Title:

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

Short title: **Chemical genomics translation 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 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.

Key words: plant growth regulator, high-throughput screening, chemical genomics, Arabidopsis, crops

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

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 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 cost 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 species 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 (Figure 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 (Figure 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 (Figure 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 (Figure 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 (Figure1). Secondly, seedlings 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 for quantitative analysis. This pipeline (Figure1) captured high-resolution images that enabled multi-parameter characterization of growth and developmental responses to chemical treatments.

Once collected, the next step was to convert the images into quantitative phenotypic information. Although the images were rich in seedling phenotypic information, the scoring was focused on traits of potential value for PGR discovery. The aim was to demonstrate the utility of this approach for a future biotechnology and agriculture approach by the translation from Arabidopsis to other dicots and monocots. Therefore root length, lateral root number and leaf area were selected as interesting traits for this study. The compounds that affected seed germination, pigmentation of leaves or caused obvious detrimental developmental abnormalities were scored but not pursued in this analysis. Thus, by evaluating the selected traits, among 10,000 screened compounds, 689 hits were selected as potential PGRs (further details are provided in Materials and Methods). This proportion of hits in a primary screening is within the range of cited values for chemical

genomics screens which predict 1 to 5% hit selection [12]. The percentage of HTS hits suggested that the chemical libraries would not have a major bias for growth modulator compounds.

The quantitative phenotypic data was then scored for fold change compared to the control for leaf growth, root growth and lateral root branching. The values range from -2 (inhibition) to +2 (stimulation) and 0 represents no change. To discover relationships between these set of data multivariate relationship analysis was performed. Thus, in a 3D scatter plot were displayed values for root growth, lateral root branching, and leaf growth phenotypic data and the diverse hit compounds. The data plotted in a certain phenotypic category are bundled together to display a single bubble for each category and sized by the count of hit compounds within each category (Figure 2A). The resulting plot reveals a variety of compounds enriched for different growth regulatory categories. Nevertheless, the overrepresented categories correspond to inhibitory phenotypes (Figure 2A). Consistently, hierarchical clustering analysis organized the compounds into phenotypically related clusters (Figure 2B). The larger clusters correspond to inhibitory phenotypes and the smaller to stimulatory growth phenotypes. Thus, 587 hits were grouped as growth inhibitors and 102 hits as growth stimulators. Both results are in concordance with the tendency of high throughput pharmacological studies to select small molecules that inhibit rather than stimulate the function of biological targets [13]. This bias toward small molecules that act as inhibitors of plant growth suggest that these mechanisms are more susceptible to disruption or down regulation than stimulation. Nonetheless, in the clustering analysis each of the main branches in the dendogram is subdivided into smaller phenotypic clusters (phenoclusters) (Figure 2B). This indicates a variety of actions and specificities among the collection of selected hits. For example, one phenocluster, is characterized by a specific leaf growth inhibition without significantly affecting root development. Another phenocluster is formed by compounds that specifically inhibit root

growth but not alter leaf growth or lateral root branching. Other phenoclusters included compounds capable of inducing stimulation of leaf growth, root branching or have an impact on general growth inhibition or stimulation (Figure 2B).

The identification of sets of compounds causing similar phenotypes makes it possible to sort the molecules by structures that are possibly targeting common or related targets or pathways. It is generally assumed that structurally similar compounds have similar biological activity [17]. As the larger phenotypic categories correspond to growth inhibitory compounds it would be interesting to investigate the structure-activity-relationship (SAR) of these hits. Nevertheless, this inhibitory phenotype category includes three main sub-categories: (1) general inhibition of growth, (2) inhibition of leaf growth and (3) inhibition of root growth (Figure 2A). Thus, we focused on examples of compounds belonging to these three main categories in the hierarchical phenotypic cluster. Using subsets of compounds exemplifying these categories, structural and physicochemical similarity relationship analysis was done (Figure 2C). Pairwise comparison was graded from zero for identical compounds to 1 for compounds that have no common substructure. Hierarchical clustering revealed a high degree of structural diversity using distance matrices. The distances between compounds ranged from 0.76 to 0.96 showing that most had little structural similarity among each other. In the heatmap similar compounds (coefficients <0.5) correspond to the self-reciprocal-comparison of each compound (Figure 2C). This data indicated that the HTS identified a large set of inhibitors with a wide range of structures among them. This could reflect the variety of biological targets that influence growth inhibition, as well as the potential of different structures to influence common target pathways.

The variety of phenotypes obtained in the Arabidopsis HTS and the structural diversity of the selected *hits* are very valuable characteristics in the search for new PGRs. As the goal of this work was to address the question of species translatability between Arabidopsis

and other plant species, the analysis was used to guide a strategy for selecting representative hits from the enriched phenotypic categories. Thus, hits that preferentially inhibited root or leaf growth and represented diverse structures from each other were used as a test set to continue the analysis and test the hypothesis of translatability from Arabidopsis to crops.

2.2 **Characterization of Arabidopsis growth regulators**

To continue analyzing root and leaf growth inhibitory compound clusters selected in the previous section were tested. The phenocluster of root growth inhibitors included ten hits and the phenocluster of leaf growth inhibitors included eight hits, named A for Agrochemicals. To further characterize these hit compounds a secondary screen was performed. A chemical was only considered a confirmed as lead compound if both a reproducible phenotype and a dose dependent effect were present.

The dose-response assay was performed considering that the concentration used in the primary screening was 15-17µM which was able to render an impact on plant growth. Arabidopsis seeds were germinated in the presence of 10, 20 and 50µM of each compound to evaluate their effects. As a result, the HTS inhibitory phenotypes of the seventeen compounds were confirmed (Figure 3A). It was also noticeable the dose-dependent effect for all compounds. The exception was A28 which effect resulted in maximum inhibition at 10µM. Identity, chemical structures and physicochemical features of the characterized Arabidopsis lead compounds are detailed in Supplementary Table 1.

The next step was evaluating the capability of the Arabidopsis lead inhibitors to induce their effect on Arabidopsis seedlings. This is an important trait as PGRs are often applied during specific developmental stages to inhibit or stimulate growth and organ formation. Arabidopsis seedlings were grown in standard media for seven days and seedlings were treated with the lead compounds at different concentrations. In these conditions the compounds continued exhibiting similar bioactivity as when applied from the seed stage

(Figure 3B). The majority of the growth inhibitors showed a response at 20µM that ranged from 70% to the same response obtained at 50µM. These results indicated that the effects on growth were inducible but saturable.

Another desirable trait for PGRs is to recover normal plant growth once the treatment is withdrawn. The recovery or reversibility refers to the loss of the induced phenotype over time due to chemical metabolism, modification, exclusion, sequestration, or other form of metabolic clearing [14]. The recovery assays indicated that all the leaf growth inhibitors recover a normal growth ratio, except A33 that showed no recovery and therefore was excluded from further analysis (Figure 3C). Also, seedlings grown in the presence of the root growth inhibitors A11, A14, A46 or A47 showed a complete recovery of growth. However, seedlings grown in the presence of the root growth inhibitors A6, A8, A31, A36 or A47 showed recovery at 10µM, while those grown at 20µM and 50µM recovered partially after seven days (Figure 3C). Probably these growth inhibitors had a slower recovery over time.

Altogether, the characterization of Arabidopsis hits allowed the selection of 16 lead compounds for further analysis. Furthermore, the secondary screen uncovered interesting insights into the action of the lead compounds such us reversibility of the action and optimal doses. The growth inhibition induced when seeds were germinated on presence of the compounds was dose dependent. Nevertheless, inducibility treatments in seedlings revealed that 20µM was approaching to saturating concentration of the growth response. Thus, the effect was dependent upon the response capacity of the seedlings which varied somewhat with the plant developmental stage. Even further, analysis of the recovery profiles by concentration of treatment indicated that 50µM had the greatest impact on recovery (Figure 3C). These data suggest that the optimal treatment concentration to achieve Arabidopsis growth inhibition is 20µM. Thus, the selected lead root or leaf growth inhibitors could be applied at different times during development and the suggested

optimal concentration to elicit effects in a reversible or partially reversible manner was 20µM. The action of the Arabidopsis lead compounds can be further tuned using varying doses of the compound to elicit mild or strong effects.

