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竹子分子生物学研究进展

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摘要: 对 2003 年以来的竹子分子生物学研究进展进行了综述,包括现代分子手段在竹子分类学研究中的开发与应用,鞭芽发育、快速生长、开花、抗逆等相关的重要功能基因研究,基因组测序和转录组测序,遗传转化体系的建立等。这些为今后竹子生物学的研究提供了依据。

关键词: 竹子; 分子生物学; 分子标记; 基因; 基因组学
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Recent Advances in Bamboo Molecular Biology

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Abstract: Bamboo species (Poaceae: Bambusoideae) possess a distinct life history characterized by a predominance of rhizome-dependent asexual reproduction and erratic flowering at intervals of 1 year to 120 years. Some bamboo species are of notable economic, ecological and social significance throughout the World. Considerable progress has been made in bamboo research in the past few years. The advances in bamboo molecular biology since 2003 were reviewed, including the development and application of modern molecular tools in the taxonomy; the cloning and characterization of key genes involved in the critical biological processes of bamboo, such as rhizome bud development, rapid growth, flowering and stress-tolerance; the accomplishment of bamboo genome and transcriptome sequencing projects; and the establishment of genetic transformation systems. The progresses in bamboo molecular biology research provide new insights into further biological studies in Bamboo.

Key words: Bamboo; Molecular biology; Molecular marker; Gene; Genomics

Bambusoideae is a subfamily of the grass family Poaceae and is further divided into nine subtribes comprising more than 80 bamboo genera and about 1400 species worldwide[1]. Bamboo species are distributed in diverse climates, while the tropics and the subtropics are their optimal habitats. Bamboo has a distinct life cycle, which is characterized by a predominant way of rhizome-dependent asexual reproduction and erratic flowering at intervals of 1 year to 120 years. Moreover, bamboo species have a strikingly rapid growth speed and have economic, cultural and ecological significance throughout the world, especially in the Asia-Pacific region. They not only have extensive utility as construction material, food source and versatile raw products, but also offer fodder resources and refuge to animal species, such as

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the giant panda.

However, severe problems such as shooting, flowering, and breeding hinder the progress of the bamboo industry. Consequently, considerable efforts have been made to address these complex issues. For instance, a variety of molecular markers, nuclear DNA and plastid DNA markers have been exploited for the construction of a comprehensive and robust bamboo phylogeny. Furthermore, a multitude of genes have been identified to understand the molecular mechanisms underlying the physiological phenomena in bamboo, such as rhizome bud development, rapid growth, flowering and stress-tolerance. Genome and transcriptome sequences generated in bamboo provide new insights into its functional and comparative genomics studies. Genetic transformation systems have been established in certain bamboo species for genetic breeding. We highlight the significant advances made onwards the year 2003 in this review and discuss the future directions in bamboo molecular biology research.

1 Molecular bamboo taxonomy

Traditionally, bamboo classification systems are primarily based on morphological features. However, there are a few marked limitations: (1) the vegetative features are less reliable for taxonomic analysis as they are easily affected by the changing environment; (2) it is hard to obtain reproductive organs, such as flowers and fruits, for taxonomic studies because of irregular flowering; (3) bamboo classification is a little confounding, since the bamboo plants are widely distributed for economic value in the Asia-Pacific region without proper identification at the species level. With the advent of the molecular era, modern molecular techniques have become effective alternatives for bamboo taxonomy. The cellular and molecular changes can be stably detectable in all tissues regardless of the growth, differentiation, developmental stage of the cell and the effects of the changing environment. Moreover, the use of molecular tools in combination with traditional morphology and/or ecological data has proven to be an ideal strategy for bamboo classification. In this chapter, various molecular tools applied to bamboo taxonomic studies are reviewed, including molecular markers, plastid DNA markers and nuclear DNA markers.

1.1 Molecular marker-based method

Molecular markers like random amplified polymorphic DNA (RAPD), amplified fragment length polymorphism (AFLP) and simple sequence repeats (SSR) are DNA segments representing the differences at the genomic level. These markers have gained rapid popularity in bamboo to elucidate evolutionary history, investigate genetic diversity, and identify germplasm resources.

