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Decoupling early development traits in Arabidopsis thaliana accessions with contrasting seed sizes

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Abstract

Seed size variation in *Arabidopsis thaliana* is highly diverse across populations and has been shown to influence establishment, fitness, and local adaptation. In addition, seed size has a strong genetic basis and correlates with geographic distribution.
In this thesis, I investigate the role of seed size in *A. thaliana*, focusing on its potential impact on germination properties and early seedling development by examining germination rate and hypocotyl length, as well as its response to abiotic factors such as temperature and light.

To explore these relationships, I conducted a series of germination assays using Swedish accessions of *A. thaliana* with contrasting seed sizes. The experiments tested germination under varying temperature and light conditions, as well as hypocotyl elongation in darkness following stratification and light exposure.

Across treatments, accessions showed variable germination responses, with no consistent correlation with seed size and environmental sensitivity. However, hypocotyl elongation was remarkably higher in big-seeded accessions, even when seed size was controlled by sorting. This suggests that while environmental conditions influence germination behavior in complex ways, seed size may also influence early seedling growth capacity, through internal resource allocation and underlying genetic differences.

These findings contribute to our understanding of how seed traits interact with environmental cues to shape early plant development. Future work could include genetic studies of hypocotyl length variation and expanded trait measurements such as root–shoot allocation, providing deeper insight into the ecological significance of seed size diversity in *A. thaliana*.

Zusammenfassung

Die Variation der Samengröße bei *Arabidopsis thaliana* ist über Populationen hinweg sehr vielfältig und beeinflusst nachweislich die Etablierung, Fitness und lokale Anpassung. Darüber hinaus hat die Samengröße eine starke genetische Grundlage und korreliert mit der geografischen Verteilung. In dieser Arbeit untersuche ich die Rolle der Samengröße bei *A. thaliana*, wobei ich mich auf ihren potenziellen Einfluss auf die Keimungseigenschaften und die frühe Entwicklung der Sämlinge konzentriere, indem ich die Keimrate und die Hypokotyllänge sowie ihre Reaktion auf abiotische Faktoren wie Temperatur und Licht untersuche.

Um diese Zusammenhänge zu erforschen, führte ich eine Reihe von Keimungstests mit schwedischen Akzessionen von *A. thaliana* mit unterschiedlichen Samengrößen durch. Die Experimente testeten die Keimung unter variierenden Temperatur- und Lichtbedingungen sowie die Elongation des Hypokotyls im Dunkeln nach Stratifikation und Lichtexposition.

Über die Behandlungen hinweg zeigten die Akzessionen variable Keimungsreaktionen, ohne eine konsistente Korrelation zwischen Samengröße und Umweltsensitivität. Die Hypokotyl-Elongation war jedoch bei großsamigen Akzessionen bemerkenswert höher, selbst wenn die Samengröße durch Sortierung kontrolliert wurde. Dies deutet darauf hin, dass, während Umweltbedingungen das Keimungsverhalten auf komplexe Weise beeinflussen, die Samengröße auch die frühe Wachstumskapazität der Sämlinge durch interne Ressourcenallokation und zugrunde liegende genetische Unterschiede beeinflussen kann.

Diese Ergebnisse tragen zu unserem Verständnis bei, wie Sameneigenschaften mit Umwelteinflüssen interagieren, um die frühe Pflanzenentwicklung zu formen. Zukünftige Arbeiten könnten genetische Studien zur Variation der Hypokotyllänge und erweiterte Merkmalsmessungen wie die Spross-Wurzel-Allokation umfassen, um einen tieferen Einblick in die ökologische Bedeutung der Diversität der Samengröße bei *A. thaliana* zu gewinnen.

1. Introduction

1.1 Seed size in plants

Seed size plays a key role in a plant's life cycle, influencing both its chances of survival and overall fitness (Li, Xu, & Li, 2019). Plant species have seed masses ranging over 11.5 orders of magnitude, with the smallest being orchid seeds weighing ~0.0001 mg up to the 20 kg seeds of the double coconut (Moles et al., 2005c).

The wide variation in seed size observed across plant species is often shaped by different evolutionary strategies and can influence geographic distribution. For example, orchid seeds rely entirely on fungi for nutrients, so they don't need to store nutrient resources in their seeds, resulting in very small seeds (Moles et al., 2005c). Some studies found a negative correlation between seed size and range size, suggesting a trade-off where smaller-seeded species tend to have broader distributions (Sonkoly et al., 2017), (Morin & Chuine, 2006). Within *Copaifera langsdorffii* (Fabaceae), small seeds tend to germinate faster and at higher rates, supporting rapid colonization in transient habitats, while bigger seeds develop into more vigorous seedlings that are better suited for establishment in stable environments (Souza & Fagundes, 2014).

Seed size within species has been a focus of research for decades, mainly due to its importance in agricultural improvement and optimization of crop yield. Larger seed size in wheat has been linked to improved agronomic performance, with yields noticeably higher compared to those from smaller seeds (Sabaghnia et al., 2015), (Tavakoli et al., 2015). A study with soybeans showed that the average seed yield of progeny from large seed plants always exceeded the yield from the small seed plants in the same conditions (Smith & Camper, 1975). Another study with tropical soybeans revealed plants of the small seed size showed higher seed germination while plants of the large seed size showed higher seed yield per plant (Adebisi et al., 2013). Aside from crop yield, seed size has also been associated with seed quality in an agricultural context. In a study with sweet corn lines, seeds qualified as large based on weight showed a higher degree of seed germination synchrony and performed better than small seeds (Kadapi

et al., 2018). Similarly in wheat, plants grown from larger seeds were shown to be more vigorous and produce greater dry matter, likely due to the higher nutrient reserves stored in the seeds (Moussavi Nik et al., 2011).

Seed size remains a critical trait affecting a plant's ability to survive, establish, and reproduce. The widespread variation observed both across and within species emphasizes its importance in shaping plant success in diverse environments. This variation often reflects underlying trade-offs, particularly between seed size and seed number as well as adaptive advantages linked to different environmental pressures,

1.2 Trade-offs and Adaptive Advantages of Seed Size

Seed size is shaped by trade-offs and advantages that help plants adapt to different environments. The main trade-off associated with seed size is seed set, as the number of seeds a plant can produce is limited by the resources available for reproduction and seed development. The classic Smith & Fretwell (1974) model suggests that natural selection favors an optimal offspring that balances the trade-off between producing many small offspring with lower individual survival chances and fewer large offspring with higher survival potential. This model can be applied to plants, (offspring referring to seeds) that vary in seed size depending on the resource availability. Building on this, the model proposed by McGinley, Temme, & Geber (1987) also incorporated variable environmental effects, such as changes in season and also included plants as model organisms. Together, these models provide a useful basic framework for understanding how plants optimize reproductive investment under different environmental conditions. Empirical studies across a range of species have since demonstrated this trade-off between seed size and seed number.

A study of canopy trees showed a strong inverse relationship between seed size and long-term annual seed production, with species producing larger seeds generating fewer seeds per tree. This trade-off followed a power law, highlighting that as seed mass increases, seed number decreases exponentially, supporting the resource allocation limitations proposed by the theoretical models (Greene & Johnson, 1994). In

another study the trade-off between seed size and seed number was supported by a cross-species analysis and further reinforced through 35 phylogenetically independent contrasts based on data from 72 plant species (Jakobsson & Eriksson, 2000).

In a study with *Arabidopsis thaliana*, the model organism of this thesis, three genomic regions were found to contain QTLs for seed size, ovule and seed number, suggesting that these loci influence seed size indirectly by constraining the number of seeds a plant produces, thereby reinforcing the trade-off between seed size and seed number in Arabidopsis (Alonso-Blanco et al., 1999). In another study, a negative correlation between seed size and seed number was observed in *Arabidopsis*, but noted that this trade-off explains only a small portion of the overall trait variation (Gnan, Priest, & Kover, 2014). These studies illustrate that while the trade-off between seed size and number is widely observed across species, its strength and genetic basis can vary, particularly within *Arabidopsis*. Understanding how this trade-off interacts with environmental pressures provides insight into the adaptive advantages of producing either small or large seeds under different ecological conditions.

