Project Title:

De novo assembly of rubber transcriptome

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Background and Mission:

Natural rubber (*cis*-1.4-polyisoprene) is an essential raw material for many industrial applications, such as heavy duty tires and medical devices. The family Euphorbiaceae is a large family consists of rubber-producing crops, with about 280 genera and 80000 species (Verheye, 2005). Among 2000 rubber producing species, *Hevea brasiliensis* (Willd, IexI A. Juss.) Mull. Arg. is the only species that gives commercially viable quantities of high quality rubber. The unique qualities of natural rubber such as longer chain length, resilience, elasticity, impact and abrasion resistance, efficient heat dispersion and malleability could not be replaced by any other synthetic rubber (Li *et al.*, 2012).

Global demand for natural rubber is increasing at an alarming rate and is predicted to be 12 million tons in 2020 (Smit and Burger, 1994). Despite the rapid increase in global demand of rubber, the production of rubber is relatively low. The target yielding potential of rubber tree is 10000 kg/per ha/ year. However, the yield potential of current rubber clones is 3000 kg per ha per year which is far below than the target potential. Thus, it is crucial to increase the rubber production in order to meet rising natural rubber demands, or else, the prices for the rubber related products will have a steep escalation. Now, the general metabolic pathway of rubber biosynthesis is clear and genes involved have been identified. However, molecular regulation of rubber biosynthesis and the functional genomics studies are still in infancy. With the detailed understanding of the molecular mechanism and the identification of key genes involved, we could improve rubber clones via genetic engineering and breeding technologies.

Research Goals:

- To evalute the gene expression profiling between control rubber clone (RRIM 600), high-latex yielding clone (PB350) and non-latex producing clone .
- To identify the key genes that regulate rubber biosynthesis.

Results & Discussions:

Latex Samples: RRIM 600 (control) VS PB350 (High-latex yield)

Genes that are up-regulated in PB350

RICC Usage Report for Fiscal Year 2013

1) Transcription factor and protein synthesis

- zinc finger ccch domain-containing protein 48-like
- zinc finger protein constans-like protein
- zinc finger protein zat9-like
- transamine
- hydrolase family protein
- probable dolichyl pyrophosphate glc1man9 c2 alpha- -glucosyltransferase-like
- probable carboxylesterase 2-like

2) Signal Transduction

- sarcoplasmic reticulum histidine-rich calcium-binc
- serine threonine-protein kinase cbk1-like
- serine threonine-protein kinase pbs1
- serine threonine-protein kinase sepa-like
- serine threonine-protein kinase tor-like
- serine threonine-protein kinase tor-like isoform 2
- phospholipase d alpha

3) Cell Structure Growth and Division

- cyclin family isoform 1
- auxin response factor 25-like
- auxin-induced protein 5ng4-like
- villin-4-like
- glucan endo--beta-glucosidase 7-like

4) Defense and stress

- disease resistance protein at3g14460-like
- disease resistance protein rga3-like

5) Protein destination and storage

- ubiquitin carboxyl-terminal
- ubiquitin carrier protein
- ubiquitin ligase e3
- ubiquitin ligase e3
- ubiquitin ligase e3
- ubiquitin-like modifier-activating enzyme atg7-like

Up-regulation in PB350

1) Transcrition factor & protein synthesis

- 60s acidic ribosomal protein
- 60s acidic ribosomal protein
- 60s ribosomal protein
- 60s ribosomal protein l13
- 60s ribosomal protein l15
- 60s ribosomal protein l19-2-like
- 60s ribosomal protein l23
- 60s ribosomal protein l29
- 60s ribosomal protein l30
- 60s ribosomal protein l30-like
- 60s ribosomal protein l32-1-like
- 60s ribosomal protein l34-like
- 60s ribosomal protein l39
- 60s ribosomal protein l44-like
- 40s ribosomal protein
- 40s ribosomal protein
- 40s ribosomal protein s13-like
- 40s ribosomal protein s13-like
- 40s ribosomal protein s15a-1-like
- 40s ribosomal protein s21
- 40s ribosomal protein s23-1
- 40s ribosomal protein s23-1
- 40s ribosomal protein s23-1
- 40s ribosomal protein s27-2-like
- 40s ribosomal protein s7-like
- 40s ribosomal protein s7-like protein
- 40s ribosomal protein s9-2-like
- 40s ribosomal protein s9-2-like
- 40s ribosomal protein sa-like
- 50s ribosomal protein
- 50s ribosomal protein chloroplastic-like
- 50s ribosomal protein chloroplastic-like
- 50s ribosomal protein l4-like
- ethylene responsive transcription factor 2b
- ethylene-responsive transcription
- ethylene-responsive transcription factor 4
- ethylene-responsive transcription factor 4-like
- ethylene-responsive transcription factor rap2-4-like
- ethylene-responsive transcription factor rap2-7-like
- ethylene-responsive transcription factor rap2-7-like

