

Germplasm resources, genetic diversity, functional genes, genetic breeding, and prospects of *Pinellia ternata* (Thunb.) Breit: a review

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Abstract

Pinellia ternata (Thunb.) Breit. is a traditional Chinese medicinal plant that has been widely used in China, Japan and Korea to relieve cough, vomiting, and inflammation. This review summarizes the recent research focus on germplasm resources, genetic diversity, functional genes, genetic breeding and prospects of *P. ternata*. The impact of germplasm resources and biogeography are the key factors of the effects of traditional Chinese medicinal materials, avoiding the medical negligence caused by using the confused medicine. Genetic diversity and genetic breeding are the basis of germplasm improvement. A virus-free technique of tissue culture is used to rapid propagation of *P. ternata*, promoting the production of seedlings without season restrictions. Functional gene research is the theoretical basis and target of germplasm improvement. Many genes, such as *PtsHSP17.2* and *PtSAD* have been confirmed to play an important role in heat stress, guiding the selection and breeding of heat-resistant and drought-resistant *P. ternata* resources. Still, some problems exist in the production of *P. ternata*, presenting a challenge in breeding and cultivation. We summarize previous studies here and propose directions for further study to advance the research in the production of *P. ternata*.

Citation: Zhang J, Luo M, Miao Y, Xu R, Wang M, et al. 2023. Germplasm resources, genetic diversity, functional genes, genetic breeding, and prospects of *Pinellia ternata* (Thunb.) Breit: a review. *Medicinal Plant Biology* 2:13 <https://doi.org/10.48130/MPB-2023-0013>

Introduction

Pinellia ternata (Thunb.) Breit. is a perennial herbaceous plant in the Araceae family. It is found in the wild in most of China, as well as in Japan and South Korea. *P. ternata* is known in China as 'Banxia', and the first records of *P. ternata* can be traced back to the 'Shen Nong's Herbal Classic' of the Han Dynasty^[1]. In the field of traditional Chinese medicine, the dried Banxia tuber often has anti-depressant, wound-healing, anti-coughing and anti-vomiting functions, and also has anti-fungal, anti-inflammatory, antioxidant, sedative-hypnotic, insecticidal activities^[2].

The *Pinellia* genus ranges in the number of chromosomes and has abundant germplasm resources. Chromosomal haplotypes 2–16 have been reported, and the basic chromosome number (x) varies greatly^[3]. The *Pinellia* genus is generally thought to have a common basic chromosome number of $X = 13$ ^[3], but not in integer multiples, so there is a possibility of other chromosome cardinalities. *Pinellia pedatisecta* and *Pinellia yaoluopingensis* are two species in which diploidy has been identified, and *P. yaoluopingensis* differs in taxonomic classification from *P. ternata* only in the presence or absence of beaded buds, making its classification somewhat controversial. The formation of *P. ternata* bulbils is involved in asexual reproduction, which means that *P. ternata* may be an asexual organism that evolved through polyploid changes resulting in descending dysploidy. Thus, the $X = 13$ chromosome set is theorized to be more primitive than the $X = 7–9$ variation^[3,4]. There is unresolved controversy around the classification of *P. ternata*, and the disagreement between its classification in the Flora Republicae Popularis Sinicae (FRPS) and Flora of China (FOC) is an

obstacle to the study of *P. ternata*. The species classification and germplasm resources are unclear and thus cannot be correlated with drug efficacy, which directly affects the dosage of medicine and its curative effect.

Reviews published in recent years have mainly reviewed pharmacological activities, processing to reduce toxicity, genetic cultivation, etc. Mao & He^[5] gave a detailed review of the germplasm resources, genetic diversity, and active ingredients of *P. ternata* and suggested that molecular identification or primary active ingredients should be used as quality markers to distinguish the panel from counterfeit products. Bai et al.^[6] has described the main components and medicinal mechanisms of *P. ternata* mainly from chemical composition, pharmacology and toxicity reviews. Pharmacological and molecular targets of *P. ternata* can be analyzed using network pharmacology. Peng et al.^[7] was more specific about the treatment of pineal and the mechanism by which the toxicity was reduced. There are many reviews of *P. ternata* which have been published in recent years, and a number of new ideas and research projects have been proposed. But there is a gap in the field of molecular genetic breeding of *P. ternata*. Therefore, this is the main focus in this paper.