The trade-off between seed size and number reflects how plants allocate limited reproductive resources, with species varying in both traits depending on their ecological strategy. Colonizing species, typically found in early successional stages, often produce many small seeds to maximize dispersal and population growth, while long-lived perennials invest in fewer, larger seeds that support seedling survival in stable, competitive environments, highlighting how dispersal potential is closely linked to seed size (Harper et al., 1970). A modeling study on annual plants further supports this link, showing that smaller seeds enable wider dispersal, while larger seeds tend to remain near the parent plant (Ezoe, 1998). The results highlight that dispersal ability is inherently tied to seed size, and that the evolutionarily stable seed size reflects a balance between dispersal potential and the competitive benefits of larger seeds. However, while smaller seeds enable a broader dispersal, larger seeds offer distinct competitive advantages that become critical during seedling establishment.

Larger seeds typically store more nutrients, allowing seedlings to rely less on external environmental resources during early growth. This internal reserve can be especially beneficial in nutrient-poor soils, where access to essential elements is limited. A study on two populations of *Banksia cunninghamii* found that seed nutrient content (nitrogen, phosphorus, and potassium) increased with seed mass, meaning larger seeds contained both greater amounts and concentrations of these nutrients. In controlled conditions, seedlings from larger seeds consistently grew larger and the benefit of large seed mass was amplified in low-nutrient availability, suggesting that larger seeds helped minimize the negative effects of nutrient stress (Vaughton & Ramsey, 2001). Further evidence for this advantage comes from a comparison of 21 species across the genera *Eucalyptus*, *Hakea*, and *Banksia*, where seedlings from larger seeds performed better under nutrient-poor conditions. This benefit was most pronounced under low nutrient availability and disappeared when soil nutrients were abundant. Larger seeds contained proportionally more nitrogen, phosphorus, and potassium, promoting rapid root development and access to deeper moisture, traits that are especially useful in dry, nutrient-poor habitats (Milberg et al., 1998). This pattern also holds true in *Arabidopsis thaliana*, where a comparison of ten genetically distinct inbred lines revealed that seedlings from larger seeds survived significantly longer under severe nutrient deprivation. Since all seedlings were supplied only with sterile distilled water, the results suggest that larger seed size confers greater tolerance to nutrient stress, likely due to increased internal reserves (Krannitz, Aarssen, & Dow, 1991).

These findings show that seed size is shaped by a balance between ecological trade-offs and adaptive benefits, with larger seeds offering advantages under nutrient-poor or competitive conditions, and smaller seeds supporting wider dispersal. Building on this, the next section explores how natural variation in seed size is evident across *Arabidopsis* accessions, including differences between coastal and inland populations in Sweden which may reflect local adaptation to contrasting environmental conditions.

1.3 Seed size variation in natural and Swedish accessions of Arabidopsis

Seed size variation is not only evident across plant species but also within species, and *Arabidopsis thaliana* provides a powerful model for studying this, since natural accessions of Arabidopsis differ markedly in seed size. Studies showing approximately a threefold difference in mean seed size across both natural accessions (Krannitz, Aarssen, & Dow, 1991) and MAGIC lines (Gnan et al., 2014), which are multi-parent populations created through repeated intercrossing and inbreeding to allow fine mapping of complex traits.

This phenotypic variation has also prompted research into the genetic basis of seed size in *Arabidopsis thaliana*. Both QTL mapping and genome-wide association studies have identified specific loci and candidate genes that contribute to natural differences in seed size across accessions. For example, a recombinant inbred line (RIL) population from a cross between the small-seeded Ler and large-seeded Cvi ecotypes (whose seeds differ nearly two-fold in mass) revealed numerous quantitative trait loci (QTLs) for seed weight. Several of these QTL co-localize with loci affecting seed number or fruit traits, suggesting that some allelic effects on seed size are mediated via maternal resource allocation, whereas other QTL act more specifically on seed developmental processes (Alonso-Blanco et al., 1999). More recently, a multi-parent MAGIC mapping population identified ~8 seed-size QTL dispersed across four chromosomes, with most of these loci distinct from those controlling seed number, indicating that seed size and number can be genetically uncoupled. The alleles for larger seeds at the majority of these QTL derived from the same founder accession, pointing to past directional selection for increased seed size in that lineage (Gnan, Priest, & Kover, 2014).

In addition to QTL mapping, association studies have helped identify specific genes underlying seed size variation. A genome-wide association study (GWAS) detected 38 loci for seed size, including one at the CYCB1;4 gene; natural allelic variation in this cell-cycle regulator significantly influences seed size, as enhanced CYCB1;4 expression

accelerates seed growth (producing larger seeds) whereas loss-of-function cycb1;4 mutants form smaller seeds (Ren et al., 2018). Similarly, fine-mapping of a major QTL from the Cvi×Ler cross led to the cloning of SSW1, which encodes an amino acid permease (AAP8) that acts maternally to promote seed filling and the *Cvi* allele of SSW1 shows higher nutrient transport activity and produces heavier, larger seeds than the *Ler* allele (Jiang et al., 2024).
In addition, environmental influences can shape the expression of seed size–related genes. For instance, epigenetic regulation of genes like *CKX2* has been shown to respond to environmental cues and since *CKX2* modulates cytokinin levels that influence cell division and organ growth, such regulation can directly impact seed development and final seed size, linking seed size to both genetic and ecological contexts (Li et al., 2013). Both genetic background and environmental conditions contribute to seed size variation in *Arabidopsis* and this is clearly reflected in the differences observed among Swedish accessions of *Arabidopsis* collected from coastal and inland regions, which are the focus of our lab's research.

One key finding published from our lab is that different wild accessions of *Arabidopsis thaliana* exhibit significant natural variation in seed size. In particular, accessions originating from colder, high-latitude climates tend to produce larger seeds compared to those from warmer, low-latitude (southern) regions. A survey of 123 Swedish *A. thaliana* lines found that seed mass correlated strongly with the climate of origin; plants from areas with lower winter temperatures had larger seeds on average. The author notes that this difference in seed size among populations is non-random and likely reflects local adaptation (Clauw et al., 2022).

Other findings from our lab show that seed size in *Arabidopsis thaliana* varies greatly among natural accessions, with seed area ranging roughly twofold (from ~0.10 to 0.20 mm^2) across different genotypes (Figure 1A). This variation exhibits clear geographic patterns: seeds from coastal populations are consistently larger than those from inland populations, as demonstrated in a panel of Swedish accessions where “beach” ecotypes had significantly larger seeds compared to inland ecotypes (Figure 17,

Appendix). This coastal–inland difference in seed size suggests local adaptation to environmental conditions in different regions. Moreover, seed size variation correlates with differences in fitness and early establishment – in a selection experiment, lines with larger seeds tended to show higher average seedling establishment and fitness (Figure 1B; Brachi et al., 2025), suggesting that the differences in seed size among accessions may help them adapt better to their local environments.

