Final Scientific Report

Cover Page

BARD Project Number: IS-4249-09

Date of Submission of the report: 11/2012

Project Title: Exploring general and specific regulators of phase transitions for crop improvement.

Investigators

Principal Investigator (PI):
Yuval Eshed

Co-Principal Investigator (Co-PI):
Sarah Hake

Collaborating Investigators:
George Chuck

Institutions

WIS, Rehovot, Israel

PGEC, USDA, Albany, CA

University of California, Berkeley CA

Keywords not appearing in the title and in order of importance. Avoid abbreviations.
Phase change, juvenile to adult transition, plant architecture

Abbreviations commonly used in the report, in alphabetical order:
corngrass – Cg, Micro RNA – miR, Shoot apical meristem – SAM

Budget: IS: 150,000$ US: 150,000$ Total: 300,000$

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Principal Investigator

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Authorizing Official, Principal Institution

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Publication Summary (numbers)

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Postdoctoral Training:

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Description Cooperation:
Regular communication and cooperation between partners was maintained throughout. The mutual visit planed to the third year was postponed to continuation period. Yet, electronic communications allowed the exchange of information in the form of raw data, images of manipulated plants and unpublished manuscripts.

We chose not to write joint manuscript as the two labs work on different experimental platforms; grasses in the US and Solanaceae in Israel. Once a clearer picture of common and unique responses of the different plants so general phase change programs will emerge, a joint publication will be pertinent.

Patent Summary (numbers)

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Appendix G6b
Abstract

The transition of plants from a juvenile to adult growth phase entails a wide range of changes in growth habit, physiological competence and composition. Strikingly, most of these changes are coordinated by the expression of a single regulator, micro RNA 156 (miR156) that coordinately regulates a family of SBP genes containing a miR156 recognition site in the coding region or in their 3’ UTR.

In the framework of this research, we have taken a broad taxonomic approach to examine the role of miR156 and other genetic regulators in phase change transition and its implication to plant development and crop improvement.

We set to:

A) Determine the common and unique factors that are altered upon juvenile to adult phase transition.

B) Determine the functions of select miR156 target genes in tomato and maize, and identify those targets that mediate phase transition.

C) Characterize the role of miR172 and its targets in tomato phase change.

D) Determine the relationships between the various molecular circuits directing phase change.

E) Determine the effects of regulated manipulation of phase change genes on plant architecture and if applicable, productivity.

In the course of the study, a new technology for gene expression was introduced – next generation sequencing (NGS). Hence some of the original experiments that were planned with other platforms of RNA profiling, primarily Affymetrix arrays, were substituted with the new technology. Yet, not all were fully completed.

Moreover, once the initial stage was completed, each group chose to focus its efforts on specific components of the phase change program. The Israeli group focused on the roles of the DELAYED SYMPODIAL TERMINATION and FALSIFLORA factors in tomato age dependent programs whereas the US group characterized in detail the role of miR156 (also termed Cg) in other grasses and in maize, its interplay with the many genes encoding miR172.
Background to the topic
Age dependent programs modify shoot branching, leaf morphology, sensitivity to day length signals and many additional plant characteristics. The Hake lab pioneered the study of miR156 and miR172 in these processes in maize. It was subsequently shown that the same factors are pivotal for the regulation of age dependent programs in Arabidopsis. In the course of this BARD project, the role of miR156 (Cg) in age dependent programs was extended to other species as well, several grasses as well as members of the Solanaceae family (potato, tomato and tobacco). However, the developmental programs regulated by miR156 and its SBP targets are not known. Moreover, in some species age dependent programs direct flowering, whereas in others they are more pronounced in the distribution of trichomes at specific sides of the leaf. Thus, it is not clear how “universal” responses to the same “universal” regulators are implicated in species-specific morphological and physiological responses. We thus established a comparative genetic and genomic experimental platform to understand the regulation of age dependent programs in several distantly related species, representative of monocot and dicot plants.