2.3 Translatability of Arabidopsis growth regulator leads to agronomically significant species

Biotechnology industry efforts to discover PGRs for key crops require both efficient and predictive surrogate screens and validation of bioactivity. There are a number of screens in Arabidopsis that have proven useful for identifying bioactive chemicals as putative growth regulators [10, 18-26]. Similarities between Arabidopsis and crop species suggest the possibility that small molecules can be translated to crops efficiently. This requires the development of a robust predictive framework in which biological outputs can be translated to other species. To date, this issue has been addressed only rarely in the literature [26]. So there has been insufficient data to assess the translatability of bioactive compounds. Thus, the translatability of the lead Arabidopsis growth regulators to agronomic crops and vegetables was characterized and quantified to inform future approaches.

A diverse spectrum of representative species of dicot and monocot families was chosen to examine translatability: Solanaceae (tomato), Apiaceae (carrot); Asteraceae (lettuce); Brassicaceae (Arabidopsis), and monocots Gramineae (wheat and turfgrass). This collection of species was selected due to their diversity and because they can be handled under ordinary laboratory growth conditions. All the lead growth inhibitor compounds were tested in both tomato and lettuce. Additionally, root growth inhibitors were tested in carrot and in the monocot maize. Meanwhile, leaf growth inhibitors were tested in turfgrass as there is a growing interest in the turfgrass management industry in regulating leaf growth [28].

Results indicated that 13 of 16 lead growth inhibitors were active in species other than Arabidopsis, indicating that their effect was translatable (representative results at 20µM

are shown in Figures 4 and 5). Among the Arabidopsis leaf growth inhibitors A1, A4, A15 and A38 showed activity in all species (Figure 4). A4 inhibited approximately 40% of leaf growth in lettuce and tomato but only 20% in turfgrass. Also, A21 showed inhibitory leaf growth activity only in tomato and lettuce. This lower rate of inhibition in the monocot turfgrass could be expected as the growth regulators were selected in a dicot plant. Nevertheless, A15 inhibited leaf growth in Arabidopsis by 23%, in lettuce and tomato 20-30% and in turfgrass by 44% (Figure 4). Additionally, A1 showed similar growth inhibition percentages in tomato, lettuce and turfgrass. These data indicated that results obtained in a dicot could be translated to a monocot plant at even higher growth regulator efficiencies. Interestingly, the compound A38 named sildenafil has been previously characterized in *Cucumis sativus* as a phosphodiesterase inhibitor increasing cGMP and consequently affecting the NO levels (Table 1) [29]. According to our results, the molecular target is conserved through different species as the compound action is effective in monocots and dicots (Figure 4). By comparison, the compound A18 was specifically active in tomato where it inhibited leaf growth by 33%. This specificity of compound action may result from distinct targets, different target selectivity, differential sensitivity among distinct species, different abilities to be taken up and transported in planta.

In the subset of root growth inhibitory lead compounds A8 and A11 were effective in all the tested species (Figure 5). Particularly, A8 caused about 60% root inhibition in lettuce and tomato but approximately 10% root growth inhibition in carrot and maize. These differences could be an expected outcome as these species exhibit distinct root architecture. Dicots such as Arabidopsis, lettuce and tomato have an allorhizic system consisting of a primary central root which may develop lateral roots [30]. Carrot has an extreme allorhizic system with a single thick, central root and very thin lateral roots [31]. Whereas monocots have a homorhizic system, a fibrous root system consistent in multiple central roots that can develop lateral roots but also shoot-born roots called crown and

brace roots [32]. These differences could also explain the root growth inhibitory activity of A36 in lettuce, tomato and carrot but not in maize (Figure 5). Nevertheless, the compound A11 showed high root growth inhibitory activity in all the species. Thus, despite differences in root system architecture, A11 could affect a common target or pathway among the different species. Even further, A6 did not affect tomato but was effective in the rest of the species (Figure 5). A14 was also active all the plant species except lettuce. The compounds A44 and A46 showed around 80% root growth inhibitory phenotypes specifically in lettuce or tomato respectively.

Among the 16 characterized lead compounds tested for translatability in other plant species A31, A47 and A28 inhibited growth only in Arabidopsis. A31 corresponded to a compound known as Trifluralin which has been previously characterized as a weed root growth inhibitor (Supplementary Table 1). Trifluralin has been used as a commercial herbicide in different crops species [33] concordantly with its lack of effect on the rest of the tested species (Figure 5). The remaining 13 compounds showed activity in species other than Arabidopsis (Figures 4 and 5). This translational data indicates that the compounds can be targeting pathways conserved between species. Considering the molecular and physiological differences between Arabidopsis and many agronomically relevant species which could result in the elimination of a significant fraction of the generated leads, we concluded that there was a remarkably efficient translation between Arabidopsis and the tested crop plant species. These results support the conclusion that HTS screening and selection performed in Arabidopsis can be efficiently translated to agronomically relevant species with a high efficiency.

2.4 Physicochemical features of the characterized Arabidopsis lead compounds.

The penetration of a bioactive compound and its distribution in the plant (bioavailability) occurs generally in a passive manner along concentration gradients and according to their physicochemical properties [34]. It has been established that compounds that fulfill the

"Lipinsky rule of five" or "Briggs rule of three" are predicted to be transported through tissues and enter cells [35, 36]. The majority of the characterized lead compounds in this study fulfill these rules (Supplementary Table 1). Only compound A36 does not meet those rules decreasing its potential for bioavailability. However, the presence of fluorine substituents in A36 can influence or enhance key physical properties as systemic movement, solubility, volatility, polarity, and penetration and could explain the observed broad activity among the different species [37]. Regarding chemical features of the bioactive compounds, weak acidic groups such as carboxylic acids, known to promote phloem mobility, are present in A1, A11, A21 and A36 [38]. The characterized lead compounds have in common the presence of substituted heterocycles (Supplementary Table 1). Compound libraries are enriched in these structures because they possess excellent biological activity and are also predominant among commercial agrochemicals [39]. Another common characteristic of the identified leads is their octanol/water partition coefficients (LogK) which are within the one to five value range predicted to reflect a high lipophilicity and therefore effective root absorption and translocation to the shoot [40].

The HTS allowed identification for the first time of the bioactivity of the compounds A1, A4, A15, A18, A21, A6, A8, A11, A14, A31, A36, A44, A46, A47 in plants. Interestingly, their closest analogs have not described as bioactive compounds in plants (Supplementary Table 1). The exception was A28 which analog is known for affecting the glycosyltranferase activity in plants (Supplementary Table 1). Nevertheless, for several of the characterized compounds it is possible to identify chemical scaffolds previously associated to plant growth regulatory activities (Supplementary Table 1). The presence of these distinctive groups in various classes of growth inhibitors could reflect the potential properties of the different moieties (Related chemical moieties with plant growth bioactivity in Supplementary Table 1). Future investigation may determine which structural elements are essential for their bioactivity. Thus, based upon analysis of the functional, structural,

and physicochemical properties we concluded that the selected leads from the HTS have the potential and novelty to become agrochemical tools for crops.

3. Conclusions

Our approach to discovering compounds modifying development and the examination of the efficiency of translation provide several significant conclusions. The chemical genomics approach in Arabidopsis was scalable and translatable which can aid the discovery of new PGRs in commercial species. As a result, the approach is facile, accurate, reliable, and robust, supporting the use of a model species for high throughput screening of PGRs for economically important crop and vegetable species. The ability to translate screening results not only from Arabidopsis but also from a dicot to a monocot plant reinforces the biological and biotechnological relevance of the approach. The added value of using Arabidopsis as a model are the numerous genomic and genetics tools available for further studies to reveal affected pathways and molecular targets of the selected lead compounds. These results highlight the conservation of molecular mechanisms controlling growth and provide a method for whole-organism chemical genomics screens to identify novel PGRs and molecular pathways modulating growth.

4. Materials and methods.

4.1 Plant material

Arabidopsis thaliana Col-0 was used. Commercial species tomato (*Solanum lycopersicum cv. Micro-Tom*); lettuce (*Lactuca sativa Longifolia*), turfgrass (*Paspalum vaginatum*) and carrot (*Daucus carota*) were used.

4.2 Seed sterilization

Chlorine gas sterilization was used for all seeds. This protocol allows sterile storage for several weeks. At the time of use seeds were hydrated in water and stratified by leaving them in darkness at 4 °C for at least 48 hours.

