Overall summary and statistics BARD Project

Number: 3767

Title: ________________________________

Affiliated Institutions:

Yuval Eshed __________________________ Weizmann Institute of Science _____________________
John Bowman __________________________ UC Davis ________________________________

Start Date of Project: 9/2005 ______________

Date of Submission of Report: 1/2009 __________

First Annual Report: 9/2006 ________

Signature ____________________________
Principal Investigator (PI)

Signature ____________________________
Institution’s Authorizing Official, Principal Institution
BARD Project Number:

Date of Submission of the report: 1/2009

Project Title:

Investigators                          Institutions

Principal Investigator (PI):

Yuval Eshed     WIS

Co-Principal Investigator (Co-PI):

John L. Bowman       UC Davis

Collaborating Investigators:

__________________________________________________________

__________________________________________________________

Signature                  Signature
Principal Investigator     Authorizing Official, Principal Institution

Keywords: Abaxial, Adaxial, Development, Transcription Factors

Abbreviations commonly KANADI-KAN, ETTIN-ETT, Auxin Response Factor – ARF, micro RNA – miRNA, PHABULOSA –PHB.

Budget:  IS: 150K$       US: 150K$       Total: 300K$
Publication Summary (numbers)

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Postdoctoral Training: Anat Izhaki (UCD) - Cooperation

Summary (numbers)

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Description Cooperation:
During the research period, extensive communication and cooperation between partners was maintained. Apart from exchange of information on a regular basis, both labs took part in the selection of genotypes to be analyzed by Affimetrix chips and for mutant screens. Genes, transgenic and mutant lines were exchanged on a regular basis. John Bowman and his Postdoc Anat Izhaki visited the Eshed’s lab twice and Yuval Eshed visited Davis once. Apart from seminars, touring the country a bit, and meeting colleagues, a few days’ meetings between the American PI, and the Eshed’s lab students took place and vice versa.

Patent Summary: None
Abstract

**Background and objectives:** Manipulation of plant organ growth is one of the primary reasons for the success of mankind allowing increasing amounts of food for human and livestock consumption. In contrast with the successful selection for desirable growth characteristics using plant breeding, transgenic manipulations with single genes has met limited success. While breeding is based on accumulation of many small alterations of growth, usually arise from slight changes in expression patterns, transgenic manipulations are primarily based on drastic, non-specific up-regulation or knock down of genes that can exert different effects during different stages of development. To successfully harness transgenic manipulation to attain desirable plant growth traits we require the tools to subtly regulate the temporal and spatial activity of plant growth genes. Polar morphology along the adaxial/abaxial axis characterizes lateral organs of all plants. Juxtaposition of two cell types along this axis is a prerequisite of laminar growth induction. In the study summarized here, we addressed the following questions:

1) Can we identify and harness components of the organ polarity establishment pathway for prolonged growth?

2) Can we identify specific regulatory sequences allowing spatial and temporal manipulation in various stages of organ development?

3) Can we identify genes associated with YABBY-induced growth alterations?

**Major conclusions and implications:**

We showed that regulated expression, both spatially and temporally of either organ polarity factors such as the YABBY genes, or the organ maturation program such as the CIN-TCPs can stimulate substantial growth of leaves and floral organs. Promoters for such fine manipulation could be identified by comparison of non-coding sequences of KAN1, where a highly conserved domain was found within the second intron, or by examination of multiple 5” regions of genes showing transient expression along leaf ontogeny. These promoters illustrate the context dependent action of any gene we examined thus far, and facilitate fine tuning of the complex growth process.

**Implications, both scientific and agricultural.**

The present study was carried out on the model organism Arabidopsis, and the broad application of its findings were tested in the tomato crop. We learned that all central regulators of organ polarity are functionally conserved, probably in all flowering plants. Thus, with minor modifications, the rules and mechanisms outlined in this work are likely to be general.
Achievements

1) Identification and harnessing components of the organ polarity establishment pathway for prolonged growth.

In the course of this study, we have analyzed the role of three types of factors in initiation and growth of Arabidopsis leaves: 1) Prime initiators of organ polarity, primarily KANADI. These factors are regulate the direction and magnitude of auxin flux into developing primordia, a morphogenetic process underlying the differentiation of primordia from the adjacent SAM (Izhaki and Bowman, 2007). 2) Factors translating primordia polarity into an “organ, not SAM” identity, and as a result, initiators of lamina growth. These factors are represented by the YABBY factors that play a role in abaxial identity, maintenance of the adaxial program and initiation of a signaling cascade back to the adjacent SAM (Goldshmidt et al., 2008; Sarojam et al., 2009). 3) Factors translating organ polarity and lamina initiation into a sequential lamina differentiation program, that entails numerous sequential steps gradually leading into organ differentiation and growth arrest. These factors are best illustrated by the CIN-TCPs, eight related factors that are activated in the fast growing lamina shortly after initial primordia expansion (Efroni et al., 2008).

