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# Gojiberry Breeding: Current Status and Future Prospects

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## Abstract

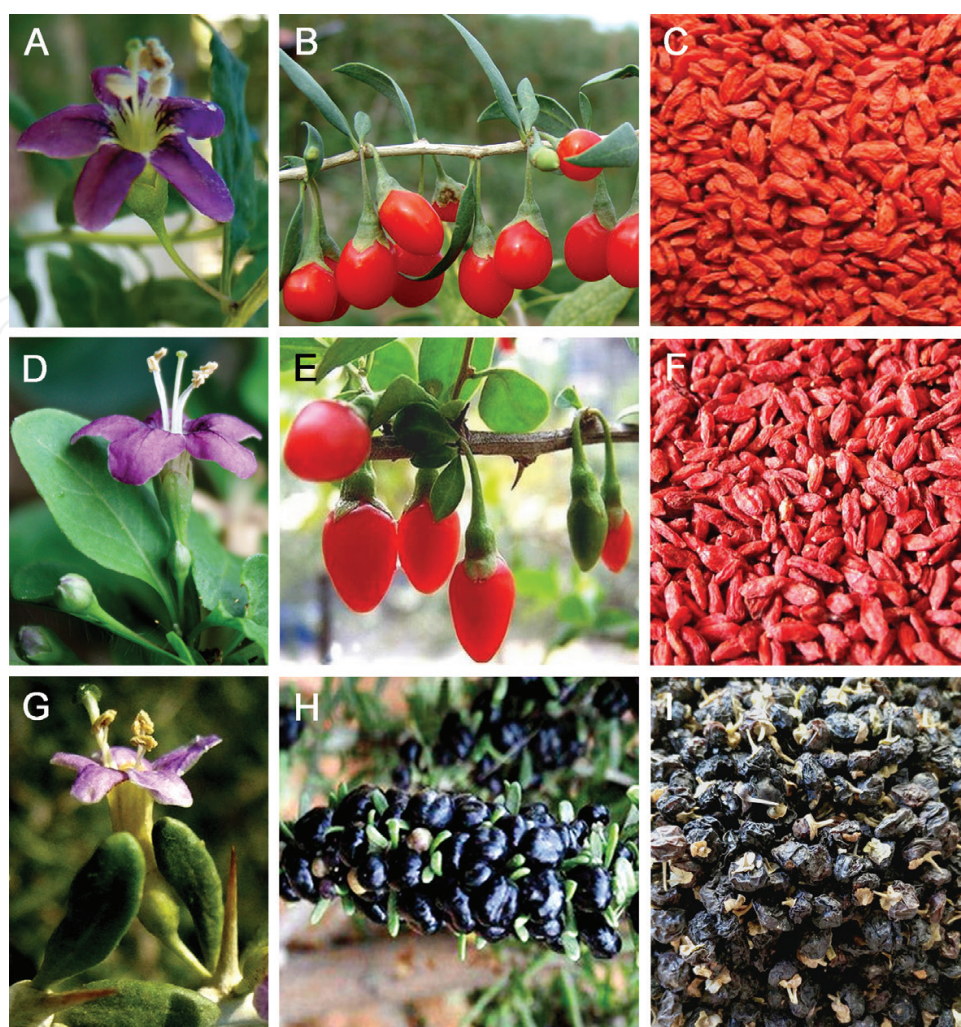
Goji, gojiberry, or wolfberry is the fruit of *Lycium barbarum* L., *L. chinense* Mill., or *L. ruthenicum* Murr. in the family Solanaceae Juss. The fruit is bright orange-red or black and is edible with a sweet and tangy flavor. Gojiberry is rich in polysaccharides, flavonoids, carotenoids, betaine, kukoamine A, sitosterol, and other compounds which have antioxidant, anti-inflammatory, and anti-neoplastic properties and have been used for the treatment of various blood circulation disorders and diabetes. Recently, there is an increased demand for high-quality gojiberry and its products because they are considered a superfruit. China is the main producer and supplier of gojiberry in the world. Thus far, limited information is available about genetic resources, breeding activities, and major cultivars of gojiberry. This chapter is intended to review the current knowledge on gojiberry germplasm resources and their relationships as well as to describe gojiberry breeding activities. Future prospects on gojiberry cultivar development are also discussed.