RAPD and AFLP have been widely used to evaluate the phylogenetic relationships among bamboo species, especially such species with controversial generic and specific assignments as *Bambusa* and *Dendrocalamus* species. The traditional circumscriptions of the genera *Bambusa* and *Dendrocalamus* have long been unsatisfactory due to their overlapping boundary between each other. Using RAPD, a thorny core *Bambusa* cluster was clearly separated from *Dendrocalamus* cluster[2]. AFLP technique provided compelling molecular evidence to resolve *Dendrocalamus* as polyphyletic[3]. Closely related species like *B. vulgaris* and *B. ventricosa* as well as *D. giganteus* and *D. asper* were successfully distinguished through RAPD approach[4].

In line with phylogenetic relationship assessment, the study on genetic diversity is another area of research in bamboo that could benefit from RAPD and AFLP. For instance, RAPD was used to evaluate genetic diversity among and within genera and species of 15 tropical woody bamboos[5]. The resultant molecular phylogeny was in congruence with the bamboo classification given by Gamble[6]. In more detail, all the *Bambusa* species except for *B. atra* were grouped in one major cluster. With a high level of genetic similarity, *B. vulgaris* and *B. striata* clustered together and were sister to *B. wamin*, hence complying with a previous finding that *B. striata* and *B. wamin* are cultivated varieties of *B. vulgaris*[7]; likewise, *B. balcooa*

was found close to *B. arundinacea*, as reported by Nayak et al.[8]. It is known that a better understanding of genetic diversity is essential for conservation and utilization of bamboo genetic resources, and AFLP proved useful in this aspect. It was found more efficient than inter-simple sequence repeat (ISSR) and sequence-related amplified polymorphism (SRAP) techniques to assess the genetic diversity assessment among 16 cultivars of *Phyllostachys praecox* (synonym *P. violascens*)[9]. Contrasting with the low genetic diversity in moso bamboo, *P. heterocycla* (Synonym *P. heterocycla*) with a predominant way of asexual reproduction[10], a high level of genetic diversity was revealed in *P. praecox*, which is cultivated through both asexual and sexual reproduction, thus indicating the significant impact of reproduction mode on the extent of bamboo genetic diversity.

Compared to bamboo phylogenetic relationship and genetic diversity studies, SSR has shown a greater potential in identification of bamboo interspecies hybrids (including *Dendrocalamus hamiltonii* × *D. latiflorus*, *Sasa tokugawana* × *S. borealis*, and four *Phyllostachys* interspecies hybrids), and even hybrids outside the genus (including *Sinobambusa tootsik* × *Pleioblastus distichus*, *Pleioblastus simoni* × *Phyllostachys praecos*, *B. textiles* × *D. latiflorus*, and *B. pervariabilis* × *D. latiflorus* / *B. textilis*)[11–14]. However, the development of SSR markers requires sequence information and is difficult for bamboo, where genomic information is rather scarce. Initially, there were several reports of the development of SSR markers for *B. arundinacea*, *S. cernua*, *S. kurilensis*, and *S. senanensis*[15–17], but the entire procedures in each case that include construction of genomic DNA or SSR-enriched libraries prior to identification of SSR makers is cumbersome and cost-intensive. Later with the ever increasing number of bamboo genomic survey sequences (GSSs) and expressed sequence tags (ESTs) available online for free, a newly emerging method, namely, *in-silico* mining of SSR became preferable and proved to be rapider, simple, and cost-effective. For instance, Sharma et al. and Tang et al. showed that both EST- and GSS-SSR markers derived from the *B. oldhamii* and *Phyllostachys heterocycla* public sequence database, respectively, had a relatively high transferability among other Bambusoideae species for genetic analysis[11,18].

