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Identification by deep sequencing and profiling of conserved and novel hickory microRNAs involved in the graft process

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Presentation Outline

- **Introduction**
- **Morphological and physiological characterizations of hickory grafting**
- **microRNA identification in grafting**
- **Conclusions**

1. Introduction

- **Hickory (*Carya cathayensis*) is a member of walnut family. It is a very well-known nut tree in eastern China and mainly found in the area of Tianmu Mountain.**



Hickory nut



Hickory Tree

155 genotypes were collected from Zhejiang, Anhui, Hunan, Guizhou, Yunan et al.

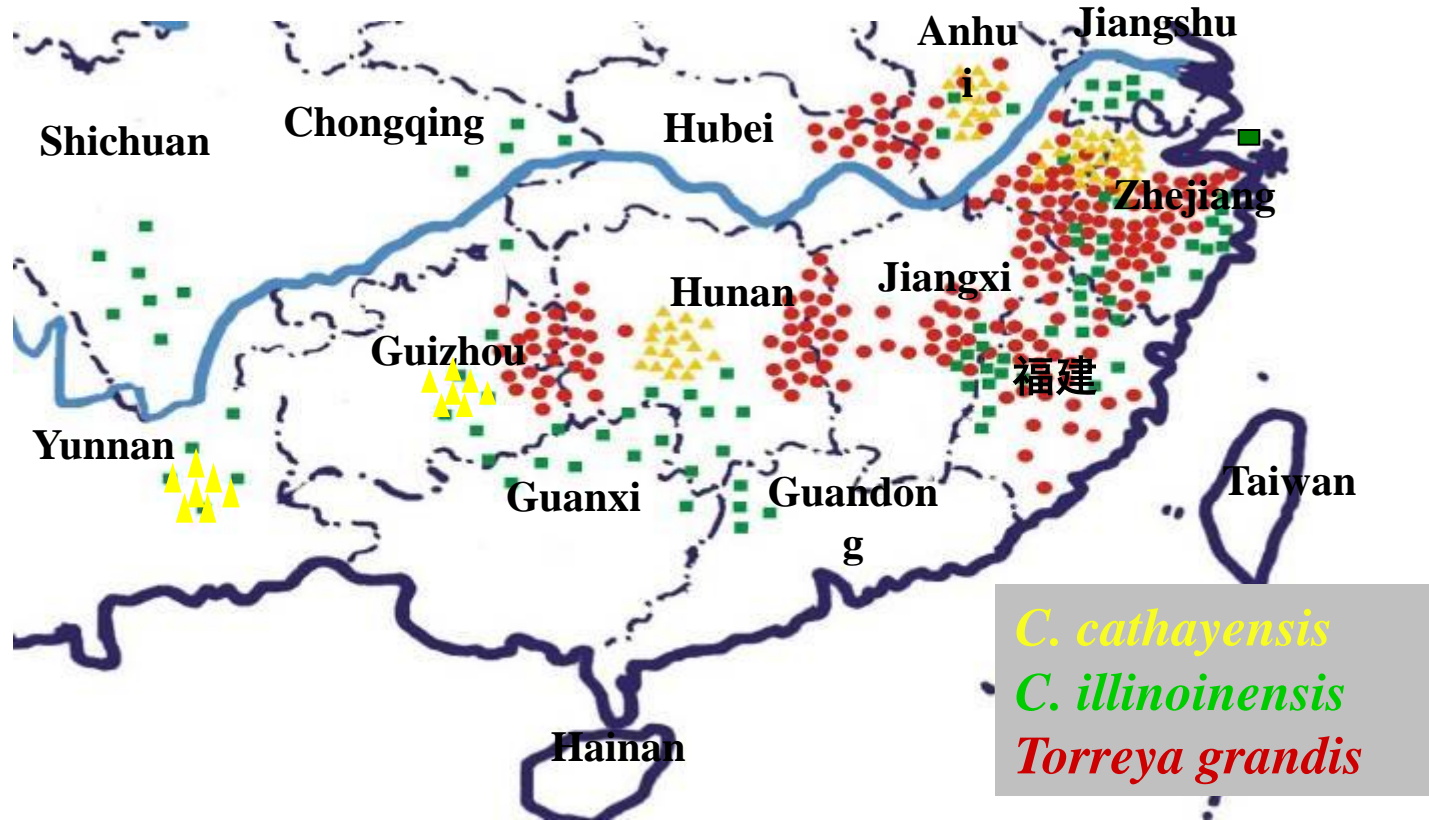


Figure 1 The geographical distribution of Hickory in China.

