Technical Report for Characterization of the Lateral Organ Boundaries Domain Genes *Ptalbd* Family in Populus Tree

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Abstract: Focusing on LBD family genes can enhance the woody plants to produce biofuel. The LBD genes are responsible for ligning that consume a lot of energy and hinder fermentation process. The genetic modification of woody plants through LBD genes might produce plants ready for biofuel production.

Keywords: biofuel, LBD, genes, tree, populous, lignin

1. INTRODUCTION

Wood cells are the key to solve ecological problems associated with pollution, producing high quality biofuel and reducing global warming. Formation, development and differentiation of wood vascular tissue are regulated by the interaction between ecological and molecular factors (Plomion et al., 2001). The molecular factors which we are interested in the current study are Lateral Organ Boundaries Domain (LBD) transcription factors. LBD genes are known to have important functions in wood formation. The availability of the *Populus trichocarpa* genome sequence (Tuskan *et al*., 2006) and the development of various molecular genetics tools made this plant a desired model to identify functional genes involved in secondary growth (Jansson and Douglas2007). Characterizing these functional candidate genes in wood formation can present an opportunity to enhance wood growth and quality of products. The lateral organ boundaries domain (LBD) proteins are a family of plant-specific transcription factors (Husbands et al., 2007) with 57 members in poplar (Zhu et al., 2007). The LBD genes are reported to play an important role in the regulation of stem secondary phloem (LBD1 and LBD4) and wood secondary development (LBD15 and LBD18) in poplar (Yordanov et al. 2010). Characterizing these genes will lead to a better understanding of their role in wood development and regulation.

2. OBJECTIVES

- The hybrid mother tree *Populus trichocarpa x Populus deltoides* cross will be sequenced to identify Single nucleotide polymorphism SNP in LBD1, 4, 15 and 18.
- Regulatory and coding region of LBD1, 4, 15 and 18 will be sequenced to identify SNPs for genetic mapping.
- Identified polymorphisms SNP in these genes will be mapped in a *Populus trichocarpa x Populus deltoides* full-sib family using PCR- RFLP.
- Analyses of wood growth traits and gene characterization will allow to associate genetic variation in genes with phenotypic traits. Diameter-height-leaf area and bud flush timing will be also estimated for two seasons.
- Associate SNP variation with phenotypic variation (Quantitative Trait Locus (QTL) mapping).

3. MATERIALS AND METHODS

3.1 Plant pedigree

A *Populus* mapping pedigree was generated by crossing the hybrid female clone 52-225 [*P. trichocarpa* (clone 93- 968) × *P. deltoides* (clone ILL-101)] with the male *P. deltoides* clone D124 (Novaes *et al*., 2009). The resulted F2 family (family 52-124) composed of 396 genotypes consist of 268 samples.

3.2. Lab work:

DNA sequencing: Primers were developed six-eight for each gene PtdLBD1, PtdLBD4, PtdLBD15 and PtdLBD18 with product size of 1000-1500bps. Leaf samples of the maternal seed parent were used for DNA isolation. Post PCR samples with successful amplification were treated with Fermentas (Exonuclease I) kit. The ABI BigDye Terminator Cycle Sequencing Ready Reaction Kit v3.1 was used for sequencing reactions and the reactions were then run on a ABI3730 DNA Analyzer. The DNA sequences were checked for each gene to identify SNPs (Single Nucleotide Polymorphisms) (Table1, 2).

Table1: Sequence obtained after direct sequencing of PCR products for LBD1, 4, 15 and 18 and number of confirmed SNPs

Sequence obtained after direct sequencing of PCR products							
Gene	5'UTR	Coding Sequence	3'UTR	Intron	Upstream	Downstream	Total
LBD1	0	514	0	339	701	0	1554
LBD4	220	174	0	582	127	0	1103
LBD15	0	412	191	0	624	220	1447
LBD18	0	453	135	1364	1652	427	4031

Table2: Number of Single Nucleotide Polymorphisms SNPs found in each gene for LBD1, 4, 15 and 18 and confirmed SNPs

	Number of SNPs							
Gene	5'UTR	Coding Sequence	3'UTR	Intron	Upstream	Downstream	Total	Status
LBD1	0	5	0	4	10	0	19	non confirmed
LBD4	0	0	0	2	0	0	2	non confirmed
LBD15	0	2	1	0	0	7	10	Confirmed
LBD18	0	0	0	4	10	0	14	Confirmed

3.3. Field work:

3.3.1. Phenotypic data measurements: Cuttings of poplar family 52-124 were planted in fall 2009 and grown in one gallon pots in the green house at Michigan Technological University. The plants were transferred to Michigan Technological University Ford center in Alberta in summer 2011. Height was measured first on 21st of Oct 2011. Height second measurements were recorded 14th of Apr 2012. Third measurements were recorded on 7th of Oct 2012. Bud flushing was recorded in 23rd of May 2012. Flushing of buds recorded from zero stage in March 1st to the stage 6 with unfolded leaves in 5th of June 2012. Bud flushing was recorded for 77 samples of terminal (apical) buds and in case the terminal bud was dead (61 samples), lateral buds were scored.

