



**REVIEW**

## Genetic Improvement in *Juglans mandshurica* and Its Uses in China: Current Status and Future Prospects

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### ABSTRACT

*Juglans mandshurica* is an economically and ecologically valuable species that is used for various construction purposes, making luxurious furniture, as food and sources of medicinal substances and landscaping because of its excellent wood, edible fruits and rich in various types of chemical compounds. In the past few decades, several genetic improvements of *J. mandshurica* were made, with a focus on the selection of improved varieties and on breeding technology. Many elite provenances and families were selected based on growth traits or wood properties. In recent years, with the increasing demand for high-quality seedlings in Chinese forestry production, the breeding goals of genetic improvement for *J. mandshurica* were redefined to include other traits, such as fruit yield and contents of medicinal component. However, the improvement processes were still slow due to the long breeding cycle and the limited use of advanced breeding technologies, resulting in the selection of fewer improved varieties. In this review, we summarized the research progresses on genetic improvements of *J. mandshurica* and other related works, and discussed research gaps and suggested future directions for genetic improvement of the species. The review provides valuable insight for the selection of improved varieties and production of excellent germplasm.

### KEYWORDS

*Juglans mandshurica*; breeding advances; genetic improvement; improved variety

## 1 Introduction

*Juglans mandshurica*, known as Manchurian walnut or northeast walnut, belongs to the genus *Juglans* (Juglandaceae). It is one of the famous “three-hardwood species” (the other two are *Fraxinus mandshurica* Rupr. and *Phellodendron amurense* Rupr.), and is distributed widely in Northeast China [1–3]. *J. mandshurica* is monoecious [4] and has extremely fast and luxuriant growth, with a thick, straight trunk that can reach 30 meters in height and 40~80 cm in diameter at breast height (DBH) under natural conditions. The detailed growth characteristics of *J. mandshurica* are shown in Fig. 1 [5,6]. This species



can be used as an excellent grafting rootstock to graft other kinds of *Juglans* species [7]. Moreover, it produces good quality timber in forest plantations because of its excellent wood traits and fast growth rate [8,9]. It is also used as food supplements due to its edible dried fruits with high nutritional content [10]. More importantly, the green peel, leaves, bark and other tissues of *J. mandshurica* can be used for medicinal purposes and have been used in the development of traditional medicines [11]. At present, studies on medicinal component have found that triterpenoids [12,13], flavonoids [14,15], quinones [16] and other compounds contained in *J. mandshurica* have great effects in treating cancer, lowering blood lipids and exert antioxidative, anti-inflammatory and other effects. Unfortunately, the wild germplasm resources of *J. mandshurica* declined seriously in the past few decades. Large numbers of trees were overharvested because of the increasing wood price and lack of protection policies [17].



**Figure 1:** Morphological characteristics of *J. mandshurica* (A: Single plant; B: Male flowers; C: Female flowers; D: Fruits; E: Green peel and kernel; F: Leaves; G: Trunk)

Research on the genetic improvement of *J. mandshurica* started in the 1980s and was mainly confined to conventional breeding. Some superior provenances and families were selected, and some wild germplasm resources were collected and preserved. However, due to the long breeding period, the research progress was relatively slow [18]. A handful of improved varieties was produced and seed orchards were established (Table 1), and few *J. mandshurica* varieties were cultivated. Strategies for research and protection of *J. mandshurica* populations have been extremely limited. With development in science and technology, molecular plant breeding techniques have been widely used in plant genetic improvement, while there have been only a few studies on molecular-assisted breeding in *J. mandshurica*, especially in recent years. People pay more attention to juglone due to its medicinal value, although the demand for *J. mandshurica* is increasing daily. Thus, there is an urgent need to cultivate high-quality *J. mandshurica* stock.

**Table 1:** Distribution of representative *J. mandshurica* improved variety bases and seed orchards

Type	Codes	Location	Longitude (E)	Latitude (N)	Altitude(m)
Improved varieties base	Gangshan	Xinbin, Liaoning	125°24'50"	41°37'53"	656
	Liumao	Jixi, Heilongjiang	130°47'07"	45°14'30"	210
	Tieli	Tieli, Heilongjiang	128°03'59"	46°59'06"	222
Seed orchard	Dailing	Yichun, Heilongjiang	128°37'46"	46°50'08"	631
	Linkou	Qingshan, Heilongjiang	130°24'09"	45°34'46"	729
	Baolongdian	Wuchang, Heilongjiang	127°46'17"	44°56'58"	210
	Linjiang	Linjiang, Jilin	127°22'58"	42°06'20"	684

Because of its high economic and ecological benefits, *J. mandshurica* has great potential for further research to develop high quality varieties. In this review, we summarized genetic improvement studies conducted to increase quality and yield of *J. mandshurica* through conventional and molecular breeding approaches. Gaps in genetic improvement were also identified and described, especially concerning medicinal substances, and new research strategies are formulated with different breeding goals for future research. This could provide a theoretical basis for the genetic improvement of *J. mandshurica*.

