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生物柴油植物膏桐高油高产品种的培育

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摘 要: 在 2005−2009 年期间，利用从中国南部和西南部收集的 80 个不同的膏桐地理种源，在西双版纳建立膏桐种质资源圃 30 亩；在圃内开展其生物学特性和农艺性状的同时，对其中表现良好的 6 个地理种源做了进一步选择和培育。结果表明：80 个种源的 2 年生时平均直径、树高和树冠分别为 7.6 cm、167 cm 和 114 cm，种子千粒重为 0.676 (0.477~0.879) kg；在这些种源中，根据单株产量和种子含油率的情况，对 6 个表现较好的种源开展了小规模试验，2 年生时平均单株产量 0.34 kg，3 年生时达 1.38 kg；在试验中，有一个种源表现尤为突出，自挂果以来种子产量逐年增加，二、三、四年生林分每公顷干种子产量分别为 964.3 kg、2 000.6 kg、2 858.7 kg，种子含油率 40％~42％。另外，还利用野外发现的膏桐突变体成功地育出了膏桐新品种。以此为父本与其他种源杂交产生的后代，种子含油率较对照提高 6 个百分点，杂种种子含油率达 41.2％。

关键词: 膏桐 *Jatropha curcas* L，地理种源，高油高产品种，生物学特性，生物柴油性质

Breeding of high-oil *Jatropha curcas* L for biodiesel production

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Abstract: Different geographic seed sources (80) of *Jatropha curcas* L. were collected in South China and planted in a germplasm resource garden to study their biological and agricultural properties. The average ground diameter, tree height and crown size of two-year old plants of the 80 sources was 7.6 cm, 167 cm and 114 cm, respectively, the average 1 000-seed weight was 0.676 (0.477−0.876) kg. The trees grew further to the average size of 12.6 cm diameter, 2.69 m height and 2.1 m crown at the 4th year. Among the 80 sources, six sources had higher oil yield (seed oil content of 40%−42%) and better behaving in expression of phenotype were selected for a small-scale trial of forestation to determine oil yield. Among them a provenance with outstand in expression of phenotype yielded 964.3, 2 000.6 and 2 858.7 kg/ha was achieved for two- three- and four-year old trees, respectively. Additionally, a new *Jatropha* mutant was found in the wild and hybridization experiments showed that its oil content increased by 6%.

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Keywords: *Jatropha curcas* L, provenance, high-oil yield cultivar, biological characteristic, biodiesel properties

Introduction

The recent oil crisis is caused by the depletion of petroleum resource and abrupt rising demand by emerging economic powers like China. Even though the present price is sharply down to about 80 US$/barrel from the peak of 147 US$/barrel due to the world financial crisis, the oil price will maintain high when the world economy is recovered. Biodiesel that is derived from renewable plant oil and animal fat can be used as an alternative to fossil oil. There are many plant seeds that can be used to extract crude oil for production of biodiesel. For example, Ruan's group successful transesterified Chinese tallow kernel oil to qualified biodiesel[1]. *J curcas*, a native shrub or tree of tropical America, is chosen as biodiesel plant because its seed kernel has high oil content (43%−59%), and the tree can resist drought and grow on barren and marginal lands without using arable lands for food crops[2]. *Jatropha* trees exist widely in many countries around the world. According to the Global Exchange for Social Investment[3], the world *Jatropha* plantation area is about 1 million ha in 2008, India with the largest area (400 000 ha) followed by China, Brazil, Zambia, Tanzania and Madagascar. One to two million hectares is expected to be planted annually in the next years all over the world. Asia is expected to prevail with more than 70% of global area developed there until 2015. Since 2006, China has planted 120 000 ha, among them Yunnan with 85 000 ha, Sichuan with 28 000 ha and Guizhou with 12 000 ha[4]. Therefore, to select and breed high oil yield variety is a key issue that depends on if *Jatropha* plantation is economic viable for biodiesel industry development and if *Jatropha* plantation can increase farmers' income. The collection and evaluation of different geographic provenances is the fundamental and first step for the breeding of high oil yield *Jatropha* variety. Systematic provenance trials and analysis of genetic resources collected from different climatic zones in India were studied[5-7]. Sirisomboon *et al*[8] studied the physical and mechanical properties of *Jatropha* fruits, nuts and kernels extensively. However, no work has been done in China for seed source collection and its evaluation for growth rate and oil yield. *Jatropha* trees were thought to be introduced from the Caribbean region through Cape Verde and Guinea-Bissau to Asia and China by Portuguese sailors 500−600 years ago[9]. The selection of best sources for oil production could be essential for a large-scale forestation in China. On the other hand, Jatropha oil has high acid value (up to 30%) because it contains a number of free fatty acids (up to 15%)[10]. It is easy to form soap from these free acids when conventional homogeneous catalyst such as NaOH is used for transesterification of *Jatropha* oil to biodiesel with methanol. Novel processing techniques are needed for biodiesel production from *Jatropha* oil. The main objective of the work is to collect geographic provenances and set up a germplasm resource garden to study and evaluate their biological characteristics and agricultural properties. The provenances that are better in expression of phenotype are selected for a small-scale plantation trial to determine the seed yield and oil content for forestation and future genetic improvement work. Another objective is to show people how biodiesel is produced through a chain from seed selection, propagation, plantation, harvesting and extraction to esterification, and it is qualified used as fossil diesel that are important for commercial production. Therefore, the crude Jatropha oil extracted from the seeds is studied using two-step process, acid esterification-base transesterification for the production of qualified biodiesel.

1 Materials and methods

1.1 Collection of different provenances

In August 2005, 80 geographical provenances of *J curcas* were collected in the wild in southern China provinces of Yunnan, Sichuan, Guizhou, Hainan and Guangxi at latitude from 15°07′00″N to 26°44′10″N, longitude from 97°49′48″E to 105°46′59″E and altitude from 250 m to 1 500 m. The 80 geographic locations were divided by about one degree of latitude or longitude (about 100 km), and at each location only one sample was collected as a geographical provenance (Table 1).

