SOIL DWELLING *STREPTOMYCES* INHIBIT *ARABIDOPSIS* SEED GERMINATION

by

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I. Abstract

Streptomyces are a cosmopolitan and economically important genus of Actinobacteria known for producing an array of secondary metabolites, including many clinically relevant antibiotics. *Streptomyces* are ubiquitous in soil environments throughout the world and have been identified as prominent members of the microbiomes of diverse plant species. The presence of *Streptomyces* around and within plants has been shown to provide a wide variety of fitness benefits including increases in disease resistance and abiotic stress tolerance. Despite recent increased interest in the benefits of these bacteria to associated plants, relatively little is known about the genetic basis for the plant growth promotion capabilities shown by *Streptomyces*, or about the traits that facilitate plant interaction. In this work plant phenotypes resulting from inoculation with isogenic bacterial cultures are quantified and correlated with the genetic background of the bacteria used. The 9 isolates of soil derived *Streptomyces* influence the early growth phenotypes of co-cultured *Arabidopsis thaliana* Columbia-0 seedlings in a manner consistent with previously identified phylogenetic clade associations. Quantification of phenotypes of seedlings growing in association with bacterial cultures, and correlation of those phenotypes with the genetic backgrounds of associated isolates, demonstrates how results from an *in vitro* plant assay can be used to elucidate genomic regions with implication for plant colonization and interaction among closely related *Streptomyces* bacteria.

II. Introduction

Plants are always observed to associate with microbes in all known natural environments(Yutin et al. 2008). Plant-microbe interactions organize into microbiomes whose composition has an impact on that plant's fitness. Benefits of soil microbes to plants include abiotic stress tolerance (Hiruma et al. 2016), increased disease resistance (Liu et al. 2017), growth promotion (Sousa and Olivares 2016; Finkel et al. 2017), and nutrient acquisition (Francis, Holsters, and Vereecke 2010; Zgadzaj et al. 2016). The obligate association of microbes with healthy plants, and the changing composition of microbiomes in response to environmental stressors, suggests that manipulation of microbial communities may be an avenue for improving agricultural productivity. In order to provide benefits throughout the life of the plant, a given strain must first be recruited to a root or seed. Much work has focused on understanding the actions of endophytic bacteria isolated from plant tissues, but the promise of growth promoting soil dwelling bacteria cannot be realized until we more clearly understand the features that make a given strain able to establish a long-term association with plant microbiomes.

A plant growing in soil will acquire a root microbiome during the germination process through recruitment of microbes from the surrounding soil and potentially from microbes already living within the seed. Soil near developing roots is altered by the plants through physical expansion of the root and secretion of mucilage and other compounds rich in sugars and secondary metabolites (Heijden and Schlaeppi 2015; Van Deynze et al. 2018). Secretions recruit soil bacteria to grow in association with the plant, and the types of compounds secreted by the plant can be modulated in response to specific environmental conditions (Y.-T. Chen, Wang, and Yeh 2017; Stringlis et al. 2018). This heavily plant influenced area of soil surrounding developing roots is called the rhizosphere (Pinton R, Varanini Z, Nannipieri P. eds. 2009). The significant fitness cost to the plant of maintaining the rhizosphere, and the ubiquity of root bacteria interaction, suggests an essential role for the rhizosphere microbiome in maintaining plant fitness. Recently, novel techniques have shown rhizosphere species compositions across varied conditions and locations all have unique microbiomes with less bacterial species diversity than the surrounding soil, but a larger biomass (Singer 2006; Bulgarelli et al. 2013). This indicates that there are mechanisms that inhibit or discourage establishment of a more diverse collection of microbes within the rhizosphere.

Microbial communities within plant microbiomes are diverse and form unique associations in and around every plant with which they interact. Given the number of bacterial families present in soil, variation is not unexpected, but there are patterns in rhizosphere bacterial populations that are consistent across plant families (Fitzpatrick et al. 2018). This suggests that some species bacteria have features that allow for easier rhizosphere recruitment and establishment. *Streptomyces* are ubiquitous soil microbes that are prominent members of diverse plant microbiomes (Liu et al. 2017; Seipke, Kaltenpoth, and Hutchings 2012). We know that the presence of *Streptomyces* in the rhizosphere can provide protection against infections (Conn, Walker, and Franco 2008), provide biofertilization (Jog et al. 2014), and growth stimulating effects (Sousa and Olivares 2016). *Streptomyces* are well known for secondary metabolite production (Barka et al. 2016), including many clinically relevant antibiotics that strongly influence microbiome composition and may affect colony establishment within the rhizosphere. Antibiotic production by *Streptomyces* species in plant associated microbiomes may help to prevent unwanted pathogen establishment in those microbiomes (Barka et al. 2016; van der Meij

et al. 2018). Some compounds produced by *Streptomyces*, such as nitric oxide (Chater 2016), have been implicated in plant development through interaction with plant hormones during abiotic stress (He, He, and Gu 2012). Some *Streptomyces* are known to produce siderophores, and to solubilize potentially important plant nutrients such as phosphates (Jog et al. 2014; Subramaniam et al. 2020), traits that have the potential to enhance the growth of associated plants.

Certain genomic features of *Streptomyces*, such as high G/C content and relatively complex linear genomes (Hopwood 2019; Paradkar et al. 2003), make laboratory manipulation comparatively challenging. Stratified sampling of a diverse population of *Streptomyces* isolates from natural soils in different locations may allow for comparison of similar genotypes adapted to different environmental conditions. This work demonstrates that *Streptomyces* can elicit variable seed and seedling phenotypes in a laboratory setting. The significant differences in plant phenotype resulting from plant growth with previously unknown soil derived *Streptomyces* isolates correlate with the previously identified genetic relationships of those bacteria, which can assist in future identification of the genetic components underlying *Streptomyces*' interaction with plants. A lab assay that produces quantifiable phenotypes from seeds co-cultured with soil derived bacteria provides a valuable screen for otherwise uncharacterized isolates. Data on phenotypes derived from laboratory screening of individual isolates, in combination with sequence information for those isolates, may further understanding of how the natural variation in genetic background allows those isolates to interact with plants.

III. Methods

Streptomyces Isolate Collection

In 2017 approximately 10g of surface soil was collected from four separate grassy areas to yield the strains focal to experiments herein. Two of which were on Mount Lemmon, at about 8000 feet of elevation, in the Santa Catalina range. Two more were from the Chiricahua Mountains, at about 9000 feet of elevation. Both mountains are in southern Arizona and are sky island mountain ecologies distinguished by their topographical isolation, and their lower temperatures compared to surrounding flatland areas. Samples of this soil were diluted in water and cultured on plates containing solid arginine-glycerol media (El-Nakeeb and Lechevalier 1963), and the resulting colonies were screened for *Streptomyces* phenotypes (e.g. colony shape and sporulation). Probable *Streptomyces* colonies were then isolated and streaked onto fresh arginine-glycerol plates, and spore preparations were made. Bacterial *rpoB* genes were amplified by PCR using previously identified primers (Andam et al. 2016), and classified based on rpoB gene sequence similarity (Caitlin Smith, unpublished data 2018). Two divergent clades, with roughly the same level of intra-clade diversity, were selected for study from a much larger collection of phylogenetically diverse strains. During lab culture, a spontaneous isolate of ad2-2 appeared and resulted in the distinct isolates ad2-2p and ad2-2w.

Media Preparation

For germination plates, 2.22g of Murashige and Skoog media with Gamborg's vitamins was used for each liter of media prepared. This was supplemented with 10g sucrose (1% w/v), 10g bacteriological agar (1% w/v), and mixed with 1L deionized water, then sterilized with a 60-minute liquid autoclave cycle. Sterile media was poured into 120mm by 120mm plates and left to cool and stored until use.

Inoculum preparation

To grow cultures of *Streptomyces* isolates, sterile 15mL culture tubes were prepared with 3mL of room temperature LB broth for each isolate. An inoculating loop was used to scrape a small amount of bacterial suspension from stock tubes stored at -80°C or, with a sterile pipette, 200µL of LB broth from a culture tube with the desired strain already present and transferred to the target broth. Liquid cultures of desired strains were kept for 5 days on a New Brunswick Scientific (Edison NJ, USA) incubator/shaker set to 27°C before the planned experiment date. After the 5-day incubation period 1mL of liquid culture per isolate was added to an empty 2mL centrifuge tube containing sterile glass beads. Bacterial cultures were homogenized in two 30 second intervals with a 5-minute break in between, on a MP Biologicals Fastprep 24 (Irvine CA, USA) set to 5.5 m/s. Homogenized cultures were transferred by sterile pipette into a new 2mL centrifuge tube and spun down at 6650 rpm for 3 minutes. Supernatant was removed by sterile pipette and volume was corrected back to 1mL with deionized water. This process was repeated 3 times, after which the optical density was measured. The OD600 of each inoculum was adjusted to 0.2.

Seed Sterilization

Immediately before inoculation, *A. thaliana* ecotype Columbia-0 seeds were surface sterilized by placing them into a 1.5mL centrifuge tube with a freshly made solution of 50%

deionized water, 50% household bleach (6% sodium hypochlorite), and 0.01% Triton X-100. Seeds in bleach solution were incubated for 10 minutes with periodic vortexing to ensure elimination of any surface microbes. After 10 minutes the bleach solution was siphoned off with a sterile pipette, replaced with sterile water, and vortexed. This process was repeated at least 4 times to ensure no bleach is present on the seeds during the inoculation process.

Seed Inoculation

Approximately 100µL of sterilized seeds were transferred into a new 1.5mL sterile centrifuge tube for all 9 isolates and 1 for a control group. 300µL of prepared inoculum for each isolate, and 300µL of sterile water for the control, was added to seeds directly before the plating process. Using a 2mL or 10mL sterile pipette, seeds were placed onto target germination plates one at a time in 12 offsetting rows of 6 seeds each, for a total of 72 seeds per plate. Each plate was sealed with parafilm and covered with heavy duty aluminum foil to prevent desiccation and light absorption. Wrapped plates were stored at 4°C for 5 days to stratify the seeds in order to normalize germination rates.