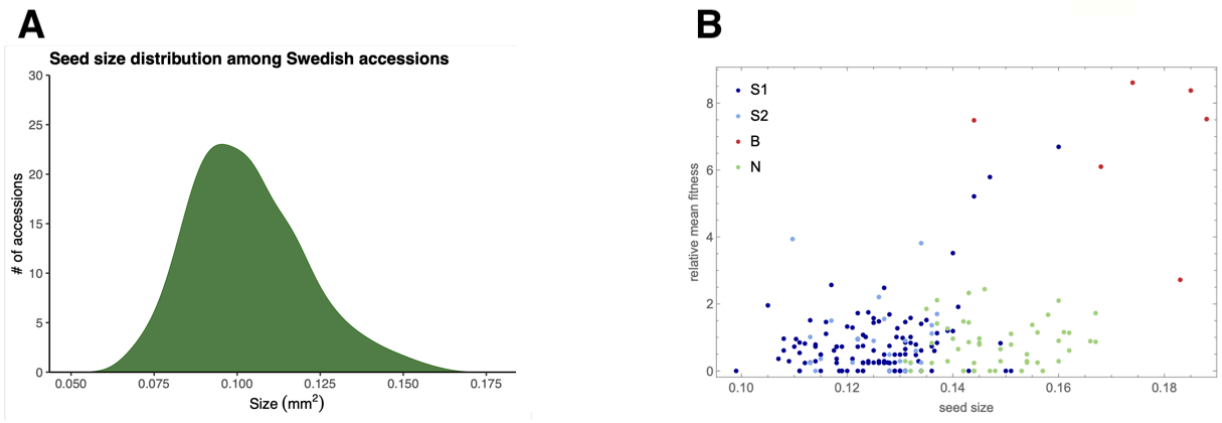


Figure 1: Natural variation in seed size among Swedish *Arabidopsis thaliana* accessions and its geographic and fitness associations (data from previous experiments conducted in the lab) (A) Seed size distribution across Swedish accessions showing a varied distribution with a majority of accessions clustered around 0.100 mm². (B) Coloured dots represent accessions from different regions: S (dark and light blue) = South, B (red) = Beach, N (green) = North. Data from Brachi et al. (2025).

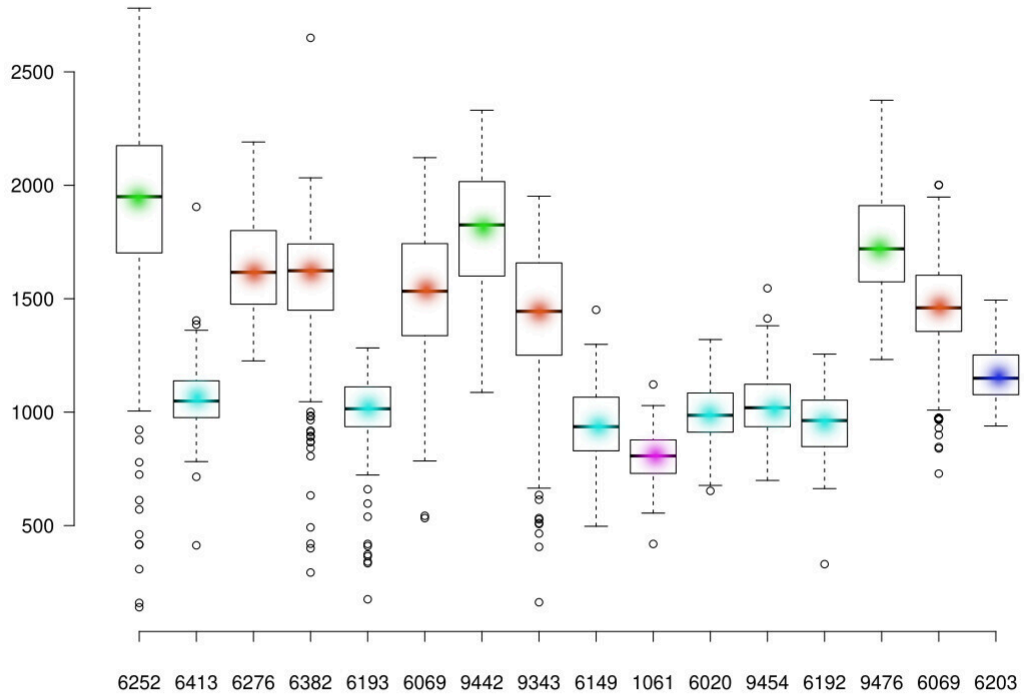


Figure 2: Box plot shows the distribution of seed area (px) for each accession as measured using the Boxeed Robot (Labdeers, Czech Republic). The data highlights substantial variation in average seed size across accessions, with some (e.g., 6252, 9442, 9476) exhibiting consistently bigger seed areas, while others (e.g., 1061, 6149, 6203) fall into the smaller size range. These differences confirm the presence of natural variation in seed size within the Swedish *A. thaliana* collection and were used to classify accessions as big- or small-seeded.

These findings highlight that seed size may play an important role in adaptation by influencing early establishment success.To better understand the variation in seed size, we investigated the potential consequences of seed size differences on two key developmental stages that may be critical for local adaptation: (i) germination properties, including the environmental regulation of germination and dormancy, and (ii) early seedling development. These stages represent the earliest phases of a plant's life cycle, where seed-intrinsic traits such as size may have the greatest impact before additional environmental or physiological factors begin to influence performance. To explore whether seed size influences these early developmental responses, I focused on germination behavior under varying environmental conditions, specifically the roles

of light and temperature as external cues, as well as the elongation of the hypocotyl during seedling growth in darkness.

1.4 Environmental factors influencing germination (role of light/temperature)

Temperature is one of the most important environmental factors influencing seed germination, as it provides a reliable cue for seasonal timing and ensures that germination occurs under conditions favorable for seedling survival. Temperatures outside the species-specific optimal range can either delay germination or inhibit it entirely.

Previous studies have shown that low temperatures can slow down or inhibit seed germination and early seedling growth across different plant species. For example, in soybean, Hatfield and Egli (1974) found that hypocotyl elongation was extremely slow at 10°C and increased progressively with higher temperatures, reaching its maximum at 30°C. Consistent with these findings, a recent study on soybean reported that germination was completely inhibited at 10°C, with germination rates increasing as temperatures rose. At low temperatures, seeds required substantially more time to germinate, and cell membrane damage, indicated by increased electrolyte leakage, further reflected sensitivity to temperature stress (Szczerba et al., 2021). In addition to low temperatures delaying or inhibiting germination, exposure to very high temperatures can also prevent germination in a condition known as thermoinhibition. For example, in *Capsicum annuum* (pepper), germination was strongly inhibited at high temperatures, with complete thermoinhibition observed at 40°C across all tested cultivars (Carter & Vavrina, 2001). The specific temperature ranges that promote or inhibit germination vary widely between species and even among populations within a species, often reflecting local adaptation to their native environments.

In *Arabidopsis thaliana*, natural variation in germination responses to temperature has been well-documented, with different accessions showing distinct germination behavior depending on their climatic origin. For example, Exposito-Alonso (2020) found that accessions from Mediterranean climates tend to germinate rapidly under mild winter conditions, whereas accessions from northern regions, such as Sweden, are adapted to

germinate in autumn and survive extended periods of cold before resuming growth in spring.

In addition to temperature, light is another critical environmental cue that seeds use to regulate the timing of germination, ensuring that emergence occurs under conditions favorable for seedling establishment. While most plant species are capable of germinating under both light and dark conditions, some require specific light environments for germination. For example, *Xyris tennesseensis* is a light-requiring species (Baskin & Baskin, 2003), whereas *Eschscholzia californica* (California poppy) shows dark-requiring light actively suppressing germination (Goldthwaite et al., 1971). These contrasting responses are mediated by the action of photoreceptors, particularly phytochromes, which sense red and far-red light and regulate hormonal pathways controlling germination. Exposure to red and far-red light is sensed by phytochrome photoreceptors, which shift into their active form and trigger germination-promoting pathways by stimulating gibberellin (GA) production and reducing abscisic acid (ABA) levels, thereby terminating dormancy and initiating embryo growth (Finch-Savage & Leubner-Metzger, 2006). The requirement for light ensures that seeds germinate only under favorable conditions, such as near the soil surface, and prevents deeply buried seeds with limited reserves from initiating germination in environments where seedlings would be unlikely to survive (Pons, 2000). In *Arabidopsis thaliana*, seed germination is highly light-sensitive, and phytochrome B (phyB) and phytochrome A (phyA) plays the dominant role as the photoreceptor that promotes germination in response to light (Shinomura et al., 1994). Mutant studies demonstrated that phyB-deficient seeds have greatly reduced germination under red light, whereas phytochrome A (phyA) can trigger germination only under prolonged far-red or extremely low-fluence light conditions, showing that Arabidopsis seeds rely on phyB for typical red light responses and rely on phyA for very-low-light or far-red light cues (Bentsink & Koornneef, 2008). Given this strong light dependence, studying light responses during germination can provide important insights into how environmental signals control early development in Swedish accessions of *Arabidopsis thaliana*. More broadly, investigating how seed size possibly interacts with light and temperature responses in Swedish accessions may reveal how

local adaptation to contrasting environments has shaped germination behavior and early seedling strategies.