There are 5 *Carya* species native to China



C. cathayensis



C. dabieshanensis



C. hunanensis



C. tonkinensis



C. kweichowensis

Figure 2 The *Carya* species in China.

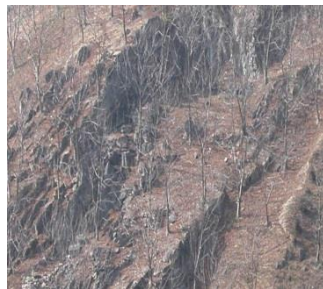
Hickory is very important economically, since its nuts are good sources of oil and nutrients.

Table 1 Comparison of the oil contents among different oil trees.

Species	<i>C. cathayensis</i>	<i>C.illinoensis</i>	<i>Torreya grandis</i>	<i>Camellia oleifera</i>
Oil content (%)	64.0~74.0	55.2~75.1	54.6~61.5	43.2~55.6
Unsaturated fatty acid (%)	88.4~95.8	80.1~84.5	74.9~79.5	83.6~86.6

The main limitation of this species is as follows:

- 1. Lack of new cultivars**
- 2. Difficulty in propogation**
- 3. Slow growth and late maturation**
- 4. Low yield and poor quality**



2. Morphological and physiological characteristics

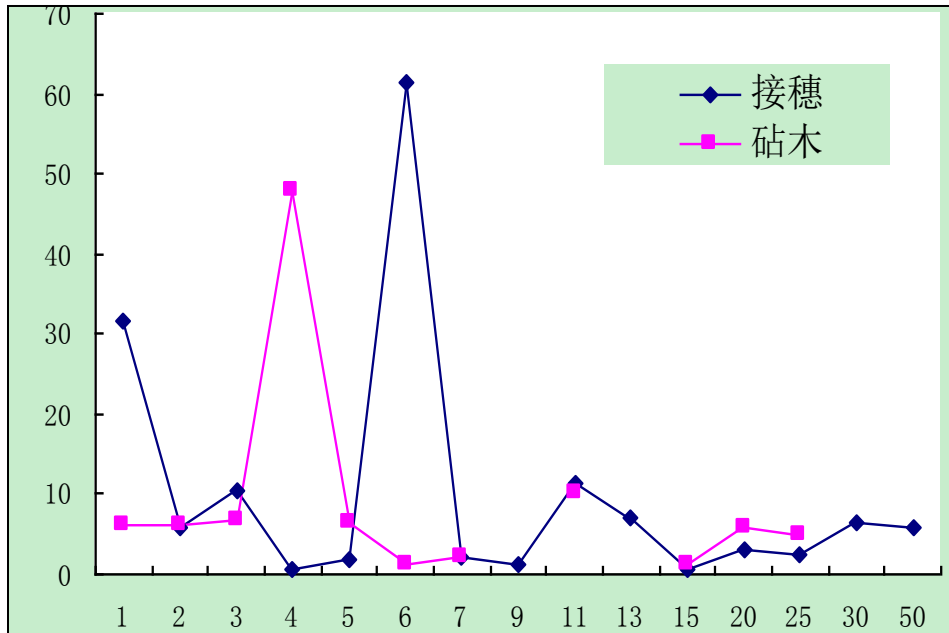


Figure 3 Auxin fluctuation during graft process

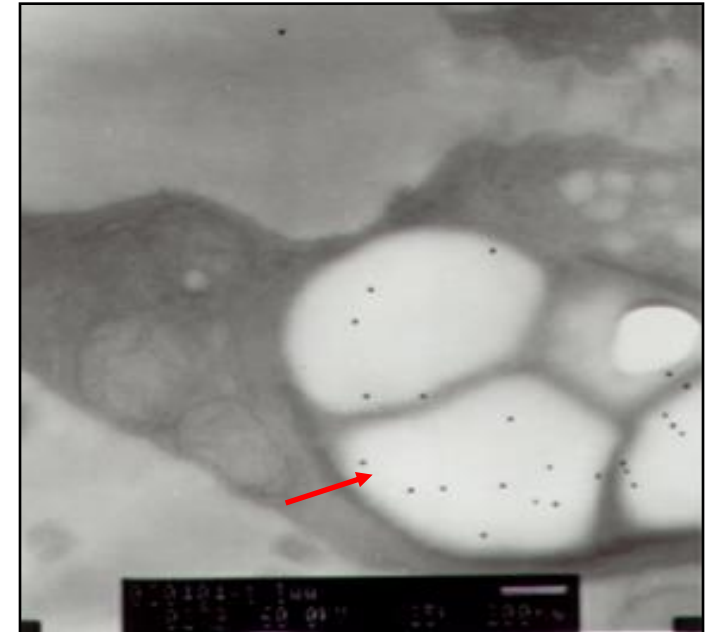


Figure 4 IAA detection using immunocolloidal gold technique

Auxin plays a key role in the graft process in *C. cathayensis*.

(Liu et al., Scientia Silvae Sinicae, 2009)

Picture representation of leaf primordia development in the Hickory grafted plants at 30 days after grafting (dag) at various treatments.



(a) Graft plants



(b) IAA applied plants



(c) NPA applied plants

Types of scion and grafting time, the main factors affecting survival rate during graft process.

Table 2 Changes of water and soluble protein contents in grafted *Carya cathayensis*

测定指标	嫁接时间	测定部位	嫁接后天数/d				
			5	9	15	25	30
含水量 /%	4月3日	穗条	47.20	44.71	48.23	48.65	49.44
