Identification of Novel Growth Regulators in Plant Populations Expressing Random Peptides

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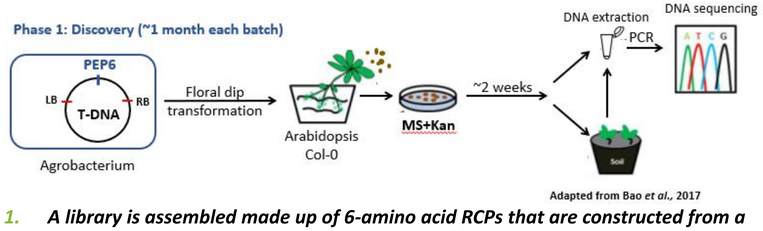
ABSTRACT

- The need for new herbicidal compounds, that selectively target certain plants as opposed to others, is steadily increasing due to the demand for environmentally friendly chemicals within agriculture, and the increasing of weeds' chemical resistance. This project utilizes rapidly screened populations of *Arabidopsis thaliana* where individual plants have been transformed with a library of a small, novel, random cyclical peptides (RCP) that have the potential to disrupt the plant's growth and development. These molecules, constructed from a degenerative DNA oligonucleotide sequence PCR reaction, could be essential for the creation of new herbicides. Currently, around 25,000 seedlings have been selectively screened on minimal media with Kanamycin, since the vector contains a Kanamycin resistance gene. Confirmation of RCP insertion into the plant is done in a two-step procedure. First, seedlings are screened under a fluorometric microscope for detection of GFP. Then, the DNA is extracted from the GFP- positive seedlings and the transgene presence and sequence is confirmed by PCR and Sanger sequencing. Almost 100 underperformers, vitrified, and GFP-positive seedlings that carry different RCP peptides have been identified to date. The next step is to re-clone these peptides into healthy plants using *Agrobacterium tumefaciens* and confirm reproducible underperforming phenotype. Finally, a specific mechanism of action for the peptides in the plant must be found along with expanding the population to identify new targets.

OBJECTIVES

- To discover potential small cyclical peptides that, when inserted into *Arabidopsis*, disrupts growth and development for the development of novel herbicides.
- Hight-Throughput strategies like Chemical Genomics allow to screen for a great number of compounds thus obtaining multiple candidates for further study.
- Confirm underperforming phenotype due to random peptide insertion using fluorometric detection and PCR amplification.

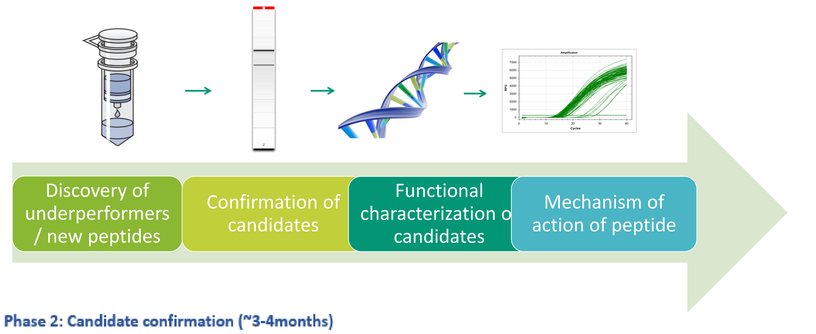
METHODS



1. ***A library is assembled made up of 6-amino acid RCPs that are constructed from a degenerative DNA oligonucleotide sequence PCR reaction.***
2. ***Library is recombined into the pK7WG2D vector and transformed into E.coli.***
3. ***Agrobacterium tumefaciens GV3101 is transformed with the binary vectors using Gateway Recombination Cloning Technology.***
4. ***Wild type (Col-0) Arabidopsis is transformed via floral dip of the Agrobacterium, and F2 seeds collected from these mutants.***
5. ***F2 mutant seeds are plated on minimal media (MS) and Kanamycin. Each Batch was 18 plates with 0.1g of total Arabidopsis seed.***
6. ***Seedlings that displayed underperforming phenotypes (pale pigmentation, stunted growth, etc.) were chosen from each plate to confirm GFP-containing insert under a UV light microscope.***
7. ***DNA extraction, PCR confirmation, and preparation for Sanger Sequencing.***
8. ***The same sequence is then re-introduced into new transgenic lines to test for recapitulation of the original phenotype (Phase 2).***
9. ***Larger KAN resistant seedlings are transplanted in soil at 20°C under 16 hour light / 8 hour dark conditions. Plants exhibiting phenotypes were tagged and monitored for atypical growth throughout their development.***

METHODS CONT.

Transformation and isolation of transgenic Arabidopsis plants



RESULTS

GFP-positive underperformers

Mutant 485 | Mutant 363 | Mutant 375

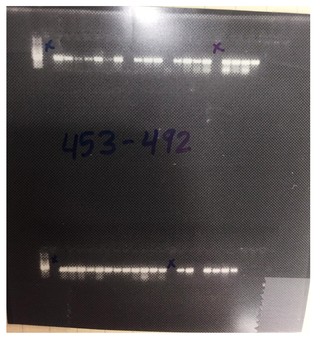
To date, 6 Batches (>25,000 seeds) have been selectively screened. Over 500 GFP-positive seedlings were isolated, and almost 100 of those have been confirmed to contain the RCP insert via PAGE and have been sent for sequencing. It is these peptide candidates that will be taken forward into Phase 2 re-cloning.

RESULTS CONT.

Transplanting of KAN-resistant seedlings to soil

Arabidopsis underperformers in soil after 2 weeks after transplanting. Red arrows indicate severe phenotype that continued in soil. DNA was extracted from these plants.

PAGE of PCR products from DNA isolation of potential peptides



An approximately 500-bp DNA fragment containing the random peptide open reading frame and part of the pK7WG2D vector sequence was amplified using primers PEP-F and PEP-R and sequenced.

The image on the left shows a PAGE showing a high amount of positive PCR products from Batch 6, mutants 453-492. Positive bands were cleaned and sent for sequencing.

DISCUSSION AND CONCLUSIONS

- The goal of this work was to test the hypothesis that overexpression of cyclical small random peptides could unveil new candidates for chemistries that modify plant biology. These trials have produced dozens of new candidates that interfere with discrete plant processes. The goal now is to increase the number of plants to be screened, screen more conditions for peptide-dependent effects, and identify the mechanisms where the characterized peptides are integrating into plant biology (Phase 3).
- Phase 3 may include immunolocalization of the peptide, in situ peptide quantification, and epitope- tagging to identify interactors in the cell.
- In this report we present a new way to potentially identify novel molecules that could modulate important processes in plants. The peptides identified may then be used to impart their effects in transgenic plants or potentially even when applied in drenches or sprays. The structure of the peptides may be a basis of drug discovery, leading to new compounds representing novel growth regulators, herbicides or developmental modulators.

References

Zhilong Bao, Maureen A. Clancy, Raquel F. Carvalho, Kiona Elliott, Kevin M. Folta. 2017. Identification of Novel Growth Regulators in Plant Populations Expressing Random Peptides. Plant Physiology Oct 2017, 175 (2) 619-627; DOI: 10.1104/pp.17.00577