1.5 Dormancy and the impact of stratification on germination

Seed dormancy is an innate seed trait that prevents germination, even under otherwise optimal conditions, until the environment is suitable for seedling survival. The ecological function of this trait is to delay germination, synchronizing seedling emergence with favorable seasons and preventing seeds from germinating at times that would be lethal to seedlings. According to Baskin and Baskin (2004), a dormant seed is one that lacks the capacity to germinate within a specified period under any combination of normal environmental conditions (such as temperature and light) that would otherwise be favorable for germination.

Multiple dormancy mechanisms have evolved across plant species to ensure that seeds germinate only when environmental conditions optimize seedling survival, reflecting the diversity of life-history strategies (Baskin & Baskin, 2004b). In *Arabidopsis thaliana*, the primary dormancy mechanism is classified as non-deep physiological dormancy, where germination is inhibited by internal physiological signals rather than by physical barriers. Dormancy in *Arabidopsis thaliana* can be broken by cold stratification, a period of moist chilling, or by after-ripening which is a period of dry storage at room temperature (Baskin & Baskin, 2001). Ecologically, the requirement for cold stratification ensures that seeds remain dormant throughout winter and germinate only when favorable conditions return in spring, so seedlings only germinate when there is higher resource availability and milder climates. As such, stratification acts as a critical environmental cue for dormancy release, coordinating germination with seasonal changes.

In Arabidopsis, the DELAY OF GERMINATION 1 (*DOG1*) gene was identified as a major quantitative trait locus controlling natural variation in seed dormancy in *Arabidopsis thaliana*, with loss-of-function mutants confirming that DOG1 is absolutely required for dormancy induction. Natural allelic variation at DOG1 largely arises from cis-regulatory polymorphisms that cause differences in gene expression between

accessions, with DOG1 transcription being seed-specific and reaching peak levels late in seed development. Although DOG1 transcripts persist in after-ripened seeds, they rapidly decline upon imbibition, suggesting that DOG1 primarily functions during seed maturation to establish dormancy (Bentsink et al., 2006). A previous study conducted in our lab on Swedish populations of *Arabidopsis thaliana* found that multiple natural alleles at the *DELAY OF GERMINATION1* (*DOG1*) locus jointly explain up to 57% of the variation in seed dormancy. This work, which involved some of the same Swedish accessions analyzed in my thesis, demonstrated through field experiments that variation at *DOG1* affects both germination timing and survival under natural conditions, highlighting its key role in local adaptation (Kerdaffrec et al., 2016). These findings illustrate that seed dormancy is a complex trait shaped by both genetic and environmental factors, with *DOG1* acting as a central regulator of natural variation in *Arabidopsis thaliana*. Building on previous work from our lab on Swedish *Arabidopsis* accessions, my thesis further explores how seed size, germination behavior, and early seedling traits vary among coastal and inland populations in response to environmental cues.

1.6 Hypocotyl Length and Early Seedling Development

In addition to regulating the timing of germination, seed traits can influence early seedling growth, which is critical for successful establishment. Hypocotyl elongation, the primary growth of the embryonic stem, provides valuable insight into a seed's early developmental capacity before substantial nutrient uptake occurs. Because hypocotyl growth largely relies on the internal reserves stored in the seed, it offers a relatively direct measure of the initial potential conferred by seed size.

Hypocotyl elongation has been widely used as a phenotypic marker to assess early seedling growth potential in plants, particularly under conditions where external nutrient input is limited. For example, a study on *Lactuca sativa* (lettuce) demonstrated that hypocotyl length responds sensitively to light and hormonal regulation even when seedlings are grown in the absence of added nutrients, making it a useful indicator of

intrinsic seed vigor (Volmaro et al., 1998). Similarly, in *Arabidopsis thaliana*, hypocotyl length has become a standard trait in early seedling phenotyping. A deep learning-based tool has even been developed to automatically quantify elongation from images of seedlings grown on nutrient-free filter paper, further highlighting its value as a standardized, resource-independent proxy for early developmental potential (Dobos et al., 2020).

Because seed size determines the quantity of stored reserves available to support early growth, it may influence variation in elongation traits such as hypocotyl length. More broadly, empirical evidence from a study on 303 angiosperm species showed that seed size was positively associated with seedling biomass growth and survival under low- to medium-light conditions, suggesting that larger seeds may confer a survival advantage by supporting greater early growth (Ma et al., 2019). Although the direct relationship between seed size and survival was variable, these findings highlight that seed size can influence early seedling traits in an environment-dependent manner, which may extend to traits such as hypocotyl elongation.

Together, these findings suggest that hypocotyl elongation serves as a marker for assessing early seedling growth potential across species. Given that bigger seeds generally possess greater internal reserves, variation in seed size may play a role in hypocotyl length. Building on this, my thesis investigates whether natural variation in seed size among Swedish *Arabidopsis thaliana* accessions corresponds to consistent differences in hypocotyl elongation, and whether these patterns reflect the initial reserve of the seed or underlying genetic differences between accessions.

2. Methods and materials

2.1 Seed Sorting and Seed Selection

Seed size measurements were obtained using the Boxeed Robot (Labdeers, Czech Republic), an automated system optimized for *Arabidopsis thaliana* dry seed samples. This system measures seed sizes ranging from 80 µm to 3 mm and records parameters such as seed area (px/mm²), seed length (mm), and seed width (mm). These measurements were used to analyze seed size variation across accessions.

Accessions were categorized based on seed size (Table 1) and Col-0 was included as a reference accession.

For two of the hypocotyl elongation experiments, seeds were additionally sorted by size, ensuring that only seeds of the exact same size were selected across all accessions, both bigger-seeded accessions and smaller-seeded accessions.

2.2 Germination and Growth Conditions

Seeds were plated on sterile petri dishes containing two layers of 47 mm Whatman® paper moistened with 2 ml of water containing 0.1% PPM™. Approximately 30 seeds of each accession were added per plate, with two replicate plates per accession.

For germination assays requiring stratification, seeds were stratified at 4°C in complete darkness for 7 days before being transferred to experimental conditions. Seeds were then exposed to 3 hours of light at 21°C, followed by incubation in continuous darkness at the required temperature, depending on the assay. Darkness was maintained by placing the plates in a box covered with black fabric and sealing the sides with tape. For the true dark condition of the assays, plates were prepared in complete darkness, briefly exposed to a far-red light lamp (15 µmol/m²s) for 10 minutes, and then transferred back to darkness for the remainder of the experiment. When measuring the germination rate or maximum germination, seeds were considered germinated when the seed coat was broken and the radicle was visible, as depicted in figure 3.

Figure 3: Criteria for germination scoring in *Arabidopsis thaliana* seeds. A seed was considered germinated when the seed coat was broken and the radicle became visible, as shown in the image.