Our studies provide a new look at organ initiation and growth, and provide a coherent framework for the role of numerous other factors acting in initiating organs. Moreover, during the course of these studies, detailed analysis of the wild type leaf transcriptome, combined with analyses of several genotypes impaired in lamina growth was made public. These include abaxialized $ANT>>KAN2$ and $fil yab3 yab5$ plants, and the adaxialized $kan1 kan2$ and $ANT>>AS2$.

Using the conceptual framework developed, several types of manipulations were used to stimulate organ growth, in an unparalleled magnitude. Slightly earlier than normal activation of either FIL or YAB3 in a primordia specific manner, using the KAN1 promoter, stimulated leaves 150% larger than normal, and same was found in all floral organs. Combined with the lack of lamina un plants lacking FIL, YAB3 and YAB5 simultaneously, it is clear that YABBY genes play a pivotal role in organ growth (Sarojam et al., 2009). In contrast, precocious activation of the primordia factors CIN-TCPs lead to premature froth arrest and minute organs. However, a delay in their activation, stimulated by a brief expression of their common negative regulator miR319 caused again, a massive increase in leaf size, up to five times more than normal leaves (Efroni et al., 2008).
2) Identification of specific promoters allowing spatial and temporal manipulation during various stages of organ development.

Approaches for identification of either abaxial-specific or stage specific cis regulatory elements were based on types of data: 1) identification of evolutionary conserved motifs and functional analysis of selected promoters, primarily, pKAN1. 2) Identification of factors expressed during specific time window along leaf ontogeny. We have concentrated on the KAN1 promoter, as this promoter allowed significant growth stimulation upon activation of either YAB3 or miR319 as described above. Comparing the 5’ region of pKAN1 from several Rosids, Aspen, Medicago and Arabidopsis failed to reveal significantly conserved domain. In a sharp contrast, A highly conserved domain of 75bp was found in the second intron of KAN1 sequences of Arabidopsis, Medicago, Aspen, Tomato and Mimulus. As these species represent nearly all members of the eudicots, this box is conserved for over 100 million years.

We have previously noted that KAN1 negatively regulate its own expression, as illustrated by the increased KAN1 RNA expression in kan1 or kan1 kan2 mutant plants. In agreement, a 6 kb of 5’ KAN1 promoter sequence could stimulate transient and abaxial expression in leaf primordia. Yet this promoter stimulated production of radial leaves upon transactivation of KAN1. When the 900bp of the second intron were added in front of the above mentioned 6kb, similar expression was evident, but no stimulation of arrested leaves was evident upon KAN1 trans activation. Thus, we conclude that this motif is responsible for the negative regulation of KAN1 by itself.

Using the dynamic expression database if leaf ontogeny, we have examined expression of 15 different promoters, and chose a subset of five that allow expression in the lamina, at given time interval following leaf initiation. These include pGH3.3, pBOL, pBLS, p7470 and p650. Using these promoter set, a window of “competence to respond”, can be defined for each and every gene. Indeed, thus far, any factor involved in leaf development has shown a different window, and the resulting approach illustrate the potential benefits of temporally based gene manipulations.

3) Identification of genes associated with YABBY-induced growth.

Here too, two approaches were successfully employed: 1) A genetic screen aimed at factors required for effects stimulated by ectopic YABBY expression. 2) Identification of factors absent in the lamina-missing fil yab3 yab5 triple mutant plants. By the first approach we have identified factors involved in YABBY mediated signaling, such as LAS (Goldshmidt et al., 2008) but also YABBY mediated growth. Here, the prime factor discovered was LUG, a previously identified YAB protein partner. We did find however that removal of LUG and its
related factor LUH from organ primordia (using time expression on an amiR-LUG/H) completely abolish lamina growth, as was found for yab mutants too. In the transcription survey, over 500 transcripts that are down regulated in the triple mutant seedlings were identified, many in common with those identified in the non-expanding \textit{phb-1d} seedlings. Most notably, all members of the CIN-TCP family were strongly down regulated in lamia-less mutant leaves. As we already demonstrated that transient delay in these TCPs expression can stimulate giant leaves (Efroni et al., 2008). Given these results, future studies will focus on the association between the YABBY and these TCPs.

**Details of cooperation: whether and how project objectives were promoted as a result of the cooperation.**

During the research, extensive communication and cooperation between partners was maintained. Apart from exchange of information on a regular basis, both labs took part in the selection of genotypes to be analyzed by Affimetrix chips and for mutant screens. Genes, transgenic and mutant lines were exchanged on a regular basis. John Bowman and his Postdoc Anat Izhaki visited the Eshed’s lab twice and Yuval Eshed visited Davis once. Apart from seminars, touring the country a bit, and meeting colleagues, a few days’ meetings between the American PI, and the Eshed’s lab students took place.

**List of publications**


**Under review:**


7/31/19 Appendix G6
Appendix

Published papers.