## 2 Conventional Breeding

### 2.1 Studies for Selection of Improved Varieties

In the process of tree breeding, the importance of improved varieties was unquestionable. Under the same geographical environment, improved varieties have higher genetic gains than unselected varieties, such as higher yields and greater resistance [19]. Generally, the improvement of superior varieties in forest trees mainly focuses on wood properties (latewood rate, basic density, fiber length, fiber width), fruit traits (high fruit yield and quality), growth traits (growth rate, tree height and DBH) or other traits to obtain high economic and ecological benefits. Currently, a variety of research methods, such as hybrid breeding [20], polyploid breeding [21,22], molecular breeding [23] and other research methods, have been used for further selection and breeding in improved varieties, which has produced many insightful results. However, researchers have mainly focused on conventional breeding to select improved *J. mandshurica* varieties [24–26]. The selection of provenances, families and clones facilitated the development of materials that accelerated the progress of genetic improvement for forest trees.

*J. mandshurica* is primarily distributed throughout Northeast China, it can also be found scattered throughout Nei Mongol, Beijing, Hebei, Shanxi, Henan, Shaanxi and other provinces (Fig. 2). Due to the local adaptation of *J. mandshurica* in these disparate environments, there are great differences in *J. mandshurica* among the various provenances. Thus, it is extremely important to carry out provenance tests,

study geographical variation and select stable and high-yielding provenances for different locations. In 1985, *J. mandshurica* seeds from eight provenances in Northeast China were collected and then used to carry out provenance tests. Two superior provenances (Kuandian and Shulan) were selected by comparative selection of growth traits, adaptive characteristics and morphological characteristics, whose genetic gain was 10.18%. At the same time, it was determined that the growth and adaptability characteristics of *J. mandshurica* were significantly correlated with longitude and latitude [24]. Moreover, four important provenance districts (Wanda Mountains, low mountains in central Jilin Province, eastern Liaoning Province, Xiaoxinganling Mountains-Songhua River) for *J. mandshurica* were identified through correlation analysis [25]. After that, researchers mainly focused on studying the genetic variation in growth and seed or other traits of *J. mandshurica*, carrying out a further study of provenance. For example, Chu et al. [26] analyzed the tree height (H) and diameter at breast height (DBH) of 45 *J. mandshurica* families (15 years) from three provenances and found that the coefficient of variation for H and DBH ranged from 35.6% to 45.35% and from 25.58% to 28.77%, respectively, indicating that there were abundant variations within and among provenances. In addition to growth traits, fruit characteristics were also investigated, and six provenances (Hunchun, Dunhua, Jiaohe, Tonghua, Huinan and Fusong) were selected according to seed traits out of 14 provenances in Jilin Province [27]. Based on fruit and seed traits, 4 superior provenances (Sanchazi, Yabuli, Tieli and Jinshantun) and 53 superior individual plants were selected [28]. In addition, 15 excellent families (XL2, XL5, etc.) were selected according to seedling height and base diameter, 10 excellent families were selected by breeding value and other conditions, and 4 families (DC12, XB1, XB4 and FC8) were selected based on the independent culling method in which both average seedling height and ground diameter were 11.18% higher than those of the other families [3]. In another study, 59 superior individual plants of *J. mandshurica* in the Changbai Mountain area were selected according to three fruit characteristics: single fruit weight, kernel weight and kernel yield [29]. These superior provenances, families, and clones could be used as the preferred materials for the establishment of large-scale plantations.

