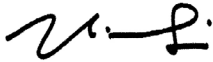# Improving Rooting of American Chestnut Cuttings

Submitted to:

The American Chestnut Foundation (TACF)

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**29 July 2022**

## A. Project title:

Improving Rooting of American Chestnut Cuttings

## B. Summary:

An efficient rooted cutting system will allow rapid and clonal propagation of American chestnut (*Castanea dentata*), accelerate the breeding process, and facilitate germplasm conservation. However, the species is notoriously recalcitrant. This project will 1) optimize the current rooting method to produce the most reliable and consistent results possible; 2) dissect adventitious root (AR) formation process via histological observation; and 3) evaluate transcriptomic expression of genes involved in AR formation. Our ultimate goals are to overcome the difficulty of rooting American chestnut cuttings and develop an easy-to apply and efficient rooted cutting system for the heritage tree.

## C. Principal Investigators (PI) and Institutional Affiliation

Haiying Liang, Ph.D., Associate Professor
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## D. During of project:

12 months from the time funding is received.

## E. Total amount requested

$10,000. Matching funds will be provided by Clemson University – see budget

## F. Short and long-term goals of the project

Short-term Goal (12 months)

- Optimize the rooting system, with the focus on the potential synergistic effects between indole-3-butyric acid (IBA) and naphthaleneacetic acid (NAA) and with *R. rhizogenes* and *Bacillus subtilis*.
- Observe AR formation process through paraffin sectioning and identify location of AR primordia.
- Analysis expression of genes involved in AR formation, including auxin signaling-responsive genes and genes associated with auxin transport and homeostasis.

Long-term Goal:

- To establish an easy-to apply vegetative propagation system that is efficient in rooting cuttings of American chestnut and its hybrids. Impacts of cutting age, collection season, and genotypes will be studied.

- Unravel the physiological and molecular mechanisms of rooting initiation in American chestnut cuttings.

## G. Narrative (no more than five (5) pages)

### 1). There is an urgent need to overcome the barriers in rooting cuttings of American chestnut, the once "perfect" heritage tree.

The American chestnut was among the largest, tallest, and fastest-growing trees in the eastern United States, covering up to 45% of the forest canopy in some areas (Keever 1953). Rich in carbohydrates and other nutrients, chestnuts were an excellent food source for thousands of years, feeding wildlife, people and their livestock. The wood was rot-resistant, straight-grained, and suitable for furniture, fencing, and building. The species was a good source of tannins and had the ability to quickly colonize burned or clearcut areas. Because of its ecological and economic importance, the American chestnut was referred to as the "perfect tree" (Freinkel 2007).

Unfortunately, due to the accidental introduction of the exotic disease, chestnut blight, in the early 1900s, the American chestnut is now widely regarded as "functionally extinct", because only a small percentage of these trees reach sexual maturity. Known as the greatest ecological disaster to strike the world's forests in history, the chestnut blight, within 40 years, virtually eliminated the American chestnut trees that once dominated the eastern half of the U.S., and had survived all adversaries for 40 million years (Brewer 1995). Another exotic disease, Phytophthora root rot, was introduced in the 1700's, and is thought to have eradicated American chestnut from low elevation forests in the Southeastern U.S. prior to the introduction of chestnut blight (Anagnostakis 2012).

Because of the great economic and ecological value of the American chestnut tree, significant efforts have been made over the century to combat the above-mentioned diseases with an aim to restore the iconic chestnut to American forests. To date, hybridizing with a blight-tolerant chestnut species followed by repeated backcrossing (Steiner et al. 2017; Clark et al. 2019) and genetic engineering (Newhouse et al. 2014) have generated promising blight-tolerant varieties. In particular, a deregulation petition for the Darling 58 transgenic variety was submitted in 2020 to USDA APHIS. Once the disease-tolerant varieties become available, a rapid and low-cost method for clonal reproduction is needed to ensure the success of the American chestnut restoration efforts.

While tissue culture-based micropropagation is possible with the American chestnut, the micropropagation approach utilizes immature embryos as explants, and plantlets are germinated through somatic organogenesis. Due to fertilization, the genetic makeups of plantlets derived from individual embryos are not the same, and somatic organogenesis takes several months to generate a plantlet. As for grafting, it is a procedure that requires much practice and skill, and the rootstock genotype may influence the phenotype of the scion and compatibility. Therefore, vegetative reproduction by rooting cuttings is preferred.