We collected branches randomly from different trees in each geological location, occasionally collected seeds if we found. All experiments were done using cutting seedlings derived from the branches except for the special mentioned comparison experiments. The *Jatropha* sources were distributed mainly over subtropics and northern tropics in the Jinsha river valley and southern Yunnan. The regions with elevation

Table 1 80 *Jatropha* seed sources collected in South China wildly and their growth rate in a germplasm resource garden

No.	Sites	Latitude	Longitude	Alt. (m)	Height (cm)			Diameter (cm)			Crown (cm)	
					1st yr	2nd yr	4th yr	1st yr	2nd yr	4th yr	2nd yr	4th yr
1#	Nahongzhai	23°37′12″N	105°36′00″E	700	120	170	289	5.2	7.3	12.4	78	173
2#	Zhongai Qiaoc	23°10′30″N	101°13′05″E	580	125	175	301	5.4	8.0	13.5	90	185
3#	Zhengyuan	23°54′00″N	100°52′48″E	1 248	260	304	350	5.7	7.7	13.0	150	246
4#	Zhongai Qiaos	23°13′06″N	101°23′30″E	900	195	203	285	4.2	6.2	12.3	83	178
5#	Huaping Qiaoa	26°31′39″N	101°15′03″E	1 000	110	152	228	6.5	9.5	12.7	80	175
6#	Sinan 360k	22°56′43″N	101°39′52″E	700	127	171	260	3.5	5.5	11.4	128	224
7#	Sinanjiangs	22°47′10″N	101°34′24″E	600	107	151	285	3.1	5.1	12.4	90	185
8#	Jiuchengc	24°45′39″N	98°12′03″E	880	115	159	285	2.8	4.8	14.0	110	206
9#	Biaocun	25°58′48″N	99°19′12″E	1 200	120	172	258	5.5	7.6	10.2	115	211
10#	Taozizhai	23°55′48″N	99°12′00″E	1 170	116	171	291	5.6	8.2	14.6	96	192
11#	Enle	23°57′48″N	100°58′48″E	1 000	188	232	276	4.0	6.0	10.2	125	221
12#	Renhe	26°44′10″N	100°50′05″E	1 500	93	132	198	6.3	8.7	11.6	116	212
13#	Jingzhen	21°57′00″N	100°30′00″E	1 176	110	159	270	5.4	8.0	13.5	108	204
14#	Zhongaiqiao2b	23°06′45″N	101°10′45″E	900	122	176	300	5.3	8.2	14.3	109	204
15#	Xiaoheijiang	23°25′10″N	101°22′05″E	820	100	151	257	5.7	8.0	13.5	95	191
16#	Huili	26°40′12″N	102°12′36″E	1 200	126	188	282	7.0	9.8	13.1	128	224
17#	Kaiyuan	23°42′00″N	103°13′48″E	1 051	115	168	252	4.8	6.7	8.9	105	201
18#	Sanjiangkou	24°24′00″N	101°54′00″E	560	156	216	324	6.5	9.5	12.7	124	220
19#	Shangyun	22°40′06″N	98°40′12″E	880	112	158	258	5.1	7.4	13.0	97	192
20#	Pingbian	22°40′48″N	103°40′12″E	1 300	112	152	228	4.8	6.7	8.9	108	204
21#	Moshao	23°59′24″N	102°00′00″N	700	124	176	264	5.4	7.6	10.2	104	200
22#	Mengsheng	23°20′06″N	99°30′12″E	950	113	165	275	5.3	7.4	13.2	83	178
23#	Sanya	18°14′13″N	109°30′47″E	102.8	90	116	174	4.6	6.7	9.0	104	200
24#	Babianjiang	23°12′06″N	101°32′12″E	930	121	170	285	6.5	9.5	16.0	140	236
25#	Niuchang	22°41′15″N	102°59′36″E	400	75	100	150	4.1	5.7	8.5	88	183
26#	Nanguocunc	23°20′06″N	101°50′30″E	1 110	164	208	290	4.4	6.4	14.5	128	224
27#	Zhongaiqiao1a	23°13′06″N	101°23′30″E	580	113	168	282	5.2	7.3	12.4	88	184
28#	Xiaoheijiangs	23°25′10″N	101°22′05″E	820	105	150	256	5.4	7.5	13.0	114	210
29#	Nanxicun	22°40′15″N	103°35′59″E	380	110	156	265	5.3	7.6	13.0	104	199
30#	Yuanjiangba1	23°35′24″N	102°00′00″N	397	110	154	275	3.1	5.1	13.0	91	186
31#	Lincangqiao	23°45′10″N	100°15′05″E	800	130	184	267	4.6	6.5	9.5	176	272
32#	Bajiaoqing	23°07′38″N	101°08′30″E	1 400	123	167	256	3.4	5.4	12.5	92	187
33#	Thakhek	17°23′59″N	104°47′59″E	418	112	152	228	5.6	8.2	11.5	127	223
34#	Gengma	23°23′00″N	99°36′00″E	1 140	115	164	246	5.4	7.6	11.2	134	230
35#	Zhongaiqiaot	23°10′00″N	101°10′30″E	1 100	124	176	299	5.8	8.5	14.6	103	198
36#	Jiucheng	24°41′24″N	97°55′48″E	827	116	169	287	6.0	8.6	14.2	100	196
37#	Bashahe	22°55′05″N	102°10′30″E	660	119	172	290	5.9	8.5	14.8	120	216
38#	Mengbicun	22°55′05″N	101°44′30″E	1 300	113	152	258	5.4	7.6	12.5	106	201
39#	Maocaoling	22°11′57″N	102°47′44″E	800	110	160	240	4.4	6.3	8.7	102	198
40#	Manban	20°10′22″N	102°59′14″E	250	88	132	280	2.6	4.6	12.4	130	226

Table 1 (continued)