In addition, given that grass genomes have coevolved, and share conserved sequences on a large scale, transferring SSR markers from related species into bamboo serves as another efficient alternative to produce new SSR markers. The use of 25 EST-SSR markers derived from rice, wheat, maize, and sorghum in genetic diversity estimation among a set of temperate bamboo collection was the earliest attempt[19]. Subsequently, the efficiency of this novel method was further verified by two other studies. Sharma et al. successfully transferred up to a total of 59 rice SSR markers and sugarcane EST-derived SSR markers into 23 bamboo species for genetic diversity studies[20]. Chen et al. performed the first systematic survey of 120 rice SSR markers in terms of their transferability into bamboo. Seven rice SSR markers were identified as species-specific to be useful for bamboo species identification[21]. These studies together suggest that SSR markers from other Poaceae species could be an alternative source for the development of bamboo SSR markers.

1.2 Plastid DNA and nuclear DNA markers-based method

Plastid DNA [mainly chloroplast DNA (cpDNA)] and nuclear DNA [mainly internally transcribed spacers (ITS) of nuclear ribosomal DNA, and granule bound starch synthase gene (*GBSSI*)] have long been employed for constructing grass as well as bamboo phylogeny. In general, high-copy cp DNA and ITS have enjoyed a greater popularity than low-copy *GSBBI* in bamboo phylogenic studies.

Previously, bamboo phylogeny was resolved through an individual application of (1) cpDNA markers including cp protein coding genes: *rbcL*, *rps4*, *ndhF*, *matK* and *rpoC2*, and noncoding cpDNA spacers: *rpl*16 and *trn*-like sequences, and (2) nuclear DNA markers, such as ITS and *GBSSI*. However, in order to obtain a more robust phylogeny, a combined

analysis of nuclear DNA and/or plastid DNA (multi-cp locus[22–23] or entire cp genome sequences[24]) data among a broad sampling of representative taxa has become a common method in recent phylogenic inferences. Guo et al. first showed that the phylogeny of the *Thamnocalamus* group and its allies was better resolved through a combined application of nuclear DNA markers (ITS and *GSBBI*) than based on an individual dataset[25]. Despite a well-established evolutionary framework of Poaceae at the family level, the internal relationships of the BEP clade comprising three subfamilies, Bambusoideae, Ehrhartoideae, and Pooideae are controversial. Based on the complete cp genome sequences, Wu et al. fully recovered the phylogeny of the as (B,P)E rather than (B,E)P or (E,P)B, with Bambusoideae and Pooideae being more closely related than Ehrhartoideae[26]. The temperate woody bamboo tribe Arundinarieae has long been a taxonomically difficult group. Recently, Zhang et al. reconstructed its phylogeny by a combined utilization of *GSBBI* and eight non-coding plastid regions along with a broad sampling of more than 100 representatives from 25 Arundinarieae genera helped unravel the complex evolution of the temperate woody bamboos, although there was a significant conflict between those recovered lineages and the current morphological classifications at both the subtribal and generic levels. As compared with the 10 lineages recovered from plastid phylogeny, the 13 lineages revealed in the nuclear phylogeny were better resolved at the generic level. In particular, the scrutiny of possible causes accounting for the discrepancies between the plastid and nuclear trees, including lack of informative characters, incomplete lineage sorting, and/or hybridization (introgression), provided new insights into elucidating the evolutionary history of Arundinarieae[27]. Thus, cpDNA and nuclear DNA data taken together are powerful resources for bamboo phylogenetic reconstructions.

Obviously, various molecular tools such as DNA markers, cpDNA and nuclear DNA markers have contributed immensely to our current understanding of bamboo phylogeny. However, the established bamboo phylogeny so far is still far from satisfying. Extensive phylogenetic studies are required to resolve taxonomic issues, and, most importantly, caution should be taken when performing these phylogenetic reconstructions. (1) An extensive taxon sampling is indispensable for phylogeny reconstruction, especially given that many genera appear to be paraphyletic or polyphyletic[28]. More specifically, in order to make sure the true phylogeny is reflected, a broad sampling of representative taxa of a genus, rather than just one or two bamboo species in previous studies, should be examined when the genus has not been confirmed to be monophyletic. (2) A multi-locus approach is necessary to obtain sufficient informative characters for phylogenetic resolution, especially when using the relatively slowly evolving plastid sequence data[27]. (3) In addition to complete lineage sorting and sufficient informative characteristics, hybridization or introgression is another critical factor that should be taken into account when conducting the combined dataset (nuclear DNA and plastid sequence data)-based phylogenetic analysis[27]. (4) Considering that molecular data alone is unable to distinguish recently formed species, it is advantageous to utilize molecular, morphological, and ecological data simultaneously to identify newly diverged taxa, such as the case of the neotropical bamboo genus *Otatea*[29]. Overall, with an extensive sampling, a combined molecular analysis of multiple nuclear loci, whole chloroplast sequences, morphological characters, and/or ecological data is perhaps the ideal way to further elucidate bamboo phylogeny.