2.3 Hypocotyl Assays and Measurement

For hypocotyl elongation assays, plates were stratified at 4°C for one week, then transferred to 21°C and exposed to 3 hours of light, followed by 7 days in complete darkness at 21°C. In one experiment, accession 1062 was treated differently, undergoing 3 days at 15°C followed by 3 days at 21°C, but this treatment was not continued in subsequent assays. After the growth period, 12–15 germinated seedlings per accession were collected at random and transferred onto square 1% agarose plates supplemented with charcoal for imaging. A ruler was included for scale, allowing hypocotyl length to be measured using Fiji software (Schindelin et al., 2012).

2.4 Data Analysis

Hypocotyl length distributions were compared across accessions, with seed-size groups analyzed separately to evaluate potential trends. Box plots and average length tables were used to summarize differences between accessions. All statistical analyses and visualizations were performed using RStudio.

3. Results

3.1 Results of Temperature Germinations Assays

3.1.1 Effect of temperatures varies between Arabidopsis Swedish accessions.

The primary objective of this study was to determine whether seed size influences germination rate in *Arabidopsis thaliana*. The seeds used in the experiments were collected from Swedish populations, which exhibit distinct differences between inland and coastal accessions. To accurately assess germination rate, it was first necessary to calibrate the temperature conditions, as germination requirements can vary among accessions. The commonly used accession Col-0 is generally recommended to be grown between 21 and 23°C (Rivero-Lepinckas et al., 2006). Since growth chambers are typically set to 21°C, this temperature is often considered the optimal condition for germination.

However, previous studies have demonstrated that germination and seedling establishment can be significantly influenced by temperature across different natural accessions (Schmuths et al., 2006). Therefore, identifying the optimal temperature and light conditions was a crucial step in tailoring experimental conditions to the specific Swedish accessions under investigation.

Accessions were selected based on seed availability, prioritizing those with sufficient quantities for the experiment. Three small-seeded accessions (1062, 6020, and 6188) and three large-seeded accessions (9343, 6016, and 6252) were included in this experiment. The seeds used in these experiments were originally collected in 2014 from *A. thaliana* plants grown in the lab. Seeds were germinated in sterile petri dishes containing moistened pads with water (0.1% PPM™) to prevent contamination. Approximately 30 seeds were sown per plate, with two replicate plates prepared for each accession at temperature conditions of 10°C, 15°C, 21°C, and 25°C. The plates were subjected to light induction at 21°C for three hours before being transferred to the corresponding temperature under darkness. Germination was monitored daily for up to 200 hours post-sowing, and the number of germinated and non-germinated seeds was

recorded at each time point. The maximum germination percentage was then calculated based on the final recorded values.

Our findings suggest that the effect of temperature on germination varies among the Swedish *Arabidopsis thaliana* accessions. Germination rate was strongly influenced by temperature, with some conditions delaying the onset of germination (Figure 4). At 10°C, germination was slow and exhibited a delayed onset compared to higher temperatures. A moderate improvement was observed at 15°C, with faster and more consistent germination. In contrast, germination at 25°C was noticeably lower than at 15°C and 21°C, as none of the accessions reached 100% germination. This suggests that while warmer temperatures generally promote faster germination, 25°C may exceed the optimal range for these accessions, potentially inducing stress.

The highest germination rates across accessions were recorded at 15°C and 21°C, indicating that these temperatures provide the most favorable conditions. The reduced germination observed at 10°C and 25°C in certain accessions suggests that while *A. thaliana* can germinate across a range of temperatures, there is an optimal window between 15°C and 21°C that maximizes germination efficiency. These results indicate that temperature plays a crucial role in regulating *A. thaliana* germination, while seed size does not appear to be a determining factor in temperature sensitivity.

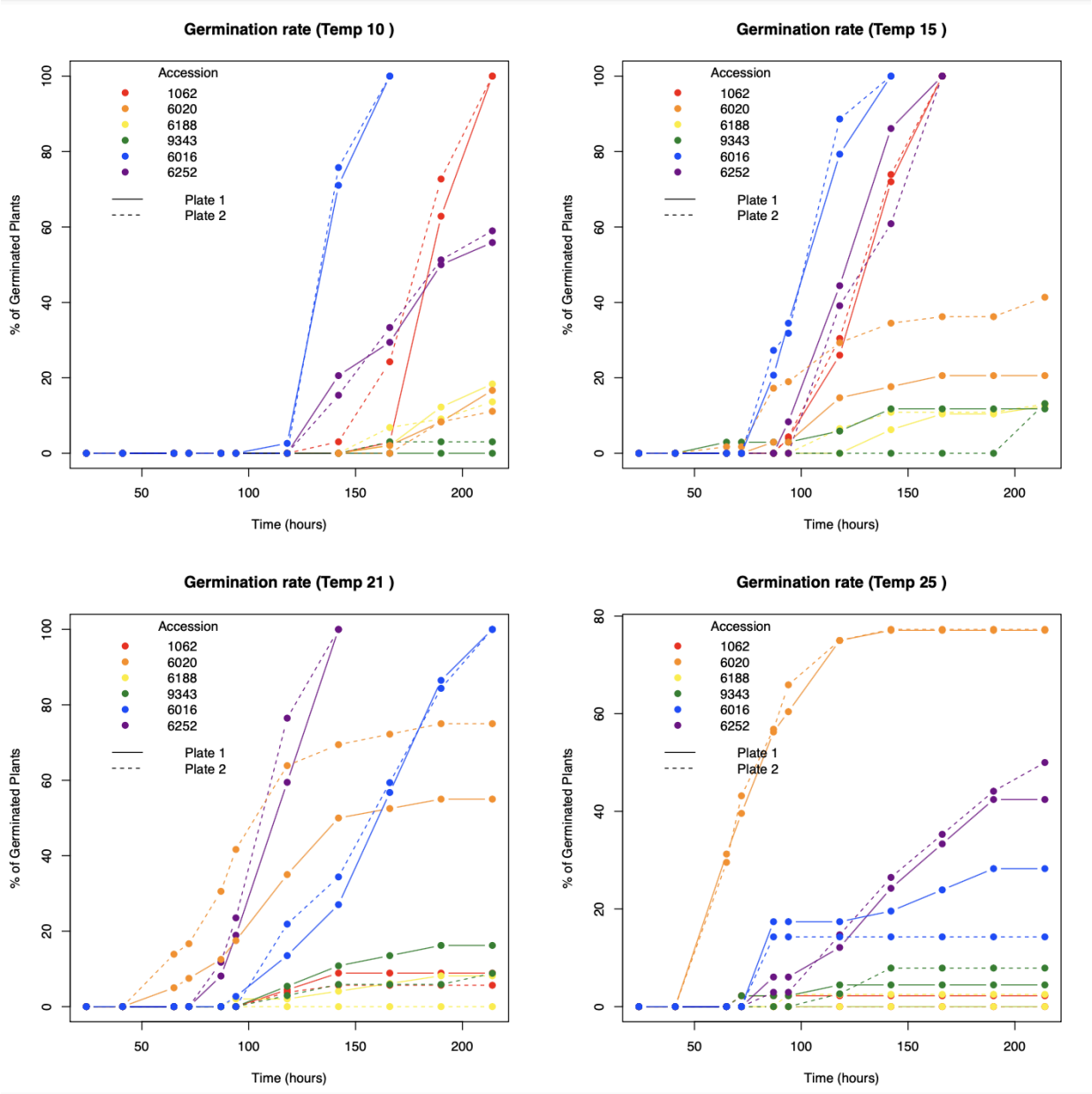


Figure 4: The effect of temperature on germination varies among Swedish *Arabidopsis thaliana* accessions. Germination was monitored at 10°C, 15°C, 21°C, and 25°C for up to 200 hours post-sowing. Colors represent different accessions, while solid and dashed lines indicate replicate plates.

3.1.2 Stratification is essential for establishing germination

Since the previous experiment indicated that germination was most efficient at 15°C and 21°C, we conducted a follow-up study focusing on these two temperatures. The experimental conditions remained the same as in the prior assay, with seeds plated on

sterile petri dishes containing moistened pads with water (0.1% PPM™). However, in this experiment, we also aimed to investigate the effect of stratification on germination. Stratification has been reported to enhance germination in *Arabidopsis thaliana* by breaking dormancy.

