581263-68579174	1089	IIIb	WRKYGQK	C2HC
NnWRKY55a	NNU_24386/XM_010254110	5	78045097-78049321	1068	IIIb	WRKYGQK	C2HC
NnWRKY55b	newGene_5274/XM_020834403	2	109823115-109826669	1083	IIIb	WRKYGQK	C2HC
NnWRKY57a	NNU_07383/XM_010260920	3	93002824-92999921	936	IIc	WRKYGQK	C2H2
NnWRKY57b	NNU_04527/XM_010259818	4	72347210-72344413	927	IIc	WRKYGQK	C2H2
NnWRKY65a	NNU_25670/XM_010250060	6	47741891-47740244	912	Ile	WRKYGQK	C2H2
NnWRKY65b	NNU_16122/XM_010243807	3	16520969-16522637	891	Ile	WRKYGQK	C2H2
NnWRKY69a	NNU_13879/XM_010260989	3	4539392-4537925	849	Ile	WRKYGQK	C2H2
NnWRKY69b	NNU_05863/XM_010269687	4	20498200-20499825	86	Ile	WRKYGQK	C2H2
NnWRKY70a	NNU_24385/XM_010253742	5	78069123-78067671	981	IIIb	WRKYGQK	C2HC
NnWRKY70b	NNU_12194/XM_010254742	2	109846840-109845383	990	IIIb	WRKYGQK	C2HC
NnWRKY72a	NNU_07209/XM_010258267	3	96330092-96327476	1731	IIb	WRKYGQK	C2H2
NnWRKY72b	NNU_07819/XM_010244080	4	76498712-76495223	1869	IIb	WRKYGQK	C2H2
NnWRKY75a	NNU_22208/XM_010250769	4	87706954-87704421	555	IIc	WRKYGQK	C2H2
NnWRKY75b	NNU_02028/XM_010252352	3	106232273-106229307	552	IIc	WRKYGQK	C2H2
/	NNU_04803/XM_010257999	5	49193574-49201749	2061	/	WRKYSEK	C2HC
/	NNU_23618/XM_010278098	2	1018601-1025115	381	/	WKYGGQK	C2H2

Note: The 6 WRKY domains marked with red capital letters are those different to the conserved “WRKYGQK” domain.

Table S2 Primers used for in the research.

Gene name	Forward primer (5'-3')	Reverse primer (5'-3')	Application
<i>NnWRKY2a</i>	CTGAAAGAAGTAAGGATGGCACT	CTTCCCTGATTGAACTGAGACC	qRT-PCR
<i>NnWRKY2b</i>	TCAAATGGCTTCTATTGCTCAG	TGTATCAGTTTCCTTGGGTGG	qRT-PCR
<i>NnWRKY4a</i>	GTTTACGATTCCGCTGGTTT	GAGGTGCTGGCACTGAAGATG	qRT-PCR
<i>NnWRKY4b</i>	CCCTTTAGTATCAATGGACCTTC	ACTCACTTCCTTTCACCTGCTT	qRT-PCR
<i>NnWRKY6a</i>	AATTCGCTTCCTAGCTCTT	TCTTCAACCTTCCAGTCCCTC	qRT-PCR
<i>NnWRKY6b</i>	TTGACCTGACCAACAACCCAC	ACTGCTTCTCATCCATGCCTC	qRT-PCR
<i>NnWRKY7a</i>	ACATCCAACACCTCCCTCCTC	GCTGCTTGCTATCAGTATCACCC	qRT-PCR
<i>NnWRKY7b</i>	AGAGGCAACAGCAGCAACACC	GGGAGGAGAAGTACCCAAAGGAC	qRT-PCR
<i>NnWRKY9</i>	GTTCTTCACATTACAGCCACA	CTTCTCCGTCTTCCAGTTGC	qRT-PCR
<i>NnWRKY12a</i>	CCTCTTTGGAATGACACCCAG	CTAGCACATCCACATCGCTTC	qRT-PCR
<i>NnWRKY12b</i>	TGGGCTTAGTGGCGACGATGT	TTCCTCCTCACCTTACCTTG	qRT-PCR
<i>NnWRKY13a</i>	TCTTCGGTGCAAAGATCGGCTAA	ACCTGGGCTCCCTCACCTTCCT	qRT-PCR
<i>NnWRKY13b</i>	GGGTACAAGACAGTGAGCGTTTG	ACCATCGTCCAAGACATCCAC	qRT-PCR
<i>NnWRKY14</i>	TTTAGCAGACATAGTCGTGGTG	GGTAGCATAGGATCTCGCGTAG	qRT-PCR
<i>NnWRKY17a</i>	CTCATCAGAGCCCTCAACACCA	GGAACCGAGAAGGACGTAGAAGC	qRT-PCR
<i>NnWRKY17b</i>	TTAGCCAGAGTCTCGCACGGAT	ACTGACTTGCCAACAGCATCCA	qRT-PCR
<i>NnWRKY20a</i>	TAAAGGCTTCCACAGGTTGA	GGGTCCGGTGCAAGTACAGAT	qRT-PCR
<i>NnWRKY20b</i>	CTCACTCATTTGCAGCCTTAT	AAATACCTGGAACCCACTATCA	qRT-PCR
<i>NnWRKY21a</i>	CCCAGTTCAGGAGATGGTTAC	GGTGTGGAGGAATTGATAGTGT	qRT-PCR
<i>NnWRKY21b</i>	AGGAGGTTGAGGAACTAACAG	TTAAGACCCAATCCAATACCA	qRT-PCR
<i>NnWRKY23a</i>	CGGAGTCGTCTGAGGTGTTGA	CTCTGCCGTTTCTGTTCTTTT	qRT-PCR
<i>NnWRKY23b</i>	GCAACCTTCGATGCCATCACC	GTCTTCTCCTGCTCCTCCTCTT	qRT-PCR
<i>NnWRKY27a</i>	GCCACTACTACCACTGCACATTCTCA	GCGAGCCCTTGATTGGTTTCT	qRT-PCR
<i>NnWRKY27b</i>	AGAGGGTTGTTGCCAGGTGC	TACGGCTGCGTTCGACTTGTT	qRT-PCR
<i>NnWRKY28a</i>	ACTACTGACATTCCGGCGACAC	TTTGGGCTTGCTCACTTCTTATT	qRT-PCR
<i>NnWRKY28b</i>	CGGGTCATGGGCTAGGAGACT	CGGCATCATCCACAGGGTAAA	qRT-PCR
<i>NnWRKY31a</i>	ACCTCAGCGCAGCTTGATG	CCAATGTGACAGTCGGAACG	qRT-PCR
<i>NnWRKY31b</i>	GACGACGGGATTTACCTAATG	TTTCAGCCTTGCGTTCTGTT	qRT-PCR
<i>NnWRKY32a</i>	TATGGTTACGAAGACACCCACT	ATTCAGTCACAAAGCCAGTATG	qRT-PCR
<i>NnWRKY32b</i>	AAGGATCAAGGATAGCGATACAG	AGGATTTCCCTTACCATTTT	qRT-PCR
<i>NnWRKY33a</i>	ATCACCCTTACGAGGGCAAAC	TGGAGCTGTAGGGACTAAAGGTAT	qRT-PCR
<i>NnWRKY33b</i>	GGATGGACAGATAACCGAGATAGT	TGCGAATGATTGGTCAGGGAT	qRT-PCR
<i>NnWRKY40a</i>	GGGGAGGAATCACTCGGTAAA	TGAAGCCCCTTCGTTGTTCT	qRT-PCR
<i>NnWRKY40b</i>	AAGATGCTAGTGTACTGGTGAGA	CAGGGAAGTTCGTTTCGTTATT	qRT-PCR
<i>NnWRKY40c</i>	ACTGAGGCAGATACAAGAAAGGGG	TGCAAAAGGATTAACTGTGGGAGA	qRT-PCR
<i>NnWRKY41</i>	AGTTCAGCCTCGGCATCTTC	CATTGTCCAGGTCCTCGGTTT	qRT-PCR
<i>NnWRKY43a</i>	GGAAATAAAGCCGAAGATGAAA	GCGATAATAACTCCTGGGAAAG	qRT-PCR
<i>NnWRKY43b</i>	ATTCCCAAAATCATACCCGACAT	GCTCGCTCTTGCTTATCTTTAC	qRT-PCR
<i>NnWRKY44a</i>	TCCCAGTTCATCTTACTTGCC	TTCGCTTACATCTTAGTTCATTGTC	qRT-PCR
<i>NnWRKY44b</i>	GCCAGAAGGTTGTGAAGGGAA	GCAATGGGCATCTCGTGGTTA	qRT-PCR
<i>NnWRKY47</i>	ATGGTCGAGACCGTAAGTGGC	TTGTGGTGATTACAGCGATGC	qRT-PCR