**Keywords:** gojiberry, *Lycium barbarum*, *Lycium barbarum* polysaccharides (LBPs), *Lycium chinense*, *Lycium ruthenicum*, Solanaceae, wolfberry

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## 1. Introduction

Gojiberry generally includes *Lycium barbarum* L., *L. chinense* Mill., and *L. ruthenicum* Murr. (Figure 1). They are deciduous woody shrubs, often thorny, spiny, and growing from 1 m to 4 m in height [1]. Stems are slender, erect or spreading, often scrambling. The leaves are gray green, fleshy, ovate, lanceolate, or subcylindric shaped and are alternately arranged,



**Figure 1.** Flower, fruit, and dried fruit appearance of *Lycium barbarum* L., *L. chinense* Mill., and *L. ruthenicum* Murr. *L. barbarum* bears royal purple flower (A) with lanceolate leaves and produces bright red berries (B) which resemble red raisins after they have been dried (C). The flower of *L. chinense* is purple (D), leaves are ovate, fresh (E) and dried (F) berries are orange-red. *L. ruthenicum* bears purple flowers that are lighter than *L. chinense* (G), leaves are sessile and succulent in a linear shape (G). This species produces dark colored berries (H) that are harvested with peduncle and sepal; thus individual dried black berries often have peduncle and sepal (I).

sometimes in fascicles. Petioles are short. Flowers are solitary or clustered in leaf axils. The corolla is funnel or bell shaped in white, green, or purple. Five stamens are structured with filaments longer than the anthers. Anthers dehisce longitudinally. The fruit is a two-chambered, usually fleshy and juicy berry and typically orange-red or black (*L. ruthenicum*). Seeds are few or many, most with over 10. In the Northern Hemisphere, flowering occurs from June to September, and berry maturation starts from August to October.

Gojiberry has been consumed as food and used as medicine for more than 4000 years in China [2]. The first mentioned “Gou Qi” was in the ancient classic “Shen Nong Ben Cao Jing” (The Classic of Herbal Medicine), a Chinese book on agriculture and medicinal plants written between 200 and 250 AD. The fruit of *L. barbarum*, *L. chinense*, and *L. ruthenicum* are commonly called “Ningxia Gou Qi,” “Gou Qi,” and “Black Gou Qi,” respectively. The fruit is a

renowned Yin strengthening agent, and the root bark, known as “Di Gu Pi,” is a cooling agent [3]. Traditional English vernacular names include boxthorn, fructus lycii, wolfberry, Chinese wolfberry, or matrimony vine [4]. Since the beginning of the century, the plant has been commonly called Goji, an appellation derived from the Chinese name “Gou Qi.”

## 2. Nutraceutical and pharmaceutical values

The berries harvested from August to October are eaten as fresh fruit, dehydrated to make dried fruit or soaked in liquor to produce *Lycium* juice. As a medicinal food, it is used as a condiment with steamed rice. Young soft leaves can also be used as a vegetable. Gojiberry has been used for its anti-aging activities, tranquilizing and thirst-quenching effects, and its ability to increase stamina [5]. Consuming gojiberry has been shown to improve health-related problems, such as diabetes, hyperlipidemia, cancer, hepatitis, immune disorders, thrombosis, and male infertility and also benefit vision, kidney, and liver function [6–8].

### 2.1. Nutraceutical value

Scientific analysis of gojiberry constituents started in the 1970s. Qian et al. [9] reviewed 142 publications from 1975 to 2016 and summarized that at least 355 compounds occur in different species of *Lycium*, which were categorized as alkaloids at 20%, sterols, steroids, and their derivatives 16%, terpenoids 11%, amides 10%, flavonoids 9%, organic acid 9%, phenylpropanoids 9%, glycerogalactolipids 6%, ligans 4%, coumarins 3%, anthraquinones 1%, peptides 1%, and others 1%.

The most intensively studied components are a group of water-soluble glycoconjugates, which are identified as arabinogalactan-proteins, commonly known as *L. barbarum* polysaccharides or LBPs. It is estimated that dried fruits comprise 5–8% of LBPs [10, 11]. Chinese Pharmacopeia [12] recommended using LBPs as an indicator for evaluation of gojiberry quality. Molecular weights of the LBPs range from 24 to 241 kDa, and they are mainly composed of six types of monosaccharides: arabinose, glucose, galactose, mannose, xylose, and rhamnose [13]. LBPs also contain galacturonic acid and 18 amino acids and share a glycan-o-ser glycopeptide structure [14]. The main chains of the glycan backbones of LBPs have been reported to be either alpha-(1–N6)-D-glucans or alpha-(1–N4)-D polygalacturonans [3, 15].

A second major group of metabolites in fruit is carotenoids [3], the content of which increases during the fruit ripening process. A total of 11 free carotenoids and 7 carotenoid esters were detected from unsaponified and saponified *L. barbarum* extracts [16]. Zeaxanthin dipalmitate was found to account for 80% of the total carotenoids [17].  $\beta$ -Cryptoxanthin palmitate, zeaxanthin monopalmitate, small amounts of free zeaxanthin and  $\beta$ -carotene are also present [3]. Seeds contain zeaxanthin (83%),  $\beta$ -cryptoxanthin (7%),  $\beta$ -carotene (0.9%), and mutatoxanthin (1.4%) [13].