To assess the influence of stratification, we introduced a cold treatment step before light induction. After plating the seeds, the plates assigned to the stratification treatment were placed in complete darkness at 4°C for seven days. Following this stratification period, the plates were subjected to light induction at 21°C for three hours, after which they were transferred to their respective final temperature conditions (15°C or 21°C) and kept in darkness for an additional seven days. In contrast to the previous experiment, where germination was monitored daily for up to 200 hours, this time we only assessed germination at the end of the incubation period by counting the number of germinated and non-germinated seeds.

The results (Figure 5) showed that stratification enhanced germination across all accessions at both 15°C and 21°C, with a comparable increase in germination percentage at both temperatures. This suggests that cold treatment effectively promotes germination regardless of whether the seeds are incubated at 15°C or 21°C. The consistent improvement across temperatures indicates that stratification is a key dormancy-breaking mechanism in these accessions. This also implies that while 15°C and 21°C were previously identified as the most favorable temperatures for germination, dormancy release via stratification remains an important factor influencing germination efficiency in *A. thaliana.*

Comparing across accessions, we observed some variability in the degree of stratification response, suggesting that different accessions may have varying dormancy mechanisms. Some accessions showed minimal differences between stratified and non-stratified conditions, while others exhibited a strong dependency on stratification for efficient germination.

Overall, these findings confirm that stratification enhances germination, and that temperature alone is not the only factor regulating germination efficiency in *A. thaliana.*

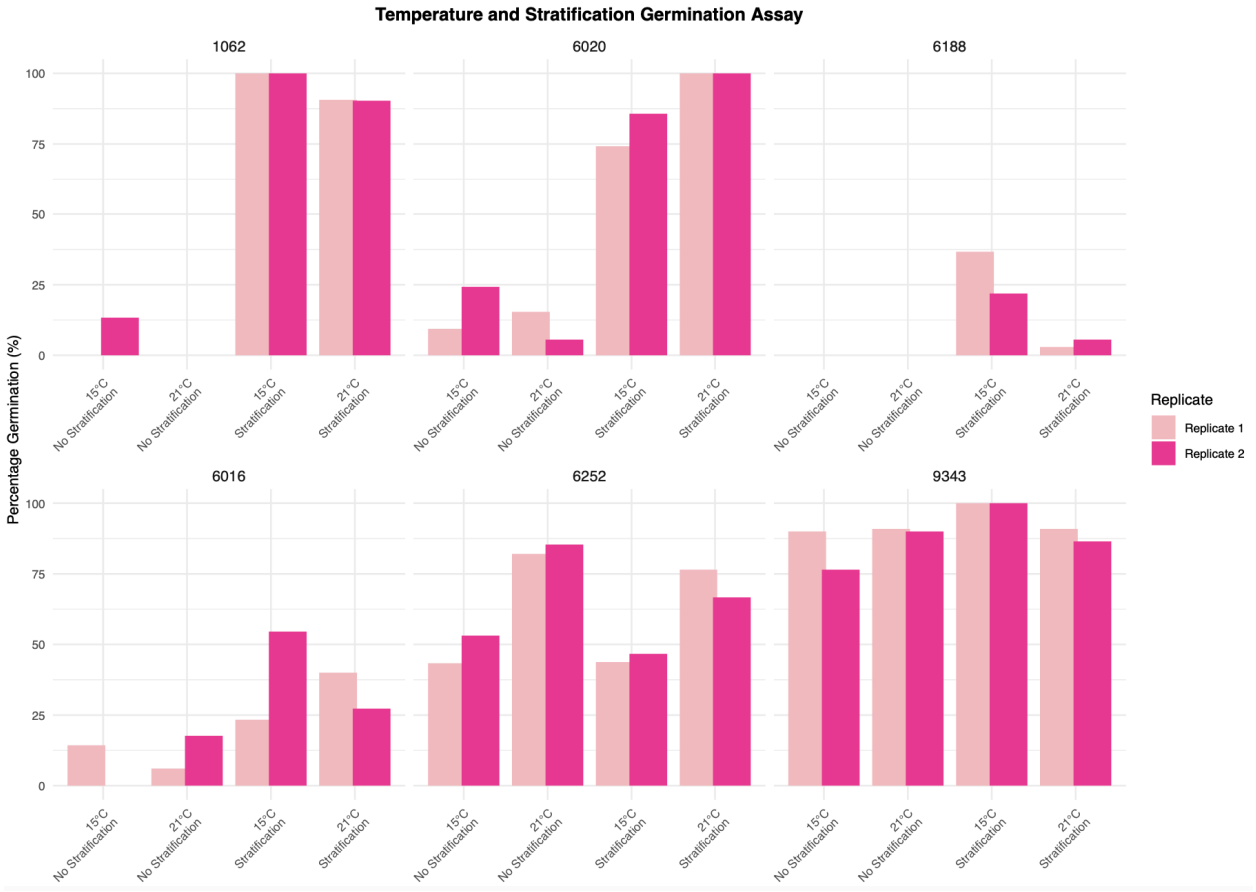


Figure 5: Stratification is essential for establishing germination. Germination was assessed at 15°C and 21°C with and without stratification. The stratification treatment was done in complete darkness at 4°C for seven days. Colors represent replicate plates. Small seed accessions are shown in the top panel, big seed accessions in the bottom panel.

3.2 Results of Light/Dark Germination Assays

3.2.1 Effect of light varies between Arabidopsis Swedish accessions.

To further investigate the factors influencing *Arabidopsis thaliana* germination, we examined the effect of light exposure on seed germination across different Swedish accessions. Light is a well-known environmental cue that regulates seed dormancy and enables germination (Holdsworth et al., 2008). In general, light can regulate the

germination response in three ways; there are light requiring seeds that germinate only upon light pulse. Light may inhibit germination in species living on fast-drying soil to ensure germination in deeper soil layers. Additionally, germination may be independent of light; species with light-neutral seeds germinate in darkness and light equally well. These categories are not mutually exclusive; seeds of some species require a light pulse for germination but inhibited by high illumination. Arabidopsis seeds (Col-0 accession) is well known as its germination requires light. However, some natural accessions can germinate efficiently in darkness, suggesting that light dependency may vary due to genetic differences or environmental adaptation (Bunsick et al., 2022; Meng et al., 2008).

The distinction between light-sensitive and light-independent germination may reflect adaptive strategies shaped by the accessions' natural habitats. Inland populations, which often experience denser vegetation and lower soil disturbance, may rely on light as a signal to ensure germination occurs near the soil surface, where seedlings have access to sufficient resources. In contrast, coastal (beach) populations are typically exposed to open, high-light environments with frequent physical disturbances. These areas often have sandy soils, where wind and water movement can regularly resurface buried seeds, reducing the reliability of light cues. As a result, beach accessions may have evolved to be less dependent on light signals, enabling germination even when seeds are initially buried. If such adaptation exists, we would expect inland accessions to show reduced germination in darkness, while beach accessions may germinate efficiently under both conditions.

We set up two experiments to assess whether the selected Swedish accessions exhibit light-dependent, light-independent or light inhibited germination and whether this dependency differs between small-seeded and large-seeded accessions.

(i) First, we applied a light pulse to induce germination. Subsequently, the seeds were kept under light or in darkness. With this experiment we assess if the germination might be inhibited by light.

(ii) Second, we compared germination with or without initial light pulse. Additionally, we applied a short far-red pulse upon imbibition to ensure that phytochromes are converted

to the inactive isoform. With this experiment we assess if the seeds require light for germination.