2 Molecular mechanism

2.1 Bamboo physiological phenomena

Within the grass family, the bamboo species are distinguished from other members by their unique physiological phenomena. Bamboos are reproduced asexually by rhizomes; they grow at a strikingly rapid speed and flower at intervals of 1–120 years. Besides, some bamboo species are capable of surviving by themselves when exposed to either biotic or abiotic

stress. Consequently, ongoing efforts have been made in bamboo towards a comprehensive understanding of the molecular bases of these elusive physiological phenomena.

2.2 Bamboo rhizome bud development

Most cultivated bamboo, which are economically vital to many countries in the tropics and subtropics, are reproduced by rhizome buds. Unlike other grass species, rhizome bud development in bamboo is a complicated process. A rhizome bud can develop into either a new rhizome or an aerial bamboo shoot; and the latter grows into a bamboo culm in a very short period. Despite some physiological mechanisms, very few efforts have been made so far to study the molecular mechanism of bamboo rhizome bud development. Rhizome bud development in bamboo can be considered as a process of shoot branching. Thus, the shoot branching genes *PpHB1* (*REV*-like) and *PpTB1* (*TB1*-like) from *Phyllostachys praecox* were the first two rhizome bud development-related genes identified in bamboo. According to their expression profiles, *PpHB1* showed a close relation to rhizome bud formation and procambial development, whereas *PpTB1* was involved in bud outgrowth[30]. Later using rice cross-species microarray hybridization, a total of six genes (including *PpHB1*, *PpRLK1*, *PpSPY*, *PpSINA*, *PpARF1* and *PpHK1*) were identified as bamboo rhizome bud development-related genes, and further cloned from *Phyllostachys praecox*. The detailed functions of *PpHB1* and *PpRLK1* in bamboo rhizome bud development were characterized after RT-PCR and *in situ* hybridization, with the former potentially involved in rhizome bud formation and procambial development whereas the latter in meristem development of the bamboo shoots[31]. Although these data is far insufficient to get the whole picture of bamboo rhizome bud development, the abovementioned studies provided a starting point for further elucidating the fascinating phenomenon.

2.3 Bamboo rapid growth

Bamboo is one of the fastest growing plants and has contributed tremendously to the economy of the tropical and subtropical countries. For example, with a striking culm elongation rate being over 100 cm d^{-1}, *Phyllostachys heterocycla* can reach a height of over 10-meters within a short period of only two to four months. At present, identification of genes[32]/ESTs[33] and proteins[34] differentially expressed in elongating culms is the focus of considerable work with the aim of unraveling molecular mechanisms of bamboo rapid growth. And these studies deemed bamboo culm elongation to be a complex network of physiological and metabolic processes, to which cell division and cell elongation contribute significantly.

A great deal of attention has been given to study bamboo cell wall (CW) biosynthesis, which is an indispensable bioactivity of cell elongation and cell division during the course of bamboo rapid growth. Given that cellulose and lignin are the two major structural components of CWs, it is not surprising that identification of genes that function in cellulose and lignin synthesis has become an important area of research in bamboo rapid growth. So far, most of such studies were carried out in *B. oldhamii*. Four sucrose synthase-coding genes (*BoSus1*–*BoSus4*) were first cloned, each with a specific role in providing substrates for the polysaccharide (such as cellulose) biosynthesis and/or energy production to support the rapid growth of *B. oldhamii*[35]. Subsequently, 10 *BoCesA* cDNAs encoding cellulose synthase were also identified in *B. oldhamii*. Gene expression studies suggested that all the 10 *BoCesA* play roles in cellulose synthesis in the primary CWs of the growing bamboo, and that at least 13 *BoCesA*, including these 10 identified *BoCesA*, may be required for cellulose synthesis in the secondary CWs during the later stages of bamboo development[36]. With respect to the genes involved in lignin synthesis, four *BoPAL* genes (*BoPAL1*–*BoPAL4*) encoding phenylalanine ammonialyase, which catalyzes the first step of phenylpropanoid and lignin biosynthesis pathway, were isolated and further expressed in *Escherichia coli*. BoPAL2 and BoPAL4 exist as tetramers, and possess both PAL and tyrosine ammonialyase activities on the expression in

