Research Article

Selection of high-oil-yield seed sources of *Jatropha curcas L.* for biodiesel production

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Method: Different geographic seed sources (in total, 80) of *Jatropha curcas L.* were collected in South China, and planted in a germplasm resource garden for the study of their biological characteristics and agricultural properties. **Results:** It was found that the average ground diameter, tree height and crown size of 2-year-old plants for the 80 sources was 0.076, 1.67 and 1.14 m, respectively, and the average 1000-seed weight was 0.676 kg (0.477–0.876 kg). The trees grew further to an average size of 0.13 m in diameter, 2.69 m in height and with a crown of 2.1 m at the fourth year. Among the 80 sources, six sources with higher oil yield and better expression of phenotype were selected for a small-scale trial, conducted to determine oil yield. A maximum value of 783 oil kg/hectare, that is, 2.7-times the national best yield, was achieved for 3-year-old trees. The oil produced from the six sources was further successfully processed to qualified biodiesel by a two-step process. **Conclusion:** High oil yield seeds were selected from the geological sources that can be further bred to meet the national requirement for large-scale plantation to provide crude *Jatropha* oil for the production of biodiesel.

The current oil crisis is caused by the depletion of petroleum resources and the abrupt rise in demand by emerging economic powers, such as China. Although the present price has sharply decreased to approximately US$80 per barrel from the peak of $147 per barrel owing to the world financial crisis, oil prices will remain high when the world economy has recovered. **Biodiesel** 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 the production of biodiesel. For example, Ruan's group successful transesterified Chinese tallow kernel oil to qualified biodiesel [1]. ***Jatropha curcas L.***, a native shrub or tree of tropical America, has been chosen as biodiesel crop because its seed kernel has a high oil content (43–59%); and the tree can resist drought and grow on barren and marginal lands without having to resort to using arable land, which is used for food crops [2]. *Jatropha* trees exist widely in many countries around the world. According to the Global Exchange for Social Investment, the world *Jatropha* plantation area was approximately 1 million hectares in 2008 [201], India has the largest area (400,000 ha), followed by China, Brazil, Zambia, Tanzania and Madagascar. In total, 1–2 million hectares are expected to be planted annually in the next few years all over the world. Asia is expected to prevail, it has been estimated that more than 70% of global area will be developed by 2015. Since 2006, China has planted 120,000 hectares, among them are Yunnan with 85,000 hectares, Sichuan with 28,000 hectares and Guizhou with 12,000 hectares [3]. Therefore, selecting and breeding a high-oil-yield variety is a key issue that depends on whether a *Jatropha* plantation is economically viable for biodiesel industry development and can increase farmers' incomes. The collection and evaluation of different geographic provenances is the fundamental and first step for the breeding of a high-oil-yield *Jatropha* variety. Systematic provenance trials and analysis of genetic resources collected from different climatic zones in India were studied [4–6]. Sirisomboon *et al.* studied the physical and mechanical properties of *Jatropha* fruits, nuts and kernels extensively [7]. However, no work has been carried out in China for seed-source collection and its evaluation

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Key terms

Biodiesel: Diesel derived from plant or animal oil.

***Jatropha curcas L.*:** Tropical biodiesel trees.

Transesterification: Exchanging the alkoxy group of an ester compound by another alcohol. One triglyceride molecule can be transesterified into three lighter alkyl ester molecules or biodiesel (Figure 2).

Biological characteristic: Organism's morphology, distribution, growth and reproduction, habitat and other properties.

Geographic seed source: Seed collected from a particular location.

for growth rate and oil yield. *Jatropha* trees were thought to be introduced from the Caribbean region through Cabo Verde and Guinea-Bissau, to Asia and China by Portuguese sailors 500–600 years ago [8]. The selection of the best sources for oil production could be essential for large-scale afforestation in China. On the other hand, *Jatropha* oil has high acid value (up to 30 mg KOH/g) because it contains a number of free fatty acids (up to 15%) [9]. It is easy to form soap from these free acids when a 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, as well as to 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 afforestation and future genetic improvement work. Another objective is to show people how biodiesel is produced in a chain (from seed selection, propagation, plantation, harvesting and extraction, to transesterification), and its' qualified use as a fossil diesel, which is important for commercial production. Therefore, the crude *Jatropha* oil is studied using a two-step process: acid esterification and base transesterification, for the production of qualified biodiesel.

Materials & methods

The experimental design route involves the initial collection of different **geographic seed sources** in the wild and subsequent planting in a nursery garden in order to breed the seedlings for growth trials in a germplasm resource garden. According to the different behaviors in growing, flowering, fruiting and seed/oil yield in the germplasm garden, the provenances with high seed yield and oil content, and better expression of phenotype, are selected (1-year-old tree branches are used to breed cutting seedlings) for a small-scale (150 m^2 for each seed source) trial afforestation. The *Jatropha* oil produced is then used for biodiesel production.

Collection of different provenances

In August 2005, 80 geographical provenances of *Jatropha curcas L.* were collected in the wild in the southern China provinces of Yunnan, Sichuan, Guizhou, Hainan and Guangxi at a latitude from 15°07′00′′N to 26°44′10′′N, a longitude from 97°49′48′′E to 105°46′59′′E and an altitude from 250 to 1500 m. The 80 geographic locations were divided by approximately 1° of latitude or longitude (~100 km). The *Jatropha* sources were distributed mainly over subtropics and northern tropics in the Jinsha river valley and southern Yunnan. The regions with elevations of 800–1200 m and annual rainfall of 800–1500 mm were suitable for *Jatropha* growing, with two periods of full flowering and fruiting. In China, there are few natural *Jatropha* forests, and most trees are grown as fences or tree boarders along the road. Therefore, a route-investigated method was used. According to the map, one seed sample consisting of five to ten trees was collected in each location as a geographical provenance that was separated by every 100 km (Table 1). The trees were selected if they were in good health, suitable flowerers and fruiters, and without diseases and pests. The average age of the trees was 5 years old, and the distance between the trees was 5–10 m. We collected cuttings (30–50 branches with a length of 20 cm and diameter of 1–3 cm) for each source, and occasionally collected seeds (2–3 kg for each sample) if they were found. However, in this research, only collected cutting sources were used as mother trees to breed seedlings. All experiments used cutting seedlings from the mother trees, except for the mentioned comparison experiments.