Since the initial temperature assays identified 15°C as a probable optimal temperature for germination across accessions, this experiment was conducted at 15°C to ensure that temperature was not a limiting factor. This allowed us to isolate the effect of light exposure on germination rates, without the influence of suboptimal temperature conditions.
(i) In the first setup we compared continuous light and light-induced dark conditions. We aimed to determine whether constant light illumination inhibits germination in these accessions and whether any patterns emerge based on their seed size classification.

To assess these differences, seeds were sown on sterile petri dishes with moistened pads with water (0.1% PPM™) and exposed to 3 hours of light at 21°C before being transferred to 15°C, where they were either maintained in continuous light or placed in dark conditions. Two replicate plates were prepared per accession for each condition, with approximately 30 seeds per plate. Germination was monitored over a period of up to 384 hours, and the maximum germination percentage was calculated based on the final count of germinated and non-germinated seeds.

The results (Figure 6) show that overall, germination rates were relatively low across accessions, with only two accessions (6252 and 9343) reaching 100% germination in both light and dark conditions. However, for these two accessions, germination was much more rapid in darkness, with both reaching 40–50% germination by the 100-hour time point, whereas under light conditions, germination was delayed, with only ~10% germination at the same time point. This suggests that, for these accessions, light may partially inhibit the germination or slow the process.

In contrast, two accessions (6016 and 6020) exhibited particularly poor germination in both conditions, with some showing almost no germination at all. This suggests that

these accessions may possess deeper dormancy or require additional environmental cues beyond light and temperature to successfully germinate. Interestingly, accession 1062 germinated better in darkness than in light, through still germinated poorly overall. The most striking difference was observed in accession 6188, which barely germinated in dark conditions but almost reached 100% germination in light. This strong light dependency highlights variation in germination responses between accessions.

Despite these differences, no clear pattern emerged regarding seed size and light sensitivity. Both small-seeded and large-seeded accessions displayed high and low germination rates in both conditions, suggesting that light dependency is not directly linked to seed size. These findings may suggest that while light is an important germination cue for some accessions, its effect is not uniform across all Swedish *A. thaliana* populations. The variability in germination success, speed, and dependency on light indicates that additional factors may be shaping the germination responses in these accessions.

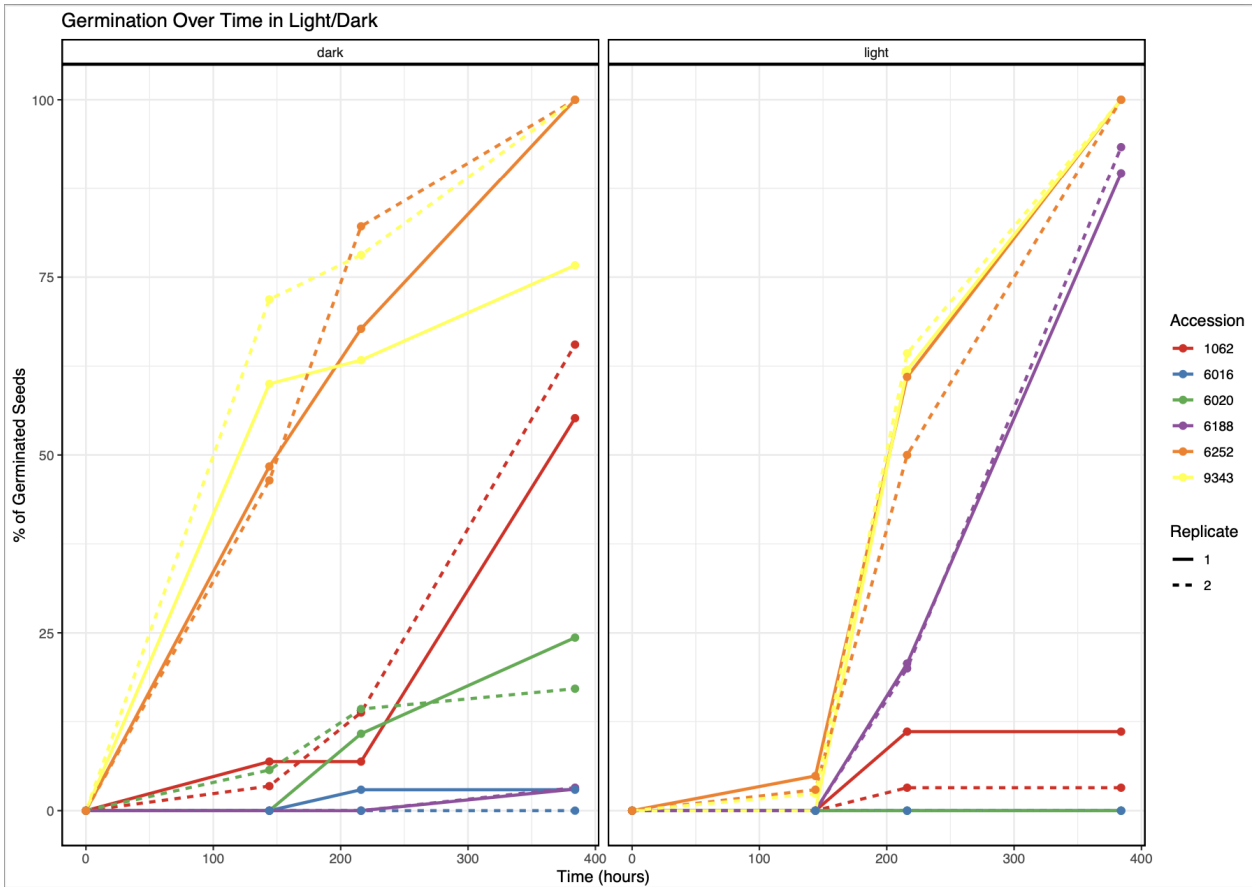


Figure 6: Effect of light varies between Arabidopsis Swedish accessions. Germination was monitored at 15°C, up to 400 hours post-sowing. Both sets of plates underwent light induction for three hours, after which they were transferred to either dark or continuous light conditions for the remainder of the experiment. Colors represent different accessions, while solid and dashed lines indicate replicate plates.

3.2.2 Stratification enhances germination in light and dark conditions.

To validate the findings from the previous light/dark assay, we repeated the experiment to confirm the consistency of the germination patterns across accessions. Based on the results of the initial temperature assays, 15°C was again selected as the experimental temperature, as it was shown to be an optimal condition for germination across multiple accessions.

In addition to testing the impact of light and dark conditions, we also introduced a new variable: stratification versus no stratification. Stratification, a commonly used

pre-treatment in seed germination studies, is known to help break dormancy. By incorporating stratification into this assay, we aimed to determine whether the combination of cold treatment and light exposure would result in increased germination rates, particularly for those accessions that previously exhibited poor germination. This approach allowed us to assess whether stratification could reduce dormancy-related barriers and whether its effects were consistent across both light and dark conditions.

To evaluate the effects of light, darkness, and stratification on germination, similar to the assay above, seeds were plated on sterile petri dishes with pads moistened using water containing 0.1% PPM™. After plating, all seeds underwent an initial 3-hour light exposure at 21°C before being transferred to 15°C, where they were either kept under continuous light or placed in dark conditions. Each condition was tested using two replicate plates per accession, with approximately 30 seeds per plate.

For the stratification treatment, seeds were kept in complete darkness at 4°C for 7 days following plating. After this cold treatment, the plates were exposed to 3 hours of light at 21°C before being transferred to 15°C under either continuous light or dark conditions. In this assay, germination was assessed only at the end of the incubation period, after 168 hours (7 days), by counting the number of germinated and non-germinated seeds.

The introduction of stratification affected germination outcomes across accessions, particularly for those that previously exhibited poor germination in the light/dark assay at 15°C without stratification (Figure 7).

Accession 6020, which had low germination rates in the previous assay, again showed low germination without stratification but germinated better with stratification under both light and dark conditions. Similarly, accession 1062, which previously had very poor germination, showed almost no germination without stratification in this experiment but reached 100% germination in both conditions after stratification, possibly indicating that cold treatment was necessary for breaking dormancy in this accession.