E. coli; whereas BoPAL1 and BoPAL3 only showed PAL activity[37]. In addition to cellulose and lignin synthesis-specific genes, other crucial loci related to CW synthesis were also isolated from bamboo, such as *BoCOMT1*[38], *BoCCoAOMT*[39], *PheC3H*[40], *C4H*[41] and *PheCYP-1*[42], thus forming a rather solid foundation for our understanding of bamboo rapid growth.

2.4 Bamboo flowering

The peculiarity and complexity of bamboo flowering at intervals ranging from 1 to 120 years and subsequent death after irregular events of sporadic or gregarious flowering have fascinated scientists for centuries. However, relatively little is known about the detailed molecular mechanism of flowering in bamboo. To decipher the process of irregular flowering in bamboo, extensive efforts have been focused on the identification of key genes related to bamboo flowering. The MADS-box genes are important players in floral meristem identity (FMI), which converts the vegetative meristem to a flowering fate, and have been thoroughly studied in model species such as *Arabidopsis*. Hitherto, a few MADS-box genes have been identified in bamboo, incluing *DlMADS18*[43], *PpMADS1* and *PpMADS2*[44] and *PeMADS1*[45]. The ectopic expression of these four *MADS* genes in *Arabidopsis* caused phenotypes such as early flowering and curled rosettes, thus indicating their involvement in bamboo floral development. Asides from MADS-box genes, other putative flowering genes were also drawn to the spotlight in bamboo, like *CONSTANS*, a gene previously reported to constitute a critical link between the FMI and the typical flowering promotion pathway in the flowering gene network[46]. Despite substantial work done in this area of research, it is striking that bamboo flowering genetics continues to be an under-researched area. With the aim of gaining insights into understanding bamboo flowering, a large-scale analysis of flowering-related ESTs and de novo sequencing of floral transcriptome were carried out in *B. oldhami*[47] and *D. latiflorus*[48], respectively. Both research led to the acceleration in identification of putative genes related to the control of flowering time and flower development. These genes will serve as a guide to further elucidate the phenomenon of bamboo flowering.

2.5 Bamboo stress-tolerance

Abiotic, as well as biotic stresses, such as drought, salinity, mineral toxicity and pathogen attack, negatively impact the growth, development and yield of bamboos. Consequently, the abiotic and biotic stress tolerance mechanisms in bamboo have been the focus of much attention for genetic improvement programs. In China, *Phyllostachys heterocycla* accounts for over two thirds of commercially cultivated bamboos because of their use in construction and food and their adaptation to harsh environmental conditions, such as drought and salinity. Recently, this species has been used to identify stress responsive genes, such as *PeZFP*[49], *PeGAPDH*[50], *PheGLU*[51] and *PpNHX1*[52]. While the former three genes lacked further functional characterization, the latter, *PpNHX1*, encoding NHX (Na^+/H^+ antiporter), was subjected to semi-quantitative RT-PCR assay to study its expression profiling in response to salt stress treatment. The *PpNHX1* was suggested as involved in protecting *Phyllostachys heterocycla* from damage caused by salt stress by maintaining the balance of pH and Na^+. All these resulting data, albeit limited, will be valuable for breeding desirable bamboo species with enhanced tolerance to stress through genetic engineering techniques.