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

Growth trials

A 2-ha germplasm resource garden was built in the Xishuangbanna tropical botanical garden at Menglun, Yunnan, which is located 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 garden is in the northern part of the Southeast Asia tropic region and has a tropical monsoon climate, with an annual average temperature of 21.5°C. There is no frost throughout the year. The hottest month of the year is June, when the average temperature is 25.5°C. The coldest month is January, at an average 14.8°C. There is a mean annual rainfall of 1557 mm, and the wet season (May–October) accounted for 87% of this amount (1335 mm) and the dry season accounted for 13% (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 needed to be reselected for high seed yield and oil content sources in the germplasm resource garden. The reselected sources were tested for their adaptability and resistance in different soils, terrains and climates to obtain what was required.

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

Number	Sites	Latitude	Longitude	Altitude (m)	Height (first, second and fourth years; m)			Diameter (first, second and fourth years; m)			Crown (second and fourth years; m)	
					First	Second	Fourth	First	Second	Fourth	Second	Fourth
1	Nahongzhai	23°37′12′′N	105°36′00′′E	700	1.20	1.70	2.89	0.052	0.073	0.124	0.78	1.73
2	Zhongai Qiaoc	23°10′30′′N	101°13′05′′E	580	1.25	1.75	3.01	0.054	0.080	0.135	0.9	1.85
3	Zhengyuan	23°54′00′′N	100°52′48′′E	1248	2.60	3.04	3.50	0.057	0.077	0.130	1.5	2.46
4	Zhongai Qiaos	23°13′06′′N	101°23′30′′E	900	1.95	2.03	2.85	0.042	0.062	0.123	0.83	1.78
5	Huaping Qiaoa	26°31′39′′N	101°15′03′′E	1000	1.10	1.52	2.28	0.065	0.095	0.127	0.8	1.75
6	Sinan 360k	22°56′43′′N	101°39′52′′E	700	1.27	1.71	2.60	0.035	0.055	0.114	1.28	2.24
7	Sinanjiangs	22°47′10′′N	101°34′24′′E	600	1.07	1.51	2.85	0.031	0.051	0.124	0.9	1.85
8	Jiuchengc	24°45′39′′N	98°12′03′′E	880	1.15	1.59	2.85	0.028	0.048	0.140	1.1	2.06
9	Biaocun	25°58′48′′N	99°19′12′′E	1200	1.20	1.72	2.58	0.055	0.076	0.102	1.15	2.11
10	Taozizhai	23°55′48′′N	99°12′00′′E	1170	1.16	1.71	2.91	0.056	0.082	0.146	0.96	1.92
11	Enle	23°57′48′′N	100°58′48′′E	1000	1.88	2.32	2.76	0.040	0.060	0.102	1.25	2.21
12	Renhe	26°44′10′′N	100°50′05′′E	1500	0.93	1.32	1.98	0.063	0.087	0.116	1.16	2.12
13	Jingzhen	21°57′00′′N	100°30′00′′E	1176	1.10	1.59	2.70	0.054	0.080	0.135	1.08	2.04
14	Zhongaiqiao2b	23°06′45′′N	101°10′45′′E	900	1.22	1.76	3.00	0.053	0.082	0.143	1.09	2.04
15	Xiaoheijiang	23°25′10′′N	101°22′05′′E	820	1.00	1.51	2.57	0.057	0.080	0.135	0.95	1.91
16	Huili	26°40′12′′N	102°12′36′′E	1200	1.26	1.88	2.82	0.070	0.098	0.131	1.28	2.24
17	Kaiyuan	23°42′00′′N	103°13′48′′E	1051	1.15	1.68	2.52	0.048	0.067	0.089	1.05	2.01
18	Sanjiangkou	24°24′00′′N	101°54′00′′E	560	1.56	2.16	3.24	0.065	0.095	0.127	1.24	2.2
19	Shangyun	22°40′06′′N	98°40′12′′E	880	1.12	1.58	2.58	0.051	0.074	0.130	0.97	1.92
20	Pingbian	22°40′48′′N	103°40′12′′E	1300	1.12	1.52	2.28	0.048	0.067	0.089	1.08	2.04
21	Moshao	23°59′24′′N	102°00′00′′N	700	1.24	1.76	2.64	0.054	0.076	0.102	1.04	2
22	Mengsheng	23°20′06′′N	99°30′12′′E	950	1.13	1.65	2.75	0.053	0.074	0.132	0.83	1.78
23	Sanya	18°14′13′′N	109°30′47′′E	102.8	0.90	1.16	1.74	0.046	0.067	0.090	1.04	2
24	Babianjiang	23°12′06′′N	101°32′12′′E	930	1.21	1.70	2.85	0.065	0.095	0.160	1.4	2.36
25	Niuchang	22°41′15′′N	102°59′36′′E	400	0.75	1.00	1.50	0.041	0.057	0.085	0.88	1.83
26	Nanguocunc	23°20′06′′N	101°50′30′′E	1110	1.64	2.08	2.90	0.044	0.064	0.145	1.28	2.24
27	Zhongaiqiao1a	23°13′06′′N	101°23′30′′E	580	1.13	1.68	2.82	0.052	0.073	0.124	0.88	1.84
28	Xiaoheijiangs	23°25′10′′N	101°22′05′′E	820	1.05	1.50	2.56	0.054	0.075	0.130	1.14	2.1
29	Nanxicun	22°40′15′′N	103°35′59′′E	380	1.10	1.56	2.65	0.053	0.076	0.130	1.04	1.99
30	Yuanjiangba1	23°35′24′′N	102°00′00′′N	397	1.10	1.54	2.75	0.031	0.051	0.130	0.91	1.86
31	Lincangqiao	23°45′10′′N	100°15′05′′E	800	1.30	1.84	2.67	0.046	0.065	0.095	1.76	2.72
32	Bajiaoqing	23°07′38′′N	101°08′30′′E	1400	1.23	1.67	2.56	0.034	0.054	0.125	0.92	1.87
33	Thakhek	17°23′59′′N	104°47′59′′E	418	1.12	1.52	2.28	0.056	0.082	0.115	1.27	2.23
34	Gengma	23°23′00′′N	99°36′00′′E	1140	1.15	1.64	2.46	0.054	0.076	0.112	1.34	2.3