The fruits also consist of vitamins including ascorbic acid (vitamin C), riboflavin, and thiamin. The content of vitamin C (42 mg/100 g) is comparable to that of fresh lemon fruits [3].



Flavonoids are another important group of compounds. Total flavonoids of *L. barbarum* var. *aurauticarpum*, a yellow fruit variety, reach up to 2.0 mg/g which is four times higher than red-fruited *L. barbarum*. Fruits of *L. ruthenicum* contain oligomeric proanthocyanidins (OPC) at 3690 mg/100 g, which surpasses blueberries at 3380 mg/100 g [18]. Furthermore, aglycones myricetin, quercetin, and kaempferol were identified after hydrolysis [19].

Gojiberry fruits contain 1–2.7% free amino acids, of which proline is the major constituent [3]. Non-proteinogenic amino acids include taurine,  $\gamma$ -aminobutyric acid, and betaine (trimethylglycine) [20, 21]. Some miscellaneous compounds, such as  $\beta$ -sitosterol and its glucoside daucosterol, scopoletin, p-coumaric acid, the dopamine derivative lyciumide A, and L-monomethyl succinate occur in fruits [22–24].

## 2.2. Pharmaceutical value

Consuming gojiberry has been shown to improve general well-being, anti-myelosuppression, and sleep quality. Five randomized clinical studies conducted in the US [25–28] and China [29, 30] indicated that daily consumption of standardized *L. barbarum* fruit juice [GoChi juice (Goji juice) 120 ml = equivalent to 150 g of fresh fruit] for 14 or 30 days improved general well-being including neurological and psychological status, cardiovascular, joint, and muscle functions as well as gastrointestinal regularity without any adverse effects.

LBP from *L. barbarum* have anti-aging and neuroprotective activities [31]. An arabinogalactan-protein (LBP-III) isolated from LBPs exhibited cytoprotective effects against stress. The protective role was mediated by reducing the phosphorylation of double-stranded RNA-dependent protein kinase (PKR) and also by decreasing the dithiothreitol (DTT)-induced LDH release and caspase-3 activity [32]. The reduction of PKR was caused by beta-amyloid peptide. It is well known that the phosphorylation state of PKR increases with age, the reduction of phosphorylation triggered by beta-amyloid peptide suggests that LBP-III from gojiberry could be a potential neuroprotective agent [33, 34]. *L. barbarum* intake was effective to control waist circumference in humans and may reduce the risks of metabolic syndrome [28].

Several experimental and clinical studies showed that *L. barbarum* exhibited anti-diabetic effects. *L. barbarum* reduced oxidation in patients with retinopathy [35]. In a randomized diabetic retinopathy study, the intake of fruit of *L. barbarum* for 3 months was shown to increase the contents of vitamin C by 61% and the activities of SOD by 87% and also to reduce serum content of lipid peroxide by about 20% compared to a control group [36]. *L. barbarum* was also effective when used for improving immunologic function of red blood cells in patients with diabetic retinopathy [36]. In a study with 44 patients with diabetic retinopathy, RBC C<sub>3</sub>b receptor rosette (RBC-C<sub>3</sub>bRR) in the patients treated with *L. barbarum* for 3 months decreased [37]. LBPs were shown to have immunomodulatory effects on patients with type-2 diabetes by reducing T8 and interleukin 6 (IL-6) by 23% and increasing T4/T8 and IL-2 by 30 and 62% compared to the normal level, respectively [38].

Gojiberry has been considered to be the richest source for zeaxanthin [39, 40] varying from 1.18 to 2.41 mg/g dried fruit [11]. Gojiberry is, thus, a natural pill for eye health. Drinking goji berry juice daily as a dietary supplementation for 90 days was reported to increase plasma

zeaxanthin and antioxidant levels significantly, which protected eyes from hypopigmentation and accumulation of oxidative stress compounds that can damage the macula [41].

There are several reviewed articles regarding chemical constituents and nutraceutical value of gojiberry as well as pharmaceutical effects of gojiberry consumption. The reader is referred to these reviews [3, 9, 11, 16, 31] for more detailed information.

### 2.3. Uses

Gojiberry has been commercialized with different names depending on the origin of plants and product types. “Ningxia Goji berry,” produced from Ningxia (Ningxia Hui Autonomous Region, which is equivalent to a Province, located in Northwestern China) and harvested primarily from *L. barbarum*, is considered to be the authentic origin. “Black Wolfberry” is the berry harvested from *L. ruthenicum*. “Himalayan Goji berry” or “Tibetan Goji berry” are also on the global functional food market; these two names are used by health food promoters for a nomenclatural marketing advantage, though commercial cultivation of the crop does not occur in those regions [42]. In addition to being an important traditional Chinese medicinal herb, commercialized products vary from dried berries in various sized bags to juices, beers, and wines. Gojiberry is also found in cookies, chocolate candies, muesli, sausages, and snack bars. Gojiberry products have been marketed online since 2002 and are termed as a “super-food” [43].