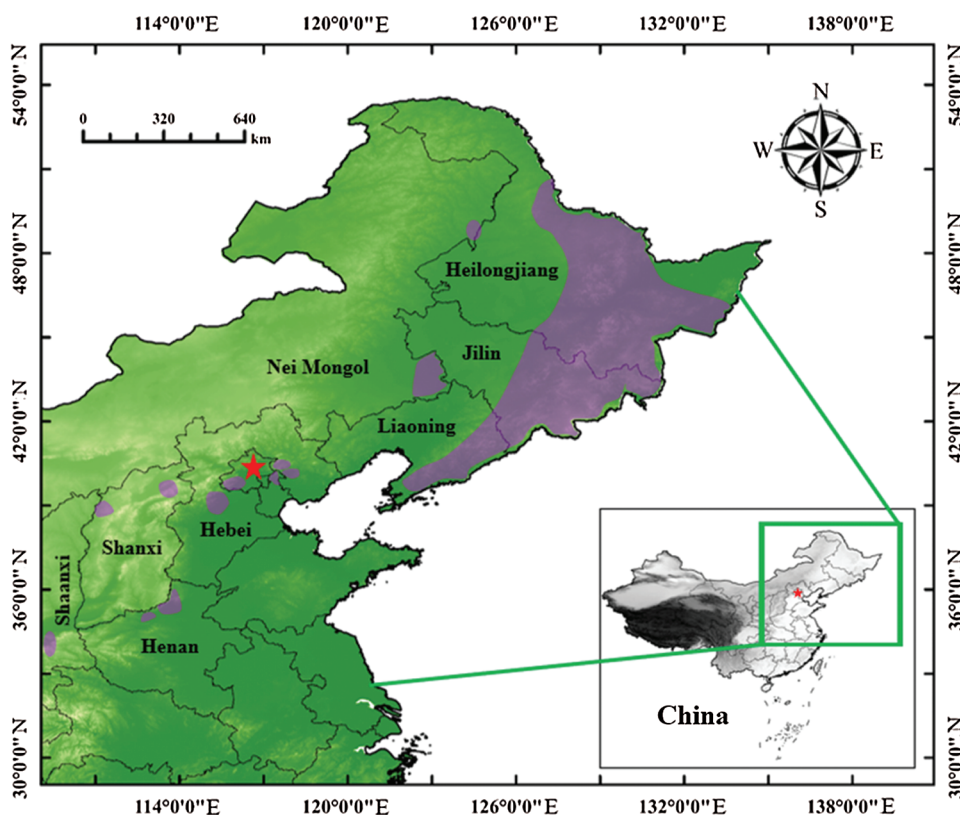


Figure 2: The main distribution areas of *J. mandshurica* in China

As the natural environment is often complex and changeable, the growth indices of seedlings of *J. mandshurica* from different provenances were often greatly different after sowing in different locations. Some representative superior materials of *J. mandshurica* were selected according to different breeding objectives in previous studies (Table 2). In future research, these superior provenances could be used in the establishment of seed orchards, clonal afforestation and so on. At the same time, they could also provide a basis for hybrid breeding, radiation breeding and molecular breeding. Most importantly, previous studies showed that the provenance selection of *J. mandshurica* based on multiple traits was mainly concerned with growth traits and fruit characters, while the selection of medicinal value was not involved. In particular, the juglone contained in *J. mandshurica* had a significant effect on cancer treatment. Future studies on the medicinal value of *J. mandshurica* may have great potential and could become a hot topic. Therefore, research on improved varieties based on their medicinal value also needs appropriate attention.

**Table 2:** Some previous studies on genetic improvement of *J. mandshurica*

Study level	Total quantity used for selection	Afforestation site	Select traits	Tree age	Name of Superior Provenances/Improved Varieties	References
Provenance	14	Changbai Mountains	Fruit	unknown	Hunchun Dunhua Jiaohe Tonghua Huinan Fusong	[27]
Provenance	14	Lushuihe	Growth	1a, 2a	4, 14, 13, 5, 8	[30]
Provenance	8	Shangzhi	Growth	1a	Shulan	[31]
Family	45	Qingshan	Growth	15a	DC1, DC3, DC4, DC5, DC7, DC10, LQ7, DC13, DC14	[26]
Family	121	Liaoning Province	Growth	2a	KD10, QY6, QY31, QY19, QY33	[32]
Family	39	Fusong	Growth	4a	JM3, JM9, JM12, JM38	[33]
Family	122	Qingshan	Growth	7a	Y4, Y14, Y15, Y19, Y21, Y22, Y30, Y31, T42, T44, T47, T54, M20	[34]
Clone	55	Taiyueshan	Growth	5a	1–6 Henan 1, Henan 2	[35]