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Number	Sites	Latitude	Longitude	Altitude (m)	Height (first, second and fourth years; m)			Diameter (first, second and fourth years; m)			Crown (second and fourth years; m)	
					First	Second	Fourth	First	Second	Fourth	Second	Fourth
35	Zhongaiqiaot	23°10´00´´N	101°10´30´´E	1100	1.24	1.76	2.99	0.058	0.085	0.146	1.03	1.98
36	Jiucheng	24°41´24´´N	97°55´48´´E	827	1.16	1.69	2.87	0.060	0.086	0.142	1	1.96
37	Bashahe	22°55´05´´N	102°10´30´´E	660	1.19	1.72	2.90	0.059	0.085	0.148	1.2	2.16
38	Mengbicun	22°55´05´´N	101°44´30´´E	1300	1.13	1.52	2.58	0.054	0.076	0.125	1.06	2.01
39	Maocaoling	22°11´57´´N	102°47´44´´E	800	1.10	1.60	2.40	0.044	0.063	0.087	1.02	1.98
40	Manban	20°10´22´´N	102°59´14´´E	250	0.88	1.32	2.80	0.026	0.046	0.124	1.3	2.26
41	Yuanjiangba2	23°35´24´´N	102°00´00´´E	397	1.12	1.63	2.75	0.059	0.070	0.130	1.33	2.28
42	Menglun	21°55´00´´N	101°16´00´´E	537	1.17	1.65	2.75	0.051	0.073	0.124	1.3	2.26
43	Binghanqiao	24°46´48´´E	97°55´48´´E	880	1.12	1.60	2.50	0.050	0.072	0.130	1	1.96
44	Zhongaiqiao1s	23°10´30´´N	101°13´05´´E	900	1.48	1.92	2.62	0.039	0.059	0.150	1.06	2.02
45	Daheishana	22°31´30´´N	102°20´03´´E	460	1.15	1.64	2.75	0.058	0.083	0.146	1.14	2.1
46	Heyuncuna	24°00´00´´N	97°49´48´´E	790	1.12	1.60	2.70	0.051	0.072	0.130	1.02	1.98
47	Reshuitang	24°46´48´´E	98°18´00´´E	1100	1.25	1.77	2.85	0.060	0.086	0.135	1.38	2.34
48	Huaping Qiaob	26°31´39´´N	101°15´03´´E	1000	1.10	1.56	2.34	0.052	0.074	0.100	1.04	2
49	Sinanjiangc	22°22´38´´N	101°50´47´´E	500	1.18	1.69	2.88	0.060	0.085	0.148	2.46	3.42
50	Menglun1s	21°55´00´´N	101°16´00´´E	537	1.30	1.84	3.13	0.052	0.072	0.120	1.09	2.05
51	Menglaba	22°35´10´´N	103°10´03´´E	325	1.15	1.71	3.85	0.053	0.075	0.134	1.1	2.06
52	Yuanmou	25°42´00´´N	101°51´00´´E	1118	0.90	1.20	1.80	0.052	0.076	0.102	1.08	2.04
53	Labaidu	22°36´05´´N	101°20´03´´E	470	1.15	1.72	2.91	0.060	0.085	0.150	1.2	2.16
54	Zhongaiqiao2a	23°12´45´´N	101°20´45´´E	1050	1.25	1.77	3.00	0.056	0.080	0.132	1.06	2.02
55	Yuanjianggaodi	23°11´24´´N	102°00´00´´E	1000	1.13	1.59	2.70	0.051	0.073	0.124	0.78	1.74
56	Niuchanglvmiao	22°41´15´´N	102°59´36´´E	400	0.90	1.16	1.74	0.065	0.094	0.125	1.1	2.06
57	Sinanjiang3s	22°15´49´´N	101°56´14´´E	600	1.15	1.63	2.77	0.063	0.089	0.152	0.8	1.76
58	Mengbicuna	22°20´06´´N	98°40´12´´E	1300	1.06	1.52	2.58	0.052	0.075	0.130	0.79	1.74
59	Cangjiangkou	25°20´10´´N	99°20´59´´E	1300	1.28	1.92	2.88	0.059	0.086	0.115	1.52	2.48
60	Louangphrabang	21°01´59´´N	102°46´59´´E	750	1.19	1.70	2.08	0.053	0.076	0.102	1.08	2.04
61	Lujiangqiao	25°10´10´´N	98°50´59´´E	720	1.48	2.16	3.24	0.060	0.086	0.113	1.24	2.2
62	Niukong	23°00´36´´N	102°03´23´´E	1080	1.09	1.54	2.62	0.056	0.080	0.135	1.04	2
63	Daheishanb	22°38´47´´N	101°44´17´´E	460	1.15	1.64	2.79	0.058	0.083	0.146	1.95	2.91
64	Lujiangshui	24°15´10´´N	99°10´10´´E	900	1.23	1.76	2.99	0.055	0.080	0.135	1.47	2.43
65	Lujiangba	24°50´10´´N	98°45´00´´E	1000	1.13	1.61	2.73	0.049	0.070	0.120	1.43	2.39
66	Zhongaiqiaos1	23°13´06´´N	101°23´30´´E	900	1.08	1.54	2.62	0.063	0.089	0.152	1.52	2.48
67	Zhefangba	24°08´45´´N	98°09´00´´E	610	1.19	1.70	2.89	0.062	0.088	0.149	1.13	2.08
68	Sinanjiangs	22°44´27´´N	101°50´47´´E	500	1.10	1.54	2.88	0.060	0.080	0.148	1.3	2.25
69	Nanguocunb	23°04´54´´N	101°42´36´´E	1100	1.20	1.71	2.91	0.058	0.084	0.152	1.13	2.08
70	Mengluns	23°50´10´´N	105°15´30´´E	576	1.18	1.70	2.89	0.064	0.092	0.157	1.03	1.98