<i>NnWRKY49a</i>	TACACCACCAAGCCCATTAG	CAAGAAAGGGTAGAAGAGGAAGA	qRT-PCR
<i>NnWRKY49b</i>	CCATCTTACCAGGCTTACCAA	TCGGCTCACATCTCCTTCTAC	qRT-PCR
<i>NnWRKY50a</i>	GATATTATGGACGACGGCTTCA	GTTGTGGGTGCCTTCGTATGT	qRT-PCR
<i>NnWRKY50b</i>	CCCATCATCTCCTAAACTTCG	TTCAGCTTGACTGCCATTCTT	qRT-PCR
<i>NnWRKY51a</i>	GGACCGAGAAGATTCGAGCTA	CAAGAGGAAGATGAATTCGAAGC	qRT-PCR
<i>NnWRKY51b</i>	AGACCGTGAGGACTCGAGGTA	GATGATGAGTGTGAATGTGAAGC	qRT-PCR
<i>NnWRKY53a</i>	TGGCTTAGACTCTGGGGAGATGATG	GTAGTTGGATTCGGATGTTGCA	qRT-PCR
<i>NnWRKY53b</i>	CGATTTAGACTCTAGGGAGATGATA	GCAGTTGGATTCGGATGTTGCT	qRT-PCR
<i>NnWRKY55a</i>	ATGTATAGGGACTTTGACATGGGTAG	TTCTCGTCGATGGAAGGGAAG	qRT-PCR
<i>NnWRKY55b</i>	GACAGAAGGAAATCTTGGGTCA	ACCTGATGATGGTGCTGTGGA	qRT-PCR
<i>NnWRKY57a</i>	CCCCACAGTTGTATTACCCAC	CAGGCACAATATCACCAAGCAGA	qRT-PCR
<i>NnWRKY57b</i>	TCACAACATACGAAGGCCAGCAT	GACATCAATCCCACCTCCCAGA	qRT-PCR
<i>NnWRKY65a</i>	CCACCACTTTCGCCAATTCCA	TCGCCCAGATCACCGAACTTATT	qRT-PCR
<i>NnWRKY65b</i>	CCGCTTCTCATTCTAACACCACC	TCTCCCAGATCACCGAACTTACTATC	qRT-PCR
<i>NnWRKY69a</i>	TAGGTGCAGCACATCTAAGGG	TTGTAGTATGGTGGCAGGGAG	qRT-PCR
<i>NnWRKY69b</i>	TGGAGGGCAGAAGAATGAAGG	GGTGTGGGTGGAGGTGTAGG	qRT-PCR
<i>NnWRKY70a</i>	CAGGAATGCAAAGAGGTGGAGA	ACCTGAGTACACGCCCGAGAT	qRT-PCR
<i>NnWRKY70b</i>	GCCAGGAAGAAATCCCAAACC	ACGAGTAGACGCCCGAAATCA	qRT-PCR
<i>NnWRKY72a</i>	CAATCACATCAATCGTAGGTGGCG	AGCTCGATGCACATCCGTTCC	qRT-PCR
<i>NnWRKY72b</i>	CGACGACCCAGGGAAATCAAG	ATTGACGAGGCCGATCTGTT	qRT-PCR
<i>NnWRKY75a</i>	GGGGAGAAGAAGATTAGAAAGC	TTGGATAGCCGTTGAACCTTGC	qRT-PCR
<i>NnWRKY75b</i>	GGCAGCAGTAGCTGTTCCATT	TGATACCGAGCTTCGTTTCAC	qRT-PCR
<i>NNU_04803</i>	GTTTCCGTCGTCTGTTGTCT	ACCTTCTCCTCGTAACCTTGA	qRT-PCR
<i>NNU_23618</i>	AGCTGAGGAAGGGTTCAGAGAA	GTTGAGATGTGGAGGAGGGATG	qRT-PCR
<i>NnACTIN</i>	CTCCGTGTTGCCCTGAAG	CCAGCAAGGTCCAACCGAAG	qRT-PCR
<i>NnWRKY44a</i>	CGGAATTCATGCCGGCCATCGATCAATTT	GCTCTAGATCACCATTTTTGCACTAGTGAATT	Dual-luciferase assay
<i>NnWRKY44b</i>	CGGAATTCATGGAGTCGACTTGTTGGAT	GCTCTAGATCACCATTTCTGCACTGTTGAATG	
<i>NnWRKY44a</i>	CCTTAATTAAATGCCGGCCATCGATCAATTT	TTGGCGCGCCACCATTTTGCCTAGTGAATT	Subcellular localization
<i>NnWRKY44b</i>	CCTTAATTAAATGGAGTCGACTTGTTGGAT	TTGGCGCGCCACCATTCTGCACTGTTGAATG	