No.	Sites	Latitude	Longitude	Alt. (m)	Height (cm)			Diameter (cm)			Crown (cm)	
					1st yr	2nd yr	4th yr	1st yr	2nd yr	4th yr	2nd yr	4th yr
41#	Yuanjiangba2	23°35′24″N	102°00′00″E	397	112	163	275	5.9	7.0	13.0	133	228
42#	Menglun	21°55′00″N	101°16′00″E	537	117	165	275	5.1	7.3	12.4	130	226
43#	Binghanqiao	24°46′48″E	97°55′48″E	880	112	160	250	5.0	7.2	13.0	100	196
44#	Zhongaiqiao1s	23°10′30″N	101°13′05″E	900	148	192	262	3.9	5.9	15.0	106	202
45#	Daheishana	22°31′30″N	102°20′03″E	460	115	164	275	5.8	8.3	14.6	114	210
46#	Heyuncuna	24°00′00″N	97°49′48″E	790	112	160	270	5.1	7.2	13.0	102	198
47#	Reshuitang	24°46′48″E	98°18′00″E	1 100	125	177	285	6.0	8.6	13.5	138	234
48#	Huaping Qiaob	26°31′39″N	101°15′03″E	1 000	110	156	234	5.2	7.4	10.0	104	200
49#	Sinanjiangc	22°22′38″N	101°50′47″E	500	118	169	288	6.0	8.5	14.8	246	342
50#	Menglun1s	21°55′00″N	101°16′00″E	537	130	184	313	5.2	7.2	12.0	109	205
51#	Menglaba	22°35′10″N	103°10′03″E	325	115	171	385	5.3	7.5	13.4	110	206
52#	Yuanmou	25°42′00″N	101°51′00″E	1 118	90	120	180	5.2	7.6	10.2	108	204
53#	Labaidu	22°36′05″N	101°20′03″E	470	115	172	291	6.0	8.5	15.0	120	216
54#	Zhongaiqiao2a	23°12′45″N	101°20′45″E	1 050	125	177	300	5.6	8.0	13.2	106	202
55#	Yuanjianggaodi	23°11′24″N	102°00′00″E	1 000	113	159	270	5.1	7.3	12.4	78	174
56#	Niuchanglvmiao	22°41′15″N	102°59′36″E	400	90	116	174	6.5	9.4	12.5	110	206
57#	Sinanjiang3s	22°15′49″N	101°56′14″E	600	115	163	277	6.3	8.9	15.2	80	176
58#	Mengbicuna	22°20′06″N	98°40′12″E	1 300	106	152	258	5.2	7.5	13.0	79	174
59#	Cangjiangkou	25°20′10″N	99°20′59″E	1 300	128	192	288	5.9	8.6	11.5	152	248
60#	Louangphrabang	21°01′59″N	102°46′59″E	750	119	170	208	5.3	7.6	10.2	108	204
61#	Lujiangqiao	25°10′10″N	98°50′59″E	720	148	216	324	6.0	8.6	11.3	124	220
62#	Niukong	23°00′36″N	102°03′23″E	1 080	109	154	262	5.6	8.0	13.5	104	200
63#	Daheishanb	22°38′47″N	101°44′17″E	460	115	164	279	5.8	8.3	14.6	195	291
64#	Lujiangshui	24°15′10″N	99°10′10″E	900	123	176	299	5.5	8.0	13.5	147	243
65#	Lujiangba	24°50′10″N	98°45′00″E	1 000	113	161	273	4.9	7.0	12.0	143	239
66#	Zhongaiqiaos1	23°13′06″N	101°23′30″E	900	108	154	262	6.3	8.9	15.2	152	248
67#	Zhefangba	24°08′45″N	98°09′00″E	610	119	170	289	6.2	8.8	14.9	113	208
68#	Sinanjiangs	22°44′27″N	101°50′47″E	500	110	154	288	6.0	8.0	14.8	130	225
69#	Nanguocunb	23°04′54″N	101°42′36″E	1 100	120	171	291	5.8	8.4	15.2	113	208
70#	Mengluns	23°50′10″N	105°15′30″E	576	118	170	289	6.4	9.2	15.7	103	198
71#	Xiyanghe	23°50′10″N	105°15′30″E	670	110	156	265	4.6	6.4	10.8	130	226
72#	Mengxingba	24°18′00″N	99°00′00″E	780	115	160	272	5.9	8.6	15.2	110	206
73#	Gasa	24°11′24″N	101°54′00″E	750	119	168	252	5.7	8.2	10.4	123	219
74#	Zhongaiqiao1c	23°10′00″N	101°10′30″E	1 100	114	164	279	6.0	8.9	15.2	128	224
75#	Heyuncunb	24°04′48″N	98°04′48″E	790	112	160	272	5.2	7.5	13.0	114	210
76#	Longtoucun	26°31′39″N	101°15′03″E	1 227	102	148	222	4.5	6.3	9.0	102	198
77#	Dacaoba	24°06′00″N	99°04′48″E	960	114	166	282	5.6	8.0	13.5	108	204
78#	Babu	23°15′00″N	104°30′30″E	700	106	152	228	6.0	8.6	11.5	79	174
79#	Ganlanba	21°55′05″N	100°50′38″E	650	145	209	357	5.0	7.4	12.7	104	200
80#	Pakse	15°07′00″N	105°46′59″E	350	100	136	204	5.4	7.3	10.2	112	208
		Aver			119	167	269	5.3	7.6	12.6	114	210
		Min			75	100	150	2.6	4.6	8.5	78	173
		Max			260	304	385	7.0	9.8	16.0	246	342

of 800−1 200 m and annual rainfall of 800−1 500 mm were suitable for *Jatropha* growth with two periods of full flowering and fruiting

A nursery garden was built to breed the seedlings of the 80 collected sources for further plantation experiments, and also for observing any pests and diseases brought from the sources.

1.2 Growth trial

A two-ha germplasm resource garden was built at Xishuangbanna tropical botanical garden, Menglun, Yunnan, locating at 21°41′ N longitude, 101°25′ E latitude and 570 m altitude for the planting test. The area is a small basin with tropical ultisols and oxisols in a hilly-gully region. The vegetation type is tropical seasonal rainforest or monsoon forest. The place is at the northern part of Southeast Asia tropic region and belongs to tropical monsoon with annual average temperature of 21.5°C. There is no frost throughout the year. The hottest month is June with average temperature of 25.5°C and the coldest month is January with 14.8°C. The mean annual rainfall is 1 557 mm, wet season (May-October) accounted for 87% with 1 335 mm and dry season accounted for 13% with 202 mm. There are differences in ecological factors between the germplasm resource garden and the locations of the geographical provenances. Therefore, all the 80 seed sources are needed to be re-selected for high seed yield and oil content sources in the germplasm resource garden. The re-selected sources are tested for their adaptability and resistance in different soils, terrains and climates to obtain what we need.

The size of plot is 40 cm×40 cm×30 cm (width× length×depth) planting pit with 1.5 m×2.5 m (line× row) tree spacing. For each provenance, 45−60 trees were planted with 3 replications (each replication with 15−20 trees according to different terrains). The planting pits were dug in a flat land before monsoon. Seedlings were planted in the raining days with inclined angles in order to let branches and crowns develop well. No chemical fertilizers and pesticides were used for the trial plantation. Growth rate, flowering, fruiting, seed yield and other data were observed and recorded at predetermined given dates.

1.3 *Jatropha* oil

The 1 000−seed weight, oil content and moisture of the natural dried seeds harvested were determined according to the Chinese national standards (GB/T 5 518−1985, NY/T4-1982 and GB/T 3 543.6–1995). Six sources with high seed yield and oil content were selected from the trial plantation in the germplasm resource garden, and planted in a small-scale (150 m^2 for each seed source). Their seeds were harvested and *Jatropha* oil was mechanically extracted (mechanical press, 6YL-60, Jingdu mechanical equipment Co. Zengzhou, China) and subsequent centrifugally separated.