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

Number	Sites	Latitude	Longitude	Altitude (m)	Height (first, second and fourth years; m)			Diameter (first, second and fourth years; m)			Crown (second and fourth years; m)	
					First	Second	Fourth	First	Second	Fourth	Second	Fourth
71	Xiyanghe	23°50′10″N	105°15′30″E	670	1.10	1.56	2.65	0.046	0.064	0.108	1.3	2.26
72	Mengxingba	24°18′00″N	99°00′00″E	780	1.15	1.60	2.72	0.059	0.086	0.152	1.1	2.06
73	Gasa	24°11′24″N	101°54′00″E	750	1.19	1.68	2.52	0.057	0.082	0.104	1.23	2.19
74	Zhongaiqiao1c	23°10′00″N	101°10′30″E	1100	1.14	1.64	2.79	0.060	0.089	0.152	1.28	2.24
75	Heyuncunb	24°04′48″N	98°04′48″E	790	1.12	1.60	2.72	0.052	0.075	0.130	1.14	2.1
76	Longtoucun	26°31′39″N	101°15′03″E	1227	1.02	1.48	2.22	0.045	0.063	0.090	1.02	1.98
77	Dacaoba	24°06′00″N	99°0448″E	960	1.14	1.66	2.82	0.056	0.080	0.135	1.08	2.04
78	Babu	23°15′00″N	104°30′30″E	700	1.06	1.52	2.28	0.060	0.086	0.115	0.79	1.74
79	Ganlanba	21°55′05″N	100°50′38″E	650	1.45	2.09	3.57	0.050	0.074	0.127	1.04	2
80	Pakse	15°07′00″N	105°46′59″E	350	1.00	1.36	2.04	0.054	0.073	0.102	1.12	2.08
Average					1.19	1.67	2.69	0.053	0.076	0.126	1.14	2.1
Minimum					0.75	1	1.5	0.026	0.046	0.085	0.78	1.73
Maximum					2.6	3.04	3.85	0.07	0.098	0.16	2.46	3.42

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

▪ *Jatropha* oil

The 1000-seed weight, oil content and moisture of the natural dried seeds harvested were determined according to the Chinese national standards (GB/T5518–1985, NY/T4–1982 and GB/T 3543.6–1995). 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 plot (150 m^2 for each seed source). Their seeds were harvested, and the *Jatropha* oil was mechanically extracted (by the mechanical press 6YL-60 [Jingdu Mechanical Equipment Co., Zengzhou, China]) and subsequently centrifugally separated.

The fatty acids of *Jatropha* oil were analyzed by gas chromatography (GC Shimadzu GC-2014), and consisted mainly 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%), which are suitable for biodiesel production [8].

▪ Biodiesel production

Owing to the high content of free fatty acids in *Jatropha* oil, it is easy to cause a saponification phenomenon when NaOH is directly used as a catalyst for the production of biodiesel, thus reducing the conversion rate of *Jatropha* oil. On the other hand, when H_2SO_4 is used as a catalyst, the transesterification process needs a longer reaction time, and the produced biodiesel is unstable. Therefore, a two-step process of acid-esterification pretreatment, and subsequent base-transesterification to biodiesel, was used for the production of biodiesel.

Acid-esterification pretreatment

In the first step, acid-esterification pretreatment **(Figure 1)** was performed in an ultrasonic reactor (AS10200BDT, Tianjin Boda Ultrasonic Cleaner Co., Ltd, Tianjin, China) with concentrated sulfuric acid as a catalyst to remove free acids. After reaction, the pretreated oil contained few free acids and some biodiesel according to the reaction.

$$RCOOH + CH_3OH \xrightarrow{H_2SO_4} RCOOCH_3 + H_2O$$

Figure 1. Acid-esterification pretreatment for biodiesel production.

Base transesterification to biodiesel

In the second step, the base transesterification reaction (Figure 2) was carried out in an ultrasonic reactor with NaOH catalyst. The aforementioned pretreated oil reacted with methanol catalyzed by NaOH to form biodiesel. After methanol was removed by distillation, the esters were extracted with ether (60 ml) and washed with distilled water. Biodiesel was obtained by distillation of the ethers. Biodiesel was analyzed by gas chromatographywith a flame ionization detector and a capillary column (Rtx-Wax; 30 m × 0.25 mm × 0.25 μm).

Results & discussion

In total, 80 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 (Figure 3) and for the 80 sources (Table 1) are provided. Waterlogging and shadiness data are listed in Tables 2 & 3. Seed weight, oil content and yield are given in Tables 4 & 5. Images of *Jatropha* fruiting are provided in Figure 4. A SPSS statistical analysis method was used to collect and analyze data [10].

▪ Growth status

Growth rate

Based on the observation from April to October 2006, both height and ground diameter of *Jatropha* trees as other typical trees increased according to the 'S-curve' (Figure 3). We chose an interval of 15–20 days to collect data because the *Jatropha* trees grew at such a rate so as to enable us to measure the data on these dates, as shown in Figure 3. Fast growth occurred in June–July and, after a short period of buffering during the middle of August, a growth peak appeared again from September to October; at the end of October, growth entered a period of stagnation. The growth rate varied very differently by source; for example, after 6 months of growing (April–October), tree height was 2.5 m (ground diameter: 0.047 m) for source 3 versus only 0.6 m (ground diameter: 0.019 m) for source 40 (Figure 3). On average, *Jatropha* trees grew faster with cutting seedlings than those with seed plantation; it was also found that the seeds from cutting plantation were heavier. Therefore, in our research, plantation used cutting seedlings unless otherwise indicated for some comparison experiments. Generally speaking, *Jatropha* trees grew very fast compared with other tree species. For trees planted on 1 April 2006, based on the data obtained on 1 April 2008, ground diameter, tree height and crown size of 2-year-old plants were 0.076 (0.046–0.098), 1.67 (1–3.04) and 1.14 (0.78–2.46) m, respectively (Table 1). After almost 4 years (March 2010), the 80 source trees had grown to an average size of 0.126 m in diameter, 2.69 m in height and with a crown size of 2.1 m. The trees have a maximum height of 3.85 m (51), maximum diameter of 0.16 m (24) and maximum crown size of 3.42 m (49).

$$\begin{array}{l} CH_2OOCR_1 \\ | \\ CHOOCR_2 \\ | \\ CH_2OOCR_3 \end{array} + 3CH_3OH \xrightarrow{NaOH} \begin{array}{l} CH_2OH \\ | \\ CHOH \\ | \\ CH_2OH \end{array} + \begin{array}{l} R_1COOCH_3 \\ R_2COOCH_3 \\ R_3COOCH_3 \end{array}$$

Figure 2. Base transesterification to biodiesel.

Growth factors

No chemical fertilizers or 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 was also found that *Jatropha* trees were sensitive to temperature, sunlight and soil moisture.

Waterlogging

Waterlogging was an instantaneous phenomenon and mainly occurred in the forest close to a 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 (Table 2). 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.