Meanwhile, bacterial disease and rising temperatures lead to declines in the production of *P. ternata*. *P. ternata* is harvested in the summer and autumn, and it is exposed to temperatures above 30 °C, which leads to rapid withering of the *P. ternata* plant^[3,8,9]. High temperature induces a higher expression of heat shock proteins in plants, and a lower expression of other proteins, affecting growth and development. Bacterial diseases are the most important diseases destroying the growth of *P.*

ternata. With the gradual rise in exports, there is an increased number of artificial *P. ternata* plantations. However, soft rot and other bacterial diseases, which are harmful to plants in hot weather, have significantly reduced the production of *P. ternata*^[10]. Diseases and improper cultivation management will directly affect crop yield and quality, resulting in extreme economic losses.

Some studies on the resources of *P. ternata* have been reported, but few focused on its germplasm resources, production, cultivation, and functional genes, and the taxonomy of the plant remains a subject of debate. This study, therefore, focuses on these topics and reviews the recent literature on *P. ternata* to provide direction for future development and practical application of *P. ternata* in germplasm resources and breeding.

Botanical description

P. ternata normally has 2–5 leaves, but occasionally only 1. The petiole is 15–20 cm long, the base is sheathed, and there are 3–5 mm diameter bead buds in or above the sheath or at the base of the leaf blade (apex of the petiole). Beady buds germinate on the parent plant or after falling into the ground. Seedling leaves are ovate-cordate to halberd-shaped, entirely simple, and measure 2–3 by 2–2.5 cm; the leaves of older plants are 3-split, lobed green, pale-backed, oblong-elliptic or lanceolate, acute at both ends and measure 3–10 by 1–3 cm; the lateral lobes are slightly shorter; entire or with inconspicuous shallow undulating concrete, lateral veins 8–10 pairs, fine, reticulated, dense, set veins 2 circles. Inflorescence petioles are 25–30 (–35) cm long, longer than petioles. The tuber globose is 1–2 cm in diameter, with fibrous roots^[1]. *P. ternata* grows below 2500 meters above sea level and is commonly found on grassy slopes, wastelands, corn fields, field edges, or in sparse forests^[11] (Fig. 1).

The *Pinellia* genus is rich in germplasm resources and widely distributed, with five species (*Pinellia peltate*, *Pinellia pedatisecta*, *Pinellia cordata*, *Pinellia integrifolia*, *Pinellia ternata*) occurring in China according to FRPS statistics, and nine species (*Pinellia peltata*, *Pinellia pedatisecta*, *Pinellia cordata*, *Pinellia integrifolia*, *Pinellia ternata*, *Pinellia polyphylla*, *Pinellia fujianensis*, *Pinellia yaoluopingensis*, *Pinellia tripartita*) described by 2010 version of FOC. *P. peltata*, *P. pedatisecta*, *P. cordata*, and *P. integrifolia* are classified clearly (Table 1), but *P. ternata* is a polyploid heterozygous species which makes its classification

controversial^[1,12]. For example, *P. yaoluopingensis* was considered a diploid *Pinellia*, but the 2010 FOC lists it as a species of the *Pinellia* genus alone. Mingwang et al.^[3] showed that *P. yaoluopingensis* lacked the bead bud structure of asexual reproduction. In terms of the degree of evolution, it was concluded that *P. yaoluopingensis* may be the product of aneuploid evolution from the sexual reproduction to the asexual reproduction stage which produced bead buds^[3,13], thus retaining its taxonomic status as a species. However, several studies report that the polyploid heterozygous species of *P. ternata* contains a diploid genome. The number of haploid chromosomes is not unique, including $X = 13$ and $X = 9$, two common haploid chromosomes numbers. In addition, the *P. ternata* leaf shape also changed during the planting process, and the species could not be completely separated by traditional leaf shape classification^[3].

The classification of species plays an active role in the study of genetic reproduction. A clearer demarcation between species will be the key to studying the genetic evolution and clinical use of *P. ternata*. Molecular techniques offer considerable improvements and overcome many of the significant challenges of phenotypic testing for a better understanding of *Pinellia* genus diversity.

Production location of research on *Materia medica*

P. ternata was first recorded in Shaanxi Province (China) during the Wei-Jin period in Shen Nong's Herbal Classic as a traditional medicine slightly used in each prescription^[14]. *P. ternata* plants were later found in the Shandong, Anhui, Jiangsu, and Zhejiang provinces^[12]. During the Tang Dynasty, *P. ternata* was grown in various regions of China, but it was produced at the highest quality in Henan, Anhui, and Jiangsu provinces^[15] (Table 2). During the Song, Ming, and Qing dynasties, *P. ternata* with the highest medicinal value was produced in Shandong Province. In the Republican period, *P. ternata* from Hubei province was regarded as a genuine medicinal material^[16–18]. In China, the origin of *P. ternata* developed from west to east and then from east to west, and now it is distributed in most areas of the country, except Xinjiang, Tibet, Qinghai, and Inner Mongolia. And some countries in East Asia such as Korea and Japan also have *P. ternata* production^[1]. Changes in the main origin of *P. ternata* have occurred throughout history, which may be related to climate change. The climate affects the quality, causing changes in the main production area.