## 2.2 Physiological Studies

Light is one of the most indispensable environmental factors that affects plant growth and development. Photosynthetic indices also play a vital role in evaluating plant growth and can reveal the mechanisms of tree growth and development and environmental adaptability. At present, the main photosynthetic characteristics concerned by scientists in the process of plant breeding include net photosynthetic rate, stomatal conductance, transpiration rate, intercellular CO<sub>2</sub> concentrations, leaf net CO<sub>2</sub> assimilation rate and so on [36,37]. In past studies, plant photosynthetic indices were investigated in many species, such as *Alnus cremastogyne* Burk. [38], *Pinus tabuliformis* [39] and *Populus tomentosa* [40]. We reviewed the literature and found that there were some studies on the photosynthetic characteristics of *J. mandshurica*. Trees are often subjected to a variety of adverse environmental conditions, which result in different adverse effects on growth and development and can even lead to death. We have already explained that the distribution range of *J. mandshurica* is extremely wide under different natural conditions, and the growth environments are relatively diverse. In particular, some populations grow in damp, sunless gullies. The photosynthetic index of *J. mandshurica* was measured under three different light environments (100%, 60% and 30%), indicating that the net photosynthetic rates of *J. mandshurica* were 16.88  $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ , 12.53  $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$  and 12.50  $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ , respectively [41]. Meanwhile, a similar conclusion emerged showing that *J. mandshurica* had strong adaptability and plasticity in different light environments [42,43]. In the seedling experiment, it was claimed that the chlorophyll content, net photosynthetic rate,

transpiration rate and stomatal conductance of *J. mandshurica* decreased significantly with the deepening of drought stress. Drought seriously affects the physiological, biochemical and photosynthetic characteristics of *J. mandshurica* [18]. Thus, the study of the photosynthetic characteristics of *J. mandshurica* can provide important basic conditions for its high quality and yield.

Other physiological studies were also carried out, such as the content of malondialdehyde (MDA), catalase (CAT), peroxidase (POD) and superoxide dismutase (SOD) of seedlings growing under different soil moisture contents. The results indicated that cultivation of *J. mandshurica* is more appropriate when the soil moisture content was 70% to 75% of the field capacity, which the best treatment to save water during irrigation [44]. Additionally, endogenous hormones play an important role in regulating processes of plant growth and development. It was found that endogenous hormones, especially ethylene (ETH) and gibberellin (GA) contents, gradually increase during the development of *J. mandshurica* fruits, which have an important guiding significance for artificially controlling the ripening of walnut fruit [45].

### 2.3 Asexual Propagation

In the breeding techniques of *J. mandshurica*, some basic research on the technology of asexual propagation has been done [46,47], however, seed sowing is still the main method in the current tree breeding practice. The application of asexual propagation system needs to be strengthened. Sexual reproduction involves seeding, but the plant is prone to variation, and it is difficult to maintain the excellent characteristics of the parent plants. In asexual reproduction (also known as vegetative reproduction), some of the parent plants were used as reproductive material for cutting, grafting, tissue culture and more to form a new individual. The desirable characteristics of the parent plants can be more easily maintained through asexual reproduction, and the process is less time-consuming. Grafting experiments of *J. mandshurica* showed that the survival rate of double tongue grafting was as high as 83%. The double tongue grafting method could obtain a larger contact surface after grafting and can make healing tissue growth faster [48]. A high-efficiency cutting system for *J. mandshurica* has also been established, with rooting rates of up to 82.2% [47,49]. In addition, plant tissue culture technology for *J. mandshurica* has also made a new breakthrough [50,51]. Browning was the greatest obstacle in *J. mandshurica* tissue culture, but new techniques have provided a solution. In one study, researchers showed that adding sodium thiosulfate and VC to the culture medium could effectively prevent the occurrence of browning [52]. Development in the asexual propagation of *J. mandshurica* could shorten the time of seedling cultivation and reduce material consumption and the influence of climate, and is thus conducive to the cultivation of high-quality seedlings for the market.

### 3 Researches on Molecular Marker-Assisted Breeding

With rapid development of molecular biology and sequencing technology in the 21st century, several molecular marker-assisted selection technologies, such as Restriction Fragment Length Polymorphism, Intersimple Sequence Repeat, Sequence-related Amplified Polymorphism, Random Amplified Polymorphic DNA, Amplified Fragment Length Polymorphism, Simple Sequence Repeats, Single Nucleotide Polymorphism, have been widely used in tree genetic breeding because of their high speed, intuitive use and low cost [53–56]. For example, fingerprints were constructed by SSR and ISSR markers in six clones of *Populus deltoides* Marsh., which provided a theoretical basis for species identification of poplar [57]; the accurate identification system of *Eucalyptus* germplasm resources was established by SSR [58]. There were similar applications in *Citrus* [59], *Pinus tabulaeformis* Carr. [60], etc. Molecular marker-assisted breeding could play an extremely important role in the research fields of phylogenetics, genome-wide association studies (GWASs), gene bank construction, fingerprint construction, gene localization and so on, solving a large number of problems in the process of breeding research [61–63].