There was no shading or waterlogging in our germplasm resource garden, or in the area for a small-scale trial afforestation (Figure 4).

Shadiness

Shadiness reduces light intensity and environment temperature, which further influences *Jatropha* growth, flowering and fruiting. In our comparison study, when x was taken to be the distance between *Jatropha* trees and a shelter (building: height 6 m and width 20 m), and y to be the growth increment, it was found that, according to the data in Table 3 using the SPSS analysis of variance (curve estimation) [10], y increased with x (3.2–6.3 m) with the following relationships:

$y_1 = 15.772x - 0.012x^3 - 47.981$

($R = 0.777$; $R^2 = 0.603$; $F = 4.556$; significance: 0.063)

where, y_1 is the maximum shoot length.

$y_2 = 8.675 - 6.796x + 0.384x^3$
($R = 0.780$; $R^2 = 0.608$; $F = 4.652$; significance: 0.060)

where, y_2 is the total accumulative shoot length.

It was found that sunlight greatly influenced fruiting and the number of female flowers, which in turn influenced the number of seeds and oil yield. More flowers and female flowers were in the branches with full sunlight without shading.

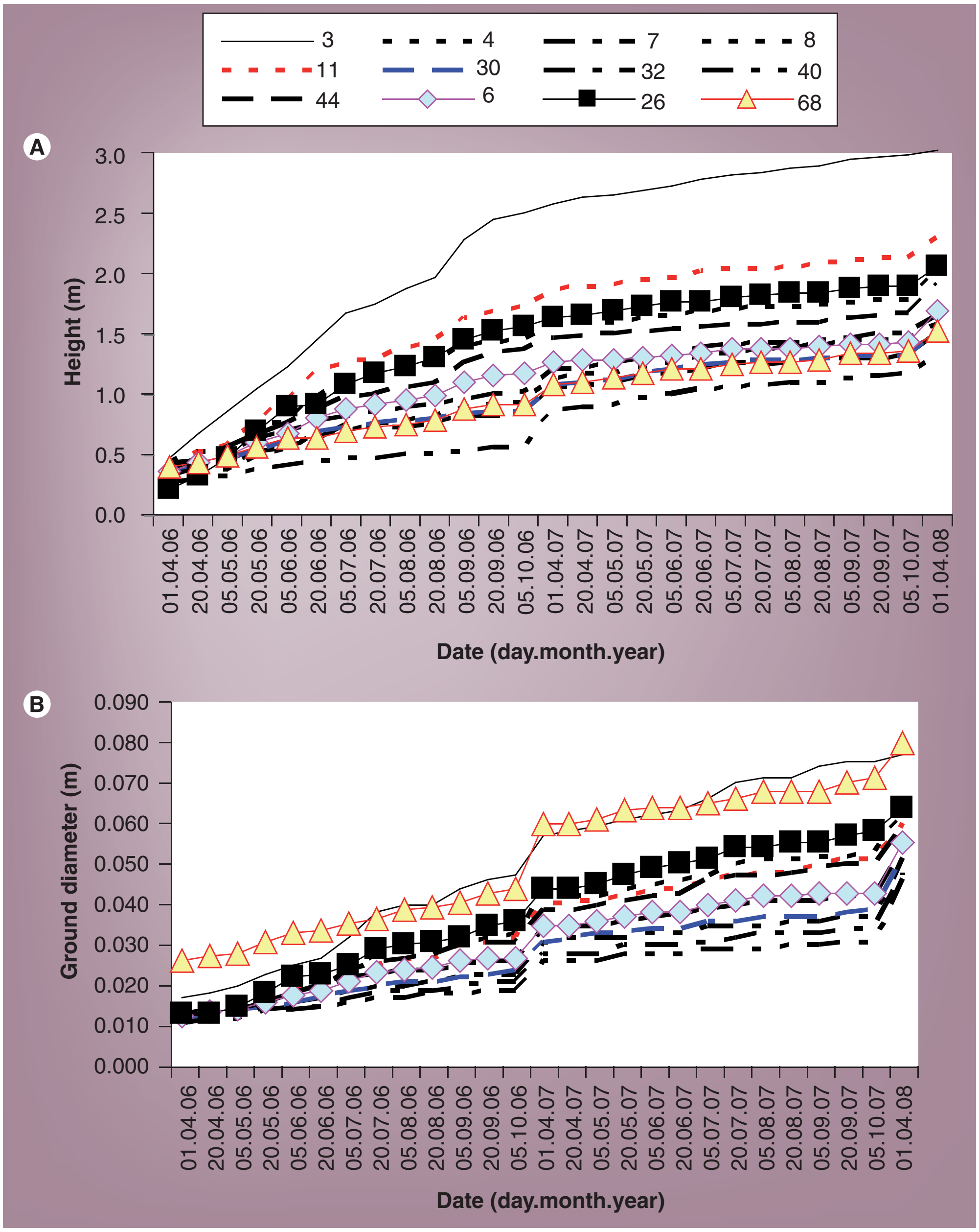

Figure 3. Growth tendency of *Jatropha* trees from the selected sources for 2 years.
(A) Height. **(B)** Ground diameter.

Seed properties & oil yield

After being planted in Spring 2006, the trees fruited in Fall of 2006 **(Figure 4)**. From 2007, all trees were fruiting and producing seeds biannually, in May–July and September–November. 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.

1000-seed weight

In August 2007, the 1000-seed weight (GB/T5518–1985) was measured for the first fruiting seeds in May–July 2007 from the 80 sources **(Table 4)**. It was 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. The moisture content of the seed is approximately 8.88% (GB/T 3543.6–1995). Our seed weight is heavier than the data reported in India [5]. In their 24 accessions, only one exceeded our average weight, but was far lower than our maximum seed weight (0.876 vs 0.692 kg/1000 seeds).

Oil content

Oil content was defined as the percentage of oil extracted by ether from cracked seeds (NY/T4–1982). The average oil content was 40.59%, with the lowest being 33.34% for source 1, and the highest being 45.66% for source 80 **(Table 4)**, which was higher than the 28–38.8% for the previous work in India [5]. Oil content of seeds from 2-year-old trees was 4% higher than that from 1-year-old trees. Oil was extracted by the mechanical press; oil extraction rate was much lower than its content. In our experiments on 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] of oil has

Table 2. Effects of waterlogging on the growth (height) and survival rate of *Jatropha* trees (source 42) for the first year.