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      *      20      *      40      *      60      *      80      *
NNU_24697 : MVRERKVMQIDLSLKLDSODESEEEEEKKEEERESVNDCEGREGHHRDGRGEAEERSGID60TSLDK-ET-CENSRKRELSALQMEMDRMKEE : 90
NnWRKY9   : MVMERKVMQIDLSLKLDSODESEEEKKEEERESVNDCEGREGHHRDGRGEAEERSGID60TSLDK-ET-CENSRKRELSALQMEMDRMKEE : 89
      *      100     *      120     *      140     *      160     *      180
NNU_24697 : NKLRRKVVVEQTHKDYDLQKLAIVQCN118RRKHHQMFSLHGGDAITTLQSKQVKKFVENDNSSACQTESEALGCEGGLGLSLSMG177 : 177
NnWRKY9   : NKLRRKVVVEQTHKDYDLQKLAIVQCN117RRKHHQMFSLHGGDAITTLQSKQVKKFVENDNSSACQTESEALGCEGGLGLSLSMG176 : 176
      *      200     *      220     *      240     *      260     *      280
NNU_24697 : AGVQCHGRDESEAEEMKSEEPVCKNFKLSELAGITSHSPNPNRKRARVSVRARC236----- : 236
NnWRKY9   : SHVQCHGRDESEAEEMKSEEPVCKNFKLSELAGITSHSPNPNRKRARVSVRARC234NDGCQWRKYGQKVAKGNPCCPRAYRYCTVAPG : 265
      *      300     *      320     *      340     *      360     *
NNU_24697 : -----VQRC240LEDMSILITTYEGTHNHPLPVGATAMASTTSSAATFMLLSRNSGA-ITSDGIISDACTE29 : 299
NnWRKY9   : CPVRKQKEIRTHKLSFVPSYHCSDCVQRC294LEDMSILITTYEGTHNHPLPVGATAMASTTSTAATFMLLSGVNSCTEIVVADGIISDACTE35 : 354
      *      380     *      400     *      420     *      440     *      460
NNU_24697 : IISYRPHSISPHSSSTTISIVNTNDHSGKIVLDLTNNSQCFSSHSSSQPQLYR359YHNAAGTI-VNLGGDLSS--SDHCEISRG : 386
NnWRKY9   : IISYRPHSISPHSSSTTISIVNTNDHSGKIVLDLTNNSQCFSSHSSSQPQLYR412WMSSNSGHHHHPAGSYGGLSTSNELGSTR- : 441
      *      480     *      500     *      520     *      540     *      560
NNU_24697 : IITDKRGRWKEETKSTENVSATSDPKK415RVAVAAAITSFINTFSTITVCFKCPPLIRDGETAGSSSSSKWVVESEPHLW*-----46 : 467
NnWRKY9   : IITDKRGRWKEETKSTENVSATSDPKK470RVAVAAAITSFINTFSTITVCFKCPPLIRDGETAGSSSSSKWVVESEPHLW*-----52 : 526
      6DK WK EE SL ENVS I SDFKE RVAVAAAITSF6 ES QP G L6L DGE AGSSS KVV

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Fig. S1 Amino acid sequence alignment of NNU_24697 and NnWRKY9. Conserved WRKY domains were marked with red lines, and conserved zinc finger domains were marked with yellow lines.