In contrast, accession 6016, which performed poorly in the previous assay, again showed very little to no germination regardless of stratification, suggesting that other factors may be preventing germination in this accession.

Among the better-performing accessions, accession 6252 exhibited moderate germination across all conditions, maintaining an overall average germination rate. Accession 9343, which had over 50% germination in both light and dark conditions without stratification, showed higher germination with stratification, reaching 100% germination in both conditions following cold treatment. This suggests that while 9343 can germinate without stratification, the cold treatment improved its germination efficiency.

These results indicate that stratification plays a role in dormancy-breaking for certain accessions, particularly 1062 and 6020. Additionally, accessions that already germinated well without stratification, such as 9343, still responded to the treatment, leading to increased germination efficiency.

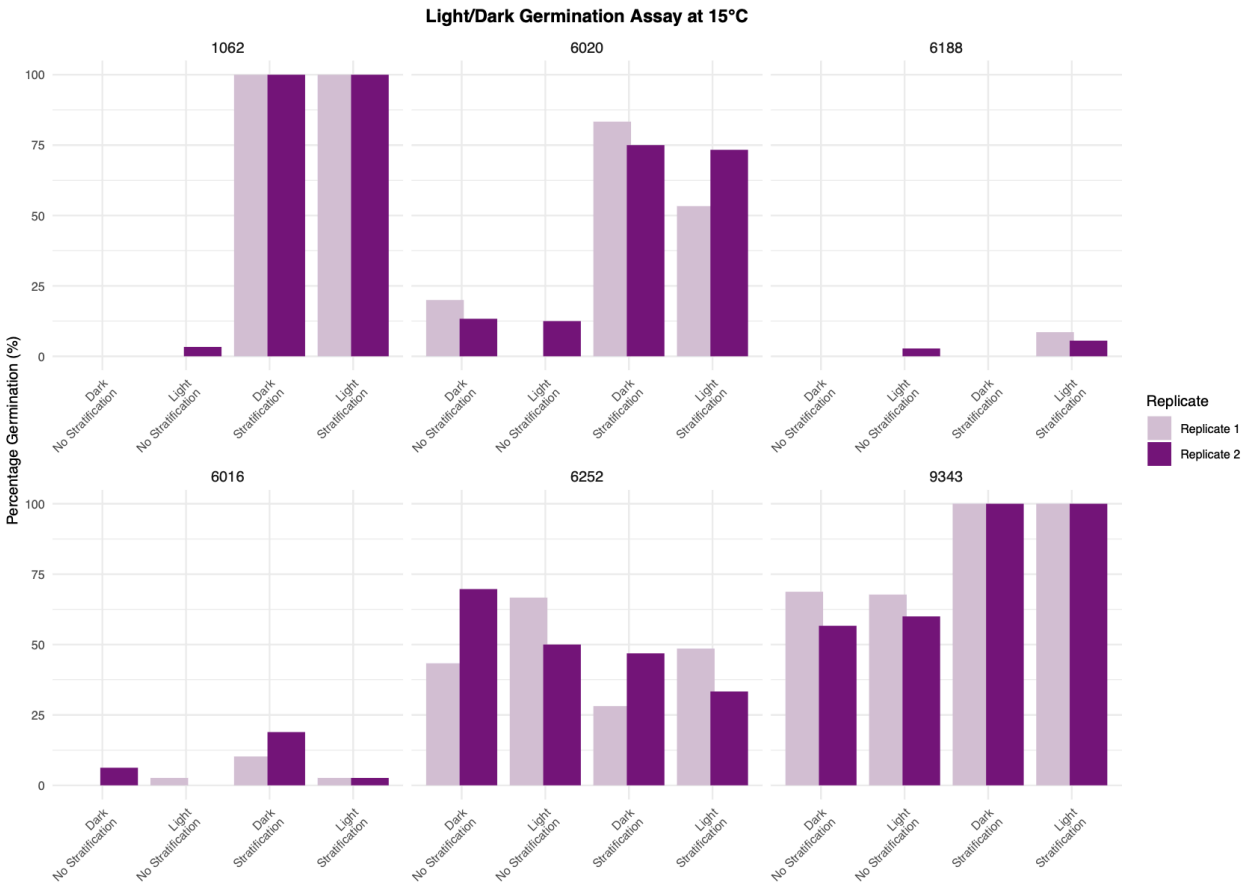


Figure 7: Germination percentages of *Arabidopsis thaliana* accessions at 15°C under light and dark conditions with and without stratification.
Bar plots show the final germination percentages for each accession under four treatment combinations: light with stratification, light without stratification, dark with stratification, and dark without stratification. Each treatment was tested in two biological replicates (light purple = replicate 1; dark purple = replicate 2). Stratification generally enhanced germination across most accessions**.** Small seed accessions are shown in the top panel, big seed accessions in the bottom panel.

3.2.3 True dark conditions suppress germination in most accessions.

Following the previous temperature assays, 21°C was identified as the most optimal temperature for germination, leading to its selection for this experiment. Additionally, previous results showed that stratification strongly enhanced germination, so all seeds in this assay underwent cold treatment before testing to ensure high germination rates across accessions.

Due to persistently low or no germination in accessions 6016 (large-seeded) and 6188 (small-seeded) in previous assays, these accessions were removed and replaced with two new accessions: 6192 (small-seeded) and 6241 (large-seeded). This substitution ensured that the study continued to assess a representative range of both small- and large-seeded accessions while focusing on accessions with measurable germination responses. Additionally, the Col-0 natural accession was included in this assay to serve as a reference, as it is widely used in *Arabidopsis thaliana* germination studies.

(ii) In the second setup we aimed to determine if germination requires light for germination.To address this, a true dark condition was introduced, in which seeds were plated in darkness, placed under a far-red light lamp (15 µmol/m²s) for 10 minutes and incubated in complete darkness, to suppress phytochrome activity. Published data showed that under true dark condition, Arabidopsis (Landsberg erecta) seeds did not germinate in true dark condition, while more than 50% germinated in dark condition without a far-red pulse. The difference between dark and true dark condition was explained by the pre-existed active form of phytochromes that reserved upon seed desiccation. Therefore, a far-red pulse upon imbibition serve as true dark condition to determine if active phytochromes are required for seed germination (Shinomura et al., 1994)

The results (Figure 8) showed that, similar to the published data in Landsberg accession, all accessions aside from 9343 exhibited 0% germination in the true dark condition, confirming that light exposure was essential for triggering germination in these accessions. Surprisingly, accession 9343 showed approximately 20% germination in both replicate plates under true dark conditions, suggesting that it may have a reduced dependency on light compared to the other accessions.

Among the accessions tested, 6192 had relatively low germination rates in both light and dark conditions, reaching a maximum of around 30% germination. In contrast, 1062 and 6020 performed well in both conditions, aligning with their stratified germination results from the previous assay, indicating that their dormancy-breaking requirements were effectively met. Accession 6252 exhibited moderate germination, with just over

50% germination in light conditions and over 75% germination in the dark. Accession 6241 showed an overall moderate germination response, without any extreme differences between conditions.

Accession 9343 performed well in both light and dark conditions, reaching near or full 100% germination, further reinforcing that this accession has a reduced dependency on light compared to the others. Col-0, included as a reference accession, exhibited 100% germination across all plates and both conditions, consistent with its well-documented ability to germinate efficiently under standard conditions.

No clear pattern emerged when comparing small-seeded and large-seeded accessions in terms of their response to light and dark conditions. Some small-seeded accessions, such as 1062 and 6020, performed well, while others, such as 6192, showed low germination. Similarly, among large-seeded accessions, 9343 germinated well in all conditions, while 6241 showed only moderate germination. This suggests that light dependency is not associated with seed size in the Swedish accessions of Arabidopsis and may instead be influenced by other factors, such as genetic background or environmental adaptation.