4.3 Chemical libraries

The chemical libraries of 10,000 compounds were provided at the Institute for Integrative Genome Biology (http://genomics.ucr.edu/), Center for Plant Cell Biology (http://cepceb.ucr.edu/), University of California, Riverside, California, USA. This library contained 2,600 compounds from the Microsource Spectrum library, 360 compounds of a bioactive pollen library [41] and 7,000 compounds from the Life Chemicals oriented library. Chemical stocks were in 100% DMSO in 96 well plate format.

4.4 Arabidopsis growth regulators HTS

Culture plates of 24-well format were used. In each well, 400µl of MS medium pH 5.7 (0.5X Murashige & Skoog salts mixture, 0.05% MES buffer) supplemented with 1% sucrose. From the library master plates, 1µL of each compound was plated into the liquid MS medium using the Biomek FX robot available at the Institute for Genome Biology Integrative UCR. Then additional 400µl of MS medium pH 5.7 supplemented with 1% sucrose was added to each well. Finally an equal amount of media with 2x gelling agent was added (0.92% Gelrite). Thus each compound reached a final concentration of 15-17µM. As compounds are dissolved in 100%DMSO, a control row was placed in each 24-well plate containing 1µl DMSO. Later 3-4 Col-0 Arabidopsis seeds were plated manually into each well. Plates were placed in a chamber with controlled conditions of temperature and photoperiod (22 °C, 16 h light /8 h dark). Plants were grown vertically for 7 days. The image acquisition process was divided into two stages. First, 7 day old vertically grown seedlings were scanned. Images were automatically acquired using a high-throughput microscope the Pathway HT (Atto Biosciences). The software was programed to find the position of each well using auto-focusing (2x objective) and four horizontal images by five consecutive rows (4x5) were taken in order to register each entire well. The rate was ≈50 sec per compound, 20 min per plate. The resulting collection of 24 reconstructed images was later processed using the freeware ImageJ to rebuild the entire plate for further

analysis. Secondly, images of the aerial organs of plants, which were left for extra 7 days for growth in a horizontal position, were taken. Image acquisition was performed on a flatbed scanner (Epson 2450) to produce image files suitable for quantitative analysis. These methods allowed obtaining high-resolution images that enable multi-parametric characterization of growth and developmental responses to chemical treatments.

4.5 Arabidopsis re-testing assays

For dose dependency assays Arabidopsis seeds were plated on 8- square-well plates over 1% sucrose MS and 0.8% agar supplemented with tested compound. Plates were placed in vertical position for 7 days or horizontal for 14 days to quantify root and leaf growth respectively. Plants were grown in temperature and photoperiod controlled conditions (22 °C, 16 h light / 8 h dark). The inducible effect of hits was tested by transferring untreated 7 days old Arabidopsis seedlings to MS media supplemented with the compound.

4.6 Tomato and lettuce assays

Tomato (*Solanum lycopersicum* cv. Micro-Tom) plants were cultivated in 1% sucrose MS and 0.8% agar. For lettuce (*Lactuca sativa longifolia)* on the other hand 0.6% of agar was used instead. The seeds were plated and incubated in temperature and photoperiod controlled conditions (22 °C, 16 h light/8 h dark) for 7 days. Plants were then transferred to new media containing the tested compounds. All the plates were scanned daily for growth quantification. Root and leaf growth was scored after seven days of recovery. For leaf area measurements, individual leafs were cut from the plant at the end of the treatment, placed stretched over a glass and scanned.

4.7 Carrot assays

Carrot (*Daucus carota*) seeds were plated in 1% sucrose MS media pH 5.8 and 0.8% agar. Seven day-old seedlings were transferred to 15ml tubes containing MS liquid media plus the tested compounds. Plants were allowed to growth for additional 15 days. For

further root growth analysis whole plants were then placed over a glass and roots were scanned.

4.8 Turfgrass assays

Seeds of turfgrass (Chepica, *Paspalum vaginatum)* were placed in phenoplates made of two glasses separated by 7 mm rubber sheets. The phenoplates contained 1% sucrose MS and 0.8% agar supplemented with the tested compounds. For seeds germination phenoplates were incubated at 28°C during 48h in darkness. Phenoplates were then placed into an incubation chamber with temperature and photoperiod controlled conditions (22 °C, 16 h light/8 h dark) for 15 days. All the phenoplates were scanned for further leaf area quantification.

4.9 Zea mays assays

Corn caryopses (Zea mays) flint were washed thoroughly in tap water, rinsed in distilled water and placed between two discs of filter paper (Watmann) and cotton soaked in distilled water containing 1 ml/L of the biocide Kathon CG (Room&Haas). The plates were then incubated in the dark at 24 °C for 48-72 hours. For the experiments were selected seeds that had emerged taproot of similar length, typically 2.0-2.5 cm. The roots were then cut under stereomicroscope with red light on filter paper moistened with a 10 mM KCl and 1 mL/L of Kathon solution. Apical segments 7.5 mm in length were used. The segments were incubated in dark for an hour in macrowells containing 500 mL of 10 mM KCl and 1 ml/ L of Kathon, to stabilize the material. Experiments were performed in triplicate and each containing 3-4 macrowell root apical segments. The different treatments were made by dissolving 2X concentration of compound in a solution of 10 mM KCl and 1 mL/L Kathon and used to supplement the media containing the root apical segments. After 96 h of incubation, the segments were measured under the stereomicroscope, using a ruler. Measures were taken in tenth of mm.

4.10 HTS Data Analysis

Images were analyzed using ImageJ (v1.47, National Institutes of Health, Bethesda, MD; http://rsb.info.nih.gov/ij/) software. The images obtained from each well using the HT Pathway microscope Atto were used to reconstruct the full 24-well plate. Measurements of root length were performed using ImageJ plugin Root Tools. The images of the aerial part of the plants obtained using the Epson scanner were also analyzed with ImageJ software to measure leaf area per well. The obtained values were compared to correspondent values of the four-wells control treatments placed in each plate. The inhibitory compounds were scored from at least 10% but less than 80% of growth inhibition to avoid detrimental developmental defects. Additionally, growth stimulatory compounds were considered those showing at least 10% of growth stimulation in the scored organs and up to 200% stimulation.

Phenotypic data was scored as growth fold of change compared to control conditions. Calculation of statistical relationships was made using the growth fold of change values in lateral root number, root and leaf growth for each hit compared to untreated controls. Multivariate relationship analysis were displayed in a bubble 3D graphical output constructed using the software TIBCO Sportfire 3.0 (http://spotfire.tibco.com/). The exploratory data analysis arrange the data in three dimension (XYZ) to help finding distinctive clusters by examining the distribution of observations [42]. The aggregated data (markers) was sized by the count of *hits* (items) within each category.

Phenotypic hierarchical clustering was constructed using growth fold of change values for lateral root number, root and leaf growth for each hit. The phenocluster was built using the web tool ClustVis (http://biit.cs.ut.ee/clustvis/) [43]. Correlations were visualized using a heatmap organized on the basis of hierarchal clustering calculated on Euclidean distances and complete agglomeration.

Chemical structure similarity analysis using the ChemMine tools (http://chemminetools.ucr.edu) was performed for inhibitory hit compounds. Hierarchical

clustering uses as input a distance matrix of all-against-all compound distances joining the most to least similar items by single linkage. The distance matrix is generated by subtracting the Tanimoto coefficient (Tc) similarity measure from one (1-Tc). Hierarchically clustering used as joining rule single linkage.

4.11 Image root and leaf analysis in agronomically significant species

Root images were analyzed using ImageJ software. Images of tomato, lettuce, corn and carrot roots were manually drawn in and each ROI was measured. Leaf area was measured using Adobe Photoshop CC 14.0. Manual color selection was used to designate colors in the image as background or leaf by selecting a specific range of colors from within the image. All pixels chosen in the color class as leaf were used in the leaf area calculation. Each photograph contained a ruler that was used to normalize pixel area. Thus quantitative area measurements were obtained for tomato, lettuce and turfgrass leafs.