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NnWRKY33a : MASSS SLE S NSHPT FSP N HMTSSFDLLGDDDK-----LSDH AAI 60 RTGA VPKFKSIPPPSLPISPP VSPSSYFAIPPGLSPE : 88
NnWRKY33b : MASSS SLE S NSHPT FSP N HMTSSFDLLGDDDAHSCKQDQITVSRGLSDH AAI 60 RTGA VPKFKSIPPPSLPISPP VSPSSYFAIPPGLSPE : 100
          MASSS SLE S NSHPT FSP N MT3SFDLL GDDDD LSDH AD RTGA VPKFKSIPPPSLPISPP 6SPSSYFAIPPGLSPE

NnWRKY33a : LLDSPVLLSASNLL SPTTG 108TLF SQA NW4 NS NYQ 6K E KN DFSFQPCQTRPAMGS SMF2SS TTI KDENSE 168 GLEQ WN5Q PTRKQ : 183
NnWRKY33b : LLDSPVLLSASNLL SPTTG 120KFL SQA NW4 NS NYQ 6K E KN DFSFQPCQTRPAMGS SMF2SS TTI KDENSE 179 GLEQ WN5Q PTRKQ : 195
          LLDSPVLLSASNLL SPTTG SQA NW4 NS NYQ 6K E KN DFSFQPCQTRPAMGS SMF2SS TTI KDENSE GLEQ WN5Q PTRKQ

NnWRKY33a : FSSGK MVKSEYAPMC FSEI SSIQANNT SNGGFQSN NCYS227 QP QS REQRR DDGYNWRKYGQKQVKGSENPRSYVKCTFPDCP KKKVERSLDG : 282
NnWRKY33b : FSSGK MVKSEYAPMC FSEI SSIQAN NCNGGFQSN NCYS238 QP QS REQRR DDGYNWRKYGQKQVKGSENPRSYVKCTYPNCP KKKVERSLDG : 293
          FSSGK MVKSEYAPMC FSEI SSIQAN Q NGGFQSN NQ QP QS REQRR DDGYNWRKYGQKQVKGSENPRSYVKCT5P1CP KKKVERSLDG

NnWRKY33a : QITEI 287 VYKGNHHPKPGSTRSSSSS---SHRICSAAPISIPD SFATRQVAS QIMESCATPEN 344 SSISMG DDF QSSCMSRSAGLEDEDEPNA : 375
NnWRKY33b : QITEI 298 VYKGNHHPKPGSTRSSSSSSSCQICPSAATLVIPIQSFATRQSS QMESCATPEN 357 SSISMG DDF QSSCMSRSAGLEDEDEPNA : 388
          QITEI VYKGNHHPKPGSTRSSSSS S IQ SAAP IPD SFATRQ Q6 ATPEN SSISMG DDF Q3SCMS48AGD EDEFIA

NnWRKY33a : KRWKTTTEGQ NEGISAAAGSRTVREPRVI 404 VQTTSIDIDLLD GEN-----420----- : 420
NnWRKY33b : KRWKKEG---ENEGISAAGSRTVREPRVI 414 VQTTSIDIDLLD GYRWRYGQKVVKGKGNPNPROGAHCVHAWVVAAPCILQKSLVSSRIT 474 ANMSSLF : 481
          KRWK 2NEGISAAGSRTVREPRV6 VQTTSIDIDLLD

NnWRKY33a : -----MGRKSYKCTHV GCPVRKHVERASHDLRAVITTYEGKH 458 NHDVPAARGSS SHIN RPTEDNRNNNTAPANNVAMAAIRPTPT : 503
NnWRKY33b : ETRLALSKSKPFVWLVCRSYKCTNAGCPVRKHVERASHDLRAVITTYEGKH 534 NHDVPAARGSS SHSSITRPSSSNNNS-DA-----TAIRPTTA : 574
          6 4SYKCT GCPVRKHVERASHDLRAVITTYEGKH NHDVPAARGSS SH RP3 NN A A AIRPT T

NnWRKY33a : NT-----FSPYSSST512NPIRVRPPITPFCCE TLEML S SHSSG GFGFS G GNSM-----F SYNOSLHIDSSV569FAAKEEPD DLFLESILC*58 : 588
NnWRKY33b : RPSNTNINONSSST588NDIHS-----QCCCT TLEMLCS-----SSFGFS G GNSM SMASSYNOSQOSDIVE639SSSAKEEPD DLFLESILC*65 : 658
          SST NPL S 2 Q P TLEML S P FGFS G GNSM SY NOS D3 AKEEP DLFLESILC

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Fig. S2 Amino acid sequence alignment of NnWRKY33a and NnWRKY33b. Conserved WRKY domains were marked with red lines, and conserved zinc finger domains were marked with yellow lines.