Data Collection and Visualization

After 5 days at 4°C, treated plates with controls were placed in a growth chamber set to 16 hours light at 22°C and 8 hours dark at 18°C set at 65% humidity. On the second and eighth day after plates were transferred to a growth chamber, at roughly the same time of day, each plate was scanned at 800dpi, two at a time, on a Canon CanoScan LiDE220 photo scanner, to acquire a digital image for further analysis and quantification.

Each image was imported into the ImageJ program for analysis. Each 2-day image was analyzed for radicle emergence, while 8-day plate images were analyzed for radicle emergence, the presence or absence of true leaves, and the angle of root elongation relative to the starting growing point of each respective root being measured. This data was imported into the RStudio program for statistical analysis running the plugin libraries ggplot2, ggpubr, plyr, and dunn.test. The data was analyzed and summarized by the non-parametric Kruskal-Wallis analysis of variance test, and further analyzed with Dunn's multiple comparison test for non-parametric, post-hoc pairwise comparison.

In total, 8 replicated assays were carried out, with each assay consisting of 9 treatment plates and 1 control plate with approximately 72 seeds analyzed per plate. During the course of the experiment plates were discarded due to desiccation of media, excessive visible contamination, or excessive force from drops of condensation that dislodged multiple seeds. All data from the 8 replicates presented here come from complete replicates of 10 total plates, with seeds from the same source that had been sterilized in unison. The order of plating for treatment and control plates was randomized by replicate to prevent any bias that may have resulted from seeds being exposed to either sterile water or inoculum for a longer interval than others within the same replicate.

IV. Results

There have been previous reports of *Streptomyces* species involvement in signaling that may affect seed germination and dormancy (Chater 2016). Radicle emergence signifies that a given seed has broken dormancy (Bewley 1997). Radicle emergence was assayed, at 2 and 8 days after placement in a controlled germination environment, to understand the effect of *Streptomyces* isolates on the dormancy of inoculated seeds. Kruskal-Wallis test analysis showed that 2 days after transfer to a growth chamber the radicle emergence rate of *A. thaliana* seeds challenged with *Streptomyces* from clade 1, 55%, is significantly different from clade 2, 74%, and the control group, 81%, with a p-value of less than 0.001 with 7 degrees of freedom (Fig. 1). The control group and clade 2 were not significantly different, p-value = 0.18 (Fig. 1). The rate of radicle emergence on day 8 follows the same pattern of significance, with the percentage of seeds completing germination being 98% for controls, which was not significantly different from the 96% rate for clade 2, p = 0.28, and different from the 78% for the first clade, p less than 0.001 at 7 degrees of freedom (Fig. 2). This shows clearly that the strains comprising Clade 1 have a partial, yet significant, effect on the germination of *A. thaliana* seeds in this assay.

True leaves are not formed within the embryonic seed but develop after a seed has broken dormancy (Boyes et al. 2001). The presence of true leaves shows that a plant has moved beyond germination, and has begun vegetative growth and development (Boyes et al. 2001). If a seed shows germination delays when growing in contact with a *Streptomyces* isolate it is possible that continued growth and development of that seedling may be inhibited by the continued growth of the associated isolate. To assay if the isolates have an inhibitory effect on the developmental progression of associated seedlings, the rate of true leaf formation was analyzed and compared using the Kruskal-Wallis analysis of variance test. The data for the presence of true leaves among treatments shows variation in a similar pattern to radicle emergence, with clade 1 isolates being significantly different than clade 2, p-value less than 0.001, and clade 2 being similar, p-value = 0.29, to controls (Fig. 3). The number of plants challenged with isolates from clade 1 with true leaves at 8 days after transfer to a growth chamber was 26%, compared to 86% and 91% for clade 2 and control respectively, with 7 degrees of freedom (Supp. fig. 4). The difference among treatments in seeds that develop true leaves, 60% between clade 1 and clade 2, contrasts sharply with the 18% difference in day 8 radicle emergence between treatments (Fig. 3).

It has been previously reported that some soil bacteria species affect the auxin gradients around developing roots (Finkel et al. 2019), increase plant auxin production, or synthesize auxins as a mechanism for growth promotion in associated plants (Sahoo, Bhardwaj, and Tuteja 2013; Li et al. 2019). *Streptomyces* species have been suspected of producing auxin and promoting auxin production in associated plants as well (Subramaniam et al. 2020). Gravitropic root growth by *A. thaliana* results in roots growing straight downward. This results from amyloplasts in root cap cells naturally orienting themselves in relation to gravity, which results in auxin distributions in nearby expanding root cells that signal those cells to grow along with gravity (R. Chen, Rosen, and Masson 1999). Disrupting this auxin gradient, naturally or otherwise, will result in agravitropic growth that doesn't neatly follow the normal elongation patterns (Zou et al. 2012). To ascertain whether *Streptomyces* isolates affect gravitropism and the developing root of newly germinated *A. thaliana* seeds, the angle of growth of the elongating primary root relative to a line perpendicular to the top and bottom of experimental plates was measured. Using the ImageJ program, a line from the furthest elongating root tip was connected

at its growth origin to a line representing growth straight down. The angle created at the intersection of these lines was measured to quantify the variability in gravitropism among treatments. Treatments from both clade 1 and 2 are significantly different from control plants in regard to the angle of root elongation, while also being significantly different from each other, all at a p-value less than 0.001 (Fig. 4). In measuring the angle of root elongation each individual seed was a discrete data point which lends statistical power to the data but may also potentially mask notable phenotypic similarities. For instance, the average root elongation angle of clade 1 is 29° +/- 23°, while the average angle of clade 2 is 8° +/- 10°, and the control is 5° +/- 8° (Supp. fig. 5). Strains from clade 1 had a significant and pervasive effect on the gravitropism of *A. thaliana* seedlings, while strains from clade 2 had a smaller effect that was more similar to the control than to clade 1 (Fig. 4).

It is clear in this assay clade 1 isolates have a significant inhibitory effect on the early developmental processes of *A. thaliana* seedlings when compared to uninoculated control seedlings, and to seedlings assayed in concert with *Streptomyces* isolates from clade 2. Within each of the clades there are minimal differences in seedling phenotype with the exception of isolate ad2-2p (Supp. tables 1-4). When first brought in the lab ad2-2 was a single isogenic isolate. During spore preparations a spontaneous white mutant appeared from strain ad2-2 referred to here as ad2-2w. This mutation changed the color of spore preparations from a faint purple color to white but did not visibly change the color or consistency of colonies growing in association with seeds and seedlings, glycerol stocks in long term cold storage, or in liquid cultures. Using the non-parametric, post-hoc pairwise Dunn's test for analysis, seedlings in association with ad2-2p were not significantly different from the control group in Day 2 germination rate (Supp. table 1), $p = 0.07$ at 9 degrees of freedom. When considering rates of radicle emergence at day 8, $p = 0.01$ with 9 degrees of freedom, the appearance of true leaves, $p = 0.01$ with 9 degrees of freedom, and angle of root elongation, $p = 0.000$ with 9 degrees of freedom, seedlings grown in association with ad2-2p are significantly different from the control (Supp. tables 2, 3, 4). There are some statistical overlaps between ad2-2p and isolates of clade 2 when analyzing day 8 germination rate and presence of true leaves, while the other isolates in clade 1, including the mutant ad2-2w are statistically similar to each other (supp. tables 2, 3, 4). When considering the day 2 rate of radicle emergence, ad2-2p elicits phenotypes more similar to clade 2 isolates than clade 1 (Supp. table 1). In this assay the phenotypes of seeds and seedlings grown in association with isolates ad2-2p and ad2-2w were similar in rate of germination 2 days after transferring to a growth chamber, but significantly different in germination at day 8, true leaf formation and angle of root elongation (Fig. 5).

V. Discussion

Plants and microbes are always found in association in nature, but the details of the mechanisms and methods implicated in plant and microbe interactions are not fully understood. In the past 30 years there has been a marked increase in research into these interactions facilitated by the rapid increase in next generation sequencing technologies (Finkel et al. 2017). Plants spend a significant amount of energy producing root secretions that result in recruitment of a unique root associated microbiome from surrounding bulk soils. (Bais et al. 2006) Plants have demonstrated modulation of root secretions in response to specific environmental conditions that result in variations to root microbiome recruitment to root surfaces. (Stringlis et al. 2018; Korenblum et al. 2020) The soil bound bacteria that would make up this microbiome is very diverse, and potentially includes tens of thousands of species. (Berendsen, Pieterse, and Bakker 2012)

The cosmopolitan and economically important genus *Streptomyces* is often well represented in the endosphere microbiome of multiple plant species (van der Meij et al. 2018; Seipke, Kaltenpoth, and Hutchings 2012). *Streptomyces* are ubiquitous in soil, (Tarkka et al. 2008; Seipke, Kaltenpoth, and Hutchings 2012) and the presence of *Streptomyces* in plant associated microbiomes has been shown to have growth promoting effects on associated plants, such as activating the innate immune system of an associated plant, which may create a more difficult environment for potential pathogens to establish disease causing relationships (Conn, Walker, and Franco 2008), nutrient mobilization (Jog et al. 2014), and modulation of hormone levels (van der Meij et al. 2018). It is not known what features of *Streptomyces* species allow them to respond to signals from plants, or what mechanisms are used by *Streptomyces* to directly or indirectly effect the growth of an associated plant. *Streptomyces* species are genetically diverse and have genomic features, such as relatively large linear chromosomes (Hopwood 2019) and high G/C content (Harrison and Studholme 2014), making genetic manipulation challenging. Work has been done to correlate genetic features of *Streptomyces* and traits involved with bioremediation, morphology, antibiotic production, and antibiotic resistance (Hopwood 2019; Subramaniam et al. 2020; Chater 2016). Variation in plant response to distinct bacterial isolates in lab assays can reveal genetic regions underlying plant association. Functional studies of *Streptomyces* in combination with genomic comparison among those studied strains can identify traits that, through selective manipulation, may improve agricultural outcomes in associated plants.