Based on molecular marker technology, recent research on *J. mandshurica* has focused mainly on genetic diversity, species identification, fingerprint and genetic structure or other aspects [64–66]. For example, Zhao et al. [67] established the optimal RAPD-PCR conditions for *J. cinerea*, *J. ailantifolia*, *J. nigra*, *J. regia* and *J. mandshurica* by polymerase chain reaction. The SRAP-PCR system of *J. mandshurica* was established for the first time and reported by Wang's research to construct successfully the fingerprint of 170 samples from 17 sampling points [68]. Then, the genetic diversity and genetic structure of *J. mandshurica* in 180 samples from six populations in different geographical distribution zones were explored successfully by ISSR technology, and 14 ISSR primers were selected. It was also found that 83.82% of the genetic variation existed within the population, and only a few variations existed in the interpopulation level [69]. Furthermore, this technology was also optimized in a better way, which will provide more convenience for further study on the genetic relationship and genetic diversity of *J. mandshurica* [70]. SSR technology was used to explore the genetic diversity of *J. mandshurica*, 20 pairs of SSR primers with more informative polymorphisms were developed, and it was determined that genetic variation mainly existed within population [71]. The genetic diversity of species or populations was due to long-term evolution, which is the most important prerequisite for the survival and evolution of any species. The higher the genetic diversity or genetic variation of a population or species is the better the ability to adapt to environmental changes and easier to expand the distribution range and exploit new environments to cope with the changing climate will be. The above studies have laid a rich theoretical foundation for the gene mapping of some important characteristics of *J. mandshurica*, including genetic diversity research, variety resource conservation, interspecific hybridization and phylogenetics.

#### 4 Medicinal Value

The medicinal value of *J. mandshurica* has been increasing in recent years. Since ancient times, *J. mandshurica* has been known as a valuable traditional oriental medicine. Historically, the bark is steeped in water to make a tea. Studies on the constituents of *J. mandshurica* husks confirmed the existence of naphthoquinones [72], alkaloids [73], coumarin [74] and other compounds, which have a strong resistance to cancer cells and could therefore potentially be used in the treatment of cancer. This is especially true for the compound juglone, which has been proven through medical practice that it has extremely strong anticancer effects [75–77]. Moreover, the content of juglone is very high in *J. mandshurica*, especially in the green peel of its fruit. For some other diseases, some of the key substances in *J. mandshurica* still play an important role. Walnut hydrolyzed peptides (WHPs) obtained from the fruit proteins of *J. mandshurica* has an obvious effect on the treatment of diabetes [78]. Similar studies have shown that additional compounds in *J. mandshurica* have effective antibacterial and antioxidation properties and strengthen the immune system [79,80]. Although these studies have made some progress in understanding the chemical composition (Table 3) and pharmacological action of *J. mandshurica*, there has been little research on the genetic improvement of the medicinal characteristics of *J. mandshurica*.

**Table 3:** Contents of some useful substances in *J. mandshurica*

Name of the material	Subordinate part	Contents	References
Anthraquinones	Green peel	3.25%	[81]
	Leaves	2.10%	
Juglone	Leaves	5.00%	[82]
	Barks	4.17%	
	Green peel	3.05%	
	Stem	3.83%	[83]

(Continued)

Table 3 (continued)			
Name of the material	Subordinate part	Contents	References
Quercetin	Barks	0.28%	[84]
Triterpenoids	Green peel	2.62%	[13]
Total flavonoids	Barks	2.20%	[85]
Protein	Kernel	34.349%	[86]
Oil	Kernel	56.42%	[86]
Unsaturated fatty acids	Kernel oil	96%	[87]

## 5 Other Studies on *J. mandshurica*

With the rapid development of the global economy, countries around world are facing a very serious energy challenge. The development of renewable energy plays a very important role in sustainable development of the national economy. Wood oil plants are becoming an important energy source due to their unique biological characteristics and significant utilization value [88]. The study showed that the oil extracted from the seed kernel of *J. mandshurica* is as high as 60.08% [89]. This demonstrated that the kernel of *J. mandshurica* has great oil production potential. At the same time, the edible value of trees, as an important part of the dietary value, has important economic significances. It is therefore important to select and breed superior *J. mandshurica* varieties with edible or oil characteristics.