		砧木	44.22	44.94	45.03	46.70	46.48
	4月23日	穗条	53.04	49.60	50.48	55.88	55.21
		砧木	53.87	53.82	52.64	54.74	51.37
蛋白质含量 /mg · g ⁻¹ FW	4月3日	穗条	47.00	54.60	67.30	48.80	38.20
		砧木	43.50	44.00	62.20	29.80	28.30
	4月23日	穗条	63.40	51.50	44.50	41.00	46.00
		砧木	34.50	42.30	26.20	26.50	27.90

Table 3 Changes of tannin contents of rootstock and scion in grafted *Carya cathayensis*

嫁接时间	测定部位	嫁接后天数/d						
		5	6	9	11	13	15	30
4月3日	穗条	21.50	25.60	25.90	26.40	26.00	26.50	25.00
	砧木	13.30	14.80	13.70	17.80	20.20	17.30	21.60
4月23日	穗条	22.80	27.10	28.00	27.90	27.60	25.50	28.50
	砧木	17.90	18.00	18.70	19.20	19.40	20.90	25.10

Table 4 Changes of polyphenol oxidase activities of rootstock and scion in grafted *Carya cathayensis*

嫁接时间	测定部位	嫁接后天数/d				
		5	7	13	20	30
4月3日	穗条	23.80	34.30	30.80	42.70	45.20
	砧木	20.30	21.00	29.40	32.90	64.40
4月23日	穗条	38.50	52.30	82.60	87.20	109.30
	砧木	32.20	38.90	62.90	66.70	74.20

(Zheng et al., J. Fujian College of Forestry, 2001)

3. microRNA identification in hickory

Table 1 Statistical analysis of small RNAs from G0, G7, and G14 libraries

	Total sRNAs	Unique sRNAs
G0 library		
Raw reads	24,178,692	
clean reads*	23,920,471	5,677,759
Mapped to genomic	5,008,672	285,487
Match known miRNAs	2,299,835	40,896
The unknown sRNAs	17,562,958	5,522,235
G7 library		
Raw reads	21,053,214	
clean reads *	20,784,923	4,626,678
Mapped to genomic	5,916,530	285,847
Match known miRNAs	2,284,689	46,774
The unknown sRNAs	13,918,918	4,428,827
G14 library		
Raw reads	25,985,000	
clean reads *	25,440,366	5,156,299
Mapped to genomic	8,241,439	307,679
Match known miRNAs	3,657,771	47,645
The unknown sRNAs	15,699,933	4,913,829

