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Breeding of a New Black Bean (*Phaseolus vulgaris* L.) Cultivar with a High Yield and Upright Growth Pattern: Longyundou 19

X.X. Meng[1], Q. Wang[2], Z.G. Yin[2], S.H. Wei[2], Y.F. Guo[2], W. Zhang[3], G.D. Yang[1]

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ABSTRACT

Background: Black bean (*Phaseolus vulgaris* L.) has been widely grown in China and is popular in the international market. However, to decrease production costs, growers require cultivars with an upright growth pattern that enables them to be harvested directly. Longyundou 19, which was developed by the Crop Resources Institute of the Heilongjiang Academy of Agricultural Sciences was released in 2020 as an upright, high-yielding black bean cultivar with resistance to anthracnose and common bacterial blight.

Methods: Cross breeding is an effective way of improving common bean varieties. Longyundou 19 was developed from the Longyundou 4 (female) × Longyundou 5 (male) hybridization and the subsequent systematic selection for upright, yield, disease resistance, quality and agronomic traits over six generations.

Result: The average yield of Longyundou 19 during yield trials from 2016 to 2019 was 1,888.5 kg ha^{-1}, which was 10.3% greater than that of the check cultivar. Disease investigations conducted in fields from 2016 to 2019, indicated Longyundou 19 exhibits some resistance to anthracnose, common bacterial blight and BCMV. This new variety combined the disease resistance and black seed coat of the female parent with the upright growth pattern of the male parent, while retaining the high yield of both parents.

Key words: Black bean, Breeding, Hybridization, Systematic selection.

INTRODUCTION

Common bean (*Phaseolus vulgaris* L.), which is a widely cultivated legume crop in China, has important nutritional and medicinal value. The proteins, starch (Gupta *et al*., 2018), oils (Sutivisedsak *et al*., 2011), lectins (He *et al*., 2013), peptides (Kumar *et al*., 2011), polyphenols (Dueñas *et al*., 2015), flavonoid and antioxidative activity (Ganesan and Xu, 2017) of common bean have recently been thoroughly investigated. Dry common beans have potential health benefits for humans because of their anti-oxidant, anti-diabetic, anti-obesity, anti-inflammatory, anti-mutagenic and anti-carcinogenic properties (Ganesan and Xu, 2017).

Additionally, black beans may help prevent cardiovascular diseases and decrease the risk of certain types of cancer. Consuming black beans may also improve digestion and the regulation of blood sugar levels. Black beans can positively affect various nervous system functions, minimize the chances of birth defects, neutralize the negative effects of sulfites and even prevent impotence (Organic Facts, 2021).

Black beans were first cultivated in Peru and Mexico approximately 8,000 years ago, but they are now cultivated worldwide, including in China (FAO, 2014). The countries currently exporting the most black bean products are Mexico, Brazil, United States (US), Guatemala, Argentina, Canada and China. The demand for black bean products has increased sharply during the COVID-19 crisis. Because of the rising cost of Chinese soybeans, current market pressures will likely cause Chinese farmers to pivot away from black bean production in favor of soybean production. Venezuelan and Cuban demands for black beans will need to be satisfied by another country, with Argentina as the most likely first option because US companies cannot export to Venezuela and Cuba. American companies might also have difficulty matching the growing demand for black beans in the Mexican market (USDBC, 2020).

In China, most black beans are imported cultivars. Long-term planting has resulted in the mixing of the cultivars. Moreover, their unpredictable growth behavior, the development of serious disease, low yields and poor quality have made it difficult for the Chinese market to meet the standards of the International market. Because of increased labor costs, upright cultivars that can be harvested directly are desired. Accordingly, in 2003, the Crop Resources Institute of the Heilongjiang Academy of Agricultural Sciences used Longyundou 4 as the female parent and Longyundou 5 as the male parent to generate a new black bean variety (Longyundou 19) *via* a systematic selection

[1]Postdoctoral Programme, Heilongjiang Academy of Agricultural Sciences, Harbin-150086, China.
[2]Crop Resources Institute, Heilongjiang Academy of Agricultural Sciences, Harbin-150086, China.
[3]Industrial Crops Institute, Heilongjiang Academy of Agricultural Sciences, Harbin-150086, China.

Corresponding Author: G.D. Yang, Postdoctoral Programme, Heilongjiang Academy of Agricultural Sciences, Harbin-150086, China. Email: ygdhouzhe2000@163.com

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over six generations. The upright growth pattern of this variety satisfies the demand of domestic black bean breeders and producers.