The *Streptomyces* genus is known to promote plant growth through induction of defense pathways (Conn, Walker, and Franco 2008), pathogen suppression (Kurth et al. 2014; Barka et al. 2016), mineral nutrient solubilization and uptake (Sousa and Olivares 2016), and growth stimulation through modification of plant hormones (Barka et al. 2016; Romano-Armada et al. 2020). Growth stimulation through phytohormone interaction by *Streptomyces* species has been linked with abiotic stress tolerance (van der Meij et al. 2018; Romano-Armada et al. 2020) Although frequently shown to interact with auxin, *Streptomyces* have also been shown to interact with salicylic acid (Chewning et al. 2019), and gibberellic acid (Solans et al. 2011). The exact nature of these interactions, and how *Streptomyces* species are involved in either the production, induction, reduction, or catabolism of phytohormones is not fully understood. Phytohormone modulation is one potential explanation for the variable plant phenotypes quantified in this work. Agravitropic root growth by *Streptomyces* associated seedlings may result from the disruption of the natural gradient of auxin that normally follows gravity, which has been observed in other

work (R. Chen, Rosen, and Masson 1999). The formation of true leaves is a product of cytokinin regulated genes (Zhang et al. 2014), while germination relies on precise levels of hormones, especially gibberellic and abscisic acid, in combination with light signals (Seo et al. 2009). These effects could all be a product of disrupted hormone gradients induced by delocalization of auxin levels in the presence of some *Streptomyces* isolates, but they could also result from the direct influence of *Streptomyces* on gibberellic acid in the case of germination inhibition (Solans et al. 2011), or cytokinin in the case of true leaf inhibition (Aldesuquy, Mansour, and Abo-Hamed 1998). Due to the difference in environmental signals as compared to plants and bacteria being grown in soil or a field, the environment present in the current study can be considered an abiotically stressed environment, specifically in regard to seedling development after germination. The environment these seedlings are developing in may elicit interaction with phytohormones by *Streptomyces*, potentially including increases in localized phytohormone levels, which could explain the agravitropic root elongation observed in associated *A. thaliana* roots. The observed interactions between bacterial colonies and developing seedlings in the present assay may utilize some of the same mechanisms responsible for the increase in *Streptomyces* activity in plants under abiotic stress in other work (Fitzpatrick et al. 2018; Romano-Armada et al. 2020).

Although in the context of the assay presented here the association with certain bacteria has negative consequences on plant health, this is not necessarily representative of the phenotype one would expect from association with any one of these isolates in a wild setting, due to the presence of other microbes and the potential ameliorating effects of microbe-microbe interactions in a wild microbiome. The soil microbiome has potentially tens of thousands of species from which the plant microbiome is recruited and established, and it has been previously shown that interactions within the microbiome will ultimately determine the effect on an associated plant in a natural setting (Finkel et al. 2019). The species complexity present can mask potentially deleterious effects of a given isolate and allow for other growth promoting effects to work on a plant. The presence of exogenous sugars and nutrients in the media also further complicates comparison of the seed and seedling phenotypes quantified in this work with the phenotypes of plants grown in a natural environment. It is possible that the pathogenic behavior of some of these isolates *in vitro* indicates an isolate likely to be recruited to the rhizosphere and eventually endosphere of a developing plant.

One potential explanation for the differences in associated seedling growth between clades of bacteria is the difference in amount of bacteria present in the prepared inoculum. Despite steps taken to control the quantity of bacteria used to inoculate seeds, there was some observed variation in the resulting colony sizes of bacteria growing in association with seeds and seedlings. Robust replication of the assay suggests that severity of observed phenotypes is not a function of any variation in bacterial quantity growing in direct association with the observed seedling, but with the genetic background of that bacteria. Additionally, there are no significant differences between the 8 assay replicates across all quantified phenotypes (Supp fig. 1). The phenotypic differences seen in 2- and 8-day germination rate, the presence of true leaves, and the angle of root elongation, are more divergent between the two identified clades as opposed to within each clade despite the potential variability in bacterial density among replicates (Supp. fig. 2, 3, 4, 5). This suggests that the genetic background of the isolates is influencing the observed phenotypes more than the quantity of bacteria in a given inoculum. The isolates used visually appeared to grow robustly in association with germinating seeds and developing seedlings. This may be due to the secretions by the developing plants, which can provide

associated bacteria with important nutrients, or molecular signals that enhance vegetative hyphae formation (Bais et al. 2006, Vives-Peris et al. 2019). If robust bacterial growth is observed in association with germinating seeds and developing seedlings, it suggests that plant derived nutrients are a potentially valuable nutrition sources for *Streptomyces*.

Among and within the clade associations there are some interesting variations in phenotypes to explore. The germination rate of seeds at day 8 follows the pattern of clade association previously identified (Smith C, unpublished data 2018). All of the strains identified as members of clade 1 have significantly different germination rates 8 days after transfer to growth chamber from all of the strains in clade 2, as well as the control (Supp fig 3, Supp table 2). This indicates that clade 1 isolates have features that effect the germination of seeds grown in association, while the isolates in clade 2 do not. This can assist in identification of genetic features involved in the observed germination defects because it correlates differential seed phenotypes with the genetic background of isolates grown in concert. The phenotypes presented go beyond germination defects. Considering the presence of true leaves on seedlings that have been in a growth chamber for 8 days, isolate ad2-2p is different from the control group as well as 1 clade 1 isolate, and 1 clade 2 isolate (Supp. table 3). Seedlings grown in association with isolate ad2-2p had a seemingly intermediate rate of true leaf formation when compared to other isolates from clade 1 and 2 (Supp. table 3). Seedlings grown with the other 4 isolates in clade 1 have similar rates of true leaf formation after 8 days in a growth chamber, but significantly different rates of true leaf formation from seedlings grown with any of the 4 clade 2 isolates (Supp. table 3). This seems to implicate something in the background of isolate ad2-2p that makes it interact differently with seedlings in this assay from the other 4 isolates in clade 1. The isolates ad2-2p and ad2-2w were originally identified as 1 isogenic isolate, but during the normal process of transfer and maintenance of lab stocks there was some event that was correlated with the visually distinct, white instead of purple, ad2-2w isolate. Additional analysis of these two isolates showed they are similar except for a 100kb stretch of genome present ad2-2p that is absent from ad2-2w (Dave Baltrus, unpublished data 2020). In measuring the angle of elongation of roots the strains in clade 1 are all similar to each other, but different from the clade 2 and control seeds, with the exception of ad2-2p, which is different from the control and all other isolates except xl-6 (Supp. table 4). There is also variation within clade 2 regarding angle of root growth of seedlings that had been transferred to a growth chamber 8 days prior, with 2 isolates, xl-6 and xl-10 being similar, and ml1-2 and ml2-9 being similar, but both pairings are otherwise different from each other as well as from the control group (Supp. table 4).

Currently there is no reliable way to predict the effect of any given strain of *Streptomyces* on an associated seed because the specific genetic features that make a bacterium effective at plant growth promotion are unknown. Due to the diversity of *Streptomyces* across the world, and the still developing understanding of the mechanisms that underlie recruitment and establishment of these bacteria in plant associated microbiomes, insight into the genetic background of soil borne *Streptomyces* may be helpful in identifying candidate species for future study into their plant growth promoting effects. This work demonstrates novel *Streptomyces* isolated from diverse bulk soil having an inhibitory effect on associated seeds and seedlings in absence of other organisms commonly present in plant microbiomes. The pathogenicity of some isolates, and the lack of pathogenicity of others, is correlated with the genetic background of those strains, and potentially indicates a propensity for plant interaction in a natural setting. However, the clade correlated pathogenicity identified in the present study could also indicate an inability of certain species to overcome the defense systems of the associated seeds. The 100kb difference

between the purple and white version of isolate ad2-2 represents a region of interest with implications for plant microbiome membership based upon the quantitative difference in plant response to these otherwise genetically indistinguishable isolates. The difference in phenotypes between the seedlings grown in association with the 2 clades represents a possible phylogenetic signal for plant recruitment and interaction, and the 100kb stretch of genes seems to contribute to this signal. This shows the utility of combining laboratory assays with genetic analysis for discovery of genes related to bacterial establishment in association with a plant microbiome.