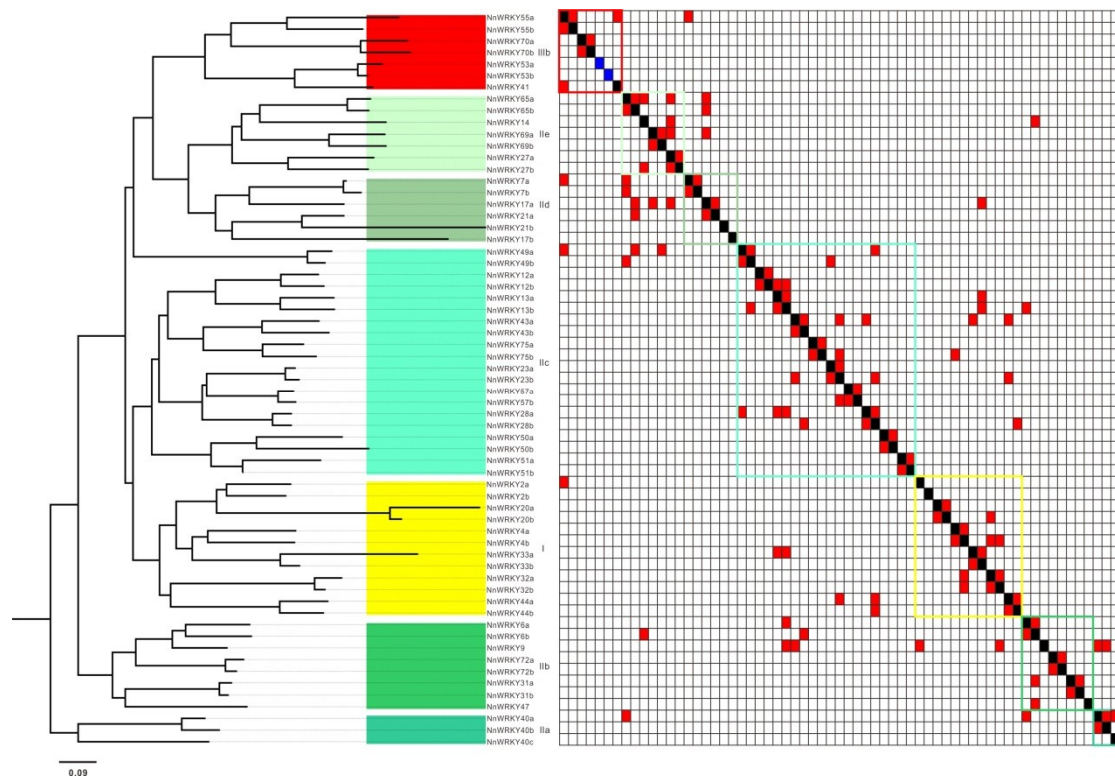
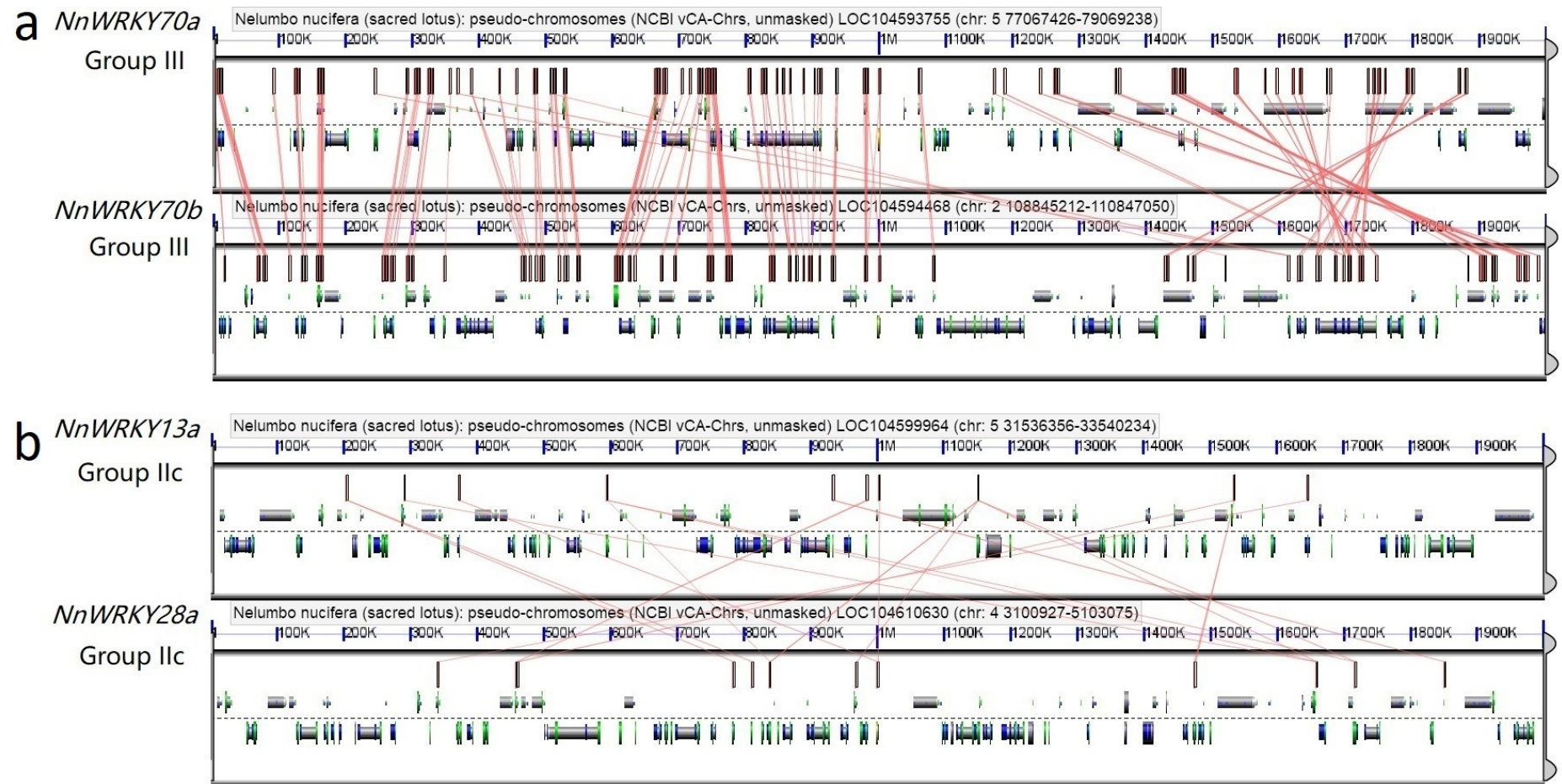