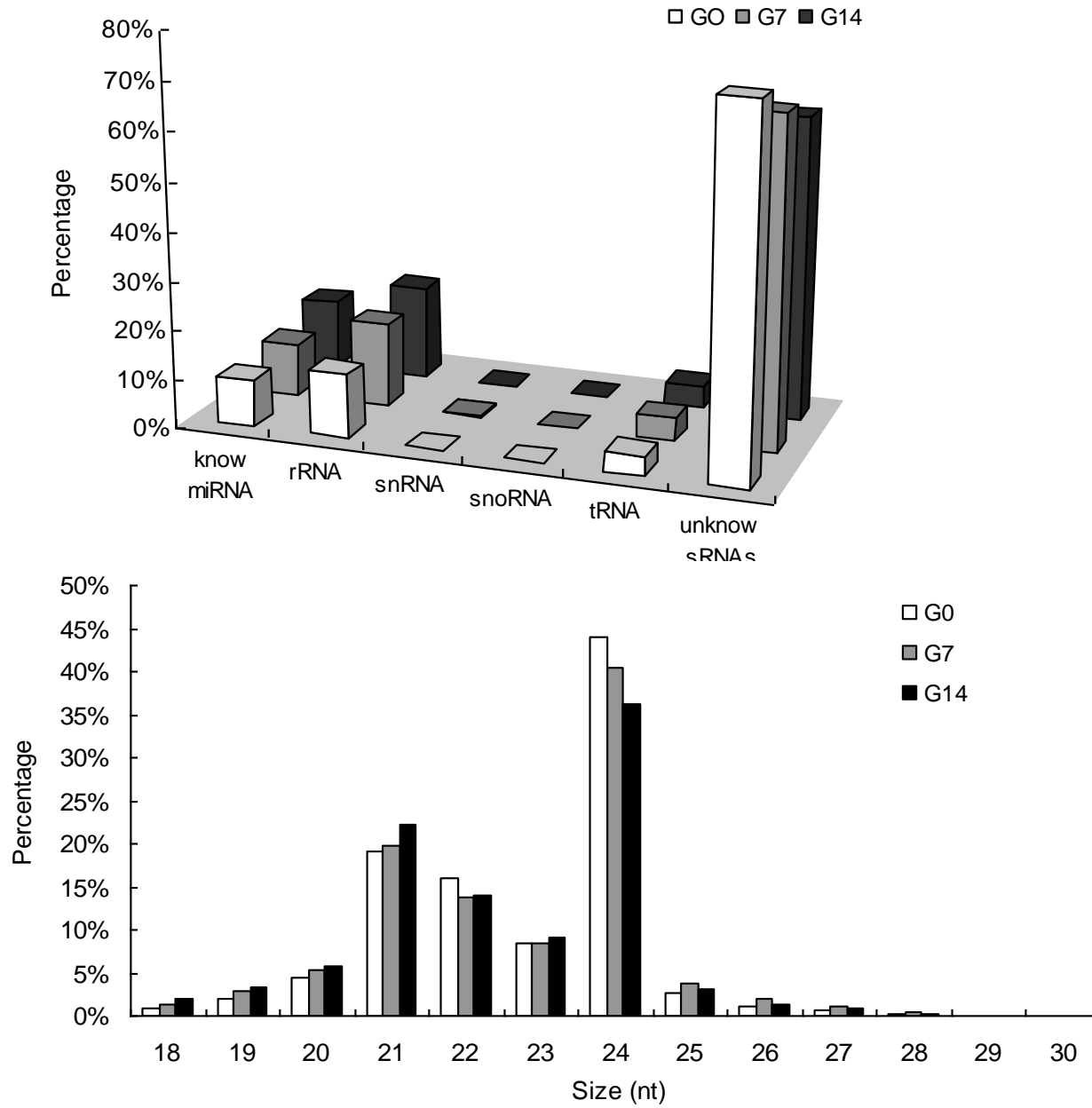


Fig. 1 Small RNA from the G0, G7, G14 libraries in hickory. a Type distribution of different sRNA reads; b size distribution of sRNA reads

