REPRODUCTIVE BIOLOGY OF PAULOWNIA ELONGATA, A MULTIPURPOSE TREE

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ABSTRACT: *Paulownia* genus is a group of fast growing trees that grow well in the southern United States. *Paulownia* (Paulowniaceae) is a genus of deciduous hardwood trees from China used for agroforestry, biomass production, land reclamation, and animal waste remediation. *Paulownia* (*Paulownia tomentosa*), or kiri, was introduced into the US during the 1800s. It quickly became naturalized over much of the eastern states. Except for its ornamental qualities, it was generally ignored as a non-native weed tree. However, since Japanese buyers have begun to buy US grown logs, *Paulownia* is now considered a premier timber species. Forestlands, an important source of cellulosic biofuels feedstock, are expected to play an important role in meeting the national biofuel target. Plants are improved continuously by using molecular or classical breeding tools and in both cases it is important to have a clear understanding of reproductive biology. Light, Fluorescent, and Scanning Electron Micrography was conducted to study pollination and subsequent steps. Variable Pressure Scanning Electron Microscopy (VP SEM: Hitachi 3400 NII) was conducted at Agricultural Research Station, Fort Valley State University. This work represents *Paulownia* floral and reproductive parts in various magnifications. Current research deals with the structure of male and female reproductive parts, pollen germination and subsequent pollen tube growth through style using fluorescent aniline blue and SEM methods. We also present freeze fracturing technique to show internal structures of reproductive parts. Results indicate a pore at the wide spindle shaped stigmatic tip followed by hollow style. Stigmatic pore opens into a cavity with internal surface lined with elongated cells that are secretory in nature. Pollen grains parachute through stigmatic pore filling up the cavity and crowding stigmatic tip. Pollen grains germinate readily and hundreds of pollen tubes with callose plugs can be visualized in the style region. Ovary contains numerous ovules attached to the placenta. Pollen tubes can be seen in the close vicinity of ovules to commence fertilization. These studies will be helpful in developing future breeding programs.

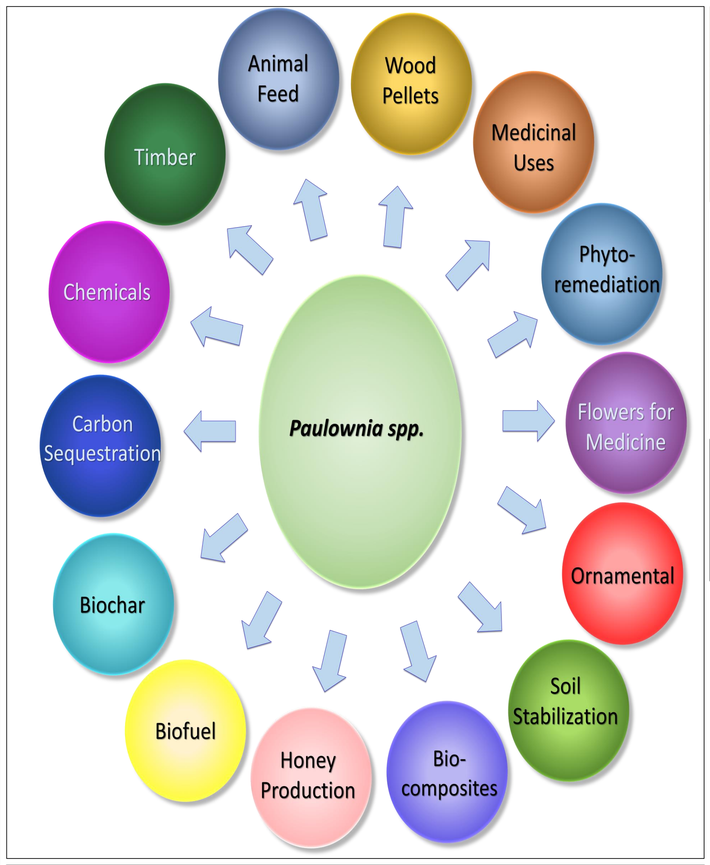

Figure 1. *Paulownia* is a fast growing multipurpose tree. We are investigating wood properties and various applications to boost rural economy in South East USA.

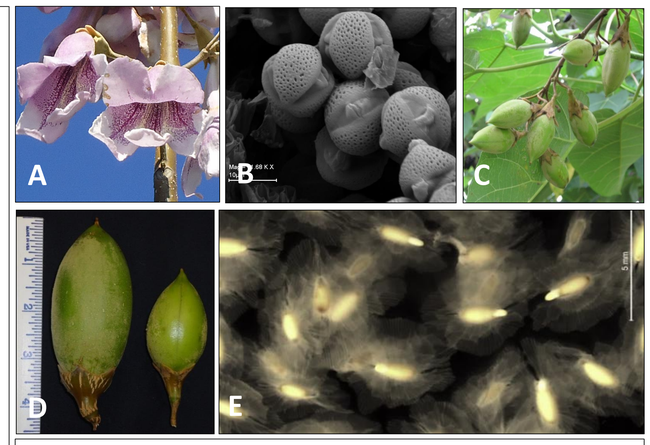

Figure 2. Paulownia in pictures. A. Flowers during bloom, B. Pollen grains, C. Green mature fruits, D. *Paulownia* spp. vary in fruit shape and size, E. Seed germination of fresh *P. elongata* seeds.