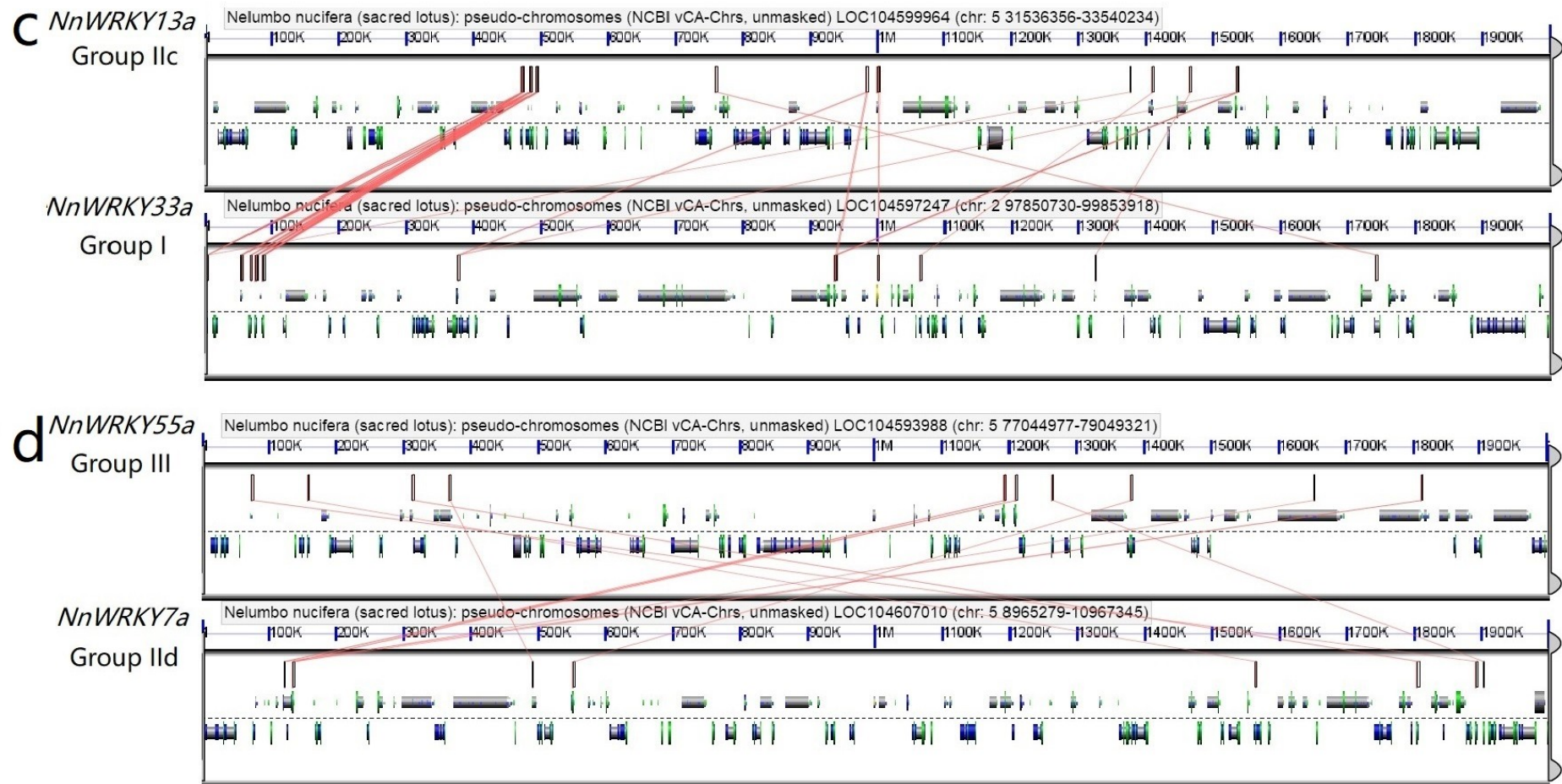