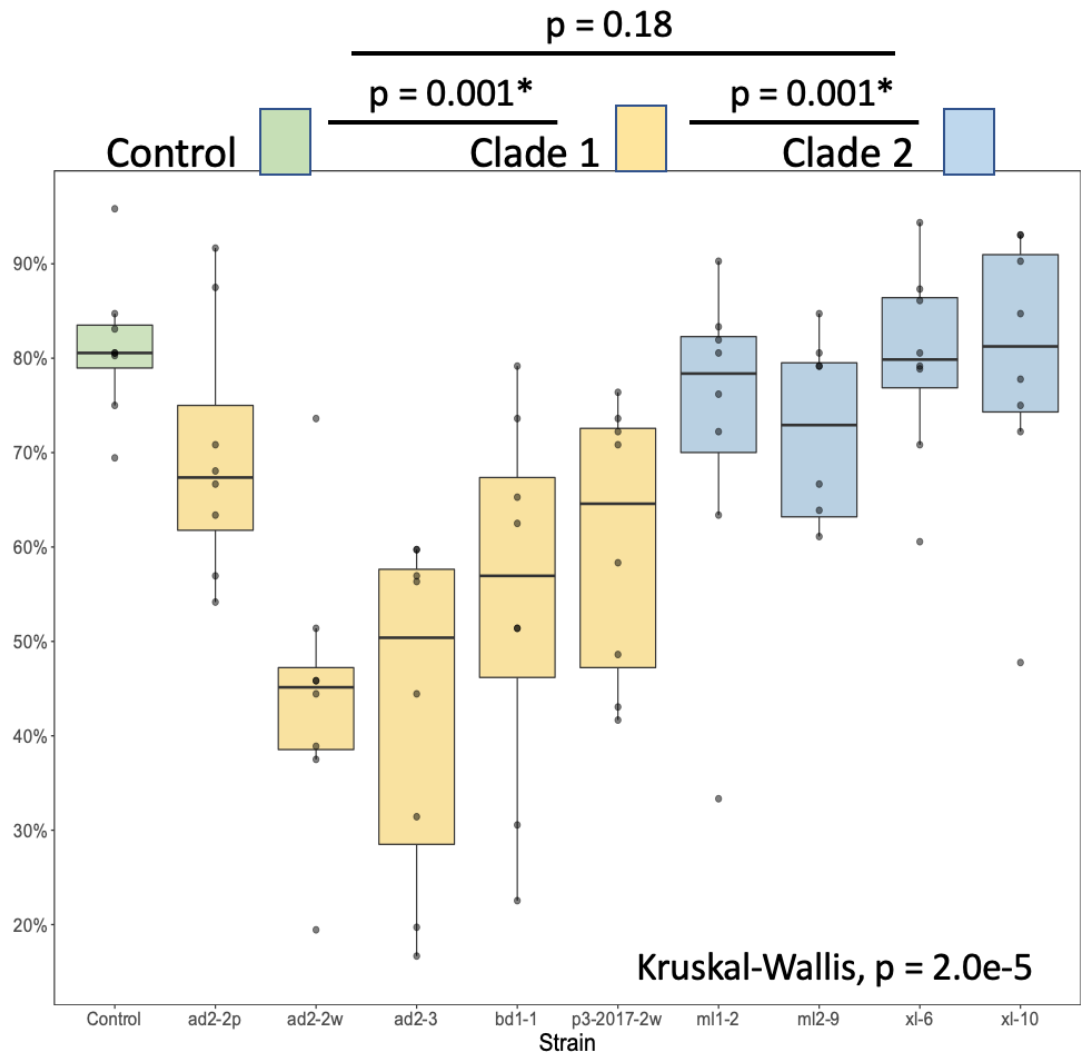


Figure 1. Rate of radicle emergence 2 days after transfer to growth chamber as a percentage of total seeds per plate. The isolate identified on the X-axis was used to inoculate the around 72 seeds on each experimental plate, except for the uninoculated control group. Each plate is represented by a point. The Y-axis is the percentage of seeds that show visible radicle emergence out of the total number of seeds on each plate. All 9 isolates and 1 control plate were grown in concert and replicated 8 times. Each box plot represents 8 plates. Colors represent clade associations identified by a distance matrix calculated using *rpoB* sequence, green is the control group, yellow are the isolates from clade 1, and blue are the isolates from clade 2. The box plots represent the data spread of quantified phenotypes of seeds grown in association with the isolate identified on the X-axis. The median of each group is represented by the bar within the colored boxes, the box above the median represents the 3rd data quartile, the box below the median shows the 2nd, the upper whisker is the 4th quartile, and the lower whiskers represent the 1st. Statistical analysis was done using the Kruskal-Wallis non-parametric analysis of variance test. Significant differences between clades were identified using a Dunn's test for post hoc analysis of non-parametric analysis of variance. The Kruskal-Wallis test gives a p-value of 2.0e^{-5} indicating significant difference among the present isolates. Dunn's test shows that phenotypes resulting from seedling association with clade 1 are significantly different from the control and clade 2, both with a p-value of 0.001. Phenotypes from clade 2 are not different from control, with a p-value of 0.18.

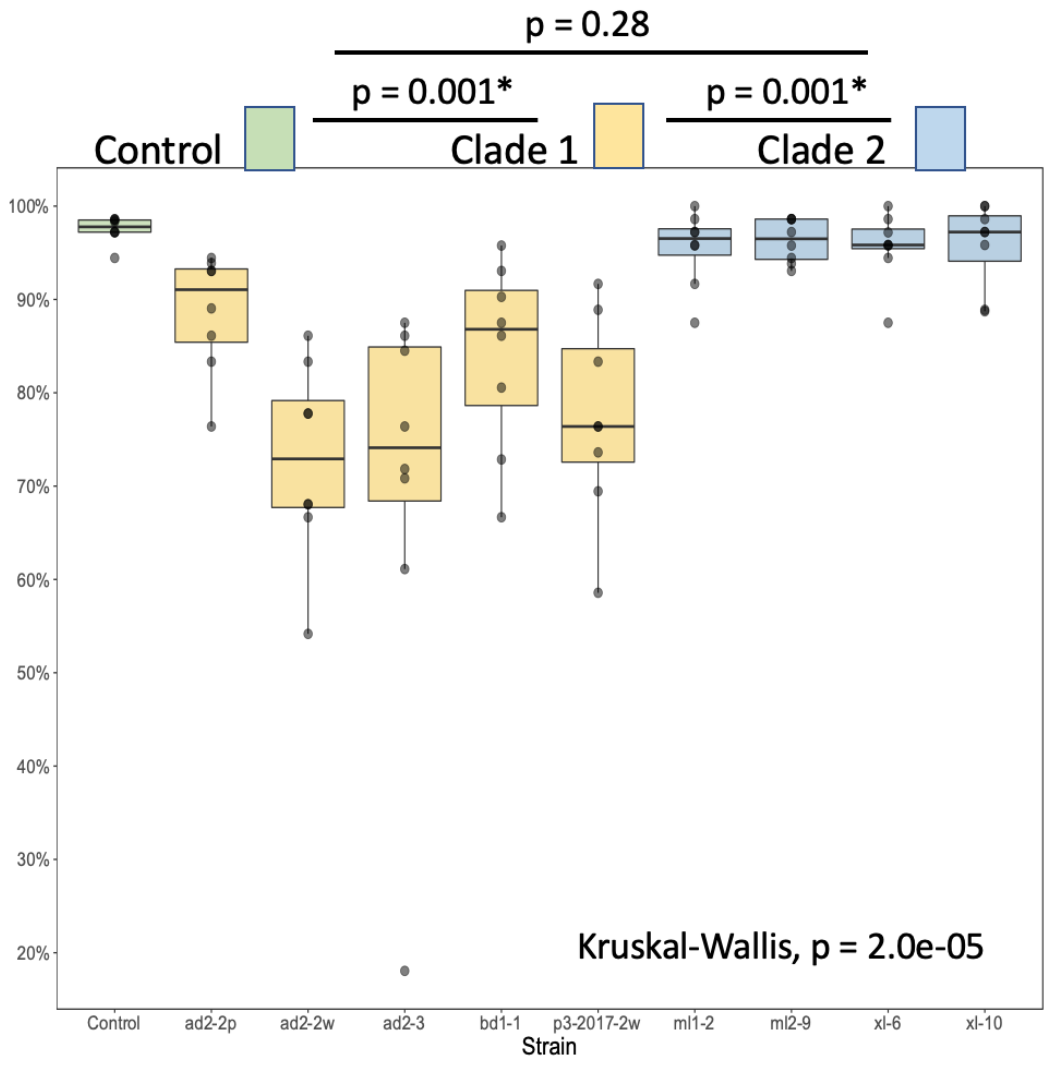


Figure 2. Rate of radicle emergence 8 days after transfer to growth chamber as a percentage of total seeds per plate. The isolate identified on the X-axis was used to inoculate the around 72 seeds on each experimental plate, except for the uninoculated control group. Each plate is represented by a point. The Y-axis is the percentage of seeds that show visible radicle emergence out of the total number of seeds on each plate. All 9 isolates and 1 control plate were grown in concert and replicated 8 times. Each box plot represents 8 plates. Colors represent clade associations identified by a distance matrix calculated using *rpoB* sequence, green is the control group, yellow are the isolates from clade 1, and blue are the isolates from clade 2. The box plots represent the data spread of quantified phenotypes of seeds grown in association with the isolate identified on the X-axis. The Kruskal-Wallis test gives a p-value of $2.0e^{-5}$ indicating significant difference among the present isolates. Dunn's test shows that phenotypes resulting from seedling association with clade 1 are significantly different from the control and clade 2, both with a p-value of 0.001. Phenotypes from clade 2 are similar to control, with a p-value of 0.28.