MATERIALS AND METHODS

The female parent (Longyundou 4) was developed by treating a native black bean variety with Co^{60}. It is a high-yielding and disease-resistant cultivar with a black seed coat and a 100-seed weight of 18.0-22.0 g. Additionally, it is a mid-maturing cultivar, with a growth period of 95-100 days. Longyundou 4 was certified by the Heilongjiang Province Coarse Cereals Committee in 1994. The male parent (Longyundou 5), which was generated in 1997 from a cross between F0637 (Yunnan cultivar) and F2179 (imported cultivar), has a relatively high yield, upright growth pattern with a white seed coat. Similar to Longyundou 4, its 100-seed weight is 18.0-22.0 g and it is also a mid-maturing cultivar, with a growth period of 90-95 days. Longyundou 5 was certified by the Heilongjiang Province Coarse Cereals Committee in 2007. The objective of this study was to breed a new high-yielding black bean cultivar with an upright growth pattern and disease resistance suitable for spring cultivation in Heilongjiang province.

Hybrid breeding was performed in Minzhu field of the Heilongjiang Academy of Agricultural Sciences (45°49′N, 126°50′E) from 2006 to 2012. Longyundou 4 and Longyundou 5 were hybridized in 2006. The F_1 and F_2 generations were planted in the field in 2007 and 2008, respectively. Two or three pods were collected from all F_1 and F_2 plants. Individual plants were selected according to their agronomic and seed traits from 2009 to 2011 (F_3-F_5). A single row with uniformly growing plants exhibiting the desired traits was selected in 2012 (F_6) and the strain was named Long 12-2632. The breeding process is presented in Fig 1. The yield trial and the variety comparison trials were conducted from 2013 to 2016. Regional trials and production trials were performed at five test sites in Heilongjiang province from 2016 to 2019. The susceptibility of the plants to diseases was assessed in Minzhu field from 2016 to 2019. Moreover, an analysis of quality-related traits was performed in 2019.

RESULTS AND DISCUSSION

Agronomic characteristics

Longyundou 19 has an upright growth pattern (Fig 2A) and produces heart-shaped leaves and purple flowers. Its plant height is about 51 cm, with 2-3 branches and roughly 12 nodes on the main stem. There are approximately 20 pods per plant, with 6-7 seeds per pod. The mature pod is yellowish-white and slightly curved (Fig 2B). The kidney-shaped seeds are black (Fig 2C) and the average 100-seed weight is 19 g. The growth period of this new cultivar is 93-96 days in spring sowings in Heilongjiang province.

Susceptibility to diseases

The susceptibility of Longyundou 19 to diseases during the flowering and pod-setting stages was investigated using plants grown in Minzhu field of the Crop Resources Institute of the Heilongjiang Academy of Agricultural Sciences from 2016 to 2019. The anthracnose, common bacterial blight

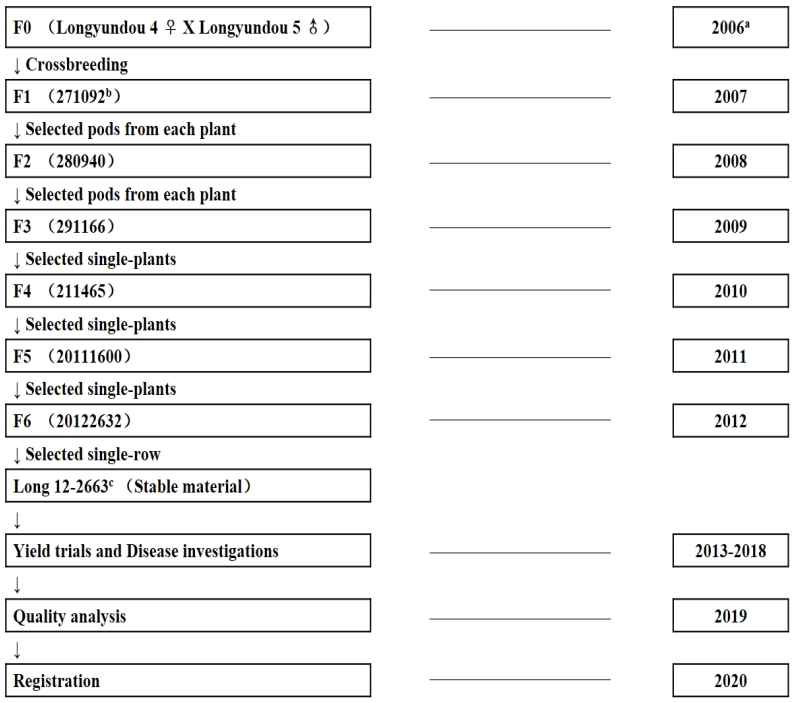


Fig 1: Breeding process of Longyundou 19.
a: Breeding year; b: Field planting number of each generation; c: Final selection.

and bean common mosaic virus (BCMV) incidence rates were slightly lower for Longyundou 19 than for the control cultivar (Longyundou 4) in 2016 and 2017. The common bacterial blight incidence rate was higher in 2018 than in the previous 2 years because of the increase in temperature and humidity. However, Longyundou 19 was more tolerant to common bacterial blight (10.4%) than Longyundou 4 (25.5%) because of its upright growth pattern Table 1.