Fig. S3 Synteny analysis for lotus (*Nelumbo nucifera*) WRKY genes. Phylogenetic tree on the left panel was based on the full length protein sequences of 63 lotus WRKYs, and was made with neighbor-joining method in MEGA7 with 1000 bootstrap replications. Clusters of different phylogenetic groups were marked with different colors. Genes marked with black boxes were queries used for searching lotus WRKY syntelogs (syntenic homologs, marked with red boxes). Genes of a phylogenetic group were framed in the same color as its cluster.





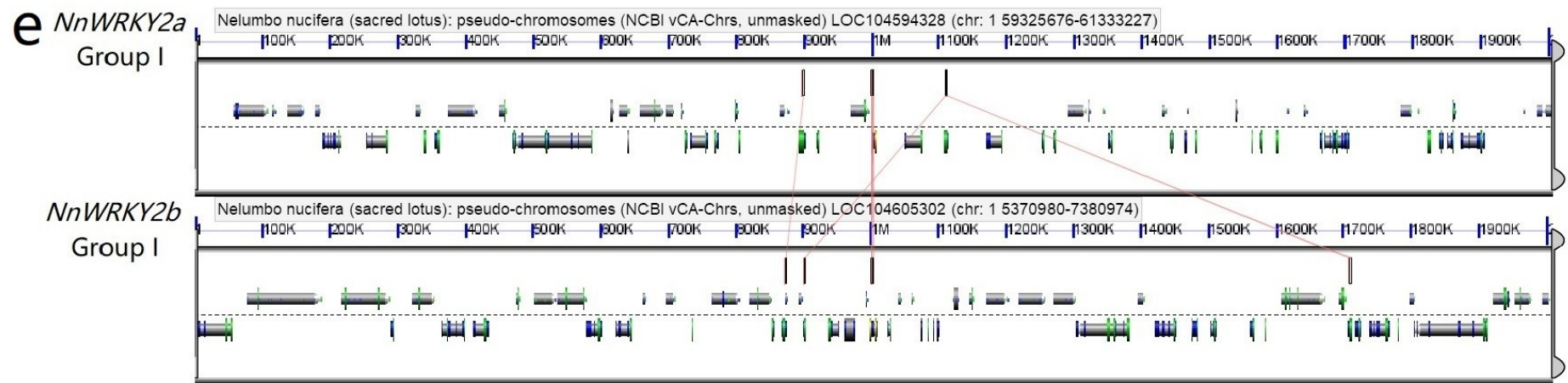


Fig. S4. Microsynteny analysis for representative lotus *NnWRKY* genes. (A) functionally paired genes with syntenic relationship; (B) syntenic genes within group IIc; (C) syntenic genes from group IIc and group I respectively; (D) syntenic genes from group III and group IIc respectively; and (E), functionally paired genes with no syntenic relationship.