4.12 Physicalchemical structure analysis

The smiles for each selected compound were uploaded in the Chemmine Tools online service (chemmine.ucr.edu) [44]. Molecular property descriptors JOELib and Open Babel were obtained. Additionally structural similarity analyses were conducted by the algorithm PubChem Fingerpint (cutoff = 0.5) for the first 10 hits. Series of MyNCBI custom filters were set up to look for plant related biological activities of the selected compounds. For bulk checking, structural-related compounds were searched using Chemicalize public web resource (www.chemicalize.org). Supplementary properties analyses were conducted in the Chemspider database (www.chemspider.com). Additionally, patent and literature-derived records from different sources were checked for each selected structure and their substructure moieties.

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Figure legends

Figure 1. Chemical genomics HTS strategy pipeline for identifying Arabidopsis growth regulators. By using a pipetting robot, chemicals from a 96-well format chemical library were rearranged to 24-well format plates. Plant media was added to the phenotype test plates and Arabidopsis Col-0 seeds were manually plated on solidified media containing 15-17μM of each chemical per well. Plants were allowed to grow in a vertical position for 7 days. Image collection was carried out using an automated microscope. Root length (red line), lateral roots (yellow arrowheads) and hypocotyl length were scored (white

line). A growth inhibitor (*) and stimulator (-) are shown as examples. The same plates were left for an additional 7 days in horizontal position. Leaf growth was documented using a plate scanner. Leaf area (red area) was quantified, leaf number (blue arrowheads) and leaf pigmentation were qualitatively scored. Examples for leaf growth inhibition (*) and stimulation (#) are shown.

Figure 2: The HTS identified a diversity of Arabidopsis growth regulators. A) Bubble 3D plot of the similarity relationship of compounds causing root growth, leaf growth and root branching growth phenotypes. Growth fold change compared to untreated controls is plotted. The zero value indicates no change, positive values indicates induction, and negative values indicates inhibition compared to control plants. The bubble plot shows the number of records at each combination of categories. The size of the bubbles represents the quantity of compounds that promotes a determined growth phenotype. Upper and lower charts display different spatial orientations of the same graph to facilitate visualization. Circled bubbles represent the bigger groups. B) The dendrogram represents a hierarchical clustering analysis (Euclidean distances) of bioactive compounds based on Arabidopsis growth phenotypes. Three different traits were considered for clustering analysis; root growth, root branching and leaf growth. Fold changes of growth compared to untreated controls was graded from -2 (maximum inhibition) to +2 (maximum stimulation). The difference was associated to a color, with closeness to red indicating growth stimulation and closeness to blue indicating growth inhibition and no change in light blue (NC). Dotted ellipses indicates examples of phenoclusters of compounds that affect growth in differential extent. Three inhibitory clusters were selected for further analysis, leaf and root growth (1), leaf growth (2) and root growth (3) inhibition. C) Functional dendrogram with clustering based on chemical similarity using a distance matrix. Zero indicates that the compounds are identical and 1 indicates compounds that are unique. Values below 0.5 indicates statistical significantly similarity. Compound distance matrix scores were plotted

for inhibitory compounds members of clusters leaf and root growth inhibition (1), leaf growth inhibition (2) and root growth inhibition (3). The score is represented by heat map (coded cyan to purple). Note that identical compounds only appear when there is a reciprocal comparison among themselves.

Figure 3: Characterization of the root and leaf growth inhibitory compounds on Arabidopsis. The dose dependence effect was tested using 10µM (with bars), 20µM (gray bars) or 50µM (black bars) of the leaf growth inhibitors, left panel (A1, A4, A15, A18, A21, A28, A33, A38) and the root growth inhibitors right panel (A6, A8, A11, A14, A31, A36, A44, A46, A47). (A) Seeds of A. thaliana (Col-0) were plated on media containing different concentrations of each chemical. Root and leaf growth inhibition percentages compared to control treatments are plotted. (B) To test the capability of the different compounds to induce leaf (left panel) or root (right panel) growth inhibition, 7 day-old Arabidopsis was exposed to each chemical. After additional 7 days, root and leaf growth rate were analyzed. Root and leaf growth inhibition compared to control treatments are plotted as percentage. (C) Arabidopsis grown seedling in A) were transferred to a regular plant media to analyze the phenotype reversibility. Root and leaf growth remaining inhibition percentage are plotted. Results are representative percentages of three independent experiments.

Figure 4: Arabidopsis leaf growth inhibitors are effective on agronomically significant models. Compounds characterized as *leads* Arabidopsis leaf growth regulators (black bars) were tested in different species. Leaf growth inhibition was evaluated in treatments with 20µM of each compound in Tomato (*Solanum lycopersicum* cv. Micro-Tom; white bars), in Lettuce (*Lactuca sativa longifolia*; light gray bars) and turfgrass (*Paspalum vaginatum*; dark gray bars). All results are expressed as percentage of leaf growth inhibition in treatments compared to control conditions. Results are

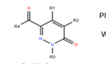
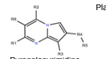
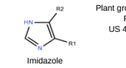
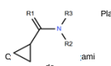
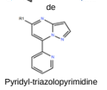
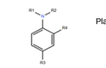
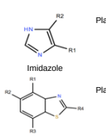
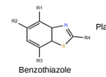
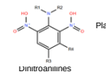
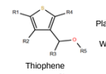
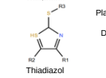
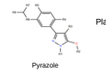
representative percentages of three independent experiments, with at least 15 seedlings per repetition. SD values are shown.

Figure 5: Arabidopsis root growth inhibitors are effective on agronomically significant models. Root growth regulators identified on the Arabidopsis HTS as *leads* (black bars) are effective on agronomical significant models. Root growth inhibition induced by 20µM of each compound was evaluated in tomato (*Solanum lycopersicum* cv. Micro-Tom; white bars), in lettuce (*Lactuca sativa longifolia*; dark gray bars), carrot (*Daucus carota*; light gray bars) and corn (*Zea mays*; white lined bars). Results are representative of three independent experiments. N per repetition = 15. The graphics displays mean and SD values.

Supplementary Table 1: Physicalchemical structural data of the analyzed growth inhibitors

Table(s)

Supplementary Table 1: Physicalchemical structural data of the analyzed lead growth inhibitors