Genetic diversity

Species genetic diversity plays an important role in adaptability to natural environments and is closely related to the evolution and genetic variation of population structure^[19]. The study of species genetic diversity is instructive for screening the high-quality germplasm resources of *P. ternata* and its polyploid complex species, as well as the evolutionary relationships among species. To study the genetic diversity of *P. ternata*, a variety of gene identification methods were used to reveal the geographical distribution of *P. ternata* in an attempt to predict the evolutionary principles and direction of germplasm resources. Various molecular markers such as SRAP (Sequence-related amplified polymorphism), TRAP (Target region

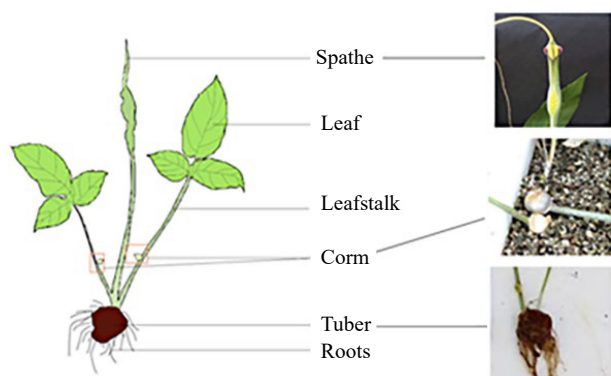


Fig. 1 The biological morphology and organs of *P. ternata*. The Spathe, corms and tubers are shown in the sketch (left) and the photo (right).

Production of *P. ternata*: a review

Table 1. Species comparison of *Pinellia* genus between Flora Republicae Popularis Sinicae (FRPS) and Flora of China (FOC).

Latin name of FRPS	Latin name of FOC	Taxonomic character	Place of origin
<i>Pinellia peltata</i>	<i>Pinellia peltata</i>	Leaf blade entire, Leaf blade peltate, ovate or oblong.	Fujian, Zhejiang
<i>Pinellia pedatisecta</i>	<i>Pinellia pedatisecta</i>	Leaf blade always pedate, leaflets 6–11; bulbils absent.	Sichuan
<i>Pinellia cordata</i>	<i>Pinellia cordata</i>	Tuber globose; leaf blade sagittate-oblong, cordate-ovate, base deeply cordate; bulbils present at base of petiole and at base of leaf blade	Anhui, Fujian, Guangdong, Guangxi, Guizhou, Hubei, Hunan, Jiangxi, Zhejiang.
<i>Pinellia integrifolia</i>	<i>Pinellia integrifolia</i>	Leaf blade ovate or oblong, base obtuse or shallowly cordate, 5–19 × 1.5–6 cm	Chongqing, Hubei, Sichuan
<i>Pinellia ternata</i>	<i>Pinellia ternata</i>	Leaf blade trisect, sometimes pedate with only 5 leaflets, Bulbils present at petiole below middle, or both at proximal part of petiole and at base of leaf blade	Widely distributed in China, excluding inner Mongolia, Qinghai, Xinjiang, and Tibet (Japan, Korea; Europe and North America)
	<i>Pinellia polyphylla</i>	Leaf blade deltoid-ovate or broadly ovate, base deeply cordate, 6–33 × 4–22 cm	Sichuan
	<i>Pinellia fujianensis</i>	Rhizome cylindrical; leaf blade broadly sagittate; bulbils at base of petiole	Fujian
	<i>Pinellia yaoluopingensis</i>	Leaf blade trisect, sometimes pedate with only 5 leaflets, leaflets oblong or lanceolate. Petiole lacking bulbils, bulbils emerging only from tuber; lateral leaflets usually bifid	Anhui, Jiangsu
	<i>Pinellia tripartita</i>	Leaf blade only deeply 3-partite, anterior lobe broadly ovate or ovate-oblong, sessile; bulbils absent.	Hong Kong (Japan)

Table 2. Record of the main planting area of *P. ternata*.