Table 2 Conserved miRNAs identified in hickory

members	miRNA	sequence(5'-3')	size(nt)	Arm	Ratio		miRNA*	star sequence(5'-3')	LP(nt)	miRNA location
					G7/G0	G14/G0				
cca-miR156a	UGACAGAAGAGAGAGAGCAC	20	5'	2.09	2.14			213	S1-contig20796:239:451	
cca-miR156b	UGACAGAAGAGAGAGAGCAC	20	5'					213	S2-contig03286:470:682	
cca-miR159a	UUGCAUAACUCGGGAGCUUC	20	3'	0.51	0.41			110	S1-contig00386:825:934	
cca-miR159b	UUGCAUAACUCGGGAGCUUC	20	3'					110	S2-contig21174:824:933	
cca-miR160	GCGUAUGAGGAGCCAUGCAUA	21	3'	2.23	2.31	UGCCUGGCUCCCUGUAUGCCA		122	S1-contig17417:140:261	
cca-miR162	UCGAUAAACCUCUGCAUCCAG	21	3'	0.57	0.72	GGAGGCAGCGGUUCAUCGACC		125	S1-contig10935:510:634	
cca-miR167	UGAAGCUGCCAGCAUGAUCUA	21	5'	0.97	1.86			105	S1-contig12781:470:574	
cca-miR171a	UGAUUGAGCCGUGCCAAUAUC	21	3'	0.97	0.82	UAUUGGCCUGGCUCACUCAGA		139	S1-contig03840:260:398	
cca-miR171b	UGAUUGAGCCGUGCCAAUAUC	21	3'					106	S1-contig04024:118:223	
cca-miR172a	AGAAUCUUGAUGAUGCUGCAU	22	3'	0.68	0.31	UAGCAUCAUCAAGAUUCACAU		140	S1-contig24571:830:969	
cca-miR172b	AGAAUCUUGAUGAUGCUGCAU	22	3'			UAGCAUCAUCAAGAUUCACAU		140	S2-contig24766:143:282	
cca-miR390a	AAGCUCAGGAGGGAUAGCGCC	21	5'	0.67	0.60	CGCUAUCUAUCCUGAGUUUCA		126	S1-contig00035:117:242	
cca-miR390b	AAGCUCAGGAGGGAUAGCACC	21	5'	0.58	0.48			113	S1-contig15630:137:249	
cca-miR397	UCAUUGAGUGCAGCGUUGAUG	21	5'	0.55	0.28			88	S2-contig20035:1:88	
cca-miR482a	UCUUUCCUAGUCCUCCAUUCC	22	3'	0.77	0.84			96	S1-contig24624:282:377	
cca-miR482b	UCUUUCCUACUCCUCCAUUCC	22	5'	0.57	0.73			320	S1-contig01252:775:1094	
cca-miR482c	UCUUUCCUACUCCUCCAUUCC	22	5'					305	S2-contig06770:136:440	
cca-miR482d	GGAAUGGGCUGUUUGGAUG	20	5'	2.96	2.10	UUCCCAAAGCCGCCAUUCCGAU		126	S2-contig16425:95:220	
cca-miR530	UGCAUUGCACCUGCACCUAU	21	5'	1.42	1.37			107	S1-contig10678:21:127	
cca-miR827	UUAGAUGCCCAUCAACGAACA	21	3'	0.54	0.28	UUUGUUGAUGGUCAUUUAAU		108	S1-contig13866:108:215	
cca-miR2118	UUGCCAAUCCACCCAUUCCAA	22	3'	0.92	1.03	GGACAUGGGUGAAUUGGUAAGG		90	S2-contig02512:106:195	