Gojiberry and its products are sold as food or food supplements in the US and in Europe [16]. These products, however, are not allowed to be promoted as drugs, and therapeutic claims are prohibited thus far. In the US, the Food and Drug Administration (FDA) warned about some Gojiberry juice distributors using marketing claims which violated the Food Drug and Cosmetic Act [3]. In the United Kingdom (UK), the Food Standards Agency in 2007 concluded that there were sufficient records of alimentary use of Gojiberry in the UK before 1997 and thus the fruit does not fall under the Novel Food legislation. In the US, however, Gojiberry is not listed on the generally regarded as safe (GRAS) list by the FDA [3].

## 3. Gojiberry production

China is the main producer and supplier of gojiberry in the world. The majority of commercially produced gojiberry comes from Ningxia with gojiberry plantations typically ranging from 40 and 400 ha. Gojiberry, primarily *L. barbarum*, has been produced along the fertile floodplains of the Yellow River for more than 700 years. Fresh fruits are uniformly orange-red, and dried fruit has a sweet-and-tangy flavor. Ningxia has earned a reputation for premium quality gojiberries. As of 2015, more than 66 million hectares were planted with gojiberry in Ningxia. The region is recognized as the largest annual harvest in China, accounting for 45% of the nation’s total yield of gojiberries. Fresh red or black berries are harvested starting in August. The harvested fruits are immediately washed and then preserved by drying them directly under full sun or through dry machines. Gojiberries are celebrated each August in Ningxia with an annual festival coinciding with the berry harvest. The celebration was originally held in Ningxia’s capital, Yinchuan City;

the festival has been moved since 2000 to Zhongning County. Furthermore, gojiberries are drought-tolerant plants. As Ningxia's borders merge with three deserts, gojiberries are also planted to control erosion and reclaim irrigable soils from desertification. Commercial volumes of gojiberry also grow in other provinces of China including Xingjiang, Hebei, Inner Mongolia, Qinghai, Gansu, and Shaanxi. Jinghe County in Xingjiang, Julu County in Hebei, and Huangjinhou in Inner Mongolia also produce high quality gojiberries

Gojiberry has also been commercially produced in the other countries including India, Korea, Japan, and other Asian countries. During the first decade of the twenty-first century, farmers in Canada and the US began cultivating gojiberry on a commercial scale to meet potential markets for fresh berries, juice, and processed products. Gojiberry has been propagated by tissue culture by Agri-starts, Inc. in Apopka, Florida, US and tissue-cultured liners are sold nationwide for production in the US and Canada. Goji farm USA celebrated the harvest of gojiberry from 8000 plants grown in Sonoma Valley of the moon region in California, US (GojiFarmUSA.com)

#### 4. Current concerns on gojiberry production

The demand for quality gojiberry fruit has become much greater than the supply. A variety of factors are implicated in the shortage of supply, which includes the availability of reliable cultivars, the lack of corresponding production protocols, disease and pest control information, appropriate methods for processing fruits, quality control, and food safety issues. Among these factors, the availability of new and reliable cultivars is a key factor. China is the only country with gojiberry breeding programs. Current breeding efforts have been largely limited to *L. barbarum*, *L. chinense*, and *L. ruthenicum*, and only a few cultivars have been released and utilized. Some of the cultivars are unstable in fruit setting, fruit size, and final yield due to seed propagation or inappropriate planting of comparable cultivars adjacently for pollination. Additionally, the evaluation of gojiberry germplasm has remained in the descriptive stage; their potential has not been fully exploited. Thus, a better understanding of the current status of gojiberry breeding is important for future development of new cultivars and for increasing its production.

#### 5. Genetic improvement of gojiberry

It has been well documented that naturally occurring variation among wild relatives of cultivated crops is an underexploited resource in plant breeding [44]. The genus *Lycium*, a member of the family Solanaceae, comprises about 80 species [2]. Most of them remain in the wild, and their reproduction systems have been studied, but their agronomic traits and nutraceutical and pharmaceutical value have barely been exploited.