HTS Lead ID	Arabidopsis Growth Regulation	Systematic IUPAC Name	Molecular Formula	Structure	Known plant growth bioactivity	Molecular Weigth	Lipinski Rule of Five	Briggs Rule of Three	LogP	LogK	Number of *HBA 1	Number of *HBA 2	Number of **HBD 1	Number of **HBD 2	Number of acidic groups	Number of aliphatic OH groups	Number of basic groups	Fraction of rotatable bonds	Number of heavy bonds	Number of heterocycles	Number of hydrophobic groups	Molar Refractivity	Number of atoms	Number of halogen atoms	Number of B atoms	Number of Br atoms	Number of Cl atoms	Number of I atoms	Number of F atoms	Number of N atoms	Number of O atoms	Number of P atoms	Number of S atoms	Number of bonds	Number of NO2 groups	Number of SO groups	Number of OSO groups	Number of SO2 groups	Polar Surface Area	Equivalent Hydrocarbure	Related chemical moieties with plant growth bioactivity	
A1	Leaf growth inhibitor	[3-(Ethoxycarbonyl)-2-furyl]methyl 6-oxo-1-phenyl-1,4,5,6-tetrahydro-3-pyridazinecarboxylate	$C_{19}N_2O_6$		ND	370	Fullfilled	Fullfilled	1.24	3.51	25	8	0	0	0	0	0	2.76E+15	29	2	0	1.09E+18	45	0	0	0	0	0	0	2	6	0	0	47	0	0	0	0	9.84E+04	35	 Pyridazinones	Plant growth inhibitor. Patent WO 2014031971 A1
A4	Leaf growth inhibitor	[2-(3-Methylphenyl)-1-pyrrolidinyl][5-(2-thienyl)-7-(trifluoromethyl)pyrazolo[1,5-a]pyrimidin-2-yl]methanone	$C_{23}F_3N_4OS$		ND	456	Fullfilled	---	3.59	5.71	23	6	0	0	0	0	0	1.39E+14	36	4	0	1.34E+18	51	3	0	0	0	0	3	4	1	0	1	55	0	0	0	0	7.87E+04	42	 Pyrazolopyrimidine	Plant growth inhibitor. Patent EP 0244097 A2
A15	Leaf growth inhibitor	(3-Fluorophenyl)[2-[(4-methylbenzyl)sulfanyl]-4,5-dihydro-1H-imidazol-1-yl]methanone	$C_{18}FN_2OS$		ND	328	Fullfilled	Fullfilled	3.46	4.49	21	4	0	0	0	0	1	2.0E-1	25	1	0	1.05E+18	40	1	0	0	0	0	1	2	1	0	1	42	0	0	0	0	5.80E+04	35	 Imidazole	Plant growth inhibitor. Patent US 4565875 A
A18	Leaf growth inhibitor	N-(5-Chloro-4-methyl-1,3-benzothiazol-2-yl)cyclopropanecarboxamide	$C_{12}ClN_2OS$		ND	267	Fullfilled	---	3.27	4.01	14	4	1	1	0	0	0	1.58E+15	19	1	0	8.29E+16	28	1	0	0	1	0	0	2	1	0	1	30	0	0	0	0	7.02E+16	23	 de ;ami	Plant growth inhibitor. Patent US3277107 A
A21	Leaf growth inhibitor	2-[[3-(3-Pyridinyl)[1,2,4]triazolo[4,3-b]pyridazin-6-yl]sulfanyl]hexanoic acid	$C_{18}N_5O_2S$		ND	342	Fullfilled	Fullfilled	2.9	2.69	22	8	0	1	1	0	0	2.69E+14	26	3	0	1.07E+18	40	0	0	0	0	0	0	5	2	0	1	42	0	0	0	0	1.21E+05	29	 Pyridyl-triazolopyrimidine	Patent CA2676736A1 Plant growth regulator
A28	Leaf growth inhibitor	3-Isobutyl-1-methyl-3,7-dihydro-1H-purine-2,6-dione	$C_{10}HN_4O_2$		Described for its analog 3-Isobutyl-1-methylxanthin: Plant growth Inhibitor, inhibits glycosyl transferase activity. [1]	222	Fullfilled	Fullfilled	1.24	1.15	17	5	1	1	0	0	0	1.18E+15	17	2	0	7.63E+06	30	0	0	0	0	0	0	4	2	0	0	31	0	0	0	0	7.27E+04	20	---	---
A33	Leaf growth inhibitor	2-Methyl-N-[4-nitro-3-(trifluoromethyl)phenyl]propanamide	$C_{11}F_3N_2O_3$		ND	276	Fullfilled	---	3.72	3.51	14	2	1	1	0	0	0	2.63E+14	19	0	0	7.36E+16	30	3	0	0	0	0	3	2	3	0	0	30	2	0	0	0	7.22E+04	23	 Halo-nitroaniline	Plant growth inhibitor. Patent US3119736 A US4643755 A
A38	Leaf growth inhibitor	5-[2-Éthoxy-5-[(4-méthyl-1-pipérazinyl)sulfonyl]phényl]-1-méthyl-3-propyl-1,4-dihydro-7H-pyrazolo[4,3-d]pyrimidin-7-one	$C_{22}HN_6O_4S$		Known as sildenafil. Inhibitor of phosphodiesterase ffecting nitric oxide level [2]	475	---	Fullfilled	2.27	2.3	37	8	2	2	0	0	1	1.94E+15	36	3	0	1.55E+18	63	0	0	0	0	0	0	6	4	0	1	66	0	0	0	2	1.23E+18	47	---	---
A6	Root growth inhibitor	1-[2-[(2-Chlorobenzyl)sulfanyl]-4,5-dihydro-1H-imidazol-1-yl]-2-(2-thienyl)ethanone	$C_{16}ClN_2OS_2$		ND	315	Fullfilled	Fullfilled	3.05	4.71	19	5	0	0	0	0	1	2.5E-1	24	2	0	1.06E+08	37	1	0	0	1	0	0	2	1	0	2	39	0	0	0	0	8.62E+04	32	 Imidazole	Plant growth inhibitor. Patent US 4565875 A
A8	Root growth inhibitor	N-(7-Methyl[1,3]thiazolo[5,4-e][1,3]benzothiazol-2-yl)pentanamide	$C_{14}N_3OS_2$		ND	305	Fullfilled	Fullfilled	3.63	4.22	19	6	1	1	0	0	0	2.27E+15	22	2	0	1.02E+18	35	0	0	0	0	0	0	3	1	0	2	37	0	0	0	0	1.11E+06	28	Benzothiazole	Plant growth inhibitor. Patent US4556411 A
A11	Root growth inhibitor	Ethyl 1-benzyl-5-[2-hydroxy-3-(4-morpholinyl)propoxy]-2-methyl-1H-indole-3-carboxylate	$C_{28}N_2O_5$		ND	452	Fullfilled	---	4.42	3.53	37	6	2	2	0	1	1	2.78E+14	36	2	0	1.60E+18	65	0	0	0	0	0	0	2	5	0	0	68	0	0	0	0	7.44E+04	56	---	---
A14	Root growth inhibitor	N-[(2Z)-3-Allyl-4-methoxy-1,3-benzothiazol-2(3H)-ylidene]-2-(2,5-dioxo-1-pyrrolidinyl)acetamide	$C_{17}N_3O_4S$		ND	359	Fullfilled	Fullfilled	1.46	1.09	23	8	0	0	0	0	0	2.22E+14	27	2	0	1.09E+08	42	0	0	0	0	0	0	3	4	0	1	44	0	0	0	0	1.09E+18	32	 Benzothiazole	Plant growth inhibitor. Patent US4556411 A
A31	Root growth inhibitor	2,6-Dinitro-N,N-dipropyl-4-(trifluorométhyl)aniline	$C_{13}F_3N_3O_4$		Known as Trifluralin. Inhibitor of weed root growth. Classified as herbicide [3]	335	Fullfilled	Fullfilled	5.49	5.31	19	1	0	0	0	0	0	3.48E+15	23	0	0	9.51E+16	39	3	0	0	0	0	3	3	4	0	0	39	4	0	0	0	8.95E+04	29	 Dinitroanilines	Plant growth inhibitor. Patent US4227913 A
A36	Root growth inhibitor	Bis[(3R,5R)-7-[2-(4-fluorophényl)-5-isopropyl-3-phényl-4-(phénylcarbamoyl)-1H-pyrrol-1-yl]-3,5-dihydroxyheptanoate] de calcium	$C_{66}H_6CaF_2N_4O_{10}$		ND	1155	---	---	5.39		81	14	6	6	0	4	0	3.33E+14	90	2	0	3.86E+18	151	2	0	0	0	0	2	4	10	0	0	158	0	0	0	0	2.02E+18	133	---	---
A44	Root growth inhibitor	4-Bromo-2-[(E)-2-nitrovinyl]thiophene	C_6BrNO_2S		ND	234	Fullfilled	Fullfilled	2.74	2.66	5	1	0	0	0	0	0	1.82E+15	11	1	0	5.23E+17	15	1	0	1	0	0	0	1	2	0	1	15	2	0	0	0	7.14E+04	11	 Thiophene	Plant growth inhibitor. Patent WO2010069880 A2
A46	Root growth inhibitor	2-[5-(Methylsulfanyl)-2,3-dihydro-1,3,4-thiadiazol-2-yl]pyridine	$C_8N_3S_2$		ND	211	Fullfilled	Fullfilled	1.23	0.99	14	5	1	1	0	0	0	1.43E+15	14	2	0	6.28E+17	22	0	0	0	0	0	0	3	0	0	2	23	0	0	0	0	8.79E+04	16	 Thiadiazol	Plant growth inhibitor. Patent DE 102007012168 A1 US4245101A US 4518414 A
A47	Root growth inhibitor	4-[3-Methoxy-4-(2-propyn-1-yloxy)phenyl]-3,6-dimethyl-1-phenyl-4,8-dihydro-1H-pyrazolo[3,4-e][1,4]thiazepin-7(6H)-one	$C_{24}N_2O_3S$		ND	434	Fullfilled	---	4.75		29	7	1	1	0	0	0	1.47E+15	34	2	0	1.44E+08	54	0	0	0	0	0	0	3	3	0	1	57	0	0	0	0	9.07E+04	45	 Pyrazole	Plant growth inhibitor. Patent US 6265351 B1

LogP: octanol-water partition coefficient
LogK: water/octanol partition coefficient
**HBA: Hidrogen bond aceptor
**HBD: Hidrogen bond donnor
ND: Non described for the compound or analogs Analogs search based on Pubchem Fingerprint similarity search (Cutoff 0.5)

[1] Hou, B., Lim, E. K., Higgins, G. S., & Bowles, D. J. (2004). N-glucosylation of cytokinins by glycosyltransferases of Arabidopsis thaliana. *Journal of Biological Chemistry* , *279* (46), 47822-47832.