3.3.2 Leaves measurements: Three measurements were recorded per sample including leaf area, width, length and number of teeth. Leaves were measured first on 2010 and the second measurement was on 2012 – 4th June.
Number of teeth in each leaf was recorded by counting teeth number on both side of leaf. Leaves length and width were recorded using **Gimp** free software (http://www.gimp.org/) from scanned images (Fig.1).
Scanned images for leaf samples were used to calculate leaf area using software **Adobe Photoshop CS6** (Fig.2).

Leaves area first by Pixel then converts pixels to cm^2 using the equation:

$$\frac{Area\ by\ Pixels}{62000} \text{ X } 4 = \ldots\ldots\text{cm}^2$$

(The method was developed by Michigan State University Dr. Thomas Sharky and Sean Weise http://www.chlorofilms.org/index.php/crpVideo/display/videoid/46).

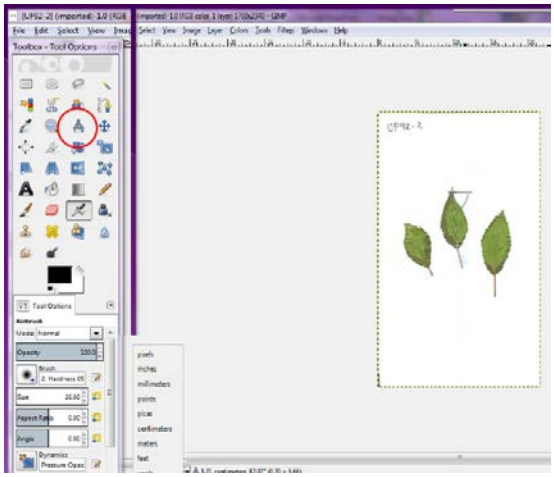

Figure1: Leaf width and length was estimated using the GIMP measure tool with centimetre.

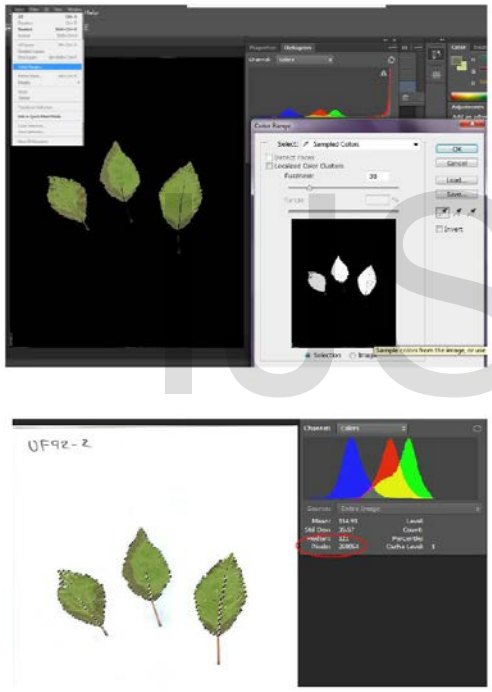

Figure2: Leaf area estimated using Adobe Photoshop CS6 with pixel.

3.3.3. Mapping the progeny: The confirmed SNPs will be used to map the progeny. Primers flanking SNPs were developed for LBD15 and LBD18. Progeny samples were amplified using these primers. The PCR reaction consisted of a denaturing step of 15 min at 95 °C, followed by 35 cycles of 1 min at 94 °C, 1 min at the annealing temperature 59 °C and 1 min at 72 °C, followed by a final elongation step of 20 min at 72 °C. 5ul of PCR product were used with 1ul **MseI** restriction enzyme+ 1ul NEBbuffer4+ 4ul did H_2O) at 37°C (at room temperature to prevent dryness) for 24hours. The excepted pattern in table3

Table 3: The expected size of PCR products after restriction with MseI for genes LBD15 AND LBD18

LBD15 and 18 SNP with restriction enzyme MseI pattern				
Gene	PCR product	Homozygous	Homozygous	Heterozygous
LBD15	248 bps	232 bps 16 bps TGAA	213 bps 19 bps 16 pbs TTAA	232 bps 213 bps 19 bps 16 bps T(TG)AA
LBD18	187bps	122 bps 65 bps CTAA	122 bps 42 bps 23 bps TTAA	122 bps 65 bpS 42 bpS 23 bpS (CT)TAA

4. CONCLUSION

The characterization of LBD family genes responsible for wood lignin might enhance the biofuel processing. The question remain is populous tree the best source for biofuel after genetic modification. Is this approach the best for biofuel production or this will threatened tree forest? Finally, we conclude that the LBD gene modifications might be the best tool for plants that are not used for food or perineal plants.

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