Another crucial issue is diseases and pest control in the forest breeding process. Diseases and pest control are an important part of forestry production to ensure stable yields and economic benefits and to maintain the ecological environment. In this regard *J. mandshurica* could be useful as extracts from leaves, exocarp and bark of *J. mandshurica* have been shown effective against many kinds of agricultural and forestry pests [90,91]. The tree still has some natural disease and is susceptible to pest invasion, such as walnut melanconis disease, *Dictyoploca japonica* Moore., *Caligula boisduvalifallax* Jordan., and *Pandemis dumetana* Treitschke [92]. In general, the application of forest pest control measures has weak effects and high cost. Therefore, selecting and breeding elite varieties that can resist pests and disease is a better way to prevent the diseases and can genetically improve *J. mandshurica*.

## 6 Research Gaps and Future Directions

Research on the genetic improvement of *J. mandshurica* was reported decades ago. However, until now, the research progress has been slow, and the main achievements in genetic improvement were provenance tests, family selection, seed orchard construction and part of the plant molecular genetic improvement. However, there were many key aspects that were not well studied, such as further collection and evaluation of germplasms, selection based on multiple traits, and the molecular mechanism of some key physiological and biophysical characteristics.

### 6.1 Germplasm Resources, Seed Orchards and Improved Variety Bases

Germplasm resources refer to many propagation materials that can be used in forest tree breeding. It is the most crucial material base for forest breeding, especially for cultivating new superior varieties and exploring the origin and evolution of species. In the past, *J. mandshurica* was harvested heavily because of its excellent wood and medicinal value. Thus, the wild germplasm resources of *J. mandshurica* have substantially declined and the species is currently listed as the third class nationally protected plant in China. Fortunately, collection and preservation of germplasm resources of *J. mandshurica* has started in recent years; for instance, the germplasm resources in eastern Liaoning Province of *J. mandshurica* were



collected. Researchers studied the variability of fruits and screened the fruit to distinguish the different morphological types; three of these (spherical, one-tip-spherical, two-tip-spherical) could be selected as the planting types and cultivated [68]. In future, concerted efforts should be made to further collect and evaluate wild germplasm resources within the natural distribution area of *J. mandshurica*.

In addition, establishment of seed orchards and improved variety bases can play a crucial role in the genetic improvement of forest trees. It not only provides convenience for the collection and transportation of seeds but also maintains the characteristics of excellent trees to improve the genetic quality of seeds. Moreover, it can quickly provide a huge number of experimental materials to obtain improved varieties with high genetic gain [93]. The existence of seed orchards provides greater convenience for genetic improvement studies and speeds up the process of seed improvement. As seeds of *J. mandshurica* collected from the wild have low genetic gain coupled with unstable seed yield and poor seed quality, it could not satisfy the actual needs for afforestation in the northeast forest region. Therefore, it is urgent to establish additional seed orchards to provide basic research materials, which then enables establishment of good seed bases based on multiple trait selection, especially for medicinal traits.

## 6.2 Molecular Breeding

In 2018, whole-genome sequencing of *J. mandshurica* was completed. The entire genome assembly was 558 Mb long (coverage = 145×) and contained 13810 scaffolds (N50 = 496.9 kb) [94]. However, the draft genomes were not yet sufficient for future research. In a recent study, a high-quality chromosome-level genome of *J. mandshurica* was reported with a genome size of 548 Mb (contig N50 = 6.49, scaffold N50 = 36.4 Mb) [95]. Hence, high-quality genome assembly and annotation of draft genomes by third-generation sequencing and Hi-C will help to better reveal some of the biological characteristics of *J. mandshurica* and to aid the process of genetic improvement by targeted genetic engineering technology. For example, improving fruit yield [96] or disease resistance [97] and other traits of great significance to forest production practices will enable higher economic benefits. However, molecular breeding studies in *J. mandshurica* are still scarce now. Genome-wide association studies, SNP marker development and mapping, QTL location, and resequencing studies have still yet to be carried out for *J. mandshurica*. However, the chloroplast DNA of *J. mandshurica* has been sequenced, and the reference chloroplast genome of *J. mandshurica* has been preliminarily constructed [98]. Chloroplast genome sequencing of *J. mandshurica* would provide strong support for fruit yield, resistance, pharmaceuticals, wood quality improvement and many other traits. Furthermore, chloroplast genome research also has great significance in the exploration of photosynthesis mechanisms and metabolic regulation of *J. mandshurica*.