The fatty acids of *Jatropha* oil were analyzed by Gas Chromatography (GC Shimadzu GC-2014), they mainly consisted of palmitic acid (C16:0; 15.18%), palmitoleic acid (C16:1; 0.99%), stearic acid (C18:0; 6.25%), oleic acid (C18:1; 41.17%), linoleic acid (C18:2; 31.25%) and linolenic acid (C18:3; 0.08%) and others (4.75%) that are suitable for biodiesel production[9].

2 Results and discussion

Eighty geographical provenances were collected in the wild in South China, and planted in the germplasm resource garden in our Institute. Growth rate for some individual selected sources and for the 80 sources were given in Fig. 1 and Table 1, respectively. Seed weight, oil content and yield will be given in Table 2 and Table 3. Images of *Jatropha* fruiting will be given in Fig. 2. A comparison photo between old and new found mutant varieties will be shown in Fig. 3.

2.1 Growth status

2.1.1 *Growth rate*

Based on observation during period of April 2006 to April 2008, both height and ground diameter of *Jatropha* trees as other typical trees increased according to the “S-curve” (Fig. 1). We chose interval of 15−20 days to collect data because the *Jatropha* trees grew to some extent that we can measure the data in these dates as can be seen from Fig. 1. Fast growing occurred in June-July, after a short period of buffering at the middle of August, a growing peak appeared again from September to October, started from the end of October, growing entered a period of stagnating. The growth rate varied very differently in sources, for example, after half year growing (April-October), tree height was 2.5 m (ground diameter of 0.047 m) for source #3 *vs.* only 0.6 m (ground diameter of 0.019 m) for source #40 (Fig. 1). Averagely, *Jatropha* trees grew faster with cutting-seedlings than those with seed plantation. In the next section, it was also found that the seeds were heavier with cutting plantation. So, in our research, plantation was using cutting-seedlings unless specially mentioned for some comparison experiments. Generally speaking, *Jatropha* trees grew very fast as compared with other tree species. After planted on April 1, 2006, based on the data determined on April 1, 2008, ground diameter, tree height and crown size of two-year old plants were 7.6 (4.6−9.8) cm, 1.67 (1.00−3.04) m and 1.14 (0.78−2.46) m, respectively (Table 1).

After almost four years (March 2010), the 80 source trees have grown to the average size of 12.6 cm diameter, 2.69 m height with 2.1 m crown size. The trees have the maximum height of 3.85 m (#51), maximum diameter of 16.0 cm (#24) and maximum crown size of 3.42 m (#49).

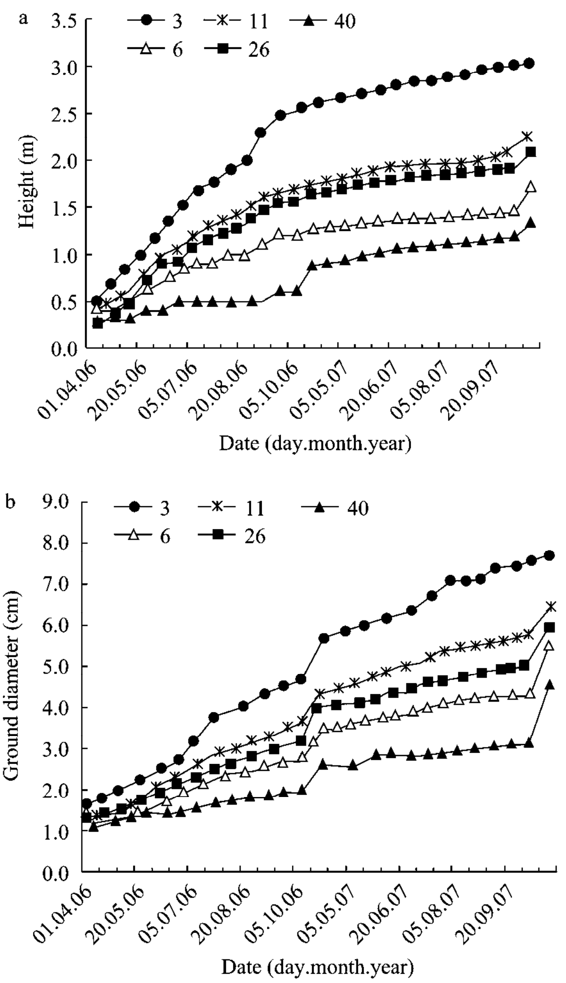

Fig. 1 Growth tendency of *Jatropha* trees from the selected sources for 2 years. (a) Height. (b) Ground diameter.

2.1.2 *Factors on growing*

No chemical fertilizers and pesticides were used in the trial plantation. Therefore, only waterlogging and shadiness were studied in some selected areas for their influences on *Jatropha* growing. It is found that *Jatropha* trees were sensitive to temperature, sunlight and soil moisture.

1) *Waterlogging*

Waterlogging was an instantaneous phenomenon and mainly occurred in the forest which is close to mountain valley and in the nursery garden for young seedlings. Our observation showed that *Jatropha* trees grew well in moist soil but waterlogging for a long time reduced not only survival rate but also growth rate. Without waterlogging, survival rate was 98% but dropped to 87% with waterlogging for 1 year old trees. Their corresponding tree height also decreased from 1.2 to 0.89 m (Fig. 4).

There are no shading and waterlogging in our germplasm resource garden and the area for small-scale trial of forestation (Fig. 2). We only selected some hilly and shading areas for the comparison experiments.

2) *Shadiness*

Shadiness reduced light intensity and environment temperature that further influenced on *Jatropha* growing (Fig. 5), flowering and fruiting. In our comparison study, if x was the distance between *Jatropha* trees and a shelter (building: height 6 m and width 20 m), and y was growth increment, it was found that y increased with x with the following relationships:

$y_1=8.675-6.796x+0.384x^3$ (R=0.780, R^2=0.608; F=4.652, $Sig.$0.060) (1)

where, y_1 was the total accumulative shoot length.

$y_2=15.772x-0.012x^3-47.981$ (R=0.777, R^2=0.603; F=4.556, $Sig.$0.063) (2)

where, y_2 was the maximum shoot length.

It was found that sunlight greatly influenced on fruiting and the number of female flowers that are depending on the number of seeds or oil yield. More flowers and female flowers were in the branches with full sunlight without shading.

2.2 Seed properties and oil yield

After planted in spring 2006, the trees bore fruit in the fall of 2006 (Fig. 2). Started from 2007, all trees bore fruit and produced seeds twice in May-July and September-November, respectively. Seed properties and oil yield were determined from the 80 source seeds planted in the germplasm resource garden in order to select good sources for trial plantation in a small-scale production of *Jatropha* oil.