21 conserved miRNAs belonging to 13 miRNA families were identified. Of which, 9 miRNA*s were detected, which had strong evidence of being bona fide miRNAs

Table 3 Novel and potential novel miRNAs identified in hickory

miRNA	miRNA sequence(5'-3')	Size	arm	Reads			LP	MFE	miRNA location
				G0	G7	G14			
cca-miRS1a	UCUGAGG GAGUUGGAGAAUUG	21	3'	16	0	0	106	-29.1	S1-contig00325:125:230
cca-miRS1a*	AAAGUCUGUCCUCCGCUUAGCUG	24	5'	1	0	0			
cca-miRS1b	UCUGAGG GAGUUGGAGAAUUG	21	3'	16	0	0	106	-29.1	S2-contig26861:50:155
cca-miRS1b*	AAAGUCUGUCCUCCGCUUAGCUG	24	5'	1	0	0			
cca-miRS2	AAUGGGAAGAUAGGAAAGAAC	21	5'	36172	47438	41174	115	-41.8	S1-contig01252:1043:1157
cca-miRS2*	UCUUCCUACUCCUCCCAUUC	22	3'	425	211	333			
cca-miRS3	AGUGGGAAGGCAGGAAAAGAA	21	5'	10171	13575	18636	90	-35.8	S1-contig24624:286:375
cca-miRS3*	UCUUCCUAGUCCUCCCAUUC	22	3'	5	5	3			
cca-miRS4	GCGUAUGAGGAGCCAUGCAUA	21	3'	0	855	0	122	-54.6	S1-contig17417:140:261
cca-miRS5	UCCCUACUCGCCC AUGCCAUA	22	3'	0	0	3430	88	-50.8	S1-contig06943:606:693
cca-miRS7	UGGUAUGGGCGAGUUGGGAAG	21	5'	0	0	43			
cca-miRS8	UCAUCGAGGUGGAGUUUGGCU	21	5'	0	0	8	68	-20.3	S2-contig02707:167:234
cca-miRS8*	UCAAAAUCGCAAGGUGGAGA	20	3'	0	0	7			
cca-miRS9	UGCUAUCUAUCCUGAGUUGC	20	3'	34	0	6	115	-60	S1-contig15630:135:249
cca-miRS10	UCAAGGUCCAAGGUUCAACAC	21	5'	0	0	5	86	-35.3	S1-contig04790:479:564
cca-miRS11	UUUGGACAAAUCAGAUGAUG	21	3'	7	21	17	144	-29.86	S2-contig15510:237:380
cca-miRS12	UUUGUUGAUGGUC AUUUA AUG	21	5'	1,390	1,078	707	87	-41.2	S1-contig13866:118:204
cca-miRS13	UUGUA GAUGUCGACGACGGAG	21	3'	6	6	0	161	-43.1	S2-contig03553:19:179
cca-miRS14	AGGUGCAGGUUUAGGUGCAA	21	3'	107	71	106	126	-43.2	S1-contig10678:3:128
cca-miRS15	GGACGGUGAUGAGUACCAGAG	21	3'	0	10	0	154	-45.59	S1-contig15644:44:197

10 novel miRNAs and their corresponding miRNA*s belonging to 8 families were identified during the graft process in hickory