Gene mapping for specific traits of *J. mandshurica* has not been performed, especially for genes related to medicinal substances, such as the synthesis of juglone. As the most important medicinal substance in *J. mandshurica* that could treat cancer [99], the synthesis mechanism of juglone is still unclear. Research on breeding for improved variety of medicinal value could become a new direction for genetic improvement of *J. mandshurica* in future. In addition, with the development of other molecular breeding techniques, such as transcriptomics, metabolomics and proteomics, future research could lead to breakthroughs to explore the molecular mechanism of medicinal substance formation. Furthermore, transgenic technology has been widely used in forestry research; for example, transgenic poplars are endowed with a toxic protein gene and eventually become resistant to diseases and pests [100]. However, transgenic research on some special genes and breeding targets has not been done in *J. mandshurica*. Therefore, the use of genetic engineering technology to improve *J. mandshurica* and produce new varieties could enable overcoming the limitation of species and interspecies hybridization bottlenecks to create new traits or varieties adapted to production.

### 6.3 Multiple-Traits Comprehensive Evaluation and Selection

Different breeding objectives in tree genetic improvement determined different breeding and selection methods [101]. For instance, to meet the demand of wood in the early stage of genetic improvement of *J. mandshurica*, the selection and breeding of improved varieties was mainly based on growth and seed traits, aiming to cultivate high-quality and fast-growing varieties to expand its wood economic benefits. Subsequently, with the development of the national economy, society has put new requirements for the development of forest research. Therefore, the multiple-trait selection of forest trees gained increasing research interest. Moreover, *J. mandshurica* also has other valuable economic traits, such as medicinal, wood and fruit traits. Previous studies have detected and identified more than 400 constituents from different plant parts of *J. mandshurica* that have medicinal potential [102], including quinones, flavonoids, steroids, triterpenoids, phenolics and more. Consequently, aiming at specific medicinal substances, the selection and breeding for the development of improved varieties of *J. mandshurica* with higher medicinal substance contents can create greater value. Some studies have shown that the content of juglone, which has the highest medicinal value, was very high in the fruit green peel of *J. mandshurica*. Therefore, it is necessary the focus on the selection and breeding towards increasing fruit yield. Because of the widely useful and excellent wood characteristics of *J. mandshurica*, it has become very popular in the wood market. Therefore, to obtain faster growth and better wood quality, it is particularly important to improve the growth and wood characteristics as much as possible in forest genetic breeding research on *J. mandshurica*. In addition, the development of other economic value of *J. mandshurica* needs to be strengthened, such as its artistic value. The kernel of the native *J. mandshurica* in northeast China was small and had many shapes. It does not reach the same high antique value as *Juglans regia*, which belongs to the same genus, *Juglans* [103].

### 6.4 Hybrid Breeding and Ploidy Breeding

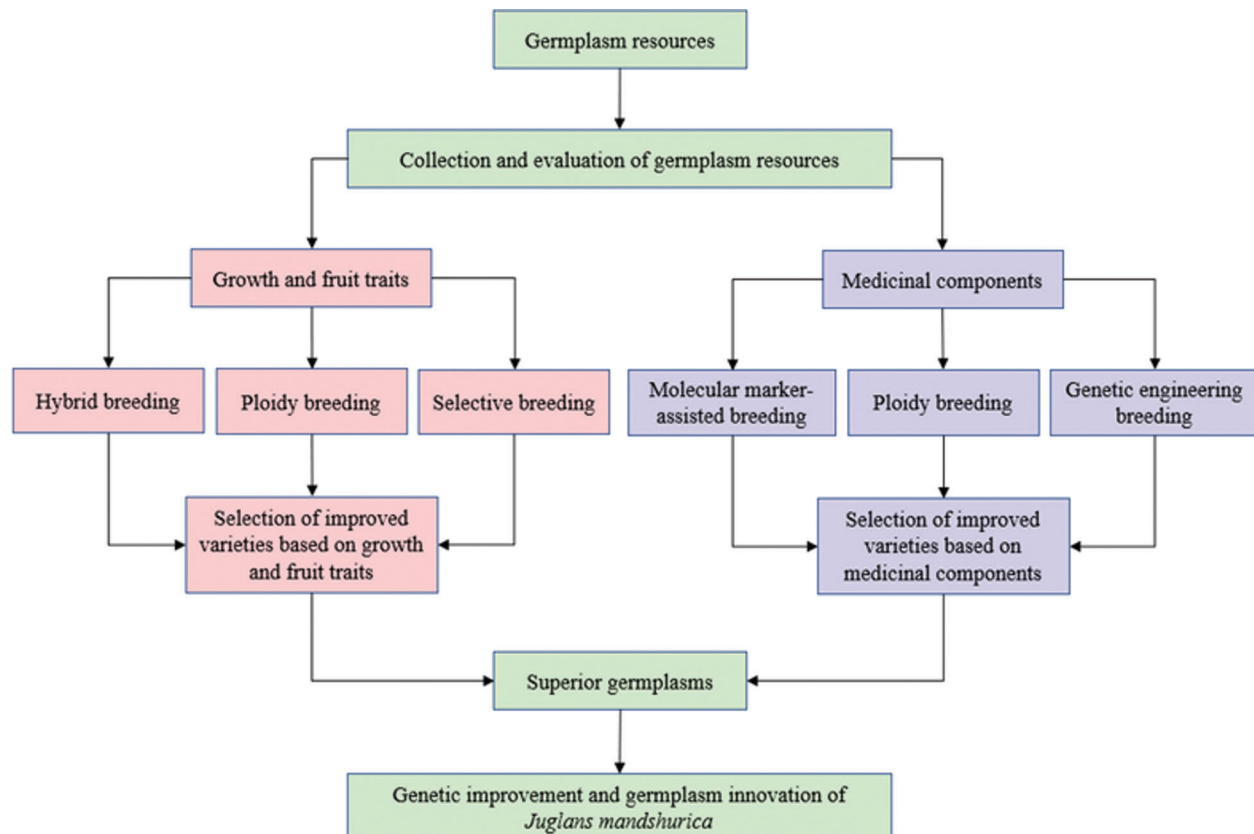
We have already shown that *J. mandshurica* has many excellent characteristics, such as good wood properties, high medicinal value and nutritious fruits. However, except for its high wood utilization rate, other characteristics have not been developed to a high degree. Therefore, according to the current social needs in research on the genetic improvement of *J. mandshurica*, it is important to solve the problems of small seeds, low fruiting and low content of medicinal substance.