### 2). Little success in rooting American chestnut cuttings with exogenous phytohormones.

Auxin is considered the "master regulator" of rooting, and indole-3-butyric acid (IBA) and 1-naphthaleneacetic acid (NAA) are commonly used, either individually or in combination (Davies et al. 2011). Currently available studies of rooting in American chestnut are mostly focused on tissue culture-regenerated plantlets. With 10 mM IBA, 0.5 g/L humic acid, and 2.0 g/L, Oakes et al. (2016) achieved an initial rooting rate of 88.5% and 44.5% for tissue-cultured shoots with *in vitro* and *ex vitro* method, respectively. However, only an average of 16.5 and 39% of plantlets survived eight weeks after acclimation. The only success on rooting cuttings was reported by Galic et al. (2014). In this study, a rooting rate of 59% and 65% was achieved for juvenile softwood and

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semi-hardwood cuttings, respectively, with 1% IBA. These cuttings were collected in July 2007. However, none survived the winter or cold storage. When using cuttings collected in April and May from a grafted plant, the same authors reported that 46% of cuttings rooted, and 15% overwintered successfully (Galic et al. 2014).

For Chinese chestnut (*C. mollissima*), Zhu et al. (1998) reported a rooting rate of at least 98% for cuttings from 1-year-old seedlings and 76% and 51%, respectively, for cuttings from 3-year-old and 6-year-old mother trees. This study was conducted in China, and the commercial rooting agents employed (PRA, HL-43, IBAN, and ABT) are not available in the U.S. market.

### 3). Current status of research on formation of adventitious roots.

Studies in other species show that there are four stages for forming adventitious roots: 1) dedifferentiation of parenchyma cells in the phloem ray area, 2) formation of root initials, 3) formation of the root primordia, and 4) elongation (Davies et al. 2011). A study with micropropagated shoots of European chestnut (*Castanea sativa*) showed very low IAA level in auxin untreated materials, which did not root (Review in Vielba et al. 2020). In Chinese chestnut microshoots, an increase of polyphenol oxidase activity and a decrease in the activities of IAA-oxidase and peroxidase-oxidase were found concomitant with the increase of auxin (Hou et al. 2010). Molecular and genetic studies on a chestnut species' adventitious root formation are scarce. Only a few genes have been studied, and they are all from *C. sativa*: *Scarecrow-like 1* (*CsSCL-1*), an ERF transcription factor gene (*CsRap2.12 like-1*), and a MYB-like 61 gene (*Cs714F2G*) (Review in Vielba et al. 2020).

### 4). Results from previous TACF support.

With the support from TACF in 2022, we are starting to establish a rooting system (Fig. 1). Key findings are:

1. It takes an average of 30 days for AR to start emerging.
2. Cuttings with buds that are ready to open are not suitable for rooting. This kind of cuttings broke buds quickly in the greenhouse before rooting could take place. New leaves then wilted and dropped.
3. Current-year's semi-lignified shoots are feasible for rooting.
4. Open chambers work better than closed ones. Leaves tend to become molded and rotten in a closed chamber with high humidity.
5. So far rooting only occurred when sphagnum moss or perlite were used as potting medium.



**Fig. 1.** Current rooting experiments. Rooting using sphagnum moss or perlite as potting medium (A). Examples of induced adventitious roots (B).

Ongoing efforts:

1.  Endogenous hormone analysis of leaf and stem. Samples were ground and sent to Clemson University's Multi-user Analytical Lab (MUAL) & Metabolomic Core on June 13, 2022. A second set of samples were sent to the Donald Danforth Plant Science Center on July 19, 2022.
2.  Rooting with inoculation of *Rhizobium rhizogenes* and *Bacillus subtilis*.

## 5). Objectives of the current proposal.

As seen above, studies to understand the adventitious root formation in American chestnut lag that of other species. While we are starting to obtain induced roots from cuttings, more efforts are needed to improve the rooting rate and understand AR formation in American chestnut at the physiological and molecular levels. There are three specific objectives for this one-year project:

*   Investigate the potential synergistic effects between IBA and NAA and with *Rhizobium rhizogenes*.
*   Identify location of AR primordia and observe AR formation process by histology.
*   Perform reverse transcription-quantitative polymerase chain reaction (RT-qPCR) to reveal the expression dynamics of genes involved in AR formation.