Degrees of water logging	Height (m)										Survival rate (%)
	Line 1	Line 2	Line 3	Line 4	Line 5	Line 6	Line 7	Line 8	Line 9	Average	
D_0	1.26	1.09	1.19	1.16	1.24	1.22	1.31	1.15	1.20	1.20	98.00
D_1	1.06	1.00	0.98	0.66	1.40	0.98	0.79	0.82	0.34	0.89	90.91
D_2	0.79	0.90	0.96	0.97	1.46	0.99	1.03	0.73	0.49	0.92	86.67
D_3	0.92	1.03	0.99	1.01	1.53	0.97	1.01	0.85	0.48	0.98	86.86
Average	1.01	1.00	1.03	0.95	1.41	1.04	1.03	0.89	0.63	1.00	90.61

Each line has 15 trees.
D_0: Without water logging; D_1: Soil with saturated water; D_2: Blocks of water logging; D_3: Large area of waterlogging.

been obtained using hexane extraction; ultrasonication followed by aqueous enzymatic oil extraction with an alkaline protease; and enzyme-assisted three-phase partitioning, respectively.

Seed & oil yield

During the first year (2006), seed yield (kg/tree) was determined from the first fruiting in order to select high-yield sources **(Table 4)**. 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 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 in determining seed yield. Oil yield was obtained by multiplying seed yield by oil content **(Table 4)**. 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 and 26, to 0.008 kg/tree for source 39. Our purpose was to produce *Jatropha* oil; therefore, six high-oil-yield sources **(Table 4)** were chosen for a small-scale trial plantation to produce crude oil as a raw material for biodiesel. The high biomass growth rate sources (e.g., 51, 24 and 49 for 4-year-old trees) were not selected. Other parameters were just as reference data for the selection of sources. For example, in **Figure 3** (or **Table 1**), source 3 grew 1.4-times faster in height (or 0.8-times larger in ground diameter) than source 30 for 1-year-old trees. However, its oil yield was only 0.035 kg/tree compared with a higher value of 0.080 kg/tree for source 30. Generally, trees with high oil yield (e.g., sources 6, 26 and 68) had moderate growth rates, as shown in **Figure 3**. The tree with the highest oil yield (source 68) was short **(Figure 3A)** but had a large ground diameter **(Figure 3B)** when compared with other trees, so it is easier for farmers to harvest these seeds. It is known that the seed yields of *Jatropha* tree become stable only after the third year of plantation, and are stabilized by the fifth year of planting. Therefore, we also measured seed yields at the second and third year **(Table 4)**, and found that seed yield increased with age. The seed yields for the

Table 3. Effect of shading on the growth of *Jatropha* trees (source 42) for the first year.

y (cm)		x (m)							
		3.2	3.6	3.8	4.5	4.8	5.2	5.6	6.3
y_1	Line 1	1.6	2.8	3.7	2.7	17.0	10.0	26.0	24.0
	Line 2	1.8	3.2	3.8	2.9	19.0	16.0	26.0	27.0
	Line 3	2.2	3.5	4.4	3.3	25.0	17.0	30.0	25.0
	Line 4	1.9	2.8	3.7	3.0	24.0	12.0	25.0	21.0
	Line 5	2.3	2.9	4.2	2.9	26.0	14.0	24.0	20.5
	Average	2.0	3.0	4.0	3.0	22.2	13.8	26.2	23.5
y_2	Line 1	1.6	3.6	5.4	4.0	15.0	25.0	33.0	70.0
	Line 2	1.8	4.1	6.3	3.8	20.0	32.0	42.0	80.0
	Line 3	2.2	4.3	6.5	4.5	24.0	36.0	45.0	82.0
	Line 4	1.9	3.6	5.6	3.9	19.0	26.0	39.0	78.0
	Line 5	2.3	4.2	6.2	4.0	20.0	31.0	40.0	81.0
	Average	2.0	4.0	6.0	4.0	20.0	30.0	40.0	78.0

Each line has 15 trees and shelter (building: height 6 m and width 20 m).
x: Distance between Jatropha trees and the shelter; y_1: Maximum shoot length; y_2: Total accumulative shoot length.

Table 4. Seed weight and 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)
	Second year	First year	Second year	Third year	First year	First year
1	0.675	0.21	0.17	0.23	33.34	0.070
2	0.564	0.16	0.19	0.16	34.50	0.055
3	0.623	0.10	0.15	0.43	35.19	0.035
4	0.715	0.10	0.10	0.39	35.55	0.036
5	0.635	0.12	0.15	0.41	35.88	0.043
6†	0.726	0.31	0.85	2.12	36.68	0.114
7	0.687	0.11	0.35	0.04	37.11	0.041
8	0.682	0.07	0.07	0.30	37.06	0.026
9	0.763	0.06	0.02	0.20	37.15	0.022
10	0.619	0.22	0.09	0.52	37.22	0.082
11	0.674	0.04	0.01	0.31	37.26	0.015
12	0.691	0.04	0.15	0.35	37.35	0.015
13	0.710	0.05	0.18	0.39	37.51	0.019
14	0.726	0.09	0.11	0.13	37.62	0.034
15	0.649	0.13	0.09	0.12	37.54	0.049
16	0.608	0.11	0.07	0.23	38.16	0.042
17	0.702	0.06	0.24	0.14	37.96	0.023
18	0.692	0.10	0.07	0.45	38.17	0.038
19	0.701	0.18	0.07	0.22	38.27	0.069
20	0.653	0.04	0.08	0.14	38.40	0.015
21	0.735	0.03	0.07	0.30	38.62	0.012
22	0.684	0.20	0.10	0.24	38.54	0.077
23	0.680	0.06	0.19	0.27	39.03	0.023
24	0.677	0.07	0.13	0.16	39.32	0.028
25	0.606	0.05	0.18	0.14	39.72	0.020
26†	0.757	0.29	0.26	1.45	39.47	0.114
27	0.720	0.05	0.06	0.28	39.57	0.020
28	0.708	0.13	0.04	0.16	39.82	0.052
29	0.709	0.17	0.07	0.18	39.79	0.068
30	0.667	0.20	0.07	0.15	39.89	0.080
31	0.652	0.03	0.07	0.21	40.26	0.012
32	0.668	0.07	0.03	0.23	40.02	0.028
33	0.610	0.10	0.20	0.30	40.20	0.040
34	0.676	0.06	0.08	0.15	40.33	0.024
35	0.477	0.15	0.01	0.25	40.22	0.060
36	0.676	0.12	0.03	0.22	40.47	0.049
37	0.731	0.11	0.06	0.19	40.55	0.045
38	0.656	0.12	0.08	0.10	40.15	0.048
39	0.677	0.02	0.04	0.19	41.13	0.008
40	0.675	0.03	0.21	0.38	40.72	0.012
41	0.618	0.15	0.25	0.26	40.88	0.061
42	0.699	0.16	0.21	0.24	40.94	0.066
43†	0.657	0.26	0.62	0.40	40.76	0.106
44	0.655	0.13	0.25	0.70	40.98	0.053
45†	0.720	0.22	0.46	0.32	40.94	0.090
46	0.721	0.10	0.16	0.21	40.90	0.041
47	0.694	0.07	0.13	0.39	41.00	0.029
48	0.627	0.05	0.13	0.34	41.22	0.021