2.2.1 *1 000-seed weight*

In August 2007, the 1000-seed weight (GB/T5 518−1985) was measured for the first fruiting seeds in May-July 2007 from the 80 sources (Table 2). It was

Fig. 2 A high-oil yield cultivar of *J. curcas*. (a) Fruiting forest. (b) Fruiting branches. (c) Near ripening fruits. (d) Ripened fruits.

Fig. 3 A new cultivar of *J. curcas* bred by natural mutant. (a) Old variety. (b) The new cultivar. (c) Fruiting of the new cultivar.

found that the average weight (dry base) was 0.676 kg, the lightest was 0.477 kg for source #35, and the heaviest was 0.876 kg for source #58. The seeds were 5% heavier from cutting plantation than that from seed raised plants. Moisture content of seed is about 8.88% (GB/T 3 543.6−1995). Our seed weight is heavier than that reported in India[6]. In their 24 accessions, only one exceeded our average weight but much lower than our maximum seed weight (0.876 vs. 0.692 kg/1 000 seed).

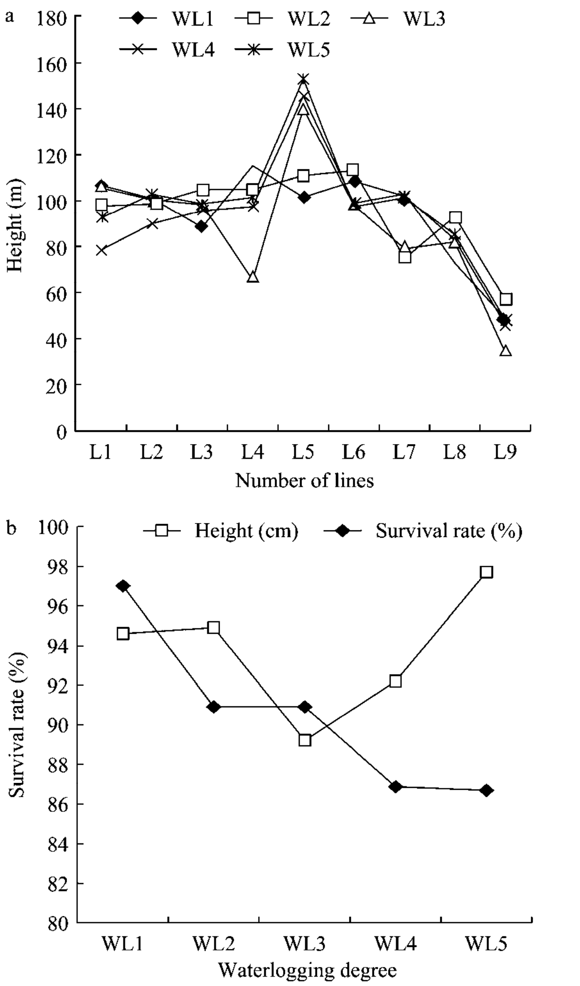

Fig. 4 Effects of waterlogging on *Jatropha* trees surviving and growing. (a) Growing status of height. (b) Survival rate of *Jatropha* trees.

2.2.2 *Oil content*

Oil content was defined as percentage of oil extracted by ether from cracked seeds (NY/T4-1982). The average oil content was 40.59% with the lowest of 33.34% for source #1 and the highest 45.66% for source #80 (Table 2) that was higher as compared to 28%−38.8% for the previous work in India[6]. Oil content of seeds from two-year old trees was 4% higher than that of one-year old trees. Practically, oil was extracted by the mechanical press, oil extracted rate was much lower than its content. In our experiments for the selected 30 high-oil-content sources with the mechanical press, only 72.4% oil was obtained from the seeds. Chemical methods can extract more oil[11], for example 98%[2], 74%[12] and 97%[13] oil were obtained by using hexane extraction, ultrasonication followed by aqueous enzymatic oil extraction with an alkaline protease and enzyme-assisted three-phase partitioning, respectively.

2.2.3 *Seed and oil yield*

At the first year (2006), seed yield (kg/tree) was determined from the first fruiting in order to select high yield sources (Table 2). The mean yield of each seed source was calculated by dividing total dry seed weight collected from all trees of the source in a year by the number of the trees of the source. An average yield of 0.12 kg/tree with a maximum of 0.31 kg/tree for source #6, and a minimum of 0.02 kg/tree for sources #39 was obtained. The maximum seed yield was 15.5 times that of the minimum. Therefore, seed sources played an important role to determine seed yield. Oil yield was obtained by multiplying seed yield with oil content, and the data were given in Table 2. The average oil yield for the 80 sources was 0.048 kg/tree. The maximum oil yield was 14 times that of the minimum, ranging from 0.114 kg/tree for sources #6&26 to 0.008 kg/tree for source #39. To produce *Jatropha* oil, six high-oil yield sources (highlighted in bold in Table 2) were chosen for a small-scale trial plantation to produce crude oil as raw material for biodiesel. The above high biomass growth rate sources (such as #51, 24 and 49 for 4 years old trees) were not selected. Other parameters were just as reference data for the selection of sources. For example, in Fig. 2 (or Table 1), source #3 grew 1.4 times faster in height (or 0.8 times larger in ground diameter) than source #30 for one-year old trees. But, its oil yield was only 0.035 kg/tree as compared to a higher value of 0.080 kg/tree for source #30. Generally, trees with high-oil yield (e.g., sources #6, 26, 68) had moderate growth rate as showed in Fig. 2. The tree with the highest-oil yield (source #68) was short (Fig. 1a) but had a large ground diameter (Fig. 1b) as compared with other trees so that it was easy for farmers to harvest the seeds. It is known that the seed yields of *Jatropha* tree start getting stable only after the 3rd year of plantation and are stabilized by the 5th year of plantation. Therefore, we also measured seed yields at the 2nd and 4th year (Table 2), and found that the seed yields increased with the growing ages. The seed yields for the selected six sources are still relative high after planted three years. But, another source (#44) performed outstandingly and had the 3rd highest seed yield among the 80 sources at the 4th year. More times are needed to observe the yield and growing behaviors of *Jatropha* trees.