Dynasty	High quality growing area	Reference
Wei-Jin period	Shaanxi Province	Supplementary Records of Famous Physicians
Wei-Jin period	Shaanxi Province, Shandong Province, Anhui Province, Zhejiang Province, Jiangsu Province, Shanghai Municipality	Collective Notes to the Canon of Materia medica
Tang dynasty	Henan Province, Anhui Province, Jiangsu Province	Qianjin Yi Fang
Song dynasty	Shandong Province	Bencao Tujing (Illustrated Classics of Materia Medica)
Ming dynasty	Shandong Province	
Qing dynasty	Anhui Province, Shandong Province	Materia Medica in Qing Dynasty
The Republican period	Hubei Province	Drug production identification

amplified polymorphism), ISSR (Inter-simple sequence repeat), ITS (Internal transcribed spacer), and cpDNA (Chloroplast DNA) have been used to investigate the relationships, germplasm resources, taxonomy, origin, and evolution of *Pinellia*^[20,21]. Genetic diversity analysis of panel germplasm resources based on ISSR markers showed that geographic factors were more important than leaf shape classification^[22]. A comprehensive analysis of the Jaccard similarity coefficient of each population of *P. ternata* showed high similarity among populations with different leaf types, with a lower similarity between different regions, suggesting that the germplasm resource groups of *P. ternata*, especially the wild type of *P. ternata*, should be mainly divided into regions and supplemented by phenotypes^[23]. RAPD (Random amplified polymorphic DNA) analyses of different populations have shown that the germplasm resources of *P. ternata* are highly mixed in both natural and cultivated populations^[24]. Some plant individuals share the same phenotype, but genotypes vary according to the composition of genetic material. RAPD and RFLP (Restriction fragment length polymorphism) analyses of *P. ternata* materials from China and Korea have revealed that genetic diversity within a species can be found in specific regions, and the geographic origin of the tubers of *P. ternata* can be easily identified^[25]. Pan et al. studied the genetic diversity and pedigree structure of wild *Pinellia chinensis*, revealing the causes of its geographical distribution pattern and speculating on the potential refuge of the species during the ice age. The ITS sequences of *Pinellia* nuclear genes from 205 individuals were analyzed by PCR (Polymerase chain reaction) amplification and sequencing^[26], revealing that *Pinellia* may have several glaciated sanctuaries in China, which are

located in southwest, central, and east China. This is consistent with the hypotheses that *Pinellia* originated in the middle and lower reaches of the Yangtze River^[27] and that *Pinellia* cell types increased from the southwest to the northeast^[28]. Based on the chloroplast genome, an adaptive evolution analysis of the three species of *Pinellia* revealed that they form a monophyletic evolutionary clade, among which *P. pedatisecta* diverged first, indicating that *P. pedatisecta* is not a basal group of *Pinellia*, while *P. peltata* may be^[29]. This differs from our prior understanding and could help to further understand the genetic diversity of the panel and better screen for high-quality germplasm resources.

Mutual introduction in different regions is the norm in the production, but it is necessary to consider the relationship between germplasm resources and geographical environment. Introduction cannot be blindly introduced and high-quality resources need to be selected according to local conditions, but there is no unified standard in the industry, it is necessary to formulate industrial standards to promote the development of *P. ternata* production.

Genetic breeding

Genetic breeding contributes to significant improvement in yield and product quality by enhancing tolerance to biological and abiotic stresses. *P. ternata* is a polyploid complex population with complex haplotypes and multiple chromosome numbers. *P. ternata* is agreed to be a population with multiple haplotypes $2n = 2x, 4x, 6x, 8x, 9x,$ and $10x$ with $X = 13$, among

which the octoploid panel with $2n = 8x$ has the highest proportion in each population and is the most widely distributed *P. ternata* population^[3,4]. The increased vigor of polyploids has made them a goal of many plant breeders over the last century, with increasingly improved plant varieties obtained by inducing polyploids and/or exploiting natural polyploids in a variety of ways^[30]. He et al.^[31] and Jia et al.^[32] reported the successive induction and identification of the 16-ploidy of *P. ternata*. Colchicine was used to induce the polyploid transformation of *P. ternata* based on the original 8-ploidy, and a *P. ternata* polyploid showed better resistance and increased yield. Pathway enrichment analysis of differentially expressed single genes (DEGs) by Gene Ontology (GO) and the Kyoto Encyclopedia of Genes and Genomes (KEGG) showed that genes involved in the 'starch and sucrose metabolism', 'purine metabolism', and 'photosynthesis' pathways were significantly induced in the *P. ternata* polyploid. Six transcription factors (MYB, WRKY, bHLH, lateral organ boundary domain (LBD), homologous domain zip (HD-ZIP), and ethylene response factor (ERF)) may play an important role in phenotypic and medicinal composition differences between 8-ploid and 16-ploid materials of *P. ternata* and may improve the resistance of 16-ploid strains to biotic and abiotic stresses^[33].