Quality-related characteristics

In 2019, Longyundou 19 quality-related traits were analyzed by the Inspection and Testing Center for Quality of Cereals and Their Production (Harbin), Ministry of Agricultural and Rural Affairs, China. The results indicated that Longyundou 19 beans had a crude protein content of 24.63%, crude fat content of 1.56% and crude starch content of 37.49%; as its crude protein content was higher than 23% and the crude fat content was lower than that of Longyundou 4, Longyundou 19 may be considered as a high-protein and low-fat cultivar Table 2.

Longyundou 19 was released by the Science and Technology Department of Heilongjiang Province in 2020 (Certificate number: 9232020Y0818).

Yield performance and adaptability

In 2013**,** a yield trial was conducted in Minzhu field of the Heilongjiang Academy of Agricultural Sciences with plot sizes of 9.75 m^2 (three rows of 5 m length) in a randomized block design, with a spacing of 0.65 m between the rows and 0.20 m between the plants in the row. A total of 78 cultivars were tested, with Longyundou 4 as the check cultivar. The average yield of Longyundou 19 was 2,146.3 kg ha^{-1}, which was 12.8% greater than that of the check cultivar (1,902.7 kg ha^{-1}).

Cultivar comparison trials were performed in Minzhu field of the Heilongjiang Academy of Agricultural Sciences in 2014 and 2015. The plot dimensions were the same as those used for the yield trial. A total of 56 cultivars were grown with Longyundou 4 serving as the check cultivar. The average yield of Longyundou 19 over 2 years was 2,217.8 kg ha^{-1}, which was 9.5% greater than that of the check cultivar (2,025.4 kg ha^{-1}).

Regional trials were carried out in 2016 and 2017 at the following five test sites in Heilongjiang province: Harbin (45°49′N, 126°50′E), Keshan (48°03′N, 125°87′E), Yinlonghe (48°45′N, 126°34′E), Baoqing (46°55′N, 133°30′E) and Jianshanjiang (47°25′N, 133°48′E). The test sites represent the main common bean-producing regions in Heilongjiang province. Each trial was conducted using randomized block design with three replications. The plot area and spacing were the same as those in the previous trials. The planting density was 156/plot. Five cultivars were tested with Longyundou 4 as the check cultivar. The average yield of Longyundou 19 was 2,123.0 kg ha^{-1}, which was 10.0% and 8.4% higher than that of the check cultivar in 2016 and 2017, respectively. Accordingly, the average increase was 9.2% (Table 3).

The production trial was conducted in 2018 at five test sites in Heilongjiang province (Harbin, Keshan, Yinlonghe, Baoqing and Jianshanjiang). The planting area of each cultivar was 333.3 m^2, with a planting density of 180,000/ha.



Fig 2: Images of a dried Longyundou 19 (A) plant, (B) pods and (C) seeds.

Table 1: Comparison of disease incidence rates between Longyundou 19 and Longyundou 4.

Year	Cultivar	Anthracnose	Common bacterial blight	BCMV
		Incidence rate		
2016	Longyundou 19	1.5%	4.5%	5.1%
2016	Longyundou 4	2.2%	6.5%	6.8%
2017	Longyundou 19	3.4%	4.5%	5.6%
2017	Longyundou 4	6.5%	5.8%	5.8%
2018	Longyundou 19	7.2%	10.4%	1.8%
2018	Longyundou 4	8.4%	25.5%	3.6%

Four cultivars were tested with Longyundou 4 as the check cultivar. The average yield of Longyundou 19 was 1,654.0 kg ha^{-1}, which was 6.9%-18.8% higher than the check yield (with an average increase of 11.3%) (Table 4).

Crossbreeding, which involves the hybridization between different parents, is suitable for introducing variability in the offspring and for breeding new varieties. Ideally, crossbreeding should integrate the ideal genes of two or more parents into one hybrid. China leads the world in the crossbreeding of rice (Yuan, 2017; Satyapal *et al*., 2015), rapeseed (Anke *et al*., 2017; Agnieszka *et al*., 2021), soybean (Liu *et al*., 2020), millet (Felix *et al*., 2019) and wheat (Simon *et al*., 2014). Yuan LongPing, who developed the first hybrid rice variety in the 1970s, set multiple world records in hybrid rice yields, thereby making substantial contributions to food security in China and worldwide. Hybrid rice is currently cultivated on 60% of the rice acreage in China. There has recently been considerable progress in hybrid wheat research (*e.g*., the "two-line" method), with many high-yielding varieties generated in China and other countries (Pushpendra *et al*., 2019). In the last few decades, breeders have attempted to develop hybrid breeding technology applicable for legumes consumed as food, including faba bean (Kul *et al*., 2021), pigeon pea (Saxena, 2009; Saxena *et al*., 2015), common bean (Kelly *et al*., 2021) and mung bean (Wang *et al*., 2022). However, common bean breeding in China was initiated relatively recently, especially crossbreeding-based research (Tang *et al*., 2014); as it is a self-pollinated crop, the artificial hybridization of common beans is difficult, resulting in a low success rate. Thus, common bean crossbreeding in China is restricted to only a few research facilities, including the Chinese Academy of Agricultural Sciences, the Heilongjiang Academy of Agricultural Sciences (Meng *et al*., 2018) and the Zhangjiakou Academy of Agricultural Sciences.

Chinese black bean exports have remained relatively high for many decades, but most black beans grown in China are vestigial varieties that produce low yields and are highly susceptible to diseases. Additionally, mechanized harvesting has been the key factor restricting the development of the common bean industry. Therefore, the Crop Resources Institute of the Heilongjiang Academy of Agricultural Sciences crossed Longyundou 4 (high yield and disease resistance) with Longyundou 5 (high yield and upright growth

Table 2: Comparison of quality-related characteristics between Longyundou 19 and Longyundou 4.

Cultivar	Crude protein	Crude fat	Crude starch
Longyundou 19	24.63%	1.56%	37.49%
Longyundou 4	24.89%	2.58%	36.95%

Note: High-protein cultivars are those with a crude protein content ≥23% according to the registration criterion for food legumes in Heilongjiang province.

Table 3: Regional trial results of Longyundou 19 during 2016 and 2017.

Year	Test site	Yield of Longyundou 19 (kg ha^{-1})	Advantage over check cultivar (%)
2016	Harbin	2057.9	13.3
	Baoqing	1965.3	10.3
	Yinlonghe	1919.1	4.1
	Jianshanjiang	2321.4	10.2
	Keshan	2188.2	11.9
Average of 2016	-	2090.4	10.0
2017	Harbin	1914.5	5.7
	Baoqing	2114.5	8.7
	Yinlonghe	2132.8	10.6
	Jianshanjiang	2272.7	6.0
	Keshan	2342.9	11.2
Average of 2017	-	2155.5	8.4
Average of 2016 and 2017	-	2123.0	9.2

Table 4: Production trial results of Longyundou 19 during 2019.

Year	Test site	Yield of Longyundou 19 (kg ha^{-1})	Advantage over check cultivar (%)
2018	Harbin	1051.0	6.9
	Baoqing	1578.6	10.6
	Yinlonghe	1014.7	8.7
	Jianshanjiang	2421.0	11.3
	Keshan	2204.8	18.8
Average	-	1654.0	11.3

pattern) to produce the new black bean variety Longyundou 19. This new variety combined the disease resistance and black seed coat of the female parent with the upright growth pattern of the male parent, while retaining the high yield of both parents. Although, the crossbreeding of common bean is extremely difficult and time-consuming, it is an effective method for increasing the yield and enhancing plant characteristics.

Future common bean breeding efforts in China may benefit from a decrease in the number of breeding cycles, perhaps by growing plants in the southern region during the winter to accelerate the process. Breeding efficiency may be improved further via a direct genetic modification. Developing more productive varieties is crucial for satisfying the needs of humans in a changing world.

CONCLUSION

On the basis of the selection of suitable parents for a hybridization and the subsequent systematic selection and yield trials, a new black bean variety (Longyundou 19) with desirable target characteristics suitable for commercial cultivation in Heilongjiang province was developed. The results of this study demonstrate that crossbreeding is a feasible and effective method for developing novel common bean varieties. To date, Longyundou 19 has been grown in Keshan, Yi'an, Baiquan, Mingshui, Nehe, Nenjiang and Baoqing in Heilongjiang province, with a cumulative planting area of 500 ha. The harvested beans have been sold to some countries in South America and the Middle East. With the changing agricultural market, the demand for high-quality cultivars with specific traits is increasing, crossbreeding is an effective way to alleviate the lack of commercial common bean varieties.

Conflict of interest: None.

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