Table 2 Seed weight & yield, oil content & yield of different *Jatropha* sources planted in the germplasm garden

Seed sources	1000-seed weight (kg)	Seed yield (kg/tree)			Oil content (%)	Oil Yield (kg/tree)	Seed sources	1000-seed weight (kg)	Seed yield (kg/tree)			Oil content (%)	Oil Yield (kg/tree)
	2nd yr	1st yr	2nd yr	4th yr	1st yr	2nd yr		2nd yr	1st yr	2nd yr	4th yr	1st yr	2nd yr
1#	0.675	0.21	0.17	0.23	33.34	0.070	41#	0.618	0.15	0.25	0.26	40.88	0.061
2#	0.564	0.16	0.19	0.16	34.50	0.055	42#	0.699	0.16	0.21	0.24	40.94	0.066
3#	0.623	0.10	0.15	0.43	35.19	0.035	43#	0.657	0.26	0.62	0.40	40.76	0.106
4#	0.715	0.10	0.10	0.39	35.55	0.036	44#	0.655	0.13	0.25	0.70	40.98	0.053
5#	0.635	0.12	0.15	0.41	35.88	0.043	45#	0.720	0.22	0.46	0.32	40.94	0.090
6#	**0.726**	**0.31**	**0.85**	**2.12**	**36.68**	**0.114**	46#	0.721	0.10	0.16	0.21	40.90	0.041
7#	0.687	0.11	0.35	0.04	37.11	0.041	47#	0.694	0.07	0.13	0.39	41.00	0.029
8#	0.682	0.07	0.07	0.30	37.06	0.026	48#	0.627	0.05	0.13	0.34	41.22	0.021
9#	0.763	0.06	0.02	0.20	37.15	0.022	49#	0.611	0.13	0.09	0.55	41.28	0.054
10#	0.619	0.22	0.09	0.52	37.22	0.082	50#	0.685	0.13	0.04	0.24	41.28	0.054
11#	0.674	0.04	0.01	0.31	37.26	0.015	51#	0.629	0.14	0.17	0.17	41.47	0.058
12#	0.691	0.04	0.15	0.35	37.35	0.015	52#	0.629	0.04	0.17	0.29	41.67	0.017
13#	0.710	0.05	0.18	0.39	37.51	0.019	53#	0.648	0.10	0.11	0.21	41.73	0.042
14#	0.726	0.09	0.11	0.13	37.62	0.034	54#	0.711	0.15	0.03	0.41	41.96	0.063
15#	0.649	0.13	0.09	0.12	37.54	0.049	55#	0.678	0.11	0.14	0.53	41.76	0.046
16#	0.608	0.11	0.07	0.23	38.16	0.042	56#	0.631	0.08	0.19	0.19	42.18	0.034
17#	0.702	0.06	0.24	0.14	37.96	0.023	57#	0.619	0.06	0.09	0.45	42.70	0.026
18#	0.692	0.10	0.07	0.45	38.17	0.038	58#	0.876	0.15	0.17	0.23	42.62	0.064
19#	0.701	0.18	0.07	0.22	38.27	0.069	59#	0.690	0.06	0.17	0.24	42.86	0.026
20#	0.653	0.04	0.08	0.14	38.40	0.015	60#	0.597	0.11	0.21	0.33	42.45	0.047
21#	0.735	0.03	0.07	0.30	38.62	0.012	61#	0.667	0.16	0.04	0.35	42.76	0.068
22#	0.684	0.20	0.10	0.24	38.54	0.077	62#	0.666	0.13	0.11	0.24	42.94	0.056
23#	0.680	0.06	0.19	0.27	39.03	0.023	63#	0.663	0.17	0.03	0.24	43.14	0.073
24#	0.677	0.07	0.13	0.16	39.32	0.028	64#	0.698	0.18	0.14	0.39	42.68	0.077
25#	0.606	0.05	0.18	0.14	39.72	0.020	65#	0.614	0.13	0.19	0.24	43.03	0.056
26#	**0.757**	**0.29**	**0.26**	**1.45**	**39.47**	**0.114**	66#	0.655	0.15	0.13	0.14	43.46	0.065
27#	0.720	0.05	0.06	0.28	39.57	0.020	67#	0.711	0.12	0.07	0.40	43.53	0.052
28#	0.708	0.13	0.04	0.16	39.82	0.052	68#	0.780	0.18	1.02	0.37	44.12	0.079
29#	0.709	0.17	0.07	0.18	39.79	0.068	69#	0.679	0.22	0.48	0.32	43.74	0.096
30#	0.667	0.20	0.07	0.15	39.89	0.080	70#	0.660	0.13	0.13	0.26	43.78	0.057
31#	0.652	0.03	0.07	0.21	40.26	0.012	71#	0.622	0.15	0.07	0.59	43.95	0.066
32#	0.668	0.07	0.03	0.23	40.02	0.028	72#	0.694	0.12	0.09	0.33	44.25	0.053
33#	0.610	0.10	0.20	0.30	40.20	0.040	73#	0.640	0.07	0.09	0.16	44.30	0.031
34#	0.676	0.06	0.08	0.15	40.33	0.024	74#	0.733	0.16	0.05	0.35	43.71	0.070
35#	0.477	0.15	0.01	0.25	40.22	0.060	75#	0.765	0.10	0.11	0.21	44.56	0.045
36#	0.676	0.12	0.03	0.22	40.47	0.049	76#	0.711	0.05	0.09	0.20	44.69	0.022
37#	0.731	0.11	0.06	0.19	40.55	0.045	77#	0.737	0.09	0.09	0.45	45.04	0.041
38#	0.656	0.12	0.08	0.10	40.15	0.048	78#	0.690	0.17	0.08	0.24	45.35	0.077
39#	0.677	0.02	0.04	0.19	41.13	0.008	79#	0.748	0.12	0.10	0.30	45.18	0.054
40#	0.675	0.03	0.21	0.38	40.72	0.012	80#	0.611	0.08	0.15	0.44	45.66	0.037
							Aver	0.676	0.12	0.15	0.32	40.59	0.048
							Min	0.477	0.02	0.01	0.04	33.34	0.008
							Max	0.876	0.31	1.02	2.12	45.66	0.114

Note: seed yield for the 1st year is from only one fruiting; those yields for the 2nd and 4th years are from two fruiting.

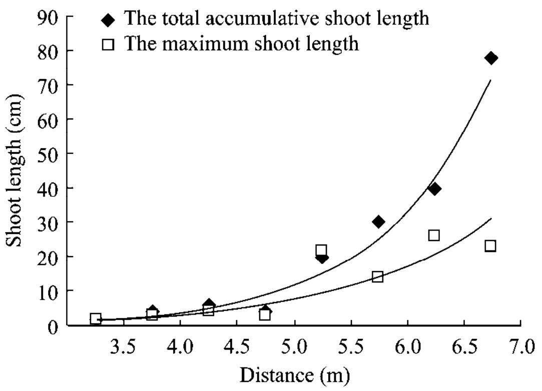

Fig. 5 An effect of shadiness on *Jatropha* growing.

The selected *Jatropha* seed source fatty acid mainly consists of five components. Its relative content is Palmitic acid 14.54%, oleic acid 32.49% and linoleic acid 35.38%, linolenic acid 8.69% and stearic acid 6.30%. It is characteristics of acid value 10.45 mg/g KOH, saponification 191.02 mg/g KOH, non-saponifcation 0.34%, average molecular weight of 932 g/mol.