Unlike other weeds and normal crop plants, *P. ternata* has a very low reproductive rate under natural conditions. It mainly relies on its bulbs to reproduce asexually, with a mature plant growing only 7-8 bulbs per year. The rapid propagation technology of *P. ternata* has been gradually developed and further promotes the differentiation of leaves *in vitro*. The cultivation of large tuber plants favors the development of large and numerous beaded buds^[34]. Xie et al.^[35] used tissue culture to study the virus-free technique on the leaves of *P. ternata* with an 80% virus-free rate, finding that the NAA (α -naphthoacetic acid) project improved the differentiation efficiency of tissue^[36]. Xu et al.^[37] selected an MS medium as the most suitable medium for *P. ternata* growth when screening basic media, and studied the hormone ratio of explant disinfection treatment and plant regeneration. Wang et al.^[38] reported that low concentrations of 2,4-dichlorophenoxyacetic acid (2,4-D), indole-3-acetic acid (IAA), and NAA were suitable for microtuber induction, while multiple concentrations of kinetin (KT) and zeatin (ZT) had no significant effect on petiole microtuber induction. When different concentrations of auxin or cytokinin were added to the MS medium, the petioles of the explants were mostly polar. Building on previous experiments on *in vitro* culture of *P. ternata*, Jie et al.^[39] performed suspension cell culture on *P. ternata* and identified the succinic acid component. There was no significant difference in succinic acid composition relative to cultured *P. ternata*, but toxicity was lower in the cell suspension. Liu et al.^[40] found the alkaloid content in cells cultured *in vitro* was much higher than in cultivated plants.

Heterosis refers to the phenomenon in which offspring of a cross between two genetically distinct parents outperform their parents in terms of growth potential, viability, fecundity, resistance to stress, yield, and quality^[41]. Conventional breeding has produced many hybrids of medicinal plants, such as peony, mint, and rehmannia^[42-44]. For the polyploid hybrid species *P. ternata*, the chromosomal ploidy of the parents should be taken into account in crossbreeding. The ploidy of *P. ternata* is complex, with two reproductive states: asexual

reproduction and sexual reproduction, with sexual reproduction dominated by asexual reproduction. It is necessary to take into account whether the breeding material is capable of sexual reproduction when conducting cross-breeding for *P. ternata*. Sun et al.^[45] pointed out that a possible cause of hybridization failure is difficulty in chromosome pairing due to inconsistency in the number of parental chromosomes, which indicates incompatibility between the two parties. Wang et al.^[46] suggested that parental affinity, which may be related to the diversity of chromosome multiplicities of the *P. ternata*, is the main factor affecting the seed set rate for hybridization. Luo et al.^[47] crossbred material from different populations of *P. ternata* for evaluation and measured the organic acid and guanosine content of the F1 generation. Maternal parentage has a greater influence than paternal parentage on the accumulation of organic acids and guanosine in the F1 generation and the accumulation of organic acids and guanosine in the tuber of *P. ternata* has a somewhat antagonistic effect^[47]. The purpose of crossbreeding was to improve agronomic traits or stress resistance, but the present study only focuses on the effective constituents, and failed to show the advantages of cross breeding. Meanwhile, the evolution of *P. ternata* tends to reproduce asexually, to increase the reproductive efficiency and promote the adaptation to the environment. This adaptability is closely related to drug efficacy and yield as well as processing. However, it contradicts crossbreeding and is a key issue to be considered in subsequent breeding work.

