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High throughput selection of novel plant growth regulators: Assessing the translatability of small bioactive molecules from *Arabidopsis* to crops

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ABSTRACT

Plant growth regulators (PGRs) have become an integral part of agricultural and horticultural practices. Accordingly, there is an increased demand for new and cost-effective products. Nevertheless, the market is limited by insufficient innovation. In this context chemical genomics has gained increasing attention as a powerful approach addressing specific traits. Here is described the successful implementation of a highly specific, sensitive and efficient high throughput screening approach using *Arabidopsis* as a model. Using a combination of techniques, 10,000 diverse compounds were screened and evaluated for several important plant growth traits including root and leaf growth. The phenotype-based selection allowed the compilation of a collection of putative *Arabidopsis* growth regulators with a broad range of activities and specificities. A subset was selected for evaluating their bioactivity in agronomically valuable plants. Their validation as growth regulators in commercial species such as tomato, lettuce, carrot, maize and turfgrasses reinforced the success of the screening in *Arabidopsis* and indicated that small molecules activity can be efficiently translated to commercial species. Therefore, the chemical genomics approach in *Arabidopsis* is a promising field that can be incorporated in PGR discovery programs and has a great potential to develop new products that can be efficiently used in crops.

1. Introduction

Agriculture faces many challenges to fulfill the growing demand for sustainable food production and ensure high-quality nutrition for a rapidly growing population. To guarantee adequate food production, it is necessary to increase the yield per area of arable land [1]. A method for achieving this goal has been the application of growth regulators to modulate plant growth. Plant growth regulators (PGR) are substances in specific formulations which, when applied to plants or seeds, have the capacity to promote, inhibit, or modify physiological traits, development and/or stress responses [2]. PGRs are used to maximize productivity and quality, improve consistency in production, and overcome genetic and abiotic limitations to plant productivity. Suitable PGRs include hormones such as cytokinins and auxins, and hormone-like compounds such as mepiquat chloride and paclobutrazol [3–5]. The use of PGRs in mainstream agriculture has steadily increased within the last 20 years as their benefits have become better understood by growers. Unfortunately, the growth of the PGR market may be constrained by a lack of innovation [2] at a time when an increase in demand for new products will require steady innovation and discovery of novel, cost-competitive, specific, and effective PGRs [4,6,7].

Application of small bioactive molecules (<500 Da) to systematically screen for novel modifiers of a biological phenomenon have gained increasing attention [8]. The approach of *Chemical Genomics* combines large-scale chemistry and biology data along with bioinformatics which is required for data mining, structure analysis, data sharing, and the extraction of useful data [9]. The effectiveness of this approach is aided by the fact that most plant endogenous

Abbreviations: PGRs, (plant growth regulators); HTS, (high-throughput screening).

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growth regulatory compounds are small molecules that modulate target proteins and/or pathways of a determinate biological process [10]. In the past decade several academic and company research initiatives undertook the systematic design and synthesis of small molecules and their subsequent use as probes for different biological processes in diverse organisms. As a result several collections of bioactive compounds became available for the research community [11]. By using diverse chemical collections researchers can screen a large number of compounds for novel activities. Bioactive chemicals can be easily administrated at any time during development and to any desired location of the organism. Therefore, the chemical action on the organism can be temporally and spatially controlled. Testing a large number of compounds to see whether they produce an appropriate effect is usually the first step in the forward chemical genomics approach [9,12]. A phenotypic assay should be as tightly correlated to the trait, and the goal pursued, as possible. A successful chemical genomics approach identifies primary "*hit*" compounds in a first round of a high throughput screen (HTS). The hits then go into a second round of screening to confirm the reproducibility and the desired dose-dependency of the biological effect. Once past this filter, a hit becomes a "*lead*". Lead compounds then undergo further rounds of chemical refinement and biological screening before finally entering trial testing [13]. Thus, to address the discovery of new PGRs for agronomically interesting species by a chemical genomics approach it is essential to establish a high throughput, simple, reliable, and robust phenotypic assay. In principle, a chemical genomic screen can be performed in any plant system. Nevertheless, large-scale phenotyping is currently a challenge for many agronomically valuable species due to large physical size or slow growth that limit assay miniaturization for HTS.