- ethylene-responsive transcription factor wri1
- ethylene-responsive transcription factor wri1
- ethylene-responsive transcription factor wri1
- eukaryotic translation initiation factor 3
- eukaryotic translation initiation factor 3
- eukaryotic translation initiation factor 5a2
- glycine-rich rna-binding
- glycine-rich rna-binding protein mitochondrial-like

- chy and ctchy and ring-type zinc finger protein

- myb family transcription factor
- myb-related protein myb4-like
- Ap2 domain transcription factor
- Ap2/Erf and b3 domain-containing transcription repressor tem1-like
- Ap2/Erf domain-containing transcription factor
- homeobox-leucine zipper protein athb-15-like
- homeobox-leucine zipper protein athb-6-like
- homeobox-leucine zipper protein hat14-like
- homeobox protein knotted-1-like 3-like
- homeobox leucine zipper family protein
- calmodulin binding
- calmodulin-like protein 5-like
- homeobox leucine zipper family protein
- homeobox protein knotted-1-like 3-like
- homeobox protein knotted-1-like 7-like
- homeobox-leucine zipper protein athb-15-like
- homeobox-leucine zipper protein athb-6-like
- homeobox-leucine zipper protein hat14-like
- dna-directed rna polymerase ii subunit rpb11-like

2) signal transduction

- probable inactive receptor kinase at1g48480-like
- probable linoleate 9s-lipoxygenase 5-like
- probable lrr receptor-like serine threonine-protein kinase at5g63710-like
- plasminogen activator inhibitor 1 rna-binding
- annexin 1
- annexin d4-like
- annexin-like protein rj4
- rac gtpase activating protein
- ran gtpase binding
- ran gtpase binding

- ran gtpase binding
- brassinosteroid receptor
- brassinosteroid receptor
- lysine histidine transporter 1-like

3) cell, structure, growth and division

- cyclin delta-3
- gibberellin-regulated protein 3
- glucan endo- -beta-glucosidase
- glucan endo- -beta-glucosidase 14-like
- glucan endo- -beta-glucosidase 2-like
- glucan endo- -beta-glucosidase 3-like
- glucan endo- -beta-glucosidase 3-like
- glucan endo- -beta-glucosidase 7-like
- profilin
- caffeic acid o-methyltransferase
- caffeoyl- o-methyltransferase

4) Defense and stress

- chloroplast small heat shock protein
- chaperone protein dnaj
- chaperone protein dnaj 10-like
- dehydrin
- disease resistance protein
- disease resistance-responsive family protein
- probable -trehalose-phosphate synthase
- s-adenosylmethionine synthase
- cytosolic class i small heat shock protein type 1
- cytosolic class i small heat shock protein type 2
- heat shock 70 kda
- heat shock factor
- heat shock factor protein hsf30-like
- heat shock protein
- heat shock protein 70
- heat shock protein binding
- heat shock protein binding
- heat stable protein 1
- heat stress transcription factor a-6b-like
- heat stress transcription factor a-6b-like

5) Rubber Biosynthesis

- 1-deoxy-d-xylulose 5-phosphate synthase (DXS)
- 3-hydroxy-3-methylglutaryl reductase (HMGR)
- diphosphomevalonate decarboxylase
- Cis-prenyl transferase
- Rubber elongation factor
- Small rubber particle protein

6) Transporters & intracellular traffic

- sucrose transporter 1
- membrane magnesium transporter-like
- adp-ribosylation factor gtpase-activating protein agd3-like
- adp-ribosylation factor-like protein 8a
- adp-ribosylation factor-like protein 8a
- mitochondrial adenine nucleotide transporter adnt1-like
- mitochondrial carnitine acylcarnitine carrier protein cacl-like