[2] Pagnussat, G. C., Lanteri, M. L., & Lamattina, L. (2003). Nitric oxide and cyclic GMP are messengers in the indole acetic acid-induced adventitious rooting process. *Plant Physiology* , *132* (3), 1241

[3] Fernandes, T. C., Pizano, M. A., & Marin-Morales, M. A. (2013). Characterization, Modes of Action and Effects of Trifluralin: A Review. , Current Research and Case Studies in Use, Dr. Andrew Price (Ed.), Chapter 19

Figure(s)

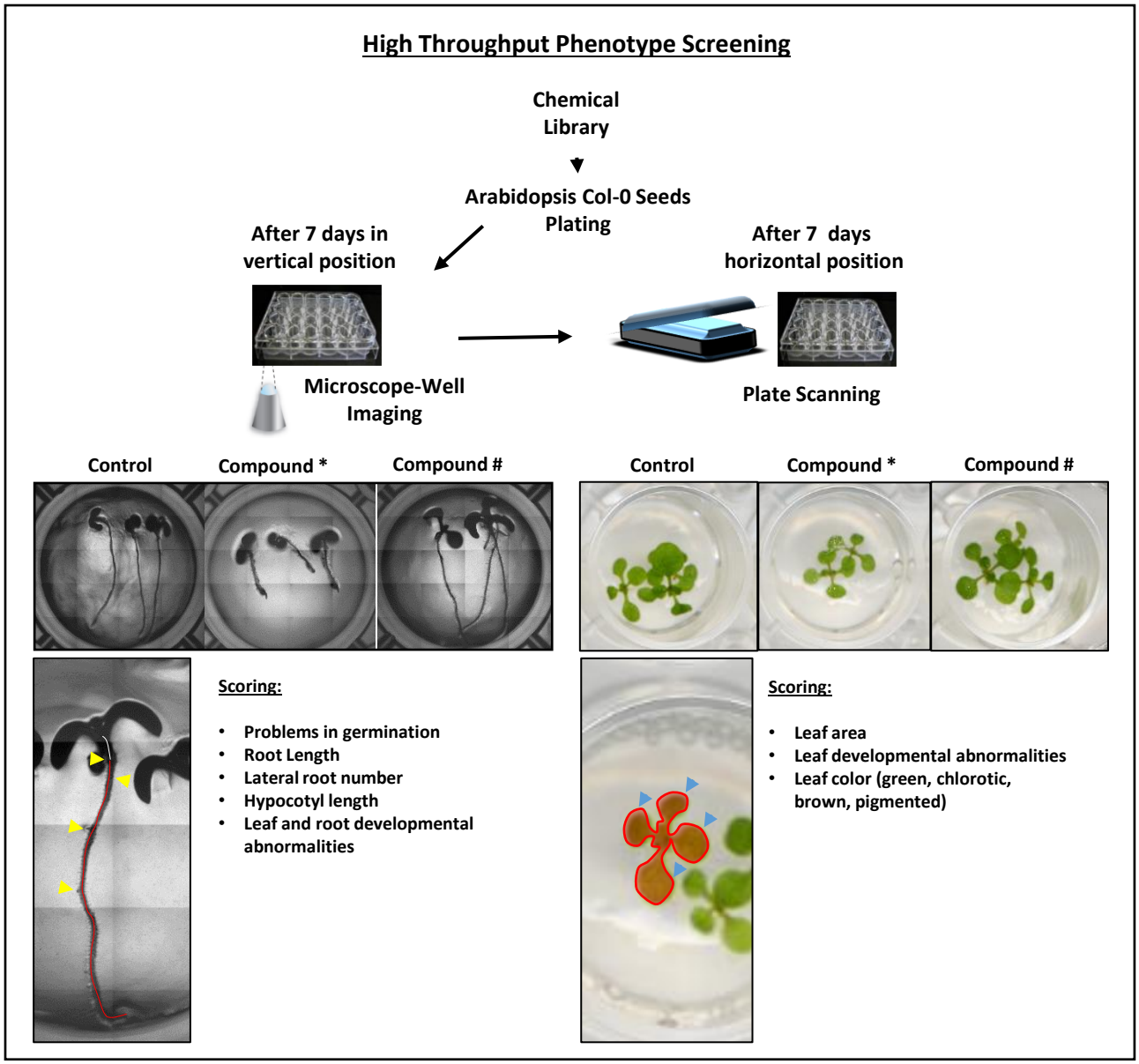


Figure 1. Chemical genomics HTS strategy pipeline for identifying Arabidopsis growth regulators.
By using a pipetting robot, chemicals from a 96-well format chemical library were rearranged to 24-well format plates. Plant media was added to the phenotype test plates and Arabidopsis Col-0 seeds were manually plated on solidified media containing 15-17µM of each chemical per well. Plants were allowed to grow in a vertical position for 7 days. Image collection was carried out using an automated microscope. Root length (red line), lateral roots (yellow arrowheads) and hypocotyl length were scored (white line). A growth inhibitor (*) and stimulator (#) are shown as examples. The same plates were left for an additional 7 days in a horizontal position. Leaf growth was documented using a plate scanner. Leaf area (red area) was quantified, leaf number (blue arrowheads) and leaf pigmentation were qualitatively scored. Examples for leaf growth inhibition (*) and stimulation (#) are shown.

Figure(s)

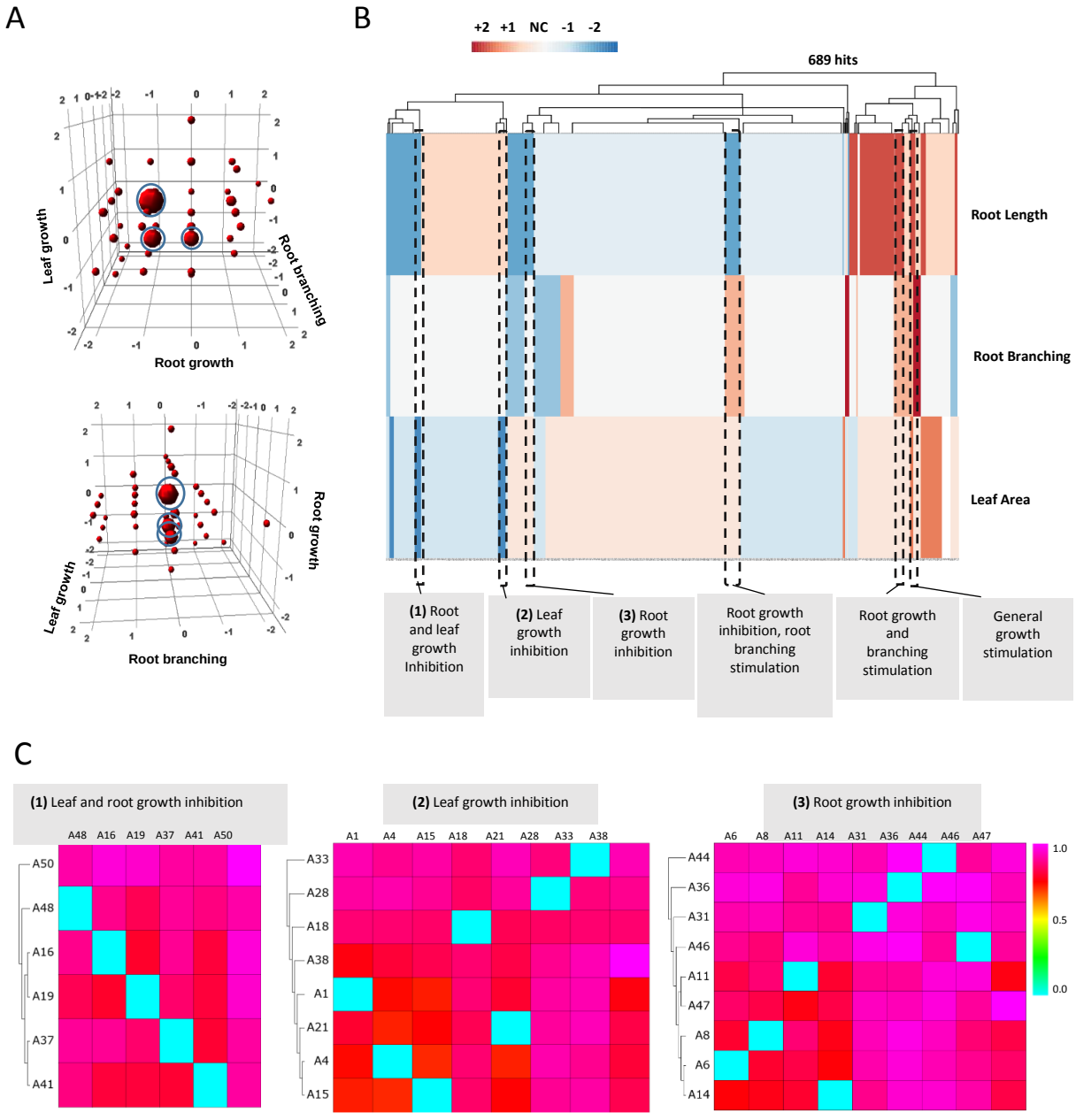


Figure 2: The HTS identified a diversity of Arabidopsis growth regulators

A) Bubble 3D plot of the similarity relationship of compounds causing root growth, leaf growth and root branching growth phenotypes. Growth fold change compared to untreated controls is plotted. 0 indicates no change, positive values indicates induction, negative values indicates inhibition compared to control plants. The bubble plot shows the number of records at each combination of categories. The size of the bubbles represents the quantity of compounds that promotes a determined growth phenotype. Upper and lower charts display different spatial orientations of the same graph to facilitate visualization. Circled bubbles represent the bigger groups. **B)** The dendrogram represents a hierarchical clustering analysis (Euclidean distances) of bioactive compounds based on Arabidopsis growth phenotypes. Three different traits were considered for clustering analysis; root growth, root branching and leaf growth. Fold changes of growth compared to untreated controls was graded from -2 (maximum inhibition) to +2 (maximum stimulation). The difference was associated to a color, with closeness to red indicating growth stimulation and closeness to blue indicating growth inhibition and no change in light blue (NC). Dotted ellipses indicates examples of phenoclusters of compounds that affect growth in differential extent. Three inhibitory clusters were selected for further analysis, leaf and root growth (1), leaf growth (2) and root growth (3) inhibition. **C)** Functional dendrogram with clustering based on chemical similarity using a distance matrix. Zero indicates that the compounds are identical and 1 indicates compounds that are unique. Values below 0.5 indicates statistical significantly similarity. Compound distance matrix scores were plotted for inhibitory compounds members of clusters leaf and root growth inhibition(1), leaf growth inhibition (2) and root growth inhibition(3). The score is represented by heat map (coded cyan to purple). Note that identical compounds only appear when there is a reciprocal comparison among themselves.

Figure(s)

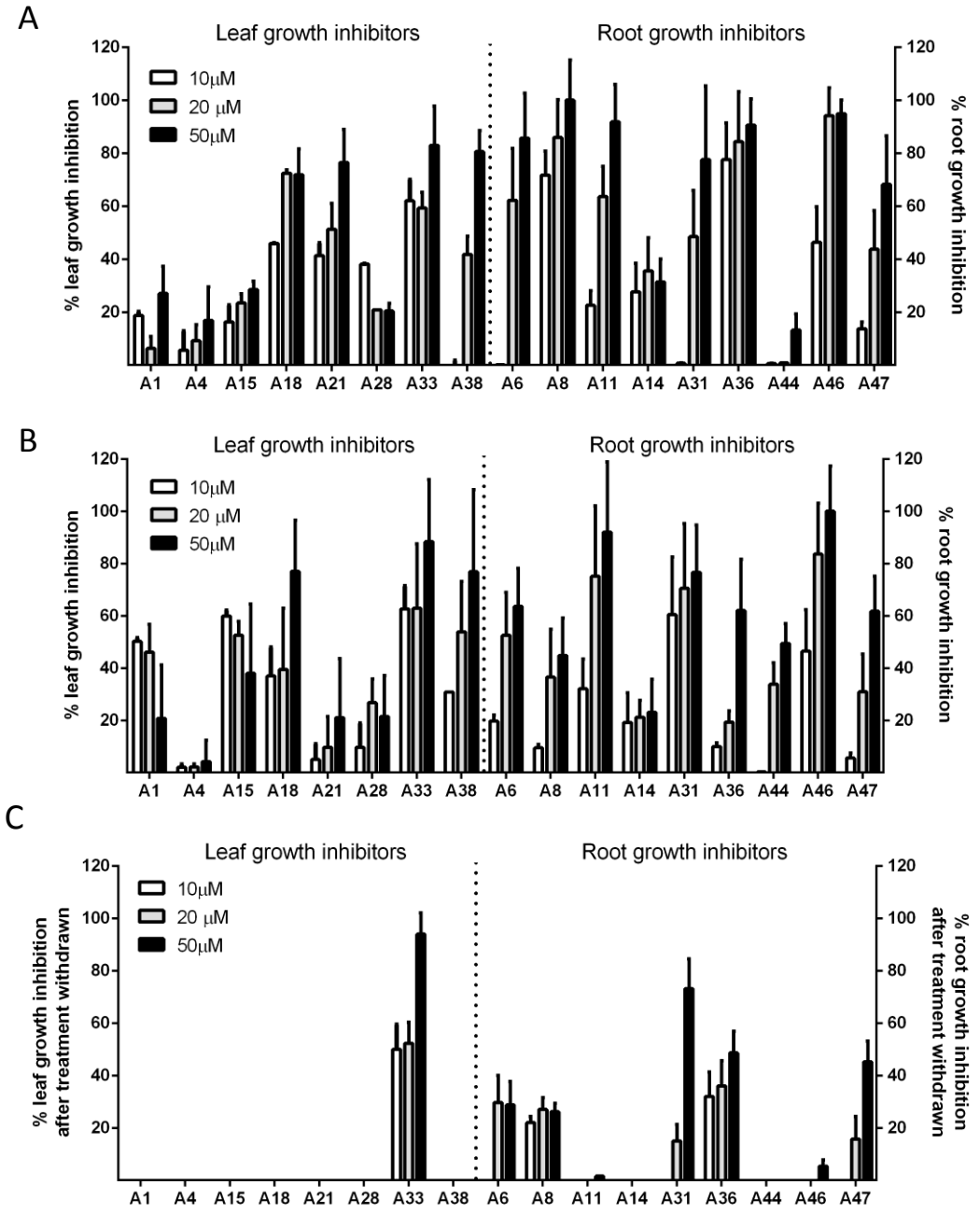


Figure 3: Characterization of the root and leaf growth inhibitory compounds on Arabidopsis. The dose dependence effect was tested using 10μM (with bars), 20μM (gray bars) or 50μM (black bars) of the leaf growth inhibitors, left panel (A1, A4, A15, A18, A21, A28, A33, A38) and the root growth inhibitors right panel (A6, A8, A11, A14, A31, A36, A44, A46, A47). (A) Seeds of *A. thaliana* (Col-0) were plated on media containing different concentrations of each chemical. Root and leaf growth inhibition percentages compared to control treatments are plotted. (B) To test the capability of the different compounds to induce leaf (left panel) or root (right panel) growth inhibition, 7 day-old Arabidopsis was exposed to each chemical. After additional 7 days root and leaf growth rate was analyzed. Root and leaf growth inhibition percentages compared to control treatments are plotted. (C) Arabidopsis grown seedling in A) were transferred to a regular plant media to analyze the phenotype reversibility. Root and leaf growth inhibition percentage after treatment withdrawn are plotted. Results are representative percentages of three independent experiments, at least 15 seedlings per repetition. SD values are shown.