Although not of agronomic significance, *Arabidopsis* offers important advantages in high throughput screening. Its small size and rapid growth simplifies the scoring of phenotypes and permits large-scale miniaturized screening which reduces costs and time. *Arabidopsis* is also one of the best characterized plant species in terms of growth-regulating molecular mechanisms which greatly enables phenotypic analysis. Despite these advantages, the translation of novel chemicals and desirable phenotypes to agronomic species has not been widely reported. Based on the mode of action of the bioactive compounds they could have effect on broad spectrum of plant species. For instance, a compound discovered in a model specie such as *Arabidopsis* may yield comparable phenotypes in agronomic species if it targets conserved pathways. This translation ability has been cited as an advantage of small molecule approaches [14,15], yet few or no published studies have addressed this. Thus, one of our objectives was to test this hypothesis. A better understanding of translatability of lead bioactive chemicals will impact the predictability of the *Arabidopsis* research in growth regulating processes and efficacy of agrochemicals. Consequently this is a potentially important route to discover and apply novel agrochemicals to economically important species. To address the question of species translatability, a chemical genomics approach was designed to first identify *Arabidopsis* growth modulators and then to test a subset of them in different plant species. In this paper a combination of automated and manual techniques are reported allowing the identification of a broad range *Arabidopsis* growth modulators. The effect of a subset of identified hits showed dose-dependent, inducible and/or reversible effect in *Arabidopsis*. These lead compounds were selected for further analysis in agronomic species, with a focus on chemicals altering root and leaf growth. Translatability of *Arabidopsis* PGRs was evaluated in tomato, lettuce, carrot, maize, and turfgrass. Some of the bioactive compounds were effective on several of the tested species while others were more specific in their effect. Overall, *Arabidopsis* chemical genomics HTS proved to be powerful for discovering new PGRs that can be translated to agronomic species for potential development as agrochemicals.

2. Results and discussion

2.1. HTS to discover growth regulators in Arabidopsis

Chemical genomics approaches rely on an appropriate HTS assay so that compounds with desired growth regulatory effects can be found if they exist in the chemical library. A rate-limiting factor for HTS success is not the speed of assays but their design; that is, establishing new simple, reliable, and robust ways of measuring biological activity *in vivo* in a high-throughput manner [10,13]. The HTS should account for several factors including (1) screening with physiologically relevant models displaying traits or biological processes that can be subject for modification; (2) screening for multiple biological traits simultaneously for comprehensive results, (3) effective miniaturization with the subsequent associated time and cost savings without sacrificing biological relevance, (4) efficient high quality and high content data collection. The first of these factors can be taken into account using a small, well-characterized model plant such *Arabidopsis* as a surrogate for studies of growth modulation in agronomically relevant species.

Several chemical genomic screens have allowed the identification of small molecules that alter *Arabidopsis* development in specific growth conditions and/or oriented to specific tissues [16]. Here, for finding novel molecules that selectively affect the development of root or aerial organs under regular growth conditions, a HTS was established by miniaturizing the phenotypic assay using *Arabidopsis* (Fig. 1). The format of 24-well microplates permitted monitoring of seedling morphological responses of root and leaf growth which was the main focus of this study. However, using this format it would also be feasible to score additional seedling phenotypes such seed germination, hypocotyl elongation, leaf bleaching or stress responses.

To efficiently measure root growth, seedlings were grown on solid media, rather than liquid, which more closely emulated field growth conditions. *Arabidopsis* seeds were manually plated on media containing 15 to 17 μM compound from a chemical library in each well. Plates were incubated vertically in a growth chamber for seven days allowing roots to grow over the agar surface to score root length and lateral root number (Fig. 1). Plates were then reoriented to a horizontal position and seedlings were allowed to grow for an additional seven days at which time the aerial tissue area was scored (Fig. 1). The HTS was designed to score the effect of 20 chemicals per plate, leaving one row of four wells as growth controls. The 24-well microplate format used for seedling growth required a minimum of sample handling and allowed automatic acquisition of quantitative data and the simultaneous monitoring of several morphological traits (Fig. 1). Using this methodology 10,000 structurally diverse compounds were assayed in a primary HTS in *Arabidopsis*.

2.2. Scoring Arabidopsis growth phenotypes

The implementation of the HTS resulted in a variety of whole organism developmental phenotypes. Thus, the imaging collection and acquisition process was divided into two stages. First, seven day-old vertically grown seedlings were automatically imaged by using the high-throughput Pathway HT microscope (Atto Biosciences). The resulting collection of 24 images was processed to rebuild the entire plate for further analysis (Fig. 1). Secondly, seedlings were grown for additional seven days in a horizontal position and images of the aerial organs were taken. Image acquisition was performed on a flatbed scanner to produce image files suitable