## 6). Approaches and methods.

Current-year's semi-lignified twigs are to be cut into segments that are 10-15 cm in length, containing at least two buds and leaves. Potting substrate will be sphagnum moss. A temperature-controlled (22-23 °C) mist room will be employed (mist is 14 minutes off and 6 seconds on).

### 6-1. Obj#1. Assess the potential synergistic effects between IBA and NAA and with Rhizobium rhizogenes on rooting efficacy.

The induced AR we have obtained so far (Fig. 1) were from cuttings treated with 0.1g/L NAA. We will test 0.5g/L NAA and combinations with 1% IBA. Experiments with individual *R. rhizogenes* and *Bacillus subtilis* strains are ongoing. In this new project, we will design combinatory experiments: NAA+ *R. rhizogenes*, IBA+ *R. rhizogenes*, NAA+ *B. subtilis*, IBA+ *B. subtilis*, NAA+ *R. rhizogenes* + *B. subtilis*, IBA+ *R. rhizogenes* + *B. subtilis*, and NAA+IBA+ *R. rhizogenes* + *B. subtilis*. USDA APHIS guidelines will be followed. Cultivation of *R. rhizogenes* will follow the protocol described in Samaan et al. (2017) with Luria Bertani medium. *Bacillus* cultures will be grown with Reasoner's 2A medium as described in González et al. (2018). The basal part (~2 cm) of freshly wounded cuttings is dipped in bacterial suspension for 24 hrs for *R. rhizogenes* (an optical density 0.6 at 600 nm wavelength) in complete darkness (Samaan et al. 2017) and for 1 min for *Bacillus* ($1 \times 10^8$ CFU mL$^{-1}$) (González et al. 2018), before being dipped in phytohormone solutions.

### 6-2. Obj#2. Understand the AR formation process by histological analysis.

Cutting samples are collected at 0, 10, 20, 30, and 40 d after rooting induction, with a one-centimeter section from the bottom of each cutting being excised and fixed in a 5:50:5 (v/v/v) formaldehyde/ethanol/acetic acid (FAA) solution overnight at room temperature. Samples will be dehydrated in an ethanol series (70, 85, 95, and 100%), infiltrated with xylene, and embedded in paraffin. Then, 15 μm-thick transverse sections will be cut with a rotatory microtome and stained with toluidine blue (Rigal et al., 2012). Untreated cuttings will be included as control.

### 6-3. Obj#3. Investigate expression dynamics of genes involved in AR formation by RT-qPCR.

Studies with model species such as Arabidopsis have identified critical genes involved in adventitious rooting. Among them are auxin signaling-responsive genes, as well as genes associated with auxin transport and homeostasis, the quiescent center maintenance, and the root

apical meristem initiation. Publicly available American chest transcriptomes will be mined for homologs of these AR genes, including *AUXIN RESPONSE FACTOR* (*ARF*), *LATERAL ORGAN BOUNDARIES-DOMAIN* (*LOB*), and *PIN- FORMED* (*PIN*). Primers will be designed with Primer3 (Untergasser et al. 2012). Stem barks from 0.5 to 1 cm basal sections of cuttings are to be frozen in liquid nitrogen at 0, 1, 5, 10, 20, 30, and 40 d after rooting induction. Total RNAs will be extracted using a QIAGEN RNeasy Plant Mini Kit (QIAGEN, CA), then are treated with a TURBO DNA-free kit (Ambion, NY) to remove any DNA carry-overs. Total cDNAs will be synthesized using a High-capacity cDNA Reverse Transcription kit (Applied Biosystems, CA). Brilliant III Ultra-Fast SYBR Green QPCR Master Mix kit (Agilent Technologies) will be used for qPCR analysis. Housekeeping genes such as β-actin, Elongation Factor-1 Alpha (EF1α), and tubulin will be chosen as the internal reference genes. The relative expression levels will be calculated using the $2^{-\Delta\Delta CT}$ method (Livak and Schmittgen 2001), and the data are presented as the mean ± SD (standard deviation) from four independent biological replicates. The SPSS software will be used to determine significant differences among various timepoints. Cuttings without rooting induction treatment will serve as control

### 6-4. Data analyses.

There will be at least 10 cuttings per treatment. The experiment will be conducted at least three times, depending on the availability of twigs. Cuttings are randomly arranged inside the mist room once a week. Rooting rate, root length, root number, and stem growth will be recorded. The data will be processed using SPSS v. 23.0 software (SPSS Inc., IL, USA). One-way analysis of variance is to be performed to identify statistically significant differences among treatments, followed by Duncan's multiple range test at $P < 0.05$.