##### 5.1. Genetic resources

*Lycium* species are distributed in temperate and subtropical regions worldwide, but are absent in both the Old and New World tropics. Areas of greatest species richness are in South

America, primarily in Argentina and Chile, with more than 30 species. There are about 21 species in Southwestern and North America, approximately 17 species in southern Africa [45], and about 10 species in Eurasia [46]. Based on the analysis of chloroplast DNA sequences, Fukuda et al. [46] proposed that *Lycium* originated from the New World. All species in southern Africa, Australia, and Eurasia have a common progenitor from the New World. Australian and Eurasian species originated once from a southern African progenitor, and *L. sandwicense* differentiated from the New World species. As a result, phylogenetic analysis showed that gojiberries (*L. barbarum*, *L. chinense*, and *L. ruthenicum*) are clustered with *L. europaceum* L. as they belong to Eurasian species. The Eurasian species are closely related to species from Australia such as *L. australe* F. Muell. and also those from southern Africa, such as *L. afrum* L., *L. cinereum* Thumb., *L. ferocissimum* Miers, *L. pilifolium* C. H. Wright, *L. prunus-spinosa* Dunal, *L. schizocalyx* C. H. Wright, and *L. villosum* Schinz. Species from North or South America as well as Pacific Island were clustered together [46].

Most *Lycium* species have perfect flowers and are bisexual or hermaphrodites. However, like some others in the family Solanaceae, *Lycium* species are generally considered to be outcrossed due to gametophytic self-incompatibility [47]. For example, allelic diversity at the self-incompatibility (S) gene in *L. andersonii* was estimated to have more than 35 alleles, and coalescence analysis showed that the S-allele lineages in this species are older than the genus as a whole, indicating that self-incompatibility is the basal condition for *Lycium* [48]. Most species are diploid with chromosome number of  $2n = 2x = 24$ . However, Miller and Venable [49] reported that three North American species, *L. californicum* Nutt. Ex Gray, *L. exsertum* A. Gray, and *L. fremontii* A. Gray are polyploids and display functional dioecy. In addition, seven species in Africa have separate male and female plants [50, 51]. Levin and Miller [52] believed that gender dimorphism (the presence of two sexual morphs in a population) evolved twice among North American *Lycium* and probably three times in Africa. Thus, gender dimorphism is more common among African *Lycium*, occurring in 7 of the 27 African species (26%) compared to only 3 of 50 American species (6%). Furthermore, gender dimorphism has been shown to be uniformly associated with polyploidy, which resulted in a proposition that polyploidy disrupts self-incompatibility of North America diploid *Lycium* species and resultant self-compatible polyploids are then subject to invasion by male sterile plants [47].

It is unknown when and how *Lycium* species were dispersed to Eurasian regions. Among the 10 Eurasian species, there are 7 species that have been naturalized in China including *L. barbarum*, *L. chinense*, *L. cylindricum* Kuang, *L. dasystemum* Pojark., *L. ruthenicum*, *L. truncatum* Y.C. Wang, and *L. yunnanense* Kuang [53], of which *L. barbarum*, *L. chinense*, and *L. ruthenicum* are the most popular species. *L. barbarum*, *L. dasystemum*, and *L. ruthenicum* are primarily distributed in northwest China including Ningxia, Gansu, Inner Mongolia, Qinghai, Xinjiang, Shaanxi, and Shanxi. *L. chinense* is largely dispensed in central and east China. *L. cylindricum* is spread in Gansu, Inner Mongolia, Ningxia, Qinghai, North Shaanxi, Xinjiang, Tibet as well as Afghanistan, Kazakhstan, Kyrgyzstan, Mongolia, Pakistan, Russia, Tajikistan, Turkmenistan, and Uzbekistan. *L. truncatum* is distributed in dry regions with altitude ranging from 800 to 1500 m including Gansu, Inner Mongolia, Ningxia, Shanxi, and Xinjiang. *L. yunnanense* is mainly situated in Yunnan, southwest China.



## 5.2. Domestication of *L. barbarum*

How *L. barbarum* became naturalized in northwest China and centralized in Ningxia, particularly Zhongning County, is unclear. One possible reason could be due to the geographical location of Zhongning, as berries of *L. barbarum* produced in this county had the highest quality. An important irrigation project during Qin (221–206 BC), Han (206 BC–220 AD), and Tang (618–907 AD) dynasties channeled water from the Yellow River to irrigate farmland on the Yinchuan Plain in Ningxia, where Zhongning County was the gateway of the irrigation project. This project created favorable conditions for *L. barbarum* production. During Ming (1368–1644) and Qing (1644–1912) dynasties as well as the China Republic (1912–1949) periods, *L. barbarum* production was largely concentrated in Zhongning County with production acreage of about 200 hectares. Growers might have consciously harvested large fruits from individual plants. The harvested seeds might have been combined or kept separately by trees and planted the next year. Such conscious practices or domestication over thousands of years might have resulted in plants differing from their wild progenitors in several morphophysiological traits. Improved traits could be associated with seed retention and germination, growth habit, fruit size and coloration, and fruit edibility and taste. The domestication resulted in the establishment of landraces. No attention was given to the number of landraces in Zhongning County until the 1960s when Mr. Guofeng Qin conducted a survey in the County and identified 10 landraces, which included “Damaye,” “Xiaomaye,” “Heyemaye,” and “Baitiaogouqi” [54]. “Damaye” was found in Mr. Zhuohan Zhang’s garden who was a gojiberry grower. He probably never realized what important role this landrace has played in gojiberry cultivar development. “Damaye” grew to a height of 1.5 m with a canopy diameter of 1.7 m in 6 years. The fruit is spheroid-shaped, and 1000 fresh fruits weigh from 450 to 510 g. A single plant produces 7–8 kg fresh fruit. “Damaye” has been considered to be the most reliable landrace as it produces large, uniform fruits with little variation over the years.