Research of functional genes in production

Many functional genes are crucial to the growth of *P. ternata* (Fig. 2). Transcriptome analysis of *P. ternata* bulb development confirmed that *GAPDH* is the best reference gene in the development stage and a variety of tissues of *P. ternata*, laying the foundation for analyzing the mechanism of bulb formation^[48]. Heat stress is an important environmental factor restricting the growth of *P. ternata*. Spermidine (SPD) and melatonin (MLT) are processed to enhance the heat tolerance of *P. ternata* under heat stress conditions, likely by regulating heat shock transcription factors with different regulatory modes. These findings suggest that MLT and SPD participate in the short duration of HSR, which is an important clue for the study of improving the heat tolerance of *P. ternata*^[49]. Tian et al.^[50] cloned the *P. ternata* heat shock protein gene *PtsHSP17.2* in transgenic tobacco and demonstrated that *PtsHSP17.2* is more highly expressed in transgenic plants with heat stress induction. This study enhances the current understanding of thermal adaptation and further strengthens the study of *P. ternata*'s genetic reproduction. Meanwhile, stearic acid desaturase genes (*PtSAD*) have been confirmed to play an important role in heat stress. High temperature induces the expression of *PtSAD*, but other stresses (drought, cold, and salt stress) do not. Overexpression of *PtSAD* increases the fraction of unsaturated fatty acids, and negative feedback regulation reduces the thermal resistance of *P. ternata*^[51]. Bacteria causes many serious diseases that have seriously affected the yield of *P. ternata* in recent years. Soft rot is a disease caused by *P. carotovorum* subsp. *Carotovorum* (*Pcc*). *P. ternata* infection, which appears as a water-stained scab, is highly transmissible and leads to tuber rot and decreased yield^[52]. pY11T-3-1, as *Ps. aeruginosa*, has a wide antagonistic spectrum and host selectivity and has an inhibitory effect on

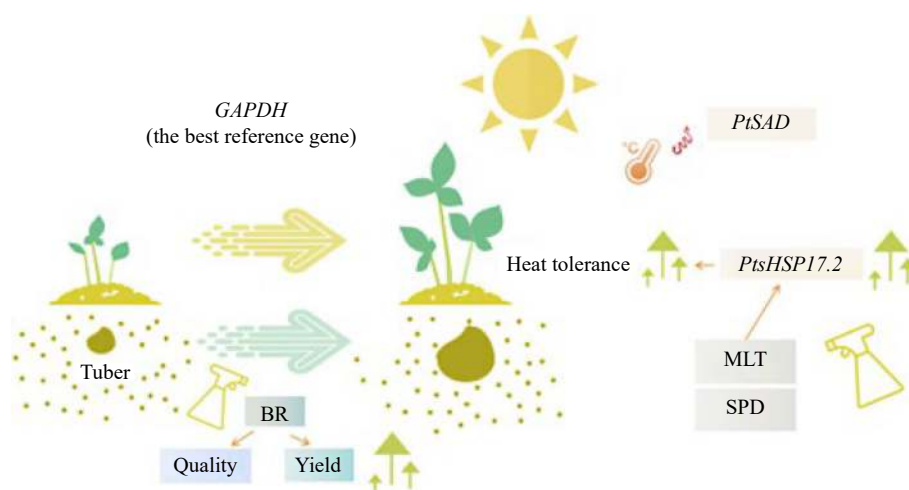


Fig. 2 Studies on functional genes in *P. ternata* production.

Pcc^[53]. Biological control is also a new trend against plant diseases in recent years, which also plays an important role in the production of *Pinellia*. The *P. ternata* (Thunb.) Breit T2 plus line system began to show symptoms at 20 h after infection with *Pcc*. During infection, genes related to growth and development were downregulated and genes related to disease resistance were reprogrammed. These results provided the initial inspiration and molecular mechanism for studying the resistance mechanism of soft rot on *P. ternata*^[54].

A hormone, Brassinolide (BR), is involved in many important physiological and biochemical regulatory processes, such as plant gene expression, reproductive development, and photosynthesis to regulate plant growth^[55,56]. A series of recent experimental studies tested the effect of BR on *P. ternata*. Guo et al.^[57] conducted BR treatment on two varieties of *P. ternata*, finding that BR improves the quality and yield of *P. ternata*. The subsequent BR treatment of *P. ternata* showed that the catabolism of starch to maltodextrin and maltose in the tuber decreased with lower expression of beta-amylase (BAM) and isoamylase (ISA), and the catabolism of cellulose to D-glucose increased with higher expression of endoglucanase (*edg*) and beta-glucosidase (BGL) genes. The photoprotective mechanism of *P. ternata* improved and the content of abscisic acid (ABA) decreased, further revealing the effect of brassinolide (BR) on the formation mechanism of the *P. ternata* tuber^[58]. In addition, the optimal harvest date of plants treated with BR was evaluated using two indexes of yield and quality change, revealing 75 days after treatment with 0.10, 0.50, and 1.00 mg/L BR to be the optimal harvest point^[59]. In recent years, there have been a number of studies on the *in vivo* regulation and expression of genes induced by exogenous hormones in order to improve abiotic stress in plants. *Pinellia* are prone to die above ground and hibernate below ground in hot and dry conditions. This pattern of growth has serious implications for *P. ternata* cultivation. How to breed and improve excellent *P. ternata* varieties, and how to improve their agronomic properties and disease resistance are also the focus of current research.