## 7). Potential pitfalls.

American chestnut cuttings are notoriously recalcitrant to rooting. However, we are starting to see some promising results with NAA treatment and using moss as supporting matrix (Fig. 1). It has been reported that *Magnolia wufengensis* cuttings pre-treated with NAA:IBA (2:1) exhibited the best rooting performance (Wang et al. 2022). We are confident that we will be able to improve the rooting efficacy in American chestnut cuttings by combining NAA with IBA and plant growth promoting rhizobacteria (PGPR). Our lab is equipped to conduct molecular biology work, and the Clemson Light Imaging Facility will assist with the histological experiment. We do not expect major pitfalls in Objective# 2 and #3.

## 8). Future plan.

We will continue to optimize treatment methods and environmental conditions to achieve high rooting and survival rates and quality growth. Ultimately, we want to understand the factors contributing to the species' recalcitrance to rooting. An integrated approach, involving histology, morpho-physiology, biochemistry, and molecular biology, will help us reach our goal. We will work with chestnut breeders and restoration projects staff to disseminate the techniques we develope.

## 9). Literature Cited.

Anagnostakis SL (2012) Chestnut breeding in the United States for disease and insect resistance. Plant Dis 96 (10):1392–1403.

Brewer LG (1995) Ecology of survival and recovery from blight in American chestnut trees (*Castanea dentata* (Marsh.) Borkh.) in Michigan. Bull Torrey Bot Club 122 (1):40–57.

Clark SL, Schlarbaum SE, Saxton AM, Baird R (2019) Eight-year blight (*Cryphonectria parasitica*) resistance of backcross-generation American chestnuts (*Castanea dentata*) planted in the southeastern United States. For Ecol Manag 433:153–161.

Davies FT, Geneve RL, Kester DE, Hartmann HT (2011) Hartmann and Kester's plant propagation: principles and practice. Prentice Hall.

Freinkel S (2007) American Chestnut. University of California Press.

Galic D, Dale A, Alward M (2014) Vegetative propagation of american chestnut. Acta Hortic 1019:99–103.

González P, Sossa K, Rodríguez F, Sanfuentes E (2018) Rhizobacteria strains as promoters of rooting in hybrids of *Eucalyptus nitens × Eucalyptus globulus*. Chil J Agric Res 78:3–12.

Hou J-W, Guo S-J, Wang G-Y (2010) Effects of in vitro subculture on the physiological characteristics of adventitious root formation in microshoots of *Castanea mollissima* cv.'yanshanhong'. J For Res 21(2):155–160.

Keever C (1953) Present composition of some stands of the former oak-chestnut forest in the southern Blue Ridge Mountains. Ecology 34(1):44–54.

Livak KJ, Schmittgen TD (2001) Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\Delta\Delta CT}$ method. Methods. 25(4):402-408.

Newhouse AE, Polin-McGuigan LD, Baier KA, Valletta KE, Rottmann WH, Tschaplinski TJ, Maynard CA, Powell WA (2014) Transgenic American chestnuts show enhanced blight resistance and transmit the trait to T1 progeny. Plant Sci 228:88–97.

Rigal A, Yordanov YS, Perrone I, Karlberg A, Tisserant E, Bellini C, et al. (2012). The aintegumenta like1 homeotic transcription factor PtAIL1 controls the formation of adventitious root primordia in poplar. Plant Physiol 160, 1996–2006.

Samaan LG, EL-Kady ME, Shalan AM (2017) *Agrobacterium rhizogenes* and B12 antioxidant mediated rooting induction and shoots proliferation on micro-cuttings of *Pyrus betulaefolia* rootstock. J Plant Prod 8(3):391–395.

Steiner KC, Westbrook JW, Hebard FV, Georgi LL, Powell WA, Fitzsimmons SF (2017) Rescue of American chestnut with extraspecific genes following its destruction by a naturalized pathogen. New For 48 (2):317–336.