3 Bamboo transposon

Transposons (transposable elements, TEs) are fragments of DNA which can move from one position to another in the genome of a single cell. These TEs can be classified into two classes: Class I elements (retrotransposons) and class II elements (DNA transposons), according to their mechanism of transposition. TEs comprise a large fraction of higher plants genomes, such as a 60% of barely genome and an estimated 85% of the maize genome, and they also contribute significantly to genome evolution[53].

Likewise, it is reasonable to speculate that TEs should be abundant in bamboo, which has a large sized genome as well. As anticipated, TEs were found relatively abundant, diverse and polyphyletic in the genome of *Phyllostachys heterocycla*[54]. This finding was confirmed by a recent report[55] wherein TEs were estimated to account for a proportion of approximately 59% of the *Phyllostachys heterocycla* genome with the long-terminal repeat elements (LTRs) (composed of 24.6% *gypsy*-type LTRs and 12.3% *copia*-type LTRs) being the most prevalent. Coupled to this discovery is a greater understanding of the evolution of TEs within the Bambusoideae genome. Retrotransposon such as Ty1-*copia*[56], and DNA transponsons such as *PIF*[57], *Pong*[58] and *Mariner*-like elements (MLEs)[59], were speculated to have undergone horizontal transfer or vertical transmission followed by different evolutionary and stochastic loss in bamboo genomes. Moreover, compared to the genome as a whole, MLEs may evolve under a different selection pressure, hence resulting in its erratic distribution pattern within the Bambusoideae subfamily: near-identical MLEs in distantly related species and diverse MLEs in closely related species[60].

TE biology is a newly developing area in bamboo, but there is an ongoing heated debate to allocate a role for TEs in the history of bamboo genome evolution. For example, many cultivars with diverse phenotypes have been produced in moso bamboo during its long cultivation history. However, a relatively low level of genetic variation was detected among these cultivars[11]. In addition, both *B. oldhamii* and *Pseudosaasa japonica* 'Akebonosuji' in our lab display stable leaf color changes in their long term *in vitro* culture. The mechanisms driving these variations are still unclear. Whether the activation of different classes of TEs is a possible mechanism illustrating variation, is a subject for further transposon research.

4 Bamboo genome and transcriptome sequencing

With the aid of next generation sequencing (NGS) technologies, genome sequencing projects have been accomplished in many model plants and cereal crops. However, bamboo genome sequencing is still in its infancy. It is until very recently that a draft bamboo genome sequence was reported[55]. Before this, sequence information available in bamboo for its genome analysis was severely lacking, and most of these limited genomic data were for *Phyllostachys heterocycla*, including 1.2 Mb GSSs composed of two BACs (GQ252886 and GQ252887) of 113.2 and 139.3 kb in length, respectively[61], and 10608 FL-cDNAs (full length cDNAs) with an average length of 1092 bp, which represent the third largest FL-cDNA collection to date among all plant species[62]. In this context, the newly generated 2.5 Gb high-quality draft genomic sequence covering 95% of the *Phyllostachys heterocycla* genome undoubtedly helped improve the situation[55]. This not only leads to the availability of the largest sequence dataset for studying the structure and function of bamboo genes, but also closes the critical missing link caused by the lack of bamboo genomic data in the grass family for comparative genomics.

Compared to whole genome sequencing, transcriptome sequencing costs relatively less and has gained a rapid popularity in bamboo species. To date, there have been five *de novo* transcriptome sequencing projects accomplished in bamboo. Two are performed in rapidly growing culms of *Phyllostachys heterocycla*, generating a temporal and spatial transcriptome profiling during the culm development[63], and a comprehensive transcriptome of a mixed sample comprising shoots or culms of different heights[64]; the other three are for *D. latiflorus*, including a floral transcriptome of developing flowers[48], a complete transcriptome of a mixed sample consisting of flowers, seeds, and different tissues (root, leaf, shoot, and stem)[65], and a small RNA library constructed from leaf tissues[66]. All of these five dataset will lead to a dramatic acceleration in gene discovery. In particular, the identification of candidate genes potentially involved in rapid culm growth and flowering have increased our understanding of the molecular mechanisms

underlying these fascinating physiological phenomena of bamboo, and provided potential gene candidates for further research.