## 5.3. Individual plant selection

An organized breeding effort on gojiberry was initiated in the 1970s at the Ningxia Research Center of Wolfberry Engineering Technology, which was renamed as National Wolfberry Engineering Research Center in 2011. The breeding center is located in Yinchuan City, Ningxia, China. This is the only federally sponsored institute devoted to gojiberry research. Initial breeding efforts were primarily focused on the selection of individual plants with vigorous growth rates, larger fruit, and higher fruit yield. Mr. Shenyuan Zhong volunteered to work in Ningxia after graduation from the Northwest Agricultural College and stayed in Zhongning County from 1965 to 1985. Mr. Zhong started individual plant selection from “Damaye” populations. A large number of individual plants were selected from “Damaye.” Seeds were harvested from each selected individual plant, and individual plant’s progenies were evaluated separately. If progenies from selected plants were variable, cloning propagation, such as rooting of cuttings, was used to stabilize the phenotypes. Using this selection method, Mr. Zhong selected 12 lines. After large-scale progeny testing, he released cultivars: “Ningqi 1” and “Ningqi 2” [54, 55]. Through mass selection, Mr. Zhong and his

associates released “Ningqi 3” in 2005. Another scientist, Mr. Zhongqin Hu at the Zhongning Wolfberry Industry Bureau made selections from “Damaye” and released “Ningqi 4” in 2005 [55]. Using “Damaye” and “Ningqi 1” as primary resources, subsequent selections resulted in the release of “Ningqi 5,” “Ningqi 6,” “Ningqi 7,” “Ningqi 8,” and “Ningqi 9.” “Ningqi 5” is a male sterile cultivar, “Ningqi 6” and “Ningqi 8” require outcrossing, while “Ningqi 7” is self-compatible. “Ningqi 9” is a tetraploid. Both “Ningqi 5” and “Ningqi 9” were selected from “Ningqi 1.”

Among the Ningqi series, “Ningqi 1” has been highly successful. It has a similar growth rate and canopy shape to “Damaye,” but it produces ellipsoid-shaped fruits with 1000 fresh fruits weighing more than 586 g, which is 15–30% greater than “Damaye.” “Ningqi 1” is particularly stable in production and is able to adapt to a wide range of environments. Its production was quickly expanded in the entire Ningxia region, subsequently Xinjiang, Gansu, Inner Mongolia, Hubei, and Shanxi, moving from northwest China to Central China in over 20 provinces. Its production acreage totaled 88,000 ha. Mr. Shenyuan Zhong, the inventor of “Ningqi 1,” “Ningqi 2,” and “Ningqi 3” was considered the father of Ningxia gojiberry. He received the National Science and Technology Progress Second Prize in China in 2005. Mr. Zhong passed away in 2012, but production of his cultivars, particularly “Ningqi 1,” has rapidly increased in China.

The success of “Ningqi 1” is closely related to the reproduction mode. As mentioned earlier, *Lycium* species are self-incompatible. Recent studies of “Damaye” and “Ningqi 1” showed that the two are self-compatible [56], which ensures the stability of agronomic traits when propagated through seed. The selection scheme used by Mr. Zhong was similar to the application of the pure-line theory [57] with a modification by vegetative propagation to fix a phenotype if needed. The finding of self-compatibility in “Damaye” and “Ningqi 1” was somewhat unexpected because *L. barbarum* has been considered to be an outcrossing species. The finding provides explanation as to why “Damaye” was the most valuable landrace and also why “Ningqi 1” was the most popular cultivar.