Table 4 Differential expression miRNAs during the graft process in hickory

family	miRNA	sequence(5'-3')	normalized reads			ratio		regulated
			G0	G7	G14	G7/G0	G14/G0	
156	cca-miR156a	UGACAGAAGAGAGAGAGCAC	9.336	19.490	19.971	2.09	2.14	up-regulated
	cca-miR156b	UGACAGAAGAGAGAGAGCAC	9.336	19.490	19.971	2.09	2.14	up-regulated
159	cca-miR159a	UUGCAUAACUCGGGAGCUUC	76.759	39.315	31.444	0.51	0.41	down-regulated
	cca-miR159b	UUGCAUAACUCGGGAGCUUC	76.759	39.315	31.444	0.51	0.41	down-regulated
160	cca-miR160	GCGUAUGAGGAGCCAUGCAUA	18.215	40.696	42.028	2.23	2.31	up-regulated
172	cca-miR172a	AGAAUCUUGAUGAUGCUGCAU	548.099	374.655	168.383	0.68	0.31	down-regulated
	cca-miR172b	AGAAUCUUGAUGAUGCUGCAU	548.099	374.655	168.383	0.68	0.31	down-regulated
390	cca-miR390b	AAGCUCAGGAGGGAUAGCACC	151.402	87.779	72.545	0.58	0.48	down-regulated
397	cca-miR397	UCAUUGAGUGCAGCGUUGAUG	137.419	74.960	38.165	0.55	0.28	down-regulated
482	cca-miR482d	GGAAUGGGCUGUUUGGGAUG	205.008	606.635	430.055	2.96	2.10	up-regulated
827	cca-miR827	UUAGAUGCCCAUCAACGAACA	5.062	2.716	1.429	0.54	0.28	down-regulated
S12	cca-miRS12	UUUGUUGAUGGUCAUUUAAUG	57.673	51.371	27.311	0.89	0.47	down-regulated

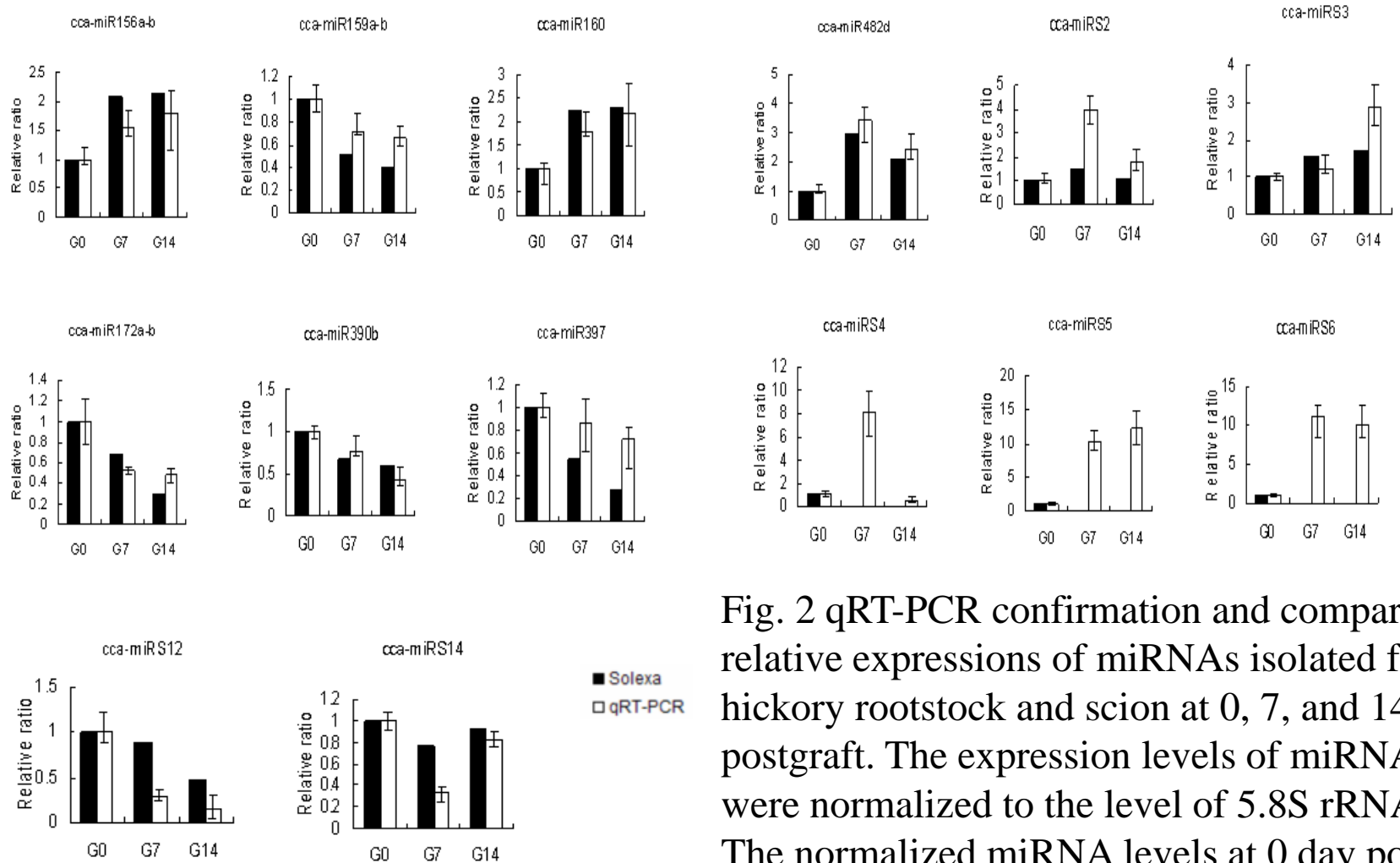
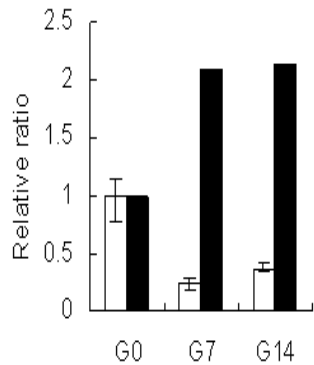
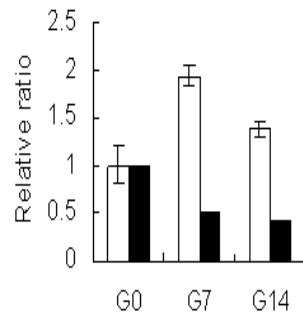