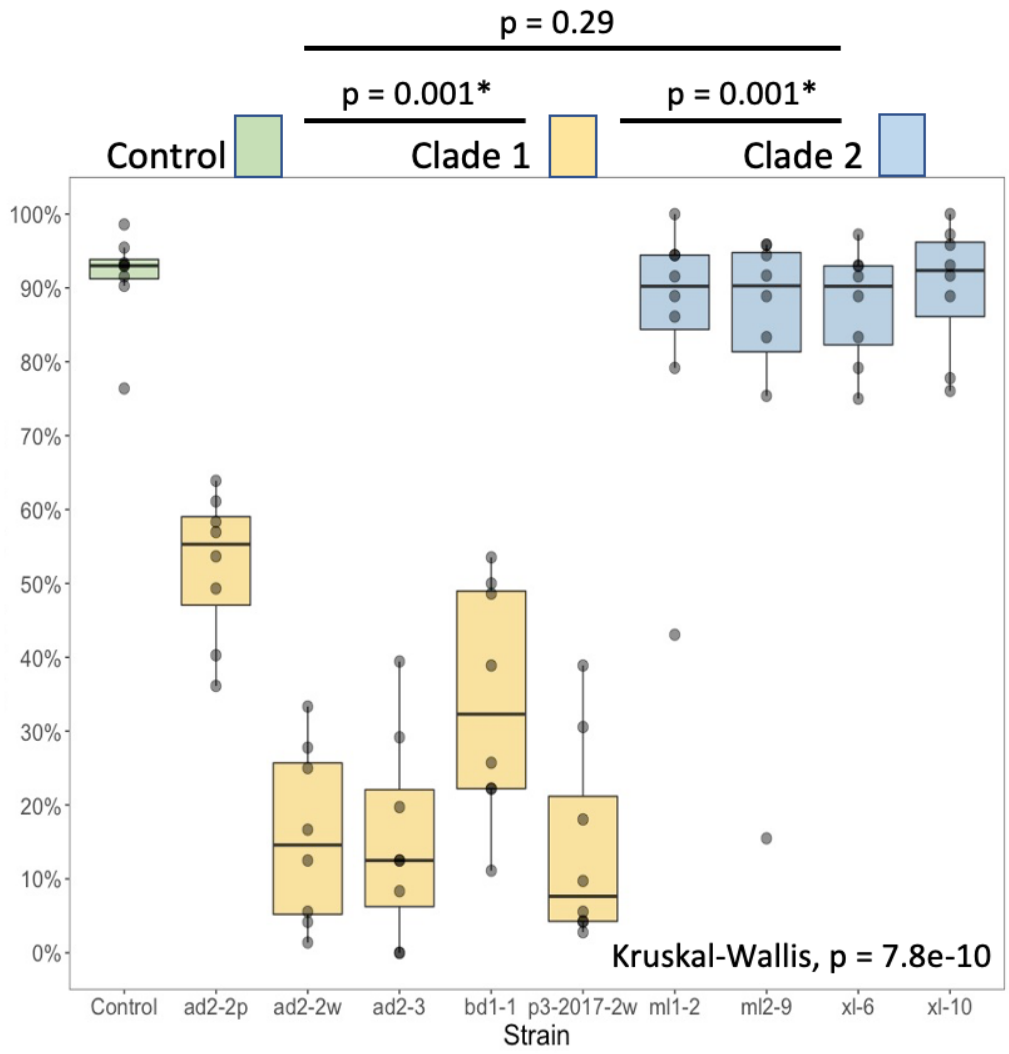


Figure 3. The rate of seedings with visible true leaves present 8 days after transfer to growth chamber as a percentage of total seeds per plate. The isolate identified on the X-axis was used to inoculate the around 72 seeds on each experimental plate, except for the uninoculated control group. Each plate is represented by a point. The Y-axis is the percentage of seeds that show visible true leaf formation out of the total number of seeds on each plate. All 9 isolates and 1 control plate were grown in concert and replicated 8 times. Each box plot represents 8 plates. Colors represent clade associations identified by a distance matrix calculated using *rpoB* sequence, green is the control group, yellow are the isolates from clade 1, and blue are the isolates from clade 2. The box plots represent the data spread of quantified phenotypes of seeds grown in association with the isolate identified on the X-axis. The Kruskal-Wallis test gives a p-value of $7.8e^{-10}$ indicating significant difference among the present isolates. Dunn's test shows that phenotypes resulting from seedling association with clade 1 are significantly different from the control and clade 2, both with a p-value of 0.001. Phenotypes from clade 2 are not different from control, with a p-value of 0.29.

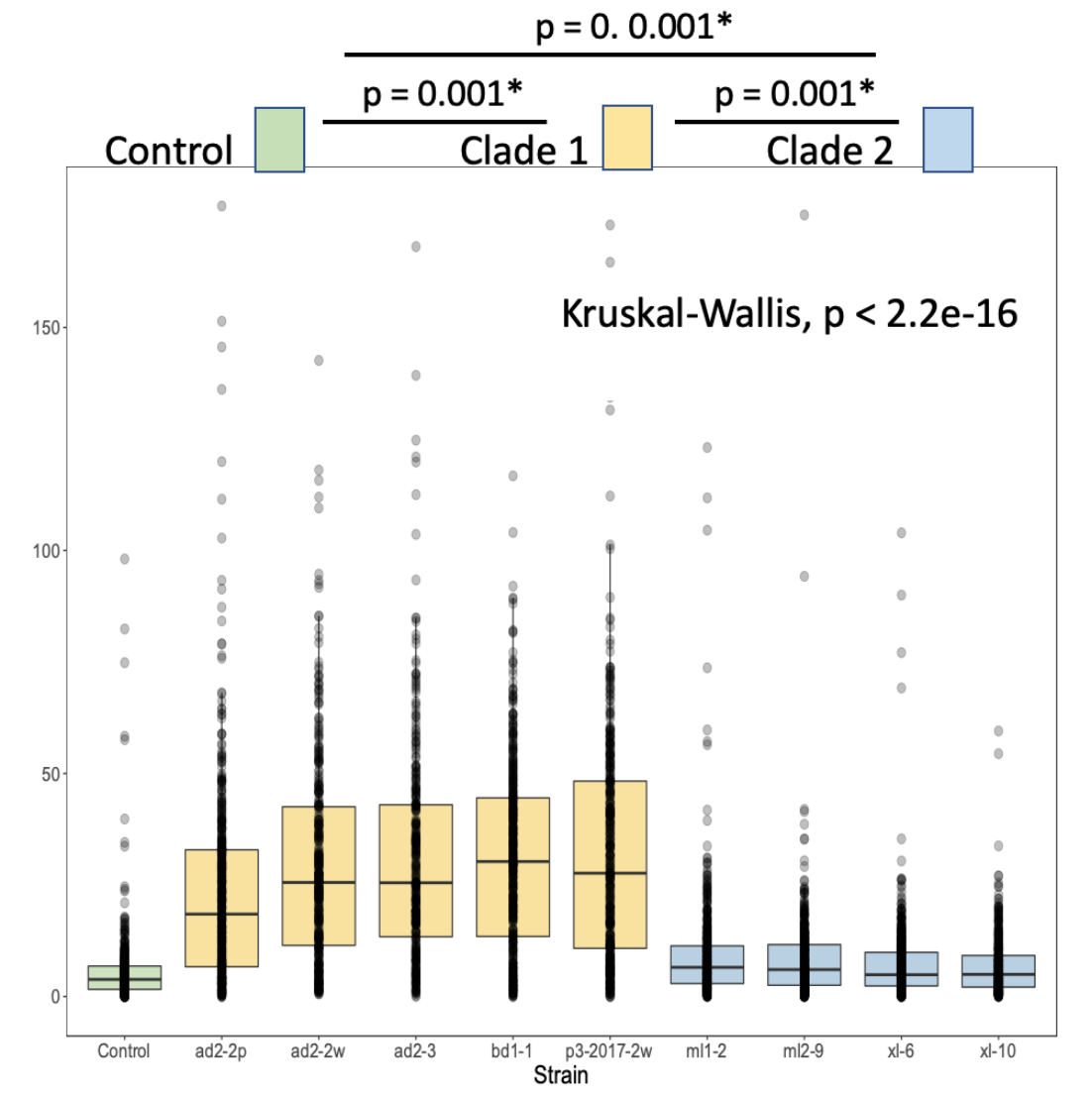


Figure 4. The Angle of root growth relative to point of origin 8 days after transfer to growth chamber. The isolate identified on the X-axis was used to inoculate the around 72 seeds on each experimental plate, except for the uninoculated control group. In this graph individual seeds are represented by a single point. The Y-axis is showing the angle of root elongation relative to its point of origin. All 9 isolates and 1 control plate were grown in concert and replicated 8 times. Each box plot represents 8 plates. Colors represent clade associations identified by a distance matrix calculated using *rpoB* sequence, green is the control group, yellow are the isolates from clade 1, and blue are the isolates from clade 2. The box plots represent the data spread of quantified phenotypes of seeds grown in association with the isolate identified on the X-axis. The Kruskal-Wallis test gives a p-value of less than $2.2e^{-16}$ indicating significant difference among the present isolates. Dunn's test shows that phenotypes resulting from seedling association with clade 1 and clade 2 are significantly different from the control and each other, all with a p-value of 0.001.

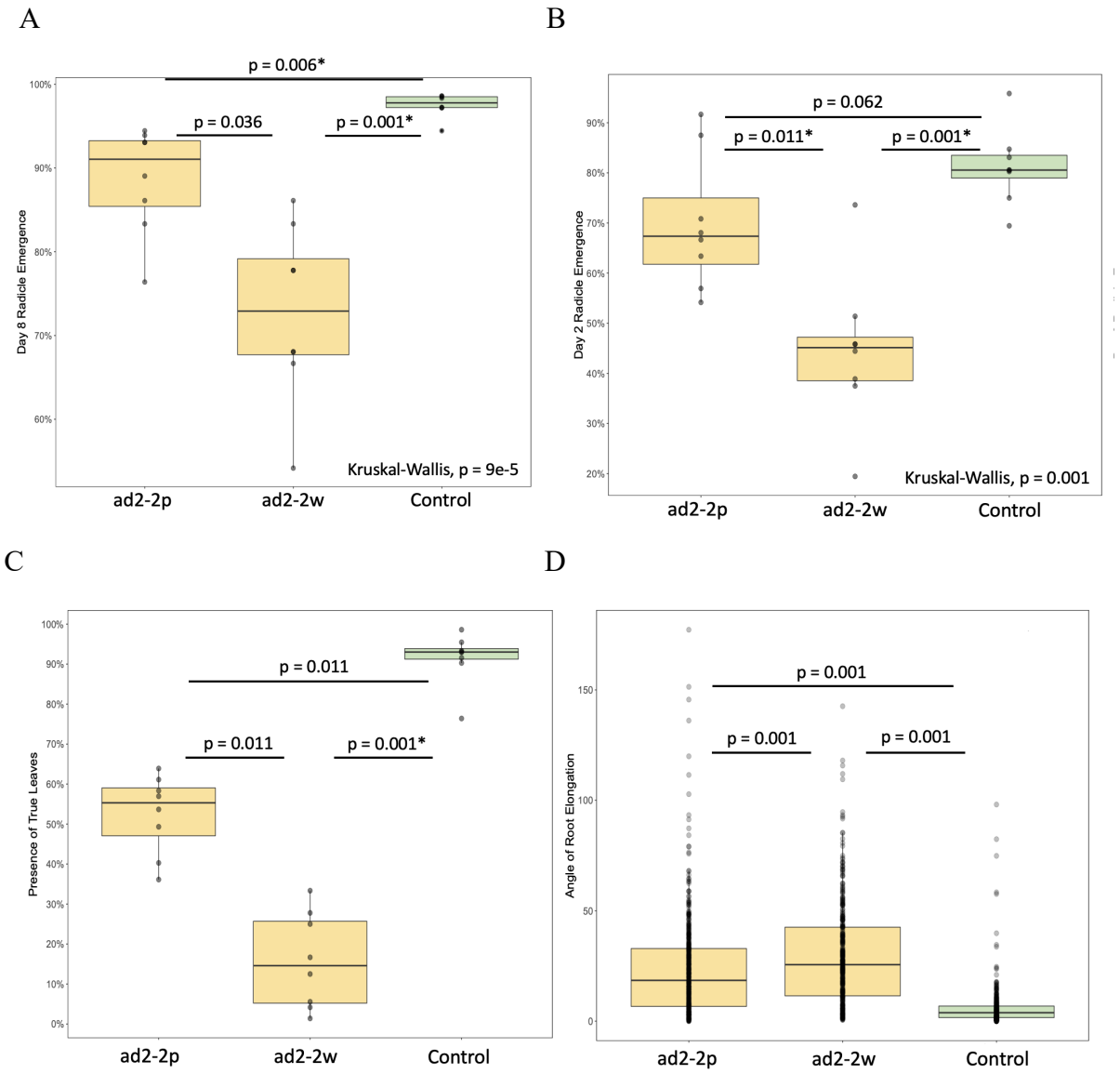
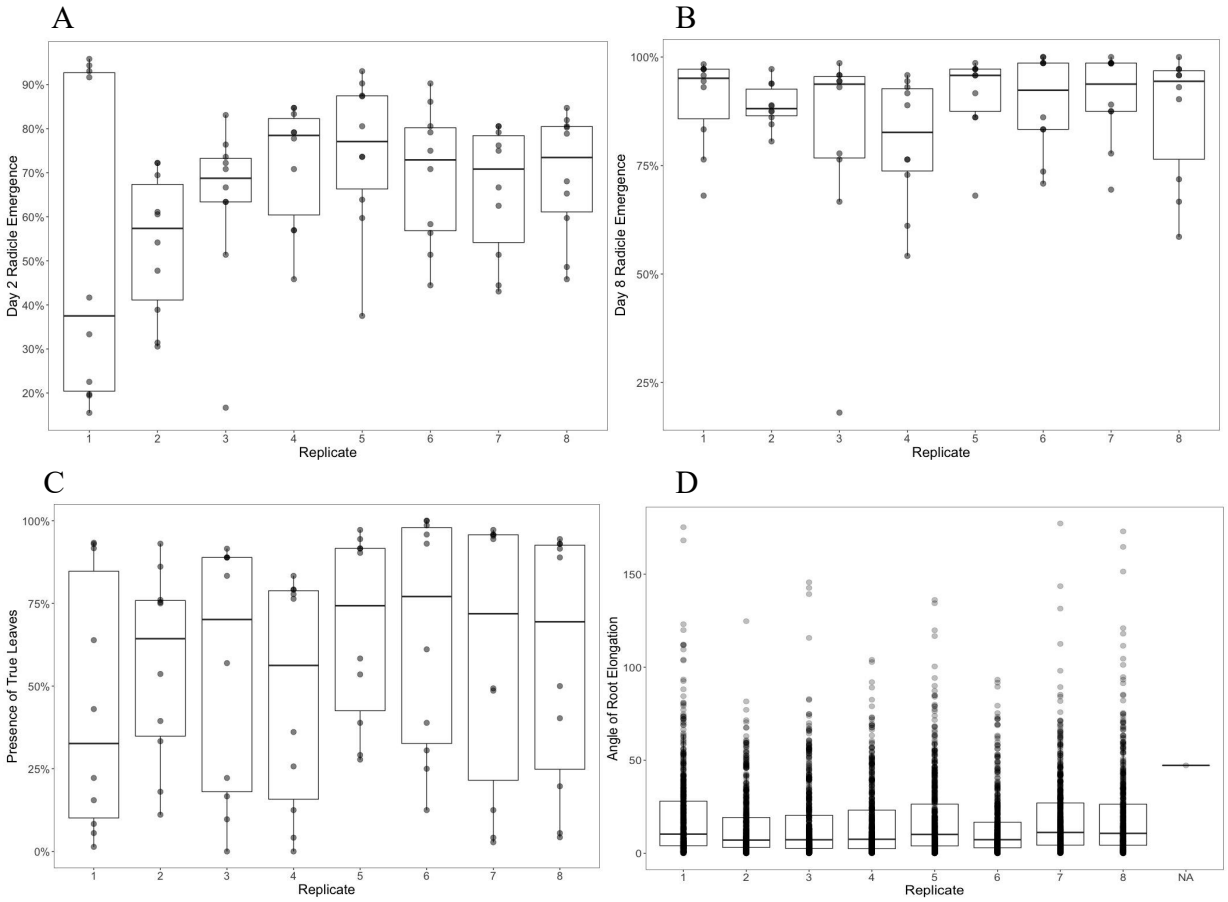
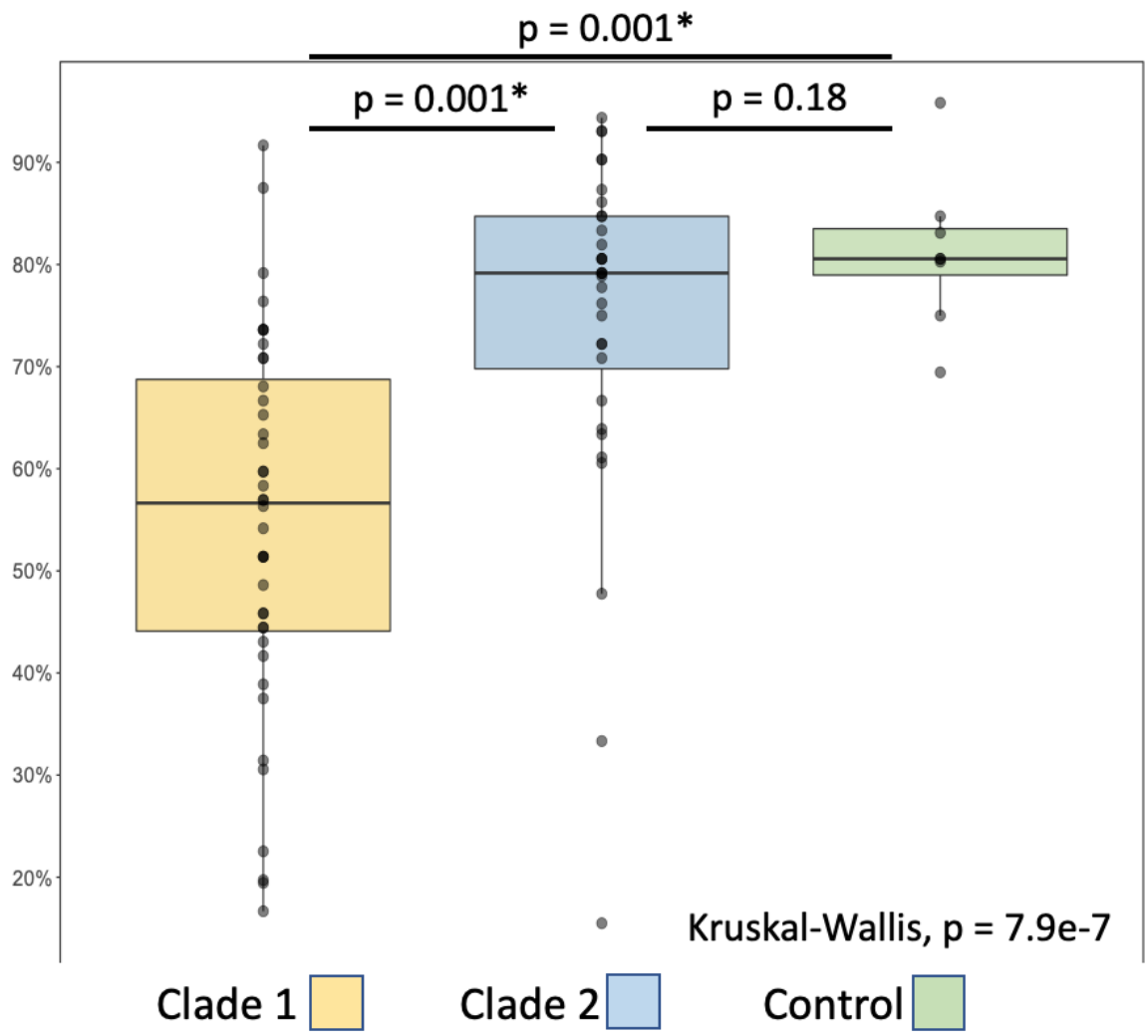


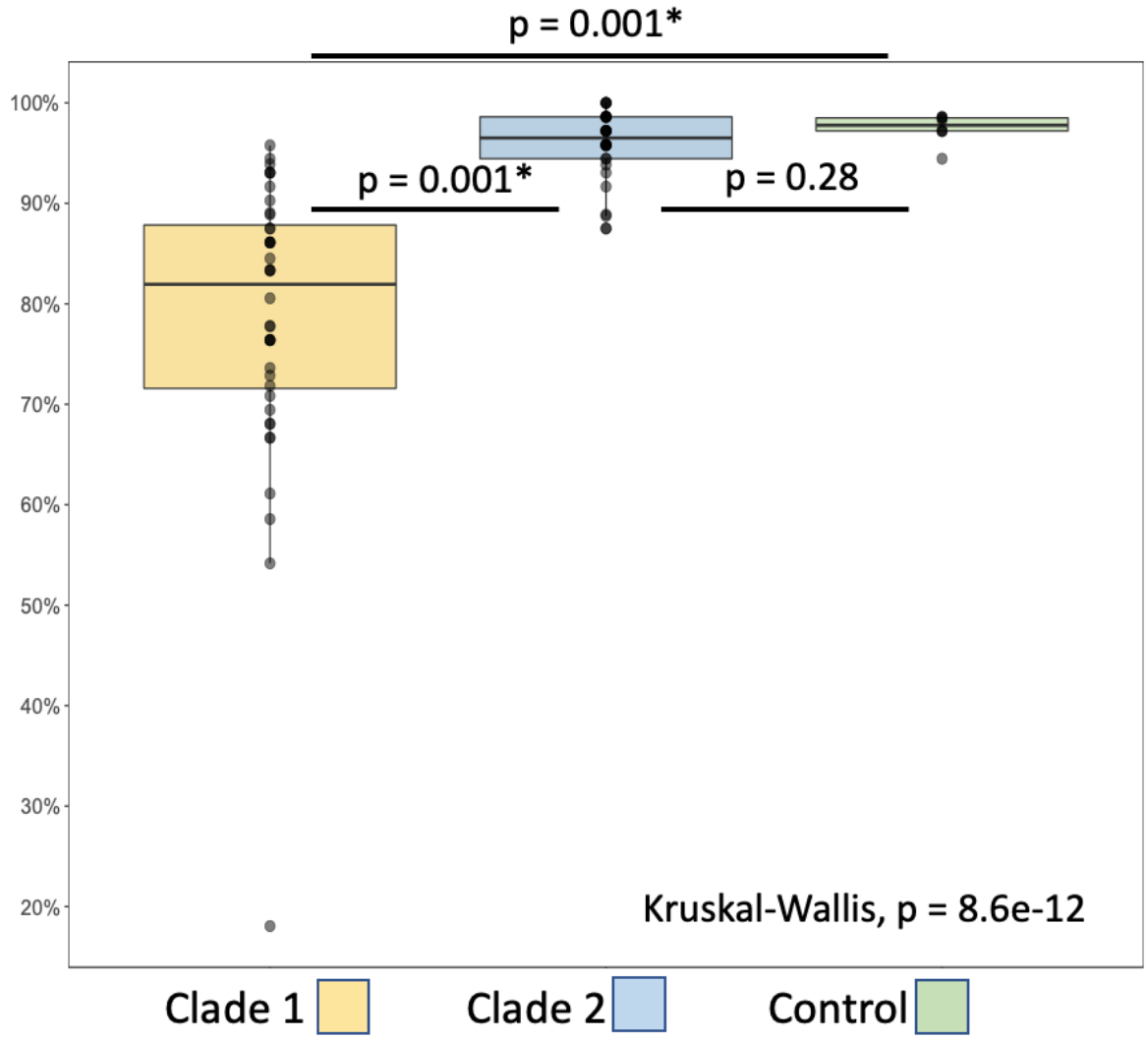
Figure 5. Comparison of plant phenotypes from association with ad2-2p with spontaneous mutant ad2-2w. A) Rate of radicle emergence 2 days after transfer to growth chamber as a percentage of total seeds per plate. The Y-axis is showing the rate of radicle emergence as a percentage of total seeds on a plate, while the X-axis is identifying the treatment used on the present seeds. B) Rate of radicle emergence 8 days after transfer to growth chamber as a percentage of total seeds per plate. The Y-axis is showing the rate of radicle emergence as a percentage of total seeds on a plate, while the X-axis is identifying the treatment used on the present seeds. C) The rate of seeds with visible true leaves present 8 days after transfer to growth chamber. The Y-axis is showing true leaf presence as a percentage of total seeds on a plate, while the X-axis is identifying the treatment used on the present seeds. D) The Angle of root growth relative to point of origin 8 days after transfer to growth chamber. The Y-axis is showing the angle of root elongation relative to its point of origin, and the X-axis is identifying the treatment used on the present seeds.



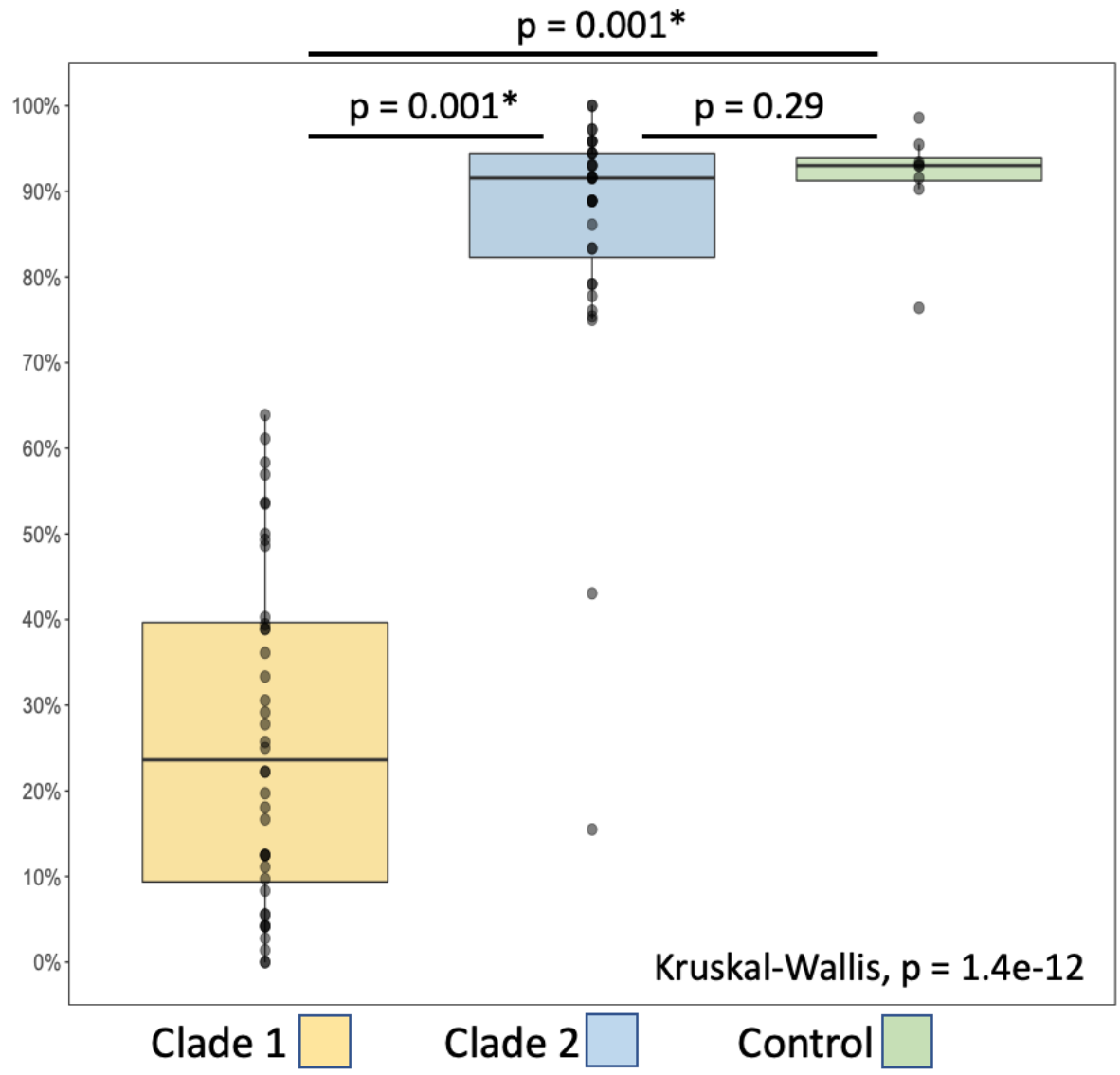
Supplementary Figure 1. Quantification of plant phenotypes organized by replicate. Each replicate includes 10 plates with around 72 seeds each co-cultured with one of 9 isolates and an uninoculated control plate grown in concert. A) Rate of radicle emergence 2 days after transfer to growth chamber B) Rate of radicle emergence 8 days after transfer to growth chamber C) Rate of true leaf formation 8 days after transfer to growth chamber D) Angle of root growth relative to point of origin 8 days after transfer to growth chamber.



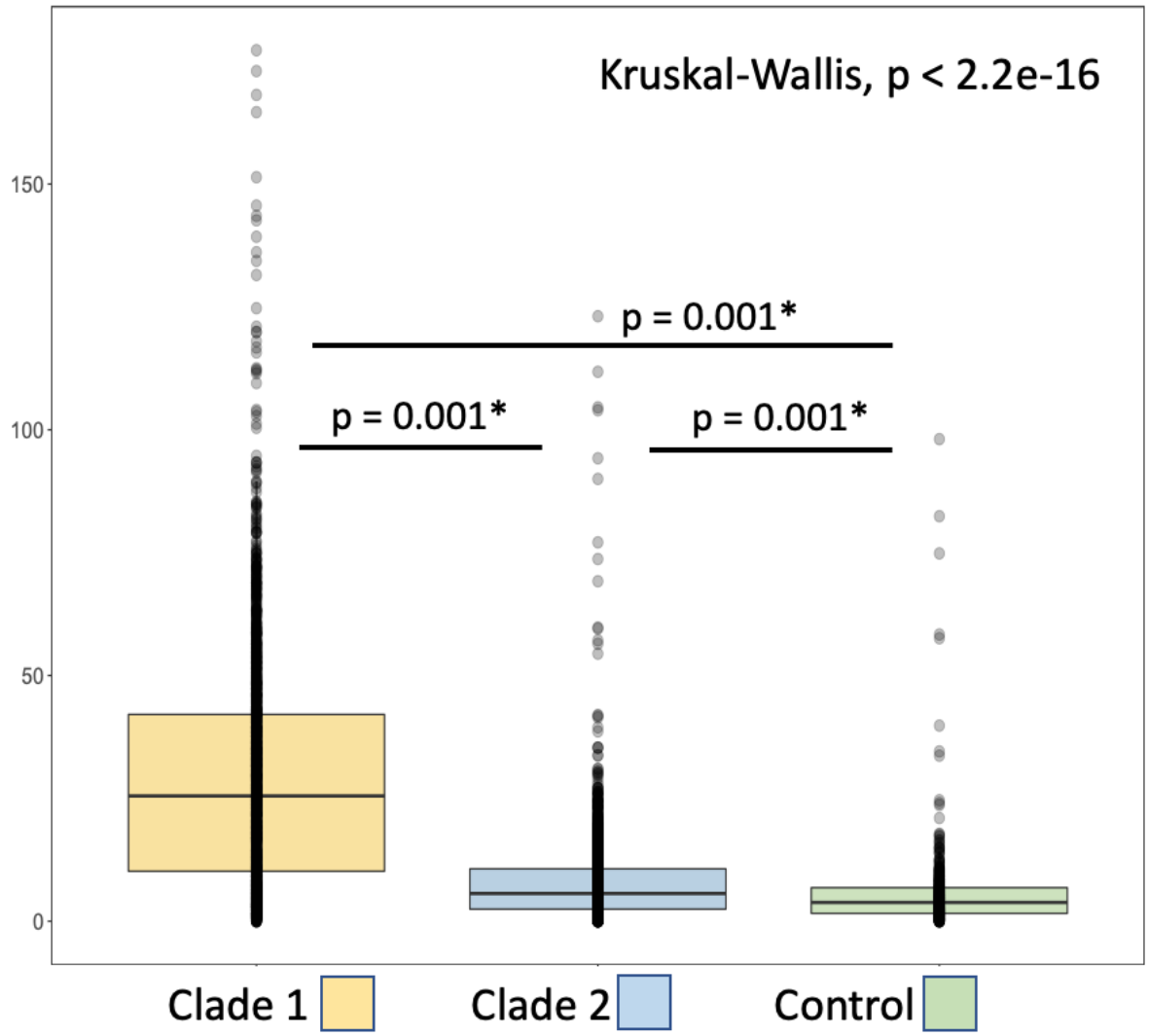
Supplementary Figure 2. Rate of radicle emergence 2 days after transfer to growth chamber organized by clade association. The Kruskal-Wallis value, $p=7.9e^{-07}$, indicates significant difference between clades in a non-parametric analysis of variance.



Supplementary Figure 3. Rate of radicle emergence 8 days after transfer to growth chamber organized by clade association. The Kruskal-Wallis value, $p=8.6e^{-12}$, indicates significant difference between clades in a non-parametric analysis of variance.



Supplementary Figure 4. The presence of visible true leaves as a percentage of all seeds present 8 days after transfer to growth chamber organized by clade association. The Kruskal-Wallis value, $p=1.4e^{-12}$, indicates significant difference between clades in a non-parametric analysis of variance.



Supplementary Figure 5. The Angle of root growth relative to point of origin 8 days after transfer to growth chamber organized by clade association. The Kruskal-Wallis value, $p < 2.2e^{-16}$, indicates significant difference between clades in a non-parametric analysis of variance.

Supplementary Table 1

Dunn's Test for Germination Rate 2 Days After Transfer to Growth Chamber

Kruskal-Wallis chi-squared = 37.7088, df = 9, p-value = 0

Comparison of x by group
(No adjustment)

Col Mean- Row Mean	ad2-2p	ad2-2w	ad2-3	bd1-1	control	ml1-2
ad2-2w	2.260021 0.0119*					
ad2-3	2.308450 0.0105*	0.048429 0.4807				
bd1-1	1.323727 0.0928	-0.936294 0.1746	-0.984723 0.1624			
control	-1.538967 0.0619	-3.798989 0.0001*	-3.847418 0.0001*	-2.862694 0.0021*		
ml1-2	-0.651101 0.2575	-2.911123 0.0018*	-2.959552 0.0015*	-1.974828 0.0241*	0.887865 0.1873	
ml2-9	-0.021524 0.4914	-2.281545 0.0113*	-2.329974 0.0099*	-1.345251 0.0893	1.517443 0.0646	0.629577 0.2645
p3-2017-	0.995485 0.1598	-1.264536 0.1030	-1.312965 0.0946	-0.328241 0.3714	2.534453 0.0056*	1.646587 0.0498
xl-10	-1.275298 0.1011	-3.535319 0.0002*	-3.583748 0.0002*	-2.599025 0.0047*	0.263669 0.3960	-0.624196 0.2662
xl-6	-1.302203 0.0964	-3.562224 0.0002*	-3.610653 0.0002*	-2.625930 0.0043*	0.236764 0.4064	-0.651101 0.2575

Col Mean- Row Mean	ml2-9	p3-2017-	xl-10
p3-2017-	1.017009 0.1546		
xl-10	-1.253774 0.1050	-2.270783 0.0116*	
xl-6	-1.280679 0.1002	-2.297688 0.0108*	-0.026905 0.4893

alpha = 0.05
Reject Ho if p <= alpha/2

Supplementary Table 2

Dunn's Test for Germination Rate 8 Days After Transfer to Growth Chamber

```
  Kruskal-Wallis rank sum test

data: x and group
Kruskal-Wallis chi-squared = 55.7924, df = 9, p-value = 0


                          Comparison of x by group
                                (No adjustment)
Col Mean-|
Row Mean |     ad2-2p     ad2-2w      ad2-3      bd1-1    control      ml1-2
---------+------------------------------------------------------------------
  ad2-2w |   1.803938
         |     0.0356
         |
   ad2-3 |   1.647776  -0.156161
         |     0.0497     0.4380
         |
   bd1-1 |   0.549258  -1.254679  -1.098517
         |     0.2914     0.1048     0.1360
         |
 control |  -2.482434  -4.286372  -4.130210  -3.031693
         |    0.0065*    0.0000*    0.0000*    0.0012*
         |
   ml1-2 |  -1.922405  -3.726343  -3.570182  -2.471664   0.560028
         |     0.0273    0.0001*    0.0002*    0.0067*     0.2877
         |
   ml2-9 |  -2.073182  -3.877120  -3.720958  -2.622441   0.409251  -0.150776
         |    0.0191*    0.0001*    0.0001*    0.0044*     0.3412     0.4401
         |
p3-2017- |   1.340837  -0.463100  -0.306938   0.791578   3.823271   3.263243
         |     0.0900     0.3216     0.3794     0.2143    0.0001*    0.0006*
         |
   xl-10 |  -2.197035  -4.000973  -3.844811  -2.746293   0.285399  -0.274629
         |    0.0140*    0.0000*    0.0001*    0.0030*     0.3877     0.3918
         |
    xl-6 |  -1.890096  -3.694034  -3.537872  -2.439355   0.592337   0.032309
         |     0.0294    0.0001*    0.0002*    0.0074*     0.2768     0.4871
Col Mean-|
Row Mean |      ml2-9   p3-2017-      xl-10
---------+---------------------------------
p3-2017- |   3.414020
         |    0.0003*
         |
   xl-10 |  -0.123852  -3.537872
         |     0.4507    0.0002*
         |
    xl-6 |   0.183086  -3.230933   0.306938
         |     0.4274    0.0006*     0.3794

alpha = 0.05
Reject Ho if p <= alpha/2
```

Supplementary Table 3

Dunn's Test Results for Presence of True Leaves 8 Days after Transfer to Growth Chamber

```
  Kruskal-Wallis rank sum test

data: x and group
Kruskal-Wallis chi-squared = 61.2099, df = 9, p-value = 0


                          Comparison of x by group
                                (No adjustment)
Col Mean-|
Row Mean |     ad2-2p     ad2-2w      ad2-3      bd1-1    control      ml1-2
---------+------------------------------------------------------------------
  ad2-2w |   1.872391
         |     0.0306
         |
   ad2-3 |   1.926196   0.053804
         |     0.0270     0.4785
         |
   bd1-1 |   0.887772  -0.984619  -1.038424
         |     0.1873     0.1624     0.1495
         |
 control |  -2.308207  -4.180599  -4.234403  -3.195979
         |    0.0105*    0.0000*    0.0000*    0.0007*
         |
   ml1-2 |  -1.845489  -3.717881  -3.771685  -2.733261   0.462717
         |     0.0325    0.0001*    0.0001*    0.0031*     0.3218
         |
   ml2-9 |  -1.608750  -3.481142  -3.534946  -2.496522   0.699456   0.236739
         |     0.0538    0.0002*    0.0002*    0.0063*     0.2421     0.4064
         |
p3-2017- |   1.996141   0.123750   0.069945   1.108369   4.304349   3.841631
         |    0.0230*     0.4508     0.4721     0.1339    0.0000*    0.0001*
         |
   xl-10 |  -2.249022  -4.121414  -4.175218  -3.136794   0.059184  -0.403532
         |    0.0123*    0.0000*    0.0000*    0.0009*     0.4764     0.3433
         |
    xl-6 |  -1.791685  -3.664077  -3.717881  -2.679457   0.516521   0.053804
         |     0.0366    0.0001*    0.0001*    0.0037*     0.3027     0.4785
Col Mean-|
Row Mean |      ml2-9   p3-2017-      xl-10
---------+--------------------------------
p3-2017- |   3.604892
         |    0.0002*
         |
   xl-10 |  -0.640271  -4.245164
         |     0.2610    0.0000*
         |
    xl-6 |  -0.182934  -3.787827   0.457337
         |     0.4274    0.0001*     0.3237

alpha = 0.05
Reject Ho if p <= alpha/2
```

Supplementary Table 4

Dunn's Test Results for Angle of Root Growth

Kruskal-Wallis chi-squared = 1553.6838, df = 9, p-value = 0

Comparison of x by group
(No adjustment)

Col Mean- Row Mean	ad2-2p	ad2-2w	ad2-3	bd1-1	Control	ml1-2
ad2-2w	-4.058046 0.0000*					
ad2-3	-4.905160 0.0000*	-0.864482 0.1937				
bd1-1	-5.105391 0.0000*	-0.765951 0.2219	0.152000 0.4396			
Control	17.78125 0.0000*	20.57549 0.0000*	21.12542 0.0000*	22.59071 0.0000*		
ml1-2	11.41903 0.0000*	14.69759 0.0000*	15.35890 0.0000*	16.38649 0.0000*	-6.565358 0.0000*	
ml2-9	12.47793 0.0000*	15.67239 0.0000*	16.31467 0.0000*	17.41177 0.0000*	-5.442859 0.0000*	1.111618 0.1332
p3-2017-	-4.675738 0.0000*	-0.482347 0.3148	0.408395 0.3415	0.277759 0.3906	-21.63168 0.0000*	-15.60693 0.0000*
xl-10	14.37750 0.0000*	17.39306 0.0000*	17.99784 0.0000*	19.17266 0.0000*	-3.100888 0.0010*	3.312013 0.0005*
xl-6	14.33565 0.0000*	17.39879 0.0000*	18.00978 0.0000*	19.25068 0.0000*	-3.640345 0.0001*	2.956597 0.0016*

Col Mean- Row Mean	ml2-9	p3-2017-	xl-10
p3-2017-	-16.60431 0.0000*		
xl-10	2.221046 0.0132*	18.34142 0.0000*	
xl-6	1.834638 0.0333	18.38182 0.0000*	-0.440294 0.3299

alpha = 0.05
Reject Ho if p <= alpha/2

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