#### 5.4. Hybridization

The performance of selected Ningqi series in the field suggested that several reproduction modes occurred in *L. barbarum*. Some are self-compatible, such as “Ningqi 1,” and “Ningqi 7.” Some were self-incompatible, such as “Ningqi 3,” “Ningqi 4,” “Ningqi 6,” and “Ningqi 8.” Whereas “Ningqi 5” showed high male sterility, and “Ningqi 9” was a tetraploid. As a result, intraspecific hybridizations have been carried out for breeding of new gojiberry cultivars. The hybridizations within *L. barbarum* included the use of self-incompatibility and male sterility and the crosses between Ningqi series cultivars with cultivars from the other regions [58]. For the use of the self-incompatible cultivars, one cultivar was planted in a row with another in a 1:1 ratio. The percentage of fruit set, 1000 fresh fruit weight, and total fruit yield per tree were evaluated for identifying the best combinations. For example, “Ningqi 6” should be planted with “Ningqi 8” in a 1:1 ratio for high fruit set. “Ningqi 5,” the male sterility line, should be planted with “Ningqi 1” or “Ningqi 4” in a 1:1 or 1:2 ratio for maximizing fruit set. Ningqi cultivars were also hybridized with cultivars from Xinjiang and other provinces. An et al. [59]

made crosses between “Ningqi 1” and “Ningqi 9” (a tetraploid) and selected a triploid cultivar, which produced fruit that had no or little seeds, contained more sugar and amino acids with better taste compared to “Ningqi 1,” and was resistant to aphid infestation.

Interspecific hybridization has also been used for developing new cultivars. Crosses between “Ningqi 1” with a wild species by Li et al. [60] resulted in the release of “Ningqi Cai 1.” It produces green, tender shoots and leaves, which is used as a vegetable, not for fruit production. This unique cultivar contains 352 g/kg protein, 18 amino acids with vitamin C at 135 mg/kg, and has been widely grown in China as a vegetable crop. Reciprocal crosses were made between either “Ningqi 1” or “Ningqi 4” with cultivars of *L. ruthenicum*, and results showed that they were highly compatible, resulting in the fruit set rate ranging from 52.38 to 91.43% [58]. Furthermore, Wang et al. [61] crossed *L. barbarum* with tomato (*Solanum lycopersicum* L.). Seven lines were selected from 21 cross combinations, and two of them flowered and produced fruit, suggesting *L. barbarum* has a wide range of crossability within the family Solanaceae.

### 5.5. Breeding through chromosome manipulation

Chromosome manipulation has been another approach for improving *L. barbarum* cultivars. In vitro culture of *L. barbarum* anthers produced haploid plants ( $2n = 12$ ), and subsequent culture of hypocotyls of the haploids caused simultaneous doubling, resulting in homozygous diploid plants [62]. In vitro culture of endosperm of *L. barbarum* by Wang et al. [63] and Gu et al. [64] resulted in the isolation of triploid plants from mixoploid populations. Colchicine treatment of in vitro cultured meristems [65] or in vitro culture of ovary [66] produced tetraploid *L. barbarum*. In general, fruits produced from triploid and tetraploid plants were larger than diploid plants. Additionally, polyploid plants had larger flowers and thicker fruit pulp than the diploid plants.

### 5.6. Biotechnological approaches

Biotechnological approaches for improving goji berry have been limited thus far. Methods for in vitro culture of existing meristems, anthers, embryos, and endosperm have been reported, and some of the methods have become well established. In vitro cultured materials were also used for inducing mutation, and some progress has been made. For example, a breeding line for resistance to *Fusarium graminearum* was produced from in vitro cultured embryonic calluses treated by  $^{60}\text{Co}$ - $\gamma$  ray under the selection pressure of crude toxin of *Fusarium graminearum* [67]. Selection of EMS-treated embryonic calluses in the presence of 1.5% NaCl resulted in the development of salt tolerant plants [68].

*Agrobacterium tumefaciens*-mediated genetic transformation was established in *L. barbarum* [69]. In attempts to improve *L. barbarum* resistance to aphids, transgenic plants containing the gene encoding snowdrop lectin (*Galanthus nivalis* agglutinin, GNA) were generated [70]. The transgenic plants showed aphid resistance and also increased fruit weight and total sugar content, but seed count decreased. Because GNA was under the control of constitutive and phloem-specific promoters, the transgene product was only detected in leaves and young

shoots, not in the fruit. Additionally, field test of the transgenic plants showed that rhizosphere microorganisms were not affected by the expression of the transgene [71].

In a study of genetic engineering of targeted genes, five carotenogenic genes from *L. barbarum*: geranylgeranyl diphosphate synthase, phytoene synthase and delta-carotene desaturase gene, lycopene beta-cyclase, and lycopene epsilon-cyclase were functionally analyzed in transgenic tobacco (*Nicotiana tabacum* L.) plants. Results showed that all transgenic tobacco plants constitutively expressing these genes and beta-carotene contents in their leaves and flowers increased [72]. These results imply that such genes could be used for improving *L. barbarum* in beta-carotene biosynthesis.