Fig. 2 qRT-PCR confirmation and comparative relative expressions of miRNAs isolated from hickory rootstock and scion at 0, 7, and 14 days postgraft. The expression levels of miRNAs were normalized to the level of 5.8S rRNA. The normalized miRNA levels at 0 day post-graft in hickory were arbitrarily set to 1

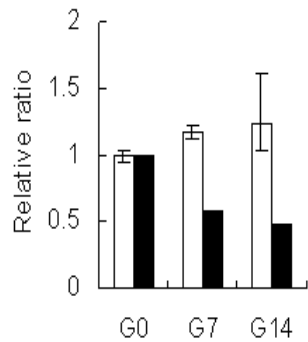
14 miRNAs and their expression trends were similar to the results obtained by Solexa sequencing



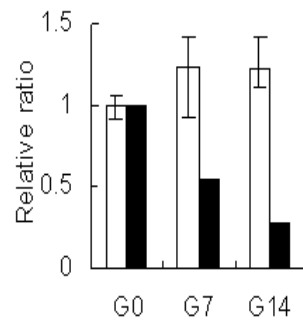
squamosa promoter-binding protein for miR156



NAC domain-containing protein for miR159



ATP binding protein for miR390b



sueroxide dismutase 4 for miR827

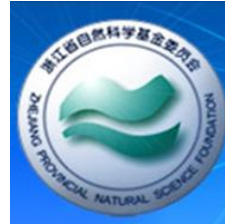
Fig. 3 The expressions of the target mRNAs (genes) of miR156, miR159, miR390b, and miR827 isolated from hickory rootstock and scion at 0, 7, 14 days post-graft. The expression levels of target mRNAs were normalized to the level of ELF1a-1 gene. The normalized target mRNAs levels at 0 day post-graft in hickory were arbitrarily set to 1. The miRNAs are in black, and their corresponding target genes are displayed in white

Four miRNA target genes (cca-miR156, cca-miR159, cca-miR390 and cca-miR827) to perform qRT-PCR. The results suggested an inverse relationship between the expression of the miRNAs and the regulation of their corresponding target genes. This evidence further validated the reliability of miRNA sequences generated by Solexa sequencing.

4. Conclusions

- **155 genotypes were collected from Zhejiang, Anhui, Hunan, Guizhou, Yunan et al. Three new varieties were selected and certified.**
- **21 conserved miRNA sequences as well as 10 novel and ten potential novel miRNAs were identified during the graft process in hickory.**

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