Seed yield for the first year is from only one fruiting, those yields for the second and fourth years are from two fruitings.
†High-oil-yeild source.

Table 4. Seed weight and yield, oil content and yield of different *Jatropha* sources planted in the germplasm garden (cont.).

Seed sources	1000-seed weight (kg)	Seed yield (kg/tree)			Oil content (%)	Oil yield (kg/tree)
	Second year	First year	Second year	Fourth year	First year	First year
49	0.611	0.13	0.09	0.55	41.28	0.054
50	0.685	0.13	0.04	0.24	41.28	0.054
51	0.629	0.14	0.17	0.17	41.47	0.058
52	0.629	0.04	0.17	0.29	41.67	0.017
53	0.648	0.10	0.11	0.21	41.73	0.042
54	0.711	0.15	0.03	0.41	41.96	0.063
55	0.678	0.11	0.14	0.53	41.76	0.046
56	0.631	0.08	0.19	0.19	42.18	0.034
57	0.619	0.06	0.09	0.45	42.70	0.026
58	0.876	0.15	0.17	0.23	42.62	0.064
59	0.690	0.06	0.17	0.24	42.86	0.026
60	0.597	0.11	0.21	0.33	42.45	0.047
61	0.667	0.16	0.04	0.35	42.76	0.068
62	0.666	0.13	0.11	0.24	42.94	0.056
63	0.663	0.17	0.03	0.24	43.14	0.073
64	0.698	0.18	0.14	0.39	42.68	0.077
65	0.614	0.13	0.19	0.24	43.03	0.056
66	0.655	0.15	0.13	0.14	43.46	0.065
67	0.711	0.12	0.07	0.40	43.53	0.052
68†	0.780	0.18	1.02	0.37	44.12	0.079
69†	0.679	0.22	0.48	0.32	43.74	0.096
70	0.660	0.13	0.13	0.26	43.78	0.057
71	0.622	0.15	0.07	0.59	43.95	0.066
72	0.694	0.12	0.09	0.33	44.25	0.053
73	0.640	0.07	0.09	0.16	44.30	0.031
74	0.733	0.16	0.05	0.35	43.71	0.070
75	0.765	0.10	0.11	0.21	44.56	0.045
76	0.711	0.05	0.09	0.20	44.69	0.022
77	0.737	0.09	0.09	0.45	45.04	0.041
78	0.690	0.17	0.08	0.24	45.35	0.077
79	0.748	0.12	0.10	0.30	45.18	0.054
80	0.611	0.08	0.15	0.44	45.66	0.037
Average	0.676	0.12	0.15	0.32	40.59	0.048
Minimum	0.477	0.02	0.01	0.04	33.34	0.008
Maximum	0.876	0.31	1.02	2.12	45.66	0.114

Seed yield for the first year is from only one fruiting, those yields for the second and fourth years are from two fruitings.
†High-oil-yeild source.

selected six sources are still relatively high after having been planted for 3 years. However, another source (44) performed outstandingly and had the third highest seed yield among the 80 sources at the third year. More data are needed to observe the yield and growing behaviors of *Jatropha* trees.

▪ Trial plantation

The six good-growing and high-oil-yield sources (6, 26, 43, 45, 68 and 69) were selected for a small-area (150 m^2 for each source) trial plantation **(Table 5)**. Here, seeds were collected twice a year after fruiting, and all data for the seeds were from the average twice harvesting in the second and third years. In 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 1445.1 kg/ha (or 1.02 kg/tree for source 68). A wide range of 313 to 12000 kg/ha for 1–9-year-old plants located in South America, India and Africa was reported, as reviewed by Achten *et al.* [11]. Their yields were scattered, mainly owing to the a large variation in age and ecological conditions. We also planted some so-claimed high-yield-seed sources from Indonesia,

Table 5. Annual seed and oil yield of the six selected *Jatropha* sources planted in a small-scale trial (150 m² for each source).

Seed sources	Area (m^2)	Seed yield (second year)		Oil yield (second year)		Seed yield (third year)	
		kg/tree	kg/hectare	kg/tree	kg/hectare	kg/tree	kg/hectare
6	150	0.85	964.26	0.40 (0.31)	377.48 (292.55)	2.12	2000.64
26	150	0.26	291.25	0.14 (0.10)	126.07 (90.05)	1.45	1305.73
43	150	0.62	883.60	0.36 (0.25)	405.40 (281.53)	0.40	450.44
45	150	0.46	517.08	0.24 (0.19)	203.93 (161.44)	0.32	271.91
68	150	1.02	1445.12	0.63 (0.45)	695.62 (496.86)	0.37	408.54
69	150	0.48	543.05	0.29 (0.21)	263.19 (190.59)	0.32	290.42
Average		0.62	774.06	0.34(0.25)	345.28 (252.17)	0.83	787.95

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

India, Laos and Surinam; however, these seed yields did not exceed our yields, owing to different climates and incorrect extrapolation. For the six sources, oil yield varyied from 126.1 (source 26) to 695.6 (source 68) kg/ha(or 0.14–0.63 kg/tree), with an average value of 345.3 kg/ha (or 0.34 kg/tree). Source 68 performed outstandingly in oil production, at 1-times higher than that of the average six sources, or 0.7-times higher than that of the second highest. However, compared with rapeseed, soybean and palm oil yield, with 975, 620 and 3850 kg/ha, respectively [14–16], 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 plantations there is one harvest. Therefore, it is very important to increase the oil yield for *Jatropha* in order for it to compete with other oil plants economically (although the yield may increase further with plant age in its 50-year life expectancy) [11]. When we also measured seed data in the third year, a slight increase for the average seed yield of the six sources was found. Seed yield for sources 6 and 26 increased greatly, while for source 68 it dropped dramatically. Seed yield for source 6 went up to 2001 kg/ha or 783 kg oil/ha.