Biodiesel that is manufactured by *Jatropha* oil is mainly composed of methyl palmitate, methyl palmitoleate, methyl stearate, methyl oleate, methyl linoleate methyl linolenate and methyl linolenate. Its relative content is methyl oleate 40.23%, methyl linoleate 32.07%, methyl palmitate 15.32%, methyl stearate 7.41%, methyl linolenate 1.72%, methyl palmitoleate 0.96% respectively.

Properties of the biodiesel is followed as acid value 0.156 mg/g KOH, density (15°C) 0.887 g/mL, viscosity (40°C) 3.89 mm^2/mL, flash point 186°C, cetane 57, and free carbinol 0.009%, free glycerol 0.19%, total Glycerin 0.24%, moisture 173 mg/kg, ash 0.023%, sulphur (S) 0.003%, color light yellow.

2.3 Trial plantation

The six well behaved and high-oil yield sources (#6, 26, 43, 45, 68, 69) were selected for a small-area (150 m^2 for each source) trial plantation (Table 3). Here, seeds were collected twice a year after fruiting and all data for the seeds were from the average twice harvesting at the second and third year. At the second year, average seed yield (dry base) was 774.06 kg/ha (or 0.62 kg/tree), varying from the minimum of 291.3 kg/ha (or 0.26 kg/tree for source #26) to the maximum of 1 445.1 kg/ha (or 1.02 kg/tree for source #68). A wide range of 313−12 000 kg/ha for one-nine years old plants located in South America, India and Africa was reported as reviewed by Achten et al.[11]. Their yields were scattered mainly due to the consequence of a big variation in age and ecological conditions. We also planted some so-claimed high-yield seed sources from Indonesia, India, Laos and Surinam; these seed yields didn't exceed our yields due to different climate and the incorrect extrapolation. For the 6 sources, oil yield varied from 126.1 (source #26) to 695.6 (source #68) kg/ha (or 0.14 to 0.63 kg/tree) with an average value of 345.3 kg/ha (or 0.34 kg/tree). The source #68 performed outstandingly in oil production with 1 times higher than that of the average 6 sources, or 0.7 times higher that of the second highest. But, as compared with rapeseed[14], soybean[15] and palm oil yield[16] with 975, 620 and 3 850 kg/ha, respectively, the highest oil yield (source #68) was 696 kg/ha. The yield is comparable to that for rapeseed and soybean, however, it should be noted that yield for *Jatropha* is annual, and for rapeseed and soybean is one harvesting. Therefore, it is very needed to increase the oil yield for *Jatropha* to compete with other oil plants economically even the yield may increase further with plant age in its 50 years life expectancy[11]. We also measured seed data at the 3rd year, a slight increase for the average seed yield of the six sources was found. But, seed yield for sources #6&26 increased greatly while #68 dropped dramatically. Seed yield for source #2 went up to 2 001 kg/ha or 964 kg oil/ha.

Table 3 Annual Seed and oil yield of the 6 selected *Jatropha* sources planted in a small-scale (150 m^2 for each source)

Seed sources	Area (m^2)	Seed yield (2nd yr)		Oil yield (2nd yr)*		Seed yield (3rd yr)		Seed yield (4th yr)	
		kg/tree	kg/ha	kg/tree	kg/ha	kg/tree	kg/ha	kg/tree	kg/ha
6#	150	0.85	964.26	0.40 (0.31)	377.48 (292.55)	2.12	2 000.64	2.68	2 858.67
26#	150	0.26	291.25	0.14 (0.10)	126.07 (90.05)	1.45	1 305.73	1.45	1 160.00
43#	150	0.62	883.60	0.36 (0.25)	405.40 (281.53)	0.40	450.44	1.56	1 040.00
45#	150	0.46	517.08	0.24 (0.19)	203.93 (161.44)	0.32	271.91	0.53	706.67
68#	150	1.02	1 445.12	0.63 (0.45)	695.62 (496.86)	0.37	408.54	0.94	877.33
69#	150	0.48	543.05	0.29 (0.21)	263.19 (190.59)	0.32	290.42	0.92	981.33
Average		0.62	774.06	0.34 (0.25)	345.28 (252.17)	0.83	787.95	1.35	1 270.67

Note: * Oil yield in the brackets are actual data extracted by the mechanical press. Seed and oil yields are on dry base.

Other comprehensive methods and techniques are needed to develop for increasing oil yield significantly, such as breeding techniques (chemical induced mutation, Co^{60} radiation, ion implantation mutation, space-flight, molecular and hybrid breeding), high-yield cultivation methods (shaping and pruning, fertilization, spacing, growth inducing agent, trace element treatment, female flower control, propagation methods-seedlings from seed and cutting) and site characteristics (climate, altitude and terrain conditions, rainfall, soil type and soil fertility) which are being studied in our institute. The maximum oil yield of 696 kg/ha, is much lower than the datum (2 100 kg/ha) set by the national government for commercial biodiesel production[17]. Therefore, at present, in order to increase the economic benefits, besides oil production, *Jatropha* should find many other applications in hedge, fertilizer, food/fodder, agrochemicals, medicines and firewood[18]. For example, we can make animal feed by detoxifying proteins, and extract curcin from seed cake as bio-pesticides[2,11,19-20]. Another key factor for plantation of *Jatropha* is for ecological benefit to control erosion and absorb carbon dioxide. forestation can improve ecological system and reduce soil erosion in the region of Jinsha river. Recently, we found an isolated tree in the wild in South China, whose characteristics of seeds, leaves, flowers and stems were very different from the old variety (Fig. 3, b&c vs. a)[9]. The mutation occurred in the whole plant including changes in color and veining of leaves (cotyledon and true leaf), anatomical characters of petioles and stems, color and size of corollas as well as flexility of branches and trunks. All the above properties were confirmed by its inherited generations through sexual and asexual reproduction. Hybridization experiments showed that oil content of the seed increased by 6% for the F-1 hybrids bred by pollening the local Yunnan species from the mutant even though there was little oil content increase for the mutant itself. One thousand seedlings were bred for the forestation in five selected places in Yunnan and Guizhou providences, and found the plants are stable in agronomic traits.

Jatropha oil was extracted from the seeds of two-year old trees of the six sources by the mechanical press with an average yield of 252 kg/ha (the maximum yield of 497 for source #68) (Table 3: data in the brackets). After separated by a centrifugal machine, the *Jatropha* oil was sent for further processing for biodiesel production.