7) Protein destination & storage

- membrane-anchored ubiquitin-fold protein
- probable e3 ubiquitin-protein ligase rnf144a-like
- ubiquitin carboxyl-terminal hydrolase
- ubiquitin carrier protein
- ubiquitin carrier protein
- ubiquitin extension protein
- ubiquitin extension protein
- ubiquitin family protein
- ubiquitin-60s ribosomal protein l40-like
- ubiquitin-conjugating enzyme
- ubiquitin-conjugating enzyme e2 19-like
- ubiquitin-conjugating enzyme e2 35
- ubiquitin-like protein 5
- ubiquitin-like protein atg12-like
- ubiquitin-like protein smt3
- ubiquitin-like protein smt3
- heat shock factor protein hsf30-like
- heat shock protein
- heat shock protein 70
- heat shock protein binding
- heat shock protein binding
- heat stable protein 1

The up regulation of sucrose transporter gene in PB350 indicated an enhanced surcrose loading to the laticifers of PB350. The efficient sucrose loading into laticifers is crucial for increasing rubber productivity.

The capability of latex regeneration between two consecutive tappings is important for latex productivity. Latex regeneration involve multiple biological process: regulation of transcription and protein synthesis, transporter& intracellular trafficking, signalling pathway. Most of the genes involved in transcription and protein synthesis are abundant in PB350, indicate that during latex regeneration in PB350, an increase gene expression and protein synthesis occurs in latex of PB350. Besides, most of the genes involved in signaling pathways are also up-regulated in latex of PB350. Stimulation of multiple signaling pathways (ethylene, jasmonate and wounding) enhance the rubber productivity.

The up-regulation of protein ubiquitin-mediated protein degradation & protein degradation related signaling pathways, indicating higher rate of protein turnover is important for higher metabolism in laticifers of PB350 which in turn contribute to high latex yield.

The expression several rubber biosynthesis genes in PB350 indicate that the high expression of these genes may incease the rubber productivity.

Conclusion

This project report markedly different yield levels of rubber trees via a global transcription comparison and analysis. The differential expression level of genes identified could give insight into the molecular mechanism underlying the regulation of rubber biosynthesis as well as indetify the genes associated with high latex yiedling. Besides, further RNA-seq analysis will be performed on various tissues on RRIM 600 to gain the overview of transcriptome of RRIM600 in order to provide a platform for the study of mechanism/enzymes determining the high molecular weight of rubber.

Schedule and Prospect for future

1) March 2014 – May 2014

   RNA-seq analysis on low latex yielding clones

2) June 2014 – August 2014

   RNA-seq analysis on various tissues

3) September 2014 – February 2015

   Full length cDNA analysis

References

Li, D., Deng, Z., Qin, B., Liu, X., and Men, Z. (2012). De novo assembly and characterization of bark transcriptome using Illumina sequencing and development of EST-SSR markers in rubber tree (Hevea brasiliensis Muell. Arg.). *BMC Genomics* **13**: 192 - 222.

Smit, H.P. and K. Burger. (1994). *Natural rubber markets: analysis and outlook.* Proc. Int. Rubber Forum, Colombo, Sri Langka. Secretariat of the International Rubber Study Group, Wembly, London.

Verheye, W. (2005). Growth and production of rubber. Soils, Plant growth and crop production,Vol II in *Encyclopedia of Life Support Systems (EOLSS)*, Developed under

the Auspices of the UNESCO, Eolss Publishers, Oxford, UK. [http://www.eolss.net].

Fiscal Year 2013 List of Publications Resulting from the Use of RICC

[Publication]

[Proceedings, etc.]

[Oral presentation at an international symposium]

1) Current status and future prospect of rubber research. 第８回長野ミーティング：生物資源の有効利用を目指して, 2nd – 4th March 2014. , Happone, Nagano, Japan.
2) Full length cDNA Analysis and Gene expression studies in *Hevea brasiliensis*. 13th December 2014. Universiti Sains Malaysia, Penang.

[Others]

1) Expression Profiling of Rubber Biosynthetic Genes based on RNA-seq Analaysis of *Hevea brasiliensis* Latex-yielding Clones. Noyori Summer School program, 6th-7th September 2013, Nichi-I Gakkan Kobe Port Island Center, Japan. (Poster presentation)