The outcomes from this project are summarized in 7 peer reviewed publications that are listed below with their respective abstracts.

1. Chen, Yiru, Yordan Yordanov, Cathleen Ma, Steven Strauss and **Victor Busov**. . (2013) DR5 as a reporter system to study auxin response in *Populus* Plant Cell Reports 32:453-463

Auxin responsive promoter DR5 reporter system is functional in Populus to monitor auxin response in tissues including leaves, roots, and stems. We described the behavior of the DR5::GUS reporter system in stably transformed Populus plants. We found several similarities with Arabidopsis, including sensitivity to native and synthetic auxins, rapid induction after treatment in a variety of tissues, and maximal responses in root tissues. There were also several important differences from Arabidopsis, including slower time to maximum response and lower induction amplitude. Young leaves and stem sections below the apex showed much higher DR5 activity than did older leaves and stems undergoing secondary growth. DR5 activity was highest in cortex, suggesting high levels of auxin concentration and/or sensitivity in this tissue. Our study shows that the DR5 reporter system is a sensitive and facile system for monitoring auxin responses and distribution at cellular resolution in poplar.

2. Rigal, Adeline, Yordan S. Yordanov, Anna Karlberg, Emilie Tisserant, Irene Perrone, Catherine Bellini, **Victor B. Busov**, Francis Martin, Annegret Kohler, Rishi Bhalerao, and Valérie Legué. (2012) The *Populus AINTEGUMENTA LIKE 1* homeotic transcription factor Pt*AIL1* controls the formation of adventitious root primordia. Plant Physiol. 160: 1996-2006

Adventitious rooting is an essential but sometimes rate-limiting step in the clonal multiplication of elite tree germplasm, because the ability to form roots declines rapidly with age in mature adult plant tissues. In spite of the importance of adventitious rooting, the mechanism behind this developmental process remains poorly understood. We have described the transcriptional profiles that are associated with the developmental stages of adventitious root formation in the model tree poplar (Populus trichocarpa). Transcriptome analyses indicate a highly specific temporal induction of the AINTEGUMENTA LIKE1 (PtAIL1) transcription factor of the AP2 family during adventitious root formation. Transgenic poplar samples that overexpressed PtAIL1 were able to grow an increased number of adventitious roots, whereas RNA interference mediated the down-expression of PtAIL1 expression, which led to a delay in adventitious root formation. Microarray analysis showed that the expression of 15 genes, including the transcription factors AGAMOUS-Like6 and MYB36, was overexpressed in the stem tissues that generated root primordia in PtAIL1-overexpressing plants, whereas their expression was reduced in the RNA interference lines. These results demonstrate that PtAIL1 is a positive regulator of poplar rooting that acts early in the development of adventitious roots.

3. Zawaski, Christine, Cathleen Ma, Steven H. Strauss, Darla French, Richard Meilan and **Victor B. Busov**. (2012) PHOTOPERIOD RESPONSE 1 (PHOR1)-like genes regulate shoot/root growth, starch accumulation, and wood formation in *Populus* J. Exp. Bot. 63: 5623-5634

This study describes functional characterization of two putative poplar PHOTOPERIOD RESPONSE 1 (PHOR1) orthologues. The expression and sequence analyses indicate that the two poplar genes diverged, at least partially, in function. PtPHOR1_1 is most highly expressed in roots and induced by short days, while PtPHOR1_2 is more uniformly expressed throughout plant tissues and is not responsive to short days. The two PHOR1 genes also had distinct effects on shoot and root growth when their expression was up- and downregulated transgenically.

PtPHOR1_1 effects were restricted to roots while PtPHOR1_2 had similar effects on aerial and below-ground development. Nevertheless, both genes seemed to be upregulated in transgenic poplars that are gibberellin-deficient and gibberellin-insensitive, suggesting interplay with gibberellin signalling. PHOR1 suppression led to increased starch accumulation in both roots and stems. The effect of PHOR1 suppression on starch accumulation was coupled with growth-inhibiting effects in both roots and shoots, suggesting that PHOR1 is part of a mechanism that regulates the allocation of carbohydrate to growth or storage in poplar. PHOR1 downregulation led to significant reduction of xylem formation caused by smaller fibres and vessels suggesting that PHOR1 likely plays a role in the growth of xylem cells.

4. Xia Ye, **Victor Busov**, Nan Zhao, Rick Meilan, Lisa M. McDonnell, Heather D. Coleman, Shawn D. Mansfield, Feng Chen, Yi Li, and (Max) Zong-Ming Cheng. (2011)**.** Transgenic *Populus* trees for forest products, bioenergy, and functional genomics. Critical Reviews in Plant Sciences. 30:5, 415-434

Species within the genus *Populus* are among the fastest growing trees in regions with a temperate climate. Not only are they an integral component of ecosystems, but they are also grown commercially for fuel, fiber, and forest products in rural areas of the world. In the late 1970s, they were designated as a bioenergy crop by the U.S. Department of Energy, as a result of research following the oil embargo. *Populus* species also serve as model trees for plant molecular biology research. In this article, we will review recent progress in the genetic improvement of *Populus*, considering both classical breeding and genetic engineering for bioenergy, as well as in using transgenics to elucidate gene functionality. A perspective for future improvement of *Populus* via functional genomics will also be presented.