Achievements
Significance of main scientific achievements or innovations
A key to success in a crop plant is a partitioning of the different stages of growth; for example, early vegetative growth permits establishment of robust plant growth with enough biomass to support the subsequent reproductive phase. Partitioning of the different stages is regulated by intrinsic signals and external cues that are translated into species-specific programs resulting in diverse shoot architectures. During our BARD funding, we showed that miR156 is a general regulator of the juvenile to adult transition (Figure 1), and its effects are universal (in monocots and dicots) but with species-specific manifestations. For example, in all plants we tested, flowering is delayed upon miR156/Cg1 over expression, but in switchgrass and potato it is completely abolished. Likewise, expression of lignin biosynthesis genes as well as lignin content is greatly reduced in “juvenilized” plants, which favors switchgrass digestibility (Chuck et al., 2011) but impairs resistance of tomato stems to fruit load.
A second scope of our study was to break down the effects of miR156/Cg1 on shoot development via characterization and exploitation of its downstream targets, which we anticipated would have more specific effects. To this end, efforts in maize were focused on isolation of mutations in specific SBP genes that were known Cg1 targets. Loss-of-function
mutations of one of these, \textit{tasselsheath4} (tsh4) resulted in de-repression of leaf primordia within the inflorescence, indicating that \textit{tsh4} primarily functions as a floral leaf repressor (Chuck et al. 2010). Knock-out mutations of a pair of duplicated \textit{SPL} target genes closely related to \textit{tsh4}, \textit{zmSBP5} and \textit{zmSBP7}, caused plants to produce extra vegetative leaves and tillers (Chuck et al. manuscript in preparation). Thus, \textit{zmSBP5} and \textit{zmSBP7} function to repress rate of leaf initiation, but only within the vegetative phase, while \textit{tsh4} represses leaf initiation within the floral phase. Consistent with this, antibodies developed to \textit{zmSBP5} and \textit{zmSBP7} localized both proteins to the peripheral zone of the meristem where leaf primordia initiate.

In tomato, analyses of four different SPLs using misexpression of miR156 insensitive forms failed to stimulate specific changes in flowering time or shoot morphology, while expression of a miR156 decoy, MIM156, resulted in a major change in cotyledon morphology and precocious formation of mature leaves suggesting that other, untested SPLs may be involved. Strikingly, the cotyledons of plants with low miR156 activity (35S:MIM156) had unique leaf-like characteristics – much larger dimensions and deep lobbing. Such a phenotype was not detected in a large-scale mutant screen of recessive seedling mutants. However, in an activation-tagging screen such a dominant mutation was identified, and analysis of the underlying gene demonstrated that activation of the FALSIFLORA (LFY ortholog) was
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responsible (MacAlister et al., 2012). Significantly, studies in Arabidopsis suggested that specific SBP genes can activate LFY. We suggest therefore that LFY regulation, at least during the seedling stage, may be a common target of the miR156 program in dicot plants.

In addition, we completed the map-based cloning of the tomato mutant dst that has a prolonged juvenile phase and delayed flowering characteristics, and found it to encode an uncharacterized pseudo kinase with a single homolog in all plant genomes examined. The characterization of this kinase, its potential role in shoot maturation and its position relative to the miR156 pathway is ongoing in Rehovot.

The microarray technology we used in the current study to assay for common miR156 targets and the use of analytical and histological methods facilitated the identification of lignin biosynthesis as a common miR156 target in dicot and monocot plants. We will continue to exploit the miR156/Cg1 over expressing lines we generated to determine the extent of miR156-dependent and independent changes, and to isolate directing age-dependent expression processes. We anticipate that adding them to the plant manipulation toolkit, we will facilitate specific changes in age dependent programs that will permit desirable changes in fruit, grains and biofuel crops.

Agricultural and/or economic impacts of the research findings

Our results demonstrate that by altering any aspect of the miR156 pathway, not only it is possible to alter plant architecture, but also biochemical properties, biomass properties, and flowering time. Indeed, the UB::Cg1 switchgrass plants fail to flower, and in doing so, accumulate starch in the stem. This starch can be obtained as an energy source without pretreatment (Chuck et al., 2011). Further experiments are aimed at reducing starch degradation in the Cg1 switchgrass plants, thus further increasing starch levels. In addition, field experiments are in progress that test different promoters driving Cg1 to find the optimal combination of increased biomass, yet improved digestibility.

In addition, overexpression of the maize Cg1 cDNA in poplar resulted in a 35% reduction in total lignin content. Since high lignin content limits the ability of biomass to be broken down into simple sugars, these transgenic plants will be easier to deconstruct and synthesized into biofuels.

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

The primary goal of the project was to identify common regulators of the miR156/Cg1...
pathway. Thus, the individual partners could not achieve this goal. Moreover, identifying common targets promoted the confidence each group had in its independent target list, and as a consequence, in follow up experiments. Significantly, this worked formed the foundation for an ongoing collaboration under the auspice of a renewed BARD grant, which we hope, will be as fruitful.

List of Publications:

