1. FINAL PUBLISHABLE SUMMARY REPORT

The plant body plan consists of three major tissues; called epidermis, ground tissue and the vascular tissues. These different tissues are established very early during embryogenesis. Although our knowledge on how these tissues are maintained and differentiated in a mature plant has increased tremendously in the past few years, virtually nothing is known about how these tissues are initially specified in the embryo. During this 'bHLH-networks' project, we gained significant insights into how one of these major tissues, the vasculature, is initiated in the embryo and how this tissue is maintained in a growing organism post-embryonically, which recently resulted in a high-impact publication in Developmental Cell (De Rybel et al., 2013) and a second story being prepared for submission.

Achievement1: *Establishing an easy and low-cost high-throughput cloning system in Arabidopsis thaliana*

During the whole of the research project, many constructs were created; highlighting the need of an efficient, quick and low-cost cloning system. As classical cloning is very time consuming and commercial alternatives, such as Gateway cloning are very costly, we started by creating a suite of 34 ligation-independent cloning (LIC) vectors for Arabidopsis research. These vectors are readily used in the host laboratory and have been made publically available. Because of the high number of material requests, we anticipate that these vectors will become increasingly used in plant research. This preparatory work resulted in a publication in Plant Physiology (De Rybel et al., 2011).

Achievement2: *A bHLH complex controls embryonic vascular tissue establishment and indeterminate growth in Arabidopsis*

Plants have the remarkable potential for sustained (indeterminate) post-embryonic growth. Following their specification in the early embryo, tissue-specific precursor cells first establish tissues, and later maintain these post-embryonically. The mechanisms underlying these processes are largely unknown. We have defined local control of oriented, periclinal cell division as the mechanism underlying both establishment and maintenance of the vascular tissue. We identified an auxin-regulated bHLH transcription factor dimer as a critical regulator of vascular development. Due to a loss of periclinal divisions, vascular tissue gradually disappears in bHLH-deficient mutants, while ectopic expression is sufficient for triggering periclinal divisions. We showed that this dimer operates independently of tissue identity, but is restricted to a small vascular domain by integrating overlapping transcription patterns of the interacting bHLH proteins. Our work reveals a common mechanism for tissue establishment and indeterminate vascular development, and provides a conceptual framework for developmental control of local cell divisions. This work was recently published in the high-impact journal Developmental Cell (De Rybel et al., 2013).

Achievement3: *A transcriptional and mathematical model for the hormonal control of tissue patterning in Arabidopsis*

Next, we identified the transcriptional targets of the bHLH dimer described above by micro-array analysis and found a cytokinin (CK) activating enzyme. Due to the tissue-specific expression of this gene, we were able to hypothesize a model in which the auxin-dependent bHLH pathway activates local CK biosynthesis, which is required for normal vascular development and maintaining the patterning in a growing organ. We are currently performing a broad range of experiments to verify this model. As some of the experiments are difficult to perform *in planta*, we started collaborating with the group of Christian Fleck (Wageningen University) to make a mathematical model of this system. At the moment, a PhD student (Milad Adibi) is working full-time to build the *in silico* model and test if our hypothetical model is sufficient to produce the hormonal interactions we observe in a growing tissue. A first draft of this work has been written down and will be submitted before the end of the year.

Achievement4: *Establishing an international collaborative network*

During the bHLH-networks project, we have established a growing international collaborative network of high-level scientists with a key interest in vascular and/or embryonic development. This allows us to exchange methodologies and materials to facilitate our research. Within Wageningen University, we collaborate with the groups of Christian Fleck (mathematical modeling) and Guido Hooiveld (micro-arrays). Internationally, we have close collaborations with the groups of Doris Wagner (ChIP analysis), Ykä Helariutta and Annelie Carlsbecker (vascular development), Tom Beeckman (FACS cell sorting), Karin Ljung and Ondrej Novak (hormone measurements) and Richard Smith (3D confocal reconstruction). We contribute our knowledge on IP-MS, FRET-FLIM and embryo development to these labs.