5. **Busov, VB**, Yordanov, Y Gou, J, Meilan, R, Ma, C, Regan, S and Steven Strauss (2010) Activation tagging is an effective gene tagging system in *Populus.* Trees Genetics and Genomes. 7: 91-101

Knowledge of the functional relationship between genes and organismal phenotypes in perennial plants is extremely limited. Using a population of 627 independent events, we assessed the feasibility of activation tagging as a forward genetics tool for *Populus*. Mutant identification after 2 years of field testing was nearly sevenfold (6.5%) higher than in greenhouse studies that employed *Arabidopsis* and identical transformation vectors. Approximately two thirds of all mutant phenotypes were not seen in vitro and in the greenhouse; they were discovered only after the second year of field assessment. The trees' large size (5-10 m in height), perennial growth, and interactions with the natural environment are factors that are thought to have contributed to the high rate of observable phenotypes in the field. The mutant phenotypes affected a variety of morphological and physiological traits, including leaf size and morphology, crown architecture, stature, vegetative dormancy, and tropic responses. Characterization of the insertion in more than 100 events with and without mutant phenotypes showed that tags predominantly (70%) inserted in a 13-Kbp region up- and downstream of the genes' coding regions with approximately even distribution among the 19 chromosomes. Transcriptional activation was observed in many proximal genes studied. Successful phenotype recapitulation was observed in 10 of 12 retransformed genes tested, indicating true tagging and a functional relationship between the genes and observed phenotypes for most activation lines. Our studies indicate that in addition to associating mapping and QTL approaches, activation tagging can be used successfully as an effective forward gene discovery tool in *Populus*.

6. Gou, J., Strauss, S.H., Tsai, C.J., Fang, K., Chen, Y., Jiang, X., and **Busov, V.B**. (2010) Gibberellins Regulate Lateral Root Formation in *Populus* through Interactions with Auxin and Other Hormones. Plant Cell **22**:623-639.

The role of gibberellins (GAs) in regulation of lateral root development is poorly understood. We show that GA-deficient (35S:PcGA2ox1) and GA-insensitive (35S:rgl1) transgenic Populus exhibited increased lateral root proliferation and elongation under in vitro and greenhouse conditions, and these effects were reversed by exogenous GA treatment. In addition, RNA interference suppression of two poplar GA 2-oxidases predominantly expressed in roots also decreased lateral root formation. GAs negatively affected lateral root formation by inhibiting lateral root primordium initiation. A whole-genome microarray analysis of root development in GA-modified transgenic plants revealed 2069 genes with significantly altered expression. The expression of 1178 genes, including genes that promote cell proliferation, growth, and cell wall loosening, corresponded to the phenotypic severity of the root traits when transgenic events with differential phenotypic expression were compared. The array data and direct hormone measurements suggested crosstalk of GA signaling with other hormone pathways, including auxin and abscisic acid. Transgenic modification of a differentially expressed gene encoding an auxin efflux carrier suggests that GA modulation of lateral root development is at least partly imparted by polar auxin transport modification. These results suggest a mechanism for GA-regulated modulation of lateral root proliferation associated with regulation of plant allometry during the stress response.

7. **Busov VB,** Brunner AM, Strauss SH. 2008. Genes for control of plant stature and form. *New Phytologist* **177:** 589-607.

Here we summarize progress in identification of three classes of genes useful for control of plant architecture: those affecting hormone metabolism and signaling; transcription and other regulatory factors; and the cell cycle. We focus on strong modifiers of stature and form that may be useful for directed modification of plant architecture, rather than the detailed mechanisms of gene action. Gibberellin (GA) metabolic and response genes are particularly attractive targets for manipulation because many act in a dose-dependent manner; similar phenotypic effects can be readily achieved in heterologous species; and induced pleiotropic effects--such as on nitrogen assimilation, photosynthesis, and lateral root production--are usually positive with respect to crop performance. Genes encoding transcription factors represent strong candidates for manipulation of plant architecture. For example, AINTEGUMENTA, ARGOS (auxin-regulated gene controlling organ size), and growth-regulating factors (GRFs) are strong modifiers of leaf and/or flower size. Plants overexpressing these genes had increased organ size and did not display negative pleiotropic effects in glasshouse environments. TCP-domain genes such as CINCINNATA, and the associated regulatory miRNAs such as miRJAW, may provide useful means to modulate leaf curvature and other foliage properties. There are considerable opportunities for comparative and translational genomics in nonmodel plant systems.