Untergasser A, Cutcutache I, Koressaar T, Ye J, Faircloth BC, Remm M, Rozen SG (2012) Primer3 - new capabilities and interfaces. Nucl Acids Res 40(15):e115

Vielba JM, Vidal N, José MCS, Rico S, Sánchez C (2020) Recent advances in adventitious root formation in chestnut. Plants 9(11):1543.

Wang Y, Khan MA, Zhu Z, Hai T, Sang Z, Jia Z, Ma L (2022) Histological, morpho-physiological, and biochemical changes during adventitious rooting induced by exogenous auxin in *Magnolia wufengensis* cuttings. Forests 13(6):925.

## H. Timeline (December 2022 – November 2023)

| Research Work | 1st quarter | 2nd quarter | 3rd quarter | 4th quarter |
|---|---|---|---|---|
| Rooting with NAA and IBA | ▮ | | | |
| Rooting with hormones and PGPR | | ▮ | | |
| Histological observation | | | ▮ | |
| RT-qPCR of rooting genes | | ▮ | ▮ | ▮ |
| Data analysis & interpretation | | ▮ | ▮ | ▮ |
| Present results and manuscript drafting | | | | ▮ |

**I. How results will be measured and reported**

Rooting rate, root length, root number, and stem growth will be recorded and compared. Results will be presented at the NE-1333 Meeting in the fall 2023. A manuscript will be drafted.

**J. Breakdown of how and when funds will be spent**

| Expense | TACF: Amount Requested |
|---|---|
| Part-time assistant salary | $3,600 |
| Part-time assistant fringe (29.2%) | $1,051 |
| Travel to attend TACF annual meeting | $800 |
| Greenhouse rental | $1,500 |
| Expendable supplies—rooting reagents, culture medium, Petri dishes, trimmers, gloves, postage, etc. | $3,049 |
| Unrecovered overhead = F&A @ 52.5%, $5,250 | 0 |
| **Total** | **$10,000** |

**Budget Justification**

- Amount Requested from TACF

The fund requested from TACF is used to cover the salary and fringe for a part-time assistant, greenhouse rental, and consumables (rooting reagents, culture medium, Petri dishes, histology service fee, reagents for RNA extraction, reverse transcriptase, and polymerase chain reactions. The greenhouse rental fee covers pots, potting mix, water, labels, fertilizers, and electricity.

- Matching funds provided by Clemson University

TACF has a policy to not pay overhead (F&A – Facilities and Administration) charges since its grants are relatively small. Therefore, this amount ($5,250) is regarded as matching funds from Clemson University. There will be Creative Inquiry (CI) undergraduates participating the project. The supply fund associated with CI students ($300 per student) will also contribute to the cost of the project.

**K. Brief Curriculum Vitae (CV) of Haiying Liang- Clemson University**

**a) Professional Preparation:**

| | | | |
|---|---|---|---|
| Beijing Forestry University, China | Forestry | B.Sc. | 1990 |
| Beijing Forestry University, China | Plant Biology | M.S. | 1993 |
| College of Environmental Science &Forestry State University of New York | Plant Science & Biotechnology | Ph.D. | 2000 |
| College of Environmental Science & Forestry State University of New York | Plant Science & Biotechnology | Postdoc | 2000~2004 |
| The Pennsylvania State University | Plant Functional Genomics | Postdoc | 2004~2006 |

**b) Appointments:**

07/2012—present: Associate professor, Clemson University, Clemson, SC
09/2006—06/2012: Assistant Professor, Clemson University, Clemson, SC.
04/2004—08/2006: Postdoctoral Fellow, The Pennsylvania State University, State College, PA.
08/2000—03/2004: Postdoctoral Fellow, College of Environmental Science and Forestry, State University of New York, Syracuse, NY.
07/1993—07/1996: Instructor, Beijing Forestry University, Beijing, China.