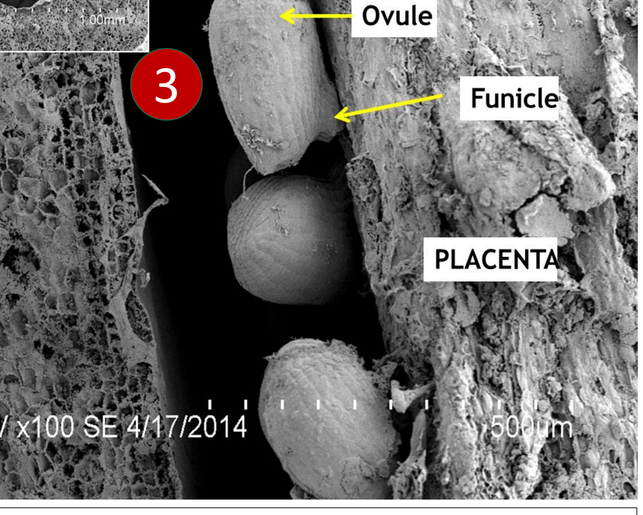

Figure 3. *Paulownia* is a beautiful ornamental tree.

***Paulownia* for biobased economy?** *Paulownia* is a fast growing, multipurpose, short rotation biomass tree (Fig. 1, 2 and 3). Total global production capacity of wood pellets in 2008 was 100 million tons. Twenty percent of wood pellet production in the U.S. was exported to Europe. An annual 25-30 % global growth over the next ten years is projected. The Wood Plastic Composite (WPC) industry consists over $2 billion of products globally. Bio-fillers are employed with thermoplastic resins to reduce the costs of production and provide unique structural products not obtainable otherwise with pure thermoplastics. *Paulownia* tree can be a source of pelletized animal feed utilizing nitrogen rich leaves, honey, bioplastics, biochar, and high value chemicals by hot water extraction of wood. State of Georgia Biobased economy- Total Jobs: 178,110; Total Value Added: $16.365 Billion; Direct Value Added: $8.238 Billion.