Figure(s)

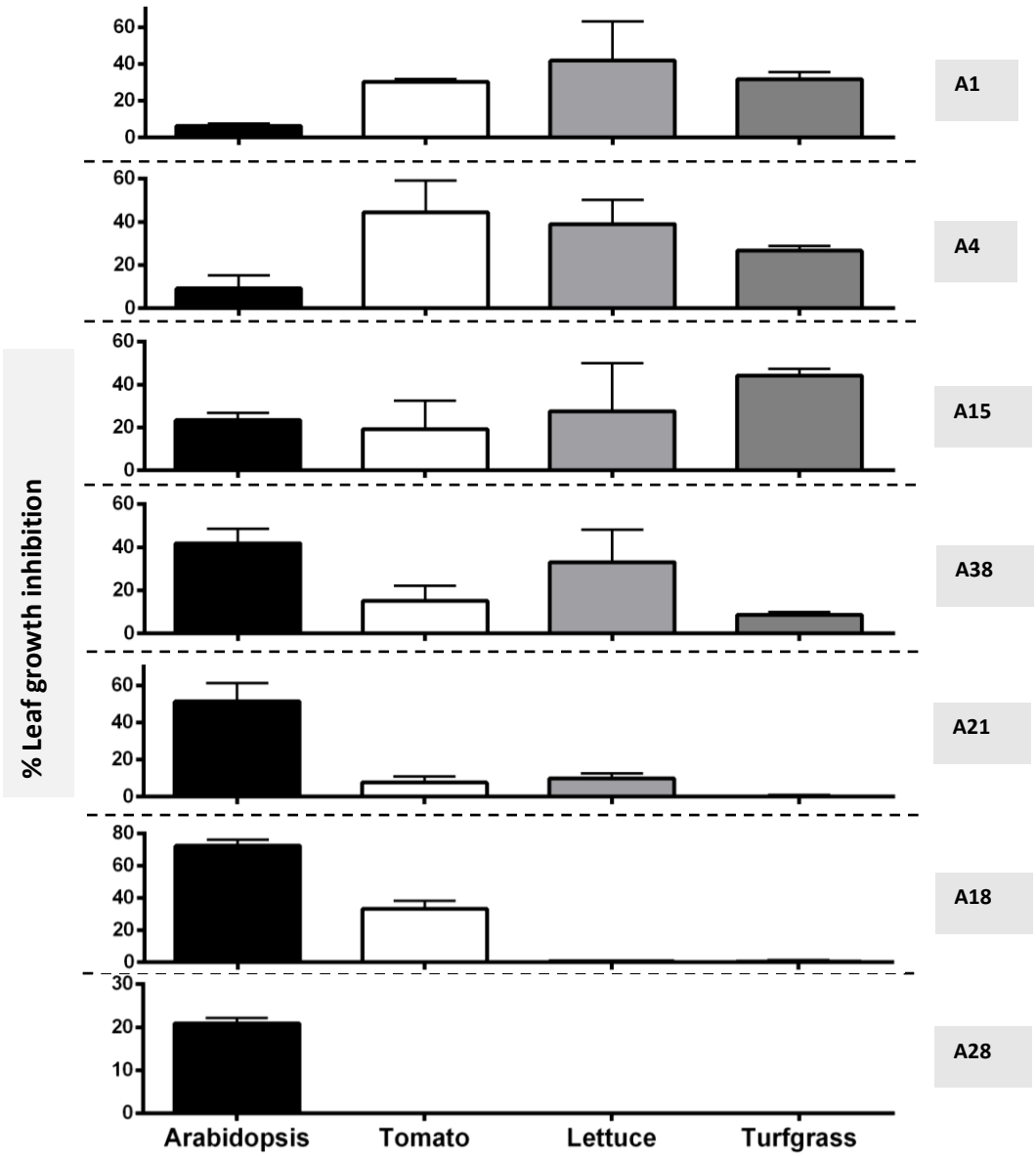


Figure 4: Arabidopsis leaf growth inhibitors are effective on agronomically significant models.
Arabidopsis leaf growth regulators characterized as leads (black bars) were tested in different species. Leaf growth inhibition was evaluated in treatments with 20µM of each compound in tomato (*Solanum lycopersicum* cv. Micro-Tom; white bars), lettuce (*Lactuca sativa longifolia*; light gray bars) and turfgrass (*Paspalum vaginatum*; dark gray bars). All results are expressed as percentage of leaf growth inhibition in treatments against leaf growth in control conditions. Results are representative of three independent experiments. Mean and SD values are displayed in the graphics. N per repetition =3.

Figure(s)

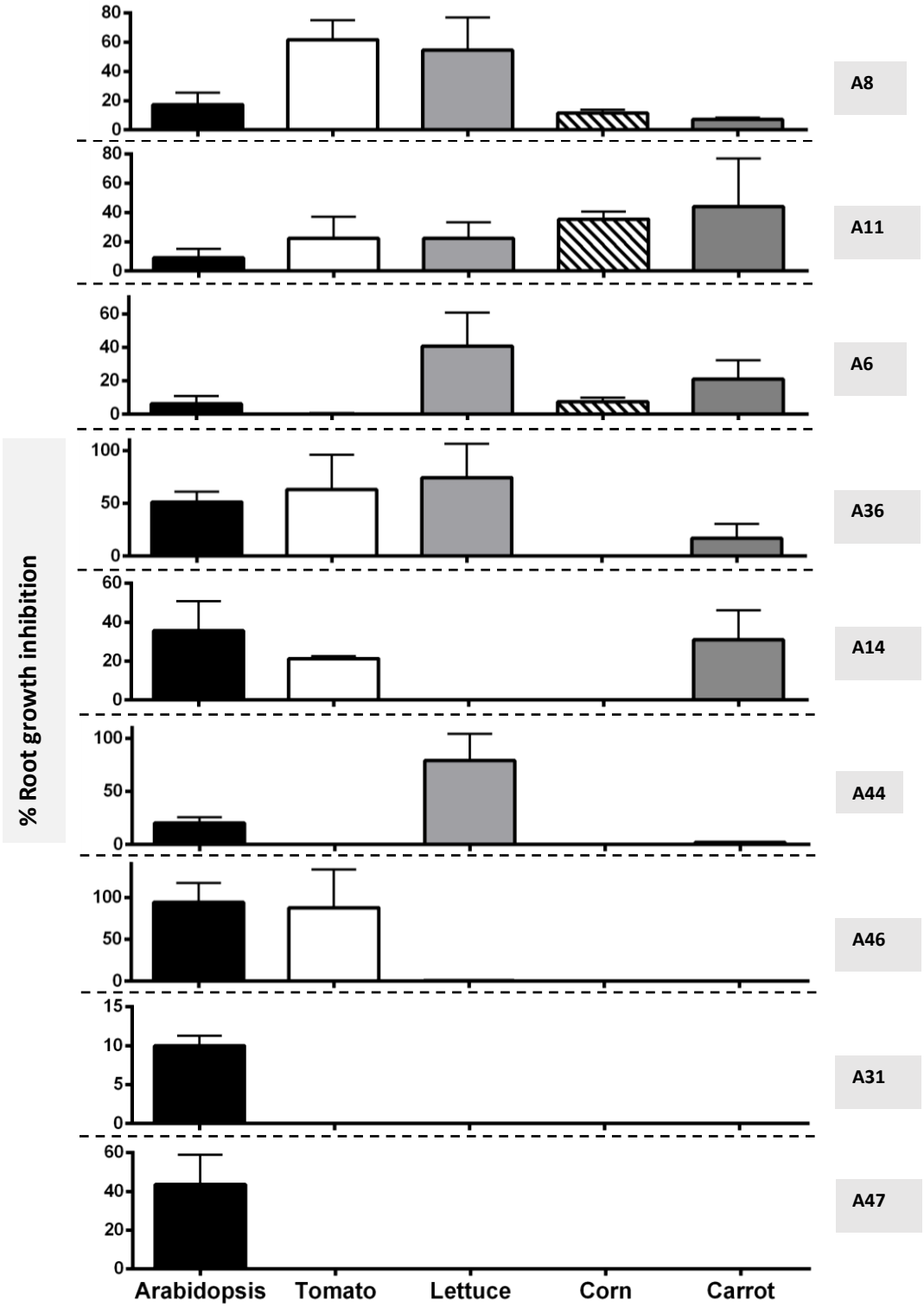


Figure 5:Arabidopsis root growth inhibitors are effective on agronomically significant models.
Root growth regulators identified in the Arabidopsis HTS as leads (black bars) were effective on agronomically significant models. Root growth inhibition induced by 20µM of each compound was evaluated in tomato (*Solanum lycopersicum* cv. Micro-Tom; white bars), lettuce (*Lactuca sativa longifolia*; dark gray bars), carrot (*Daucus carota*; light gray bars) and corn (*Zea mays*; white lined bars). Results are representative of three independent experiments. The graphics displays mean and SD values. N=3 experiments.

Graphical Abstract

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