3 Conclusion

Eighty *Jatropha* seed sources were collected, and their plantation trials in a germplasm resource garden were conducted. It was found that *Jatropha* trees grew very fast but growth rate and oil yield varied greatly in sources. Six sources with high-oil yield were chosen for a small-area trial plantation to produce crude oil. At the second year, the maximum oil yield of 696 kg/ha (or 1 445 kg/ha seed yield) was achieved that was approximately 2 times of the national best yield (750 kg/ha seed yield[21]) but still only about 28% of the yield (5 250 kg/ha seed yield[22]) required for the "eleventh-five" five year plan by the government for commercial production. In addition, a new cultivar of *J curcas* had been bred by natural mutant that fund in the wild. As a male parent, when the mutant hybrid with other cultivars of *Jatropha*, seed oil content of the offspring is increase 6% and is up to 41.2%

The seed yields increased with ages during the period of 2007−2010. The yields for the selected six sources are still relatively high. But, another source performed outstandingly at the 4th year. Maximum oil yield of 964 kg/ha (2 001 kg/ha seed yield) was obtained at the 3rd year for source #2. More times are needed to observe the yield and growing behaviors of *Jatropha* trees.

We can conclude that high oil yield seed sources were collected, selected, and successfully cultivated to produce high quality of *Jatropha* oil. Further work is needed to study the changes of *Jatropha* growing characteristics and oil yield with plant ages. Oil yield needs to increase by 2.7 times to reach the national target that can be improved by using appropriate site characteristics, breeding and cultivated techniques.

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REFERENCES

[1] Gao YY, Chen WW, Lei HW, *et al.* Optimization of transesterification conditions for the production of fatty acid methyl ester (FAME) from Chinese tallow kernel oil

with surfactant-coated lipase. *Biomass Bioenergy*, 2009, **33**: 277−282.

[2] Gubitz GM, Mittelbach M, Trabi M. Exploitation of the tropical oil seed plant *J curcas*. *Bioresour Technol*, 1999, **67**: 73−82.

[3] GEXSI: global market study on Jatropha (2008) http://www.jatropha-platform.org/documents/GEXSI_Global-Jatropha-Study-Presentation_RSB-v5.pdf1.

[4] Biomass energy office, State Forest Bureau of China. Work report on forestry biomass energy. 2008, Issue **1/3**.
国家林业局林木生物质能源领导小组办公室. 林木生物质能源工作简报. 2008 年第 **1/3** 期.

[5] Ginwal HS, Rawat PS, Srivastava RL. Seed source variation in growth performance and oil yield of *J curcas* L in central India. *Silvae Genet*, 2004, **53**: 186−192.

[6] Kaushik N, Kumar K, Kumar S, *et al*. Genetic variability and divergence studies in seed traits and oil content of *J curcas* accessions. *Biomass Bioenergy*, 2007, **31**: 497−502.

[7] Ranade SA, Srivastava AP, Rana TS, *et al*. Easy assessment of diversity in *J curcas* plants using two single-primer amplification reaction (SPAR) methods. *Biomass Bioenergy*, 2008, **32**: 533−540.

[8] Sirisomboon P, Kitchaiya P, Pholphoa T, *et al*. Physical and mechanical properties of *J curcas* L fruits, nuts and kernels. *Biosystems Eng*, 2007, **97**: 201−207.

[9] Yang CY, Tang JW, Peng DP, *et al*. Biology, Ecology and Breeding Techniques of *J curcas* L. Kunming: Yunnan Science & Technology Press, 2008.
杨成源, 唐建维, 彭代平, 等. 膏桐生物、生态学及其新品种选育技术. 昆明: 云南科技出版社, 2008.

[10] Berchmans HJ, Hirata S. Biodiesel production from crude *J curcas* seed oil with a high content of free fatty acids. *Bioresour Technol*, 2008, **99**: 1716–1721.

[11] Achten WMJ, Verchot L, Franken YJ, *et al*. *Jatropha* bio-diesel production and use. *Biomass Bioenergy*, 2008, **32**: 1063−1084.

[12] Shah S, Sharma A, Gupta MN. Extraction of oil from *J curcas* L. seed kernels by combination of ultrasonication and aqueous enzymatic oil extraction, *Bioresour Technol*, 2005, **96**: 121−123.

[13] Shah S, Sharma A, Gupta MN. Extraction of oil from *J curcas* L. seed kernels by enzyme assisted three phase partitioning. *Ind Crops Prod*, 2004, **20**: 275−279.

[14] Huang XQ. Crop Germplasm Resources in Yunnan (Oilseed Crop). Kunming: Yunnan Science & Technology Press, 2008.
黄兴奇. 云南农作物种质资源 (油料篇). 昆明: 云南科技出版社, 2008.

[15] Si W. Cost and profit analysis and prospect for soybean production in China. *Soybean Sci Tech*, 2008, **5**: 1−4.
司伟. 中国大豆生产成本与效益分析及展望. 大豆科技, 2008, **5**: 1−4.

[16] Lu K. Increase palm oil yield by united plantations berhad in Malaysia. *Inf Glob Trop Agr*, 2007, **2**: 15−16.

[17] China Green Times. http://www.fbioenergy.gov.cn/ nycy/detail3-265.aspx. 2006-10-16.
中国绿色时报. 中国林业生物质能源. http://www.fbioenergy.gov.cn/nycy/detail3-265.aspx. 2006-10-16.

[18] Kaushik N, Kumar K, Kumar S. Potential of *J curcas* for biofuels. *J Biobased Mater Bioenergy*, 2007, **1**(3): 301−314.

[19] Deng X, Fang Z, Zhang CZ, *et al*. An environmental friendly bio-pesticide and its making method from *Jatropha* curcin: CN, 101473856-A, 2009.
邓欣, 方真, 张绍志, 等. 一个环境友好型小桐子生物农药及其制作方法: CN, 101473856-A, 2009.

[20] Deng X, Fang Z, Yang CY, *et al*. A novel process for continuous extraction of curcin from *Jatropha* seeds: CN, 101463073-A, 2009.
邓欣, 方真, 杨成源, 等. 一个连续提取小桐子毒素的新工艺: CN, 101463073-A, 2009.

[21] Shi ZM, Li Y, Luo YX. Studies on cultivation and development of energy plant *J. curcas*. *J Yunnan Teacher Training Univ*, 1992, **12**(2): 31−37.
施宗明, 李云, 罗玉兴. 能源植物小桐子开发利用和栽培研究. 云南师范大学学报, 1992, **12**(2): 31−38.

[22] National Development and Reform Commission. Renewable Energy Development for the ‘Eleventh-Five’ Five-year Plan. Fagainengyuan document 610, China (2008).
国家发改委. 可再生能源发展“十一五”规划. 发改委能源文库 610 号, 2008.