Other comprehensive methods and techniques must be developed to increase oil yield significantly, such as using breeding techniques (chemically induced mutation, Co^{60} radiation, ion implantation mutation, and 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 for 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 (or 783) kg/ha, is much lower than the datum (2100 kg/ha) set by the national government for commercial biodiesel production [202]. Therefore, at present, in order to increase the economic benefits in addition to oil production, *Jatropha* should find many other applications in hedge, fertilizer, food/fodder, agrochemicals, medicines and firewood [17]; for example, we can make animal feed by detoxifying proteins, and extract curcin from seed cake as biopesticides [2,11,101,102]. Another key factor for plantation of *Jatropha* is for ecological benefit to control erosion and absorb CO_2. Afforestation can improve the ecological system and reduce soil erosion in the region of the Jinsha river.

Jatropha oil was extracted from the seeds of 2-year-old trees of the six sources by the mechanical press, with an average yield of 252 kg/ha (the maximum yield was 497 kg/ha for source 68) (Table 5). After separation by a centrifugal machine, the *Jatropha* oil was sent to be further processed for biodiesel production.

Biodiesel production

According to the Chinese National Standard GB9104.2–88 and GB164–64, the acid value (AV) and saponification value (SV) of *Jatropha* oil were measured as 10.45 mg KOH/g and 191.02 mg KOH/g, respectively. Therefore, the molecular weight was calculated as 932 g/mol by the formula [18]:

$$M = (56.1 \times 1000 \times 3) / (SV - AV)$$

Figure 4. Fruiting of the *Jatropha* trees planted in the germplasm resource garden.

Acid-esterification pretreatment

After reaction for 25 min at 65°C, a mixture of *Jatropha* oil, methanol and sulfuric acid formed an upper layer, or pretreated oil, whose acid value was reduced to only 1.2 KOH/g. The pretreated oil was unstable, and after storage for 15 days, some solid flocs precipitated. Therefore, it should be processed to biodiesel before decomposition.

Base transesterification

The pretreated oil was transesterified to fatty acid methyl esters or biodiesel at a conversion rate of 95.7%. The biodiesel was a clear or yellowish liquid, with a density of 0.883g/cm^3 and an acid value of 0.2 KOH/g, which meets the German biodiesel standard (DIN V 51606: 1997). It is very stable without any color change or solid precipitation after storage for 1 year.

Conclusion

In total, 80 *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 between sources. Six sources with high oil yield were chosen for a small area trial plantation to produce crude oil. In the second year, the maximum oil yield of 696 kg/ha (or 1445 kg/ha seed yield) was achieved, which was approximately twice the national best yield (750 kg/ha seed yield [19]), but still only approximately 28% of the yield (5250 kg/ha seed yield [20]) required for the '11th 5-year development guidelines' set out by the Chinese government for commercial production. *Jatropha* oil extracted from the six seed sources was pretreated with sulfuric acid in order to remove the free acids, and was further successfully transesterificated to qualified biodiesel.

The seed yields increased with age over the 4 years. The yields for the selected six sources are still relatively high; however, another source performed outstandingly in the third year. Maximum oil yield of 783 kg/ha (2001 kg/ha seed yield) was obtained in the third year for source 6. More research regarding times is needed to observe the yield and growing behaviors of *Jatropha* trees.

We can conclude that high-oil-yield seed sources were collected and selected and, subsequently, successfully cultivated to produce *Jatropha* oil that was further processed to high-quality biodiesel. Further work is needed to study the changes of *Jatropha* growing characteristics and oil yield with plant ages. Oil yield must increase 2.6-times to reach the national target, which can be improved upon by using appropriate site characteristics, breeding and cultivation techniques.

Future perspective

The latest data from this summer (the fourth year) showed that the seed yield for the trial plantation reached approximately 3000 kg/ha in one harvesting for source 6. It is expected that the yield for the whole year could reach 4000 kg/ha, which is still slightly lower than the national target of 5250 kg/ha. However, we think that based on our new selected source 6 and others, after 3–5 years, new high-oil seed sources will be bred through traditional and biological methods that can meet the Chinese national requirement for large-scale plantation. We plan that after

Executive summary

Collection of different provenances

- A total of 80 wild geological seed sources were collected from south west China at altitudes ranging from 250 to 1500 m.
- The 80 geographic locations were divided by approximately 1° of latitude or longitude (~100 km). They were distributed mainly over subtropics and northern tropics in the Jinsha river valley and southern Yunnan.

Growth status

- Fast growing occurred in June–July and, after a short period of buffering at the middle of August, a growing peak appeared again from September to October; at the end of October, growing entered a period of stagnating.
- The growth rate varied very differently in sources. The average ground diameter, tree height and crown size of 2-year-old plants for the 80 sources was 0.076, 1.67 and 1.14 m, respectively.

Seed properties & oil yield

- The average 1000-seed weight was 0.676 kg (0.477–0.876) and the average oil content was 40.59% (33.34–45.66%) for 2-year-old plants.
- Seed yield varied very differently in sources. An average seed yield of 0.12 (0.02–0.31) and oil yield of 0.048 kg/tree (0.008–0.114) was obtained for the 80 sources at the first year.

Trial plantation

- The six good-growing and high-oil-yield sources were selected for trial plantation.
- Oil yield varied from 126.1 to 695.6 kg/ha with an average value of 345.3 kg/ha at the second year.
- A maximum value of 783 oil kg/ha, that is 2.7-times of the national best yield, was achieved for 3-year-old trees.
- It is expected that the seed yield for the fourth year will be doubled and reach 4,000 kg/ha or 1566 kg/ha oil yield.

Biodiesel production

- A two-step process – acid esterification and base transesterification – was successfully used for the production of qualified biodiesel from *Jatropha* oil with high free fatty acid content.

3–5 years of multiregional tests, seed yield could reach 5250 kg/ha. From 2016–2020, large-scale intensive plantation of 1 million ha will be carried out to produce 5 million tons of seed.

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