**c) Publications in the last four years:**

1. (2022) L Guo, J Shen, C Zhang, Q Guo, **H Liang**, X Hou. Characterization and bioinformatics analysis of ptc-miR396g-5p in response to drought stress of Paeonia ostii. Non-coding RNA Research. June 20.
2. (2022) C Shen, Q Li, Y An, Y Zhou, Y Zhang, F He, L Chen, C Liu, W Mao, X Wang, **H Liang**. The transcription factor GNC optimizes nitrogen use efficiency and growth by driving the expression of nitrate uptake and assimilation genes in poplar. Journal of Experimental Botany, May 8.
3. (2022) O Gailing, M Staton, SE Schlarbaum, MV Coggeshall, J Romero-Severson, **H Liang** & J E Carlson Progress and Prospects of Population Genomics of North American Hardwoods. In: Population Genomics. Springer, Cham.
4. (2021) B Lei, CJ Frost, T Xu, JR Herr, JE Carlson, **H Liang**. Poplar allene oxide synthase 1 gene promoter drives rapid and localized expression by wounding. Biotechnology Journal International, 25(5): 16-28.

5. (2021) W Lu, E Wang, W Zhou, Y Li, X Song, J Wang, M Ren, D Yang, S Huo, Y Zhao, **H Liang**. Morpho-histology, endogenous hormone dynamics, and transcriptome profiling in *Dacrydium Pectinatum* during male cone development. Forests. Nov;12(11):1598.

6. (2021) X Wei, S Wu, X Liang, K Wang, Y Li, B Li, J Ma, **H Liang**. Paclobutrazol modulates endogenous level of phytohormones in inducing early flowering in *C. tamdaoensis*, a golden Camellia species. HortScience (in press).

7. (2021) KX Li, K Liu, Y Chen, X Huang, W Liang, B Li, Y Shen, **H Liang**. Comprehensive transcriptome and metabolome analysis of *Lithocarpus polystachyus* leaf revealed key genes in flavonoid biosynthesis pathways. Journal of the American Society for Horticultural Science. 1(aop):1-11.

8. (2020) S Li, **H Liang**, L Tao, L Xiong; W Liang, Z Shi, Z Zhao. Transcriptome sequencing and differential expression analysis reveal molecular mechanisms for starch accumulation in chestnut. Forests 11:388.

9. (2020) Y-Y Xia, D-X Wang, B-Q Hao, Z-P Jiang, G-C Chen, **H Liang**. Nitrogen fertilizer mitigates water loss and restores pigment composition in camellia oleifera, an oilseed crop. Journal of Soil and Plant Biology 2020:106-112.

10. (2019) X Wang, **H Liang**, D Guo, L Guo, X Duan, Q Jia X Hou. Integrated analysis of transcriptomic and proteomic data of tree peony (*P. ostii*) seed reveals key developmental stages and candidate genes related to oil biosynthesis and fatty acid metabolism. Horticulture Research. 6, Article number: 111

11. (2019) HY Zhao, **H Liang**, YB Chu, N Wei, MN Yang, CX Zheng. Effects of salt stress on chlorophyll fluorescence and the antioxidant system in *Ginkgo biloba* L. seedlings. HortScience 54(12):2125-2133

12. (2018) XJ Wei, J Ma, K Wang, XJ Liang, JX Lan, Y-J Li, K-X Li, **H Liang**. Early flowering induction in golden camellia seedlings and effects of paclobutrazol. HortScience 53 (12):1849-1854.

**d) Courses taught:**
1. GEN3000 (Fundamental Genetics)
2. GEN3020 (Molecular & General Genetics)
3. GEN 4930 (Undergraduate Senior Seminar)

**e) Synergistic Activities:**
1. Academic editor for Horticulturae (2021 to present).
2. Served in the University's Graduate and Undergraduate Integrity Hearing Boards (2015-2021).
3. Chaired (2019-2022) or serve (2017-present) in the Clemson University Genetics and Biochemistry Department's Graduate committee; Chaired the Clemson University Genetics and Biochemistry Department's curriculum committee for three years.

4. Program Committee member for the 2013 Southern Forest Tree Improvement Conference and recipient of a USDA conference award for a career development workshop and supporting students and postdoctoral fellows to attend the conference.
5. Held Visiting Professorships at Henan University of Science & Technology and Guangxi Forestry Research Institute in China.
6. A science fair judge for local and region schools for more than 10 years. A frequent manuscript reviewer for various journals, including Plant Physiology, Tree Physiology, BMC Genomics, Plant Cell, Tissue and Organ Culture, PLoS ONE, and Horticulture Research.

**L.  A Conflict of Interest or Commitment (COI or COC) statement.**

There is no known COI or COC regarding this project.