## 6. Retrospect and prospect

There is a growing interest in gojiberry around the world, but some questions about *Lycium* species are still unanswered. *Lycium* species originated in South and North America, but how and when they were dispersed to Africa and Eurasian regions remain unclear. How has *L. sandwicense* been distributed across different island archipelagos? Why has *L. barbarum* been domesticated mainly in northwest China, and how has it become an important medicinal plant known as Ningxia gojiberry? A total of 355 compounds have been identified thus far [9], do any other compounds remain to be discovered? Nutraceutical and pharmaceutical value of important compounds require further investigation.

A fundamental question from a breeding point of view, however, is the reproduction modes of *Lycium* species. Almost all reports in the literature described that diploid *Lycium* species are outcrossed due to their gametophytic self-incompatibility. Miller and Venable [47] used North American species to show that gender dimorphism has evolved in polyploid, self-compatible taxa from co-sexual, self-incompatible diploids. They proposed that polyploidy is a trigger of unrecognized importance for the evolution of gender dimorphism, which operates by disrupting self-incompatibility and leading to inbreeding depression. Subsequently, male sterile mutants invade and increase because they are unable to inbreed. Research results from China, however, showed that *L. barbarum* is diploid, no occurrence of dimorphism, and land-races range from self-compatible to self-incompatible. The primary reason for the dominance of “Damaye” is its self-compatibility. The most popular cultivar selected from “Damaye,” “Ningqi 1,” is also widely produced and reproduced due to its self-compatibility. Was *L. barbarum* originally a self-compatible species being dispensed to Eurasian regions? Baker’s Law [73–75] stated that self-compatible species are more likely to be successful island colonizers than obligate out-crossers that require pollen transfer between plants (i.e., self-incompatible species). In support of this law, a higher frequency of self-compatibility, as opposed to self-incompatibility, has been documented in island flora. Although the region where *L. barbarum* naturalized is not an island, its surroundings may be similar to an isolated region. Another possibility could be that *L. barbarum* is a self-incompatible species, and adaptation to northwest China caused switching to self-compatibility. The transition from self-incompatibility to self-compatibility has occurred often in the history of Solanaceae [76]. Thus, the reproduction



modes in *L. barbarum* should be further investigated as the modes are fundamentally important for new cultivar development as documented in this article.

Significant progress has been made in *L. barbarum* breeding compared to other emerging fruit crops, such as pawpaw (*Asimina triloba* Dunal.), quince (*Cydonia oblonga* Mill.), or blue honeysuckle (*Lonicera caerulea* L.) [42]. The *Lycium* story is that a South and North America species was naturalized in northwest China and the domestication of this species produced more than 10 landraces. Selection from the landraces resulted in the release of a series of cultivars. Its reproduction modes, cross-ability, self-incompatibility, male sterility, and phylogenetic relationships with other species have been revealed. Regeneration and transformation have been developed. From a local, traditional medicinal plant, it has now received increasing attention as an important nutraceutical and pharmaceutical crop. However, *L. barbarum* and its relatives in the genus require further attention:

1. Genetic potential of other species should be exploited. Current research has been largely focused on *L. barbarum*, and to a certain extent on *L. chinense* and *L. ruthenicum*. Attention should be expanded to species from South and North America as well as Africa. Fruit constituents of those species should be analyzed and those producing valuable compounds should be used for breeding purposes. Their reproduction modes, cross-ability, and corresponding breeding schemes should be developed. Since *L. barbarum* has been shown to be able to cross with tomato, it is believed that *L. barbarum* could be easily crossed with other *Lycium* species. With the use of other species, it is anticipated that new cultivars with more desirable traits could be developed for commercial production.
2. Breeding objectives should include not only fruit size and yield but also disease and pest resistance, valuable compound content, and adaptability. Early maturity should also be important. Constituents of fruits or leaves should be systematically analyzed, and compounds with beneficial functions or negative effects should be clearly identified. Breeding schemes should be designed to maximize production of beneficial compounds and minimize those with negative effects.
3. Effective methods for breeding of *Lycium* species should be developed. Current methods are based on individual plant selection and to some extent the use of self-incompatibility or male sterility. Individual plant selection continues to be useful but it should be accompanied with *in vitro* shoot culture to produce a large number of clones for commercial production. Pure lines derived from simultaneous doubling of anther cultured haploids should be tested to determine if homozygosity is an option for improving goji berry productivity. If not, methods for maximizing heterozygosity should be evaluated to increase fruit production. Furthermore, molecular marker technologies should be incorporated into breeding schemes to increase breeding efficiency.

With the increased recognition of their roles as functional foods, plants in the genus *Lycium* will draw more attention for systematic research. It is anticipated that the potential of this small fruit crop will be fully exploited and valuable products that benefit human health and well-being will be developed and utilized.

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