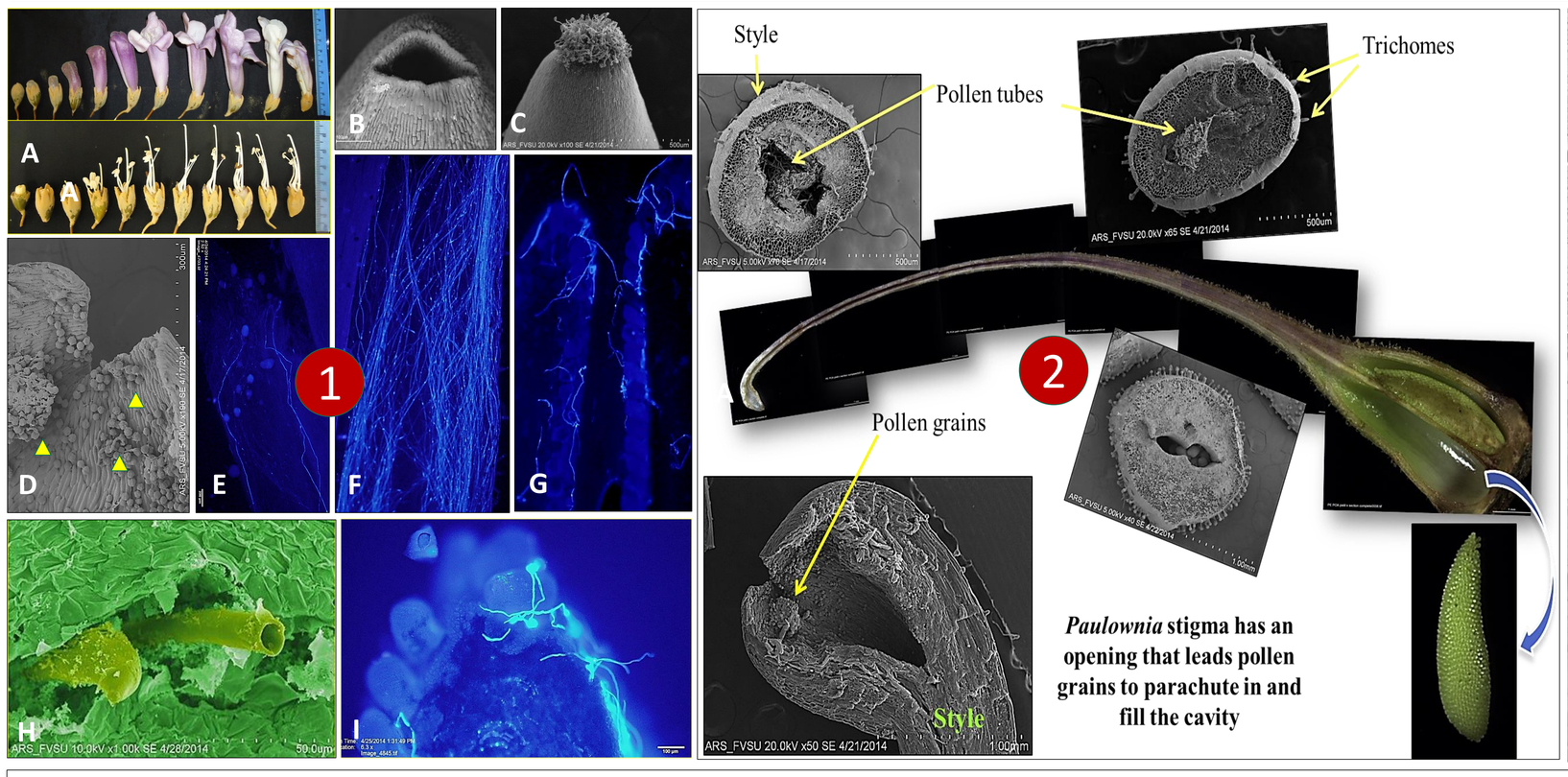

Figure 4. Aspects of reproductive biology. 1. A. Stages in flower development to show relative growth of anther and ovary. B-C. SEM of stigma tip at pre- and post- anthesis stage. Note pollen grains at post-anthesis stage. D. LS of stigma to reveal cavity that is filled by pollen grains. E. Same as in D but with aniline blue staining. F-G. Pollen tube growth in style and ovary. H. Close up of pollen tube growth in transmission tissue in the style. I. Pollen tubes in the vicinity of ovules. 2. Structure of a fully developed pistil of P. elongate. 3. Cross section of ovary exhibiting ovary wall, placenta and arrangement of ovules.

Light Microscopy (LM): Pistils were cut longitudinally using a fine scalpel. Photographs were taken using Leica MZ10F microscope with DFC450C camera at various magnifications.

Fluorescent Microscopy (FM): Fixation of pistils were done in 3:1 EtOH acetic acid (v/v) soln. The pistils were left in a capped tube at room temperature (RT) overnight. Alkaline treatment was carried out by replacing the fixative with 8N NaOH overnight at RT. Washing of pistils were done 4X with D H_2O carefully (tissue is very soft at this stage) to remove NaOH completely. Replaced D H_2O with 0.01% Decolorized Aniline Blue Stain (DABS) and incubated for at least 24 hr in dark at RT. Observation was carried out under epifluorescence microscope using UV light source and DAPI excitation filter @ 365 nm after pistils were placed on the glass slide with few drops of DABS and 10% glycerol.

Scanning Electron Microscopy (SEM): Primary fixing was done in 2% glutaraldehyde in Sorensen's Phosphate Buffer Saline (PBS, pH 7.2) for 2 hr at RT in the fume hood. Secondary fixation was done in 1% osmium tetroxide in PBS for 1 hr at RT. The tissues were dehydrated in ascending ethanol series, followed by critical point drying (CPD) using critical point dryer (EM CPD300, Leica Microsystems, MA). Samples were mounted on aluminum stubs using double sided carbon tape, and sputter coated with gold (Brearley et al. 2014). Coated samples were viewed under variable pressure scanning electron microscope (VP-SEM Hitachi 3400 NII, Hitachi High Technologies America Inc., CA). Digital images were taken at various magnifications running beam at 8-10kV.

Results and Discussion: Paulowniaceae is a monogeneric family with seven species under *Paulownia* genus, not much is known about its reproductive biology. Microscopic techniques (LM, FM and SEM) were employed to study structural and developmental aspects of reproductive organs of *Paulownia elongata.* Successful pollination resulting in pollen deposition on stigmatic pore and germination was observed using FM and SEM (Fig. 4.1. B-D). The stigmatic tip contains a pore that opens in a wide cavity followed by a hollow style (Fig. 4.2.). Pollen tubes growth is clearly seen in hollow style towards ovary using FM and SEM (Fig 4.1. E-I). Freeze fracturing of style at different parts from stigma to ovary help track pollen tube growth (Fig 4.2.). Stigmatic pore opens into a cavity with internal surface lined with elongated cells that are secretory in nature. Pollen grains germinate readily and hundreds of pollen tubes with callose plugs can be visualized with aniline blue staining in the style region. Ovary contains numerous ovules attached to the placenta. Pollen tubes can be seen in the close vicinity of ovules to commence fertilization.

Literature Cited: TA Brearley, BN Vaidya, N Joshee, 2014. Cytokinin, Carbon Source, and Acclimatization Requirements for in vitro propagation of *Scutellaria barbata* D. Don and *Scutellaria race*mosa Pers. Am J Plant Sci 5: 3662-3672.

Acknowledgements: Molecular characterization of wood, biomass utilization, and reproductive biology of Paulownia: A fast growing bioenergy crop. Evans Allen project. 2013-2017. USDA NIFA GEOX 5215, PI.: N. Joshee.