In recent years, the research and application direction of *P. ternata* is inclined to the anti-insect application of lectin, which is effective and widely used. However, there are few gene mining and functional gene verification of *P. ternata* itself.

Therefore, the follow-up research should focus on the molecular mechanism of *P. ternata* itself, and the use of gene editing or overexpression to verify the function of functional genes is the focus of future research.

Estimation of economic value

P. ternata tends to grow in a warm, moist, shaded environment and can be planted in forests, among fruit trees, or with other crops. Strong light leads to the phenomenon of lodging: the leaves wither, the buds fall, and then new plants grow and develop^[60]. According to statistics from the General Administration of Customs (General Administration of Customs of the People's Republic of China. www.customs.gov.cn), *P. ternata* exports reached 169.581145 million yuan in 2022, ranking among China's top ten export medicinal materials exports in 2020^[61]. As a traditional Chinese medicinal substance as not a source of food plant, and it is relevant for the treatment of Covid-19. Its export price has been rising rapidly since 2019, up 56.55% (Fig. 3), but the annual export volume is gradually decreasing. Japan was the largest exporter of *P. ternata* in 2022, accounting for 51% of the total annual exports, followed by Korea with 23% and the Taiwan region of China with 15% (Fig. 4). *P. ternata* is exported to Asia, North America, and even some countries and regions in Australia. Since the Covid-19 global outbreak, the demand for *P. ternata* at home and abroad is increasing, wild resources are scarce, and disease outbreak has been frequenting in artificial planting. Therefore, it is urgent to improve the quality and productivity to maintain the sustainable development of *P. ternata* industry. The export quality



Fig. 3 The volume of *P. ternata* exports and total export trade over the past six years.

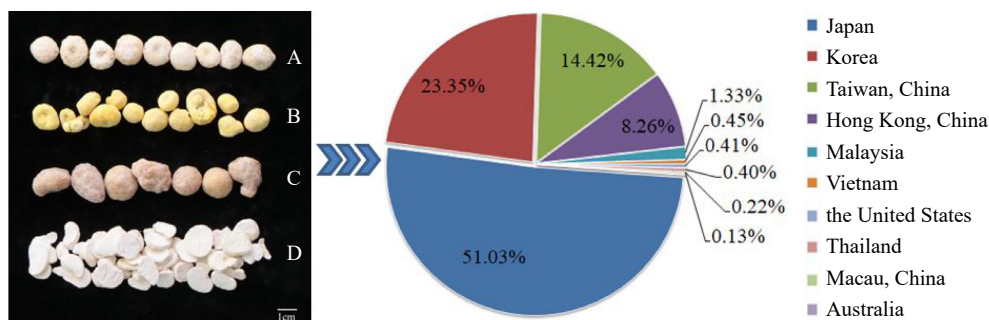


Fig. 4 The proportion of *P. ternata* (left) exports of major countries and regions in 2022 (right). (A) Pinelliae Rhizoma; (B) Pinelliae Rhizoma Praeparatum (Fa-Banxia); (C) Pinelliae Rhizoma Praeparatum Cum Zingibere et Alumine (Jiang-Banxia); (D) Pinelliae Rhizoma Praeparatum Cum Alumine (Qing-Banxia).

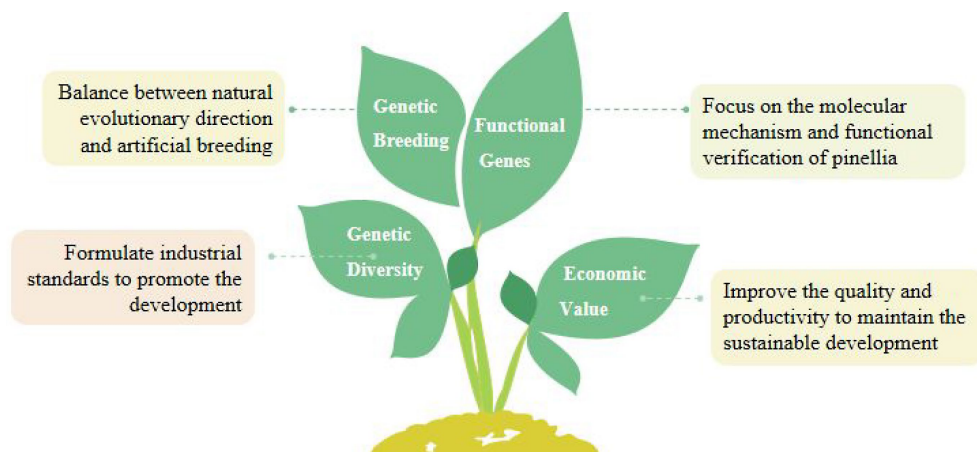


Fig. 5 The solution of *P. ternata* is proposed for breeding and production at present.