5 Bamboo genetic transformation

Genetic transformation is an active area of research in bamboo. First, cash bamboo species necessitate desirable traits to develop new cultivars, such as resistance to low temperature and improved pulping characteristics. In bamboo, as in many plant species, traditional breeding is the most common approach to develop elite interspecies hybrids. For instance, *D. hamiltonii* × *D. latiflorus* and *B. textiles* × *D. latiflorus*, which are more adaptable in response to chilling, and more palatable and nutritious than their respective parents, were just two typical products of developing elite hybrid bamboo species by crossbreeding[67–68]. However, this approach is difficult in bamboo species due to their prolonged vegetative phase and predominant way of asexual reproduction. In addition, extensive molecular genetics studies have generated an increasing body of bamboo genes as reviewed above. However, researches about the detailed functions of these genes are relatively lacking. So, if an efficient and convenient transformation method was developed in bamboo, this will not only promote bamboo gene functional research, but also offer the alternative way for traditional bamboo breeding. Nevertheless, great effort in *Agrobacterium tumefaciens*-mediated transformation (ATMT) and bamboo regeneration over the last decade only lead to two successfully established genetic transformation systems so far, for *D. latiflorus* and *D. farinosus*, respectively[69–71].

Zhuo et al. were the first to apply an alternative transformation procedure based on *A. tumefaciens* transfer DNA (T-DNA) for an economically valuable bamboo species, *D. latiflorus* which is cultivated extensively because of their young delicious shoots and mature culms used for construction[69]. Recently, with the aim of developing transgenic bamboos with increased tolerance to low temperature stress, the ATMT approach was used to transform *D. latiflorus* again. Transformants with the modification of *codA* gene that can increase the resistance of plants to low temperature stress were successfully obtained after using PCR and RT-PCR assays for verification[70]. Similarly, in order to develop transgenic bamboo plants with modified lignin biosynthesis to facilitate pulp and paper industry, another optimized transformation system was constructed in *D. farinosus*, a widely used bamboo raw material for the production of pulp and paper. Suppression of endogenous *4CL* gene expression level was achieved in both transgenic callus and their regenerated bamboo planlets with the ATMT approach[71]. Although the overall transformation efficiency of these two newly constructed methods is relatively low, all these data showed that transgenic bamboo plants could be produced successfully through the ATMT approach. Moreover, it is known that there are a variety of factors affecting the callus transformation efficiency of bamboo, including suitable explants, *Agrobacterium* density, concentrations of selection agent (hygromy) and acetyl eugenol (AS), precultivation time, inoculation time, co-cultivation period, and culture medium composition. Thus, the optimization of transformation conditions that came with the development of ATMT transformation methods in *D. latiflorus* and *D. farinosus* will be helpful for the construction of the efficient and stable transformation system in bamboo, thereby paving the way for bamboo genetic improvement programs as well as bamboo gene functional research.

6 Conclusion and directions

Bamboo species are important inhabitants of tropical and temperate forests and are notable for their economic, cultural and ecological significance worldwide. As reviewed above, considerable progress has been achieved in bamboo research in the past decade. In particular, the recently accomplished bamboo genome sequencing project has served to guide our understanding of the fascinating phenomena in bamboo, such as rapid growth, flowering and so forth and

has helped close a major gap in grass comparative genomics. Nevertheless, bamboo phylogeny remains unclear and genetic breeding and genomics research in bamboo still lags far behind other model plants and crop species. And, a great deal of attention should be needed to focus on these subjects as follows. (1) Advanced molecular tools are required to determine the appropriate taxonomic level to construct a comprehensive and robust bamboo phylogeny. A multidisciplinary approach combining these tools with morphological data is desirable in bamboo taxonomy. (2) Compared to traditional breeding methods, genetic engineering offers an alternative avenue for bamboo genetic improvement programs. Consequently, the establishment and optimization of efficient genetic transformation systems are highly desirable and will benefit bamboo breeding programs and gene functional research. (3) In addition to the genome sequencing project of *Phyllostachys heterocycla*, whole genome as well as transcriptome sequencing should be extended to other bamboo species of interest to boost the evolutionary and functional studies in bamboo genes and genomes.

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