The goal of hybrid breeding is to obtain offspring with the desired traits of both the male and female parents [104]. This breeding method plays an important role in the creation of new genotypes and has added significantly to the economic value of poplars [105], *eucalyptus* [106] and more. In addition, polyploid breeding strengthens some traits or weakened other traits [107]. For example, the new polyploid rice was created by chromosome doubling, cross breeding and self-breeding, which increased rice productivity and quality [108]. Similarly, because the fruit of natural *J. mandshurica* in Northeast China was relatively small, the fruit yield was low, while *J. mandshurica* from Hebei had larger fruits and higher yields. High fruit yielding *J. mandshurica* trees that could be suitable for cultivation in the northeast could be obtained by crossing. The increase in fruit yield due to hybridization, in turn, enhances the medicinal value as juglone is mostly found in the green peel of the fruit. Chromosome doubling provides an opportunity for the breeding of improved varieties with high medicinal substance content as it might double the medicinal substance content. Therefore, the application of cross breeding and ploidy breeding is advised for improving varieties of *J. mandshurica* with large seeds, high yield, high content of medicinal substances and rapid growth.

## 7. Conclusions and Recommendations

*J. mandshurica*, a native broad-leaved species in northeast China with immense ecological and economic benefits. It plays an extremely important role in wood, food production, medicine, landscaping and other purposes. Some achievements have been made through conventional breeding and molecular breeding, and some superior provenances and families have been selected. However, the breeding goals of *J. mandshurica* have mainly focused on growth traits in past research, which need more attention towards its medicinal substances. Related breeding methods, such as cross-breeding or ploidy breeding, have not been carried out, and the creation of germplasm resources of *J. mandshurica* based on new breeding goals has been still relatively slow. Moreover, molecular breeding should be emphasized. Due to the lack of high-quality genome sequencing data, some crucial studies of *J. mandshurica*, such as molecular evolution, gene composition or gene regulation, cannot be carried out. These key studies will allow future research into traits needed to shorten the breeding cycle of this species.

Finally, we suggested genetic improvement strategies for *J. mandshurica* by combining the existing research findings and considering future research directions (Fig. 3). As a first step, further collection and evaluation of germplasm is necessary to capture as much genetic variation as possible. Using hybrid breeding, ploidy breeding and selective breeding of these new germplasm collections, improved varieties with good growth, wood quality and fruit yield will be selected. For selecting improved varieties for higher medicinal contents of *J. mandshurica*, the newly collected germplasms should undergo selection based on molecular marker-assisted breeding, ploidy breeding and genetic engineering technologies. These studies could provide new insight for genetic improvement research on *J. mandshurica*. Combining conventional breeding with molecular breeding, the cycle of genetic improvement would be greatly shortened and both the accuracy and process of genetic improvement in *J. mandshurica* will be greatly increased.



**Figure 3:** Genetic improvement strategies for *J. mandshurica*

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