standard is very important in the export trade. At the same time, it is necessary to develop export processed products and deep-processed products to support the development of related industries. Industry and development of *P. ternata* should be closely related to genetic breeding. The development of the cultivation cannot be separated from the collection, exploitation and utilization of resources. The adaptability of the *P. ternata* should be coordinated with the local climate, and the yield and quality should also be taken into account, which are inseparable from the work of genetic breeding.

Conclusion and prospects

P. ternata is a plant commonly used in traditional Chinese medicine for over 2,000 years to relieve phlegm and cough. This review synthesizes and reviews in detail the research on genetic breeding, genetic diversity, production and planting, and functional gene research of *P. ternata* (Fig. 5).

P. ternata, as a medicinal material, is often mixed with plants of the same genus. However, *P. pedatisecta* Schott and *P. ternata* are the only species of the *Pinellia* genus that are used as medicines and listed in Chinese Pharmacopoeia^[2]. *P. ternata* tubers are often indistinguishable when mixed with tubers from the same genus confusing both doctors and patients. In the chemical separation of *P. pedatisecta* Schott, a monomer compound, triglochinic acid, can be used to effectively distinguish tubers mixed with others from the same genus^[62]. In addition, the ITS2 sequence can be used to effectively and stably identify *P. ternata* and its counterfeits^[63]. However, there

are a variety of plants under the taxonomy of *Pinellia* and the classification is inconsistent. Some plants have not been reported, so there is an error in identification which brings serious problems for drug identification. More recent studies suggest that more existing germplasm resources should be collected for research and discussion^[64]. The lack of germplasm resources seriously restricts the process and development of *P. ternata* research.

Breeding is the key means of solving the problem of production. Molecular breeding has become the best choice to solve the problem of species breeding. The genome is the key to revealing the origin, evolution, and genetic breeding of a species. Unfortunately, *P. ternata* often appears as a polyploid heterozygote, especially 7-ploid and 8-ploid, making it complex and difficult to study. Researchers can look to solutions to similar problems in different species which could be applied in *P. ternata* to construct the genome. In a similar situation involving the peanut genome, multiple resources were collected to establish the peanut reference genome for resequencing, providing new insights into the genetic evolution of the plant^[65]. For crucian carp with multiple ploidies, the separation of characters through traditional hybridization can reveal the origin of the species^[66,67]. As for *P. ternata*, traditional breeding methods, such as self-cross, backcross, or cross may reveal the evolutionary process and direction of *P. ternata* and provide ideas for its genetic breeding. At the same time, the use of modern molecular genetics may be able to explain the path of *P. ternata* evolution, such as gene editing or overexpression could be used to identify gene function, and molecular marker could be designed to screen target resources.

Production of *P. ternata*: a review

In addition, more research should be done to relieve plant disease by molecular biological application. The demand for *P. ternata* is increasing gradually, and the problems of production and quality need to be solved. Firstly, varieties with high yields and resistance to diseases should be selected and cultivated by breeding. Secondly, natural active pesticides, such as antagonists, natural active molecules, etc., should be developed based on existing research to prevent the breeding of diseases combined with cultivation management. Future research should also focus on the discovery of disease-resistant and insect-resistant resources, the exploitation of functional genes under environmental stresses, and the utilization of functional genes and molecular biological techniques to benefit *P. ternata* production and processing.

Author contributions

Conceptualization: Liu D; performed experiment: Zhang J, Luo M, Xu J and Wang M; supervision: Liu D, Miao Y; writing preparation: Zhang J, Luo M; funding acquisition: Liu D, Luo M. All authors have read and agreed to the published version of the manuscript.

Acknowledgments

Supported by the Funds for Key Program for Traditional Chinese Medicine of Hubei University of Chinese Medicine (Grant No. 2022ZZXZ001), the Postdoctoral innovation research position in Hubei Province, the special fund for the construction of modern agricultural industrial technology system (grant no. CARS-21), the third batch of industrial technology system projects in Hubei Province (grant no. HBHZD-ZB-2020-005), and key project at central government level (Grant No. 2060303).

Conflict of interest

The authors declare that they have no conflict of interest.

Dates

Received 31 March 2023; Accepted 19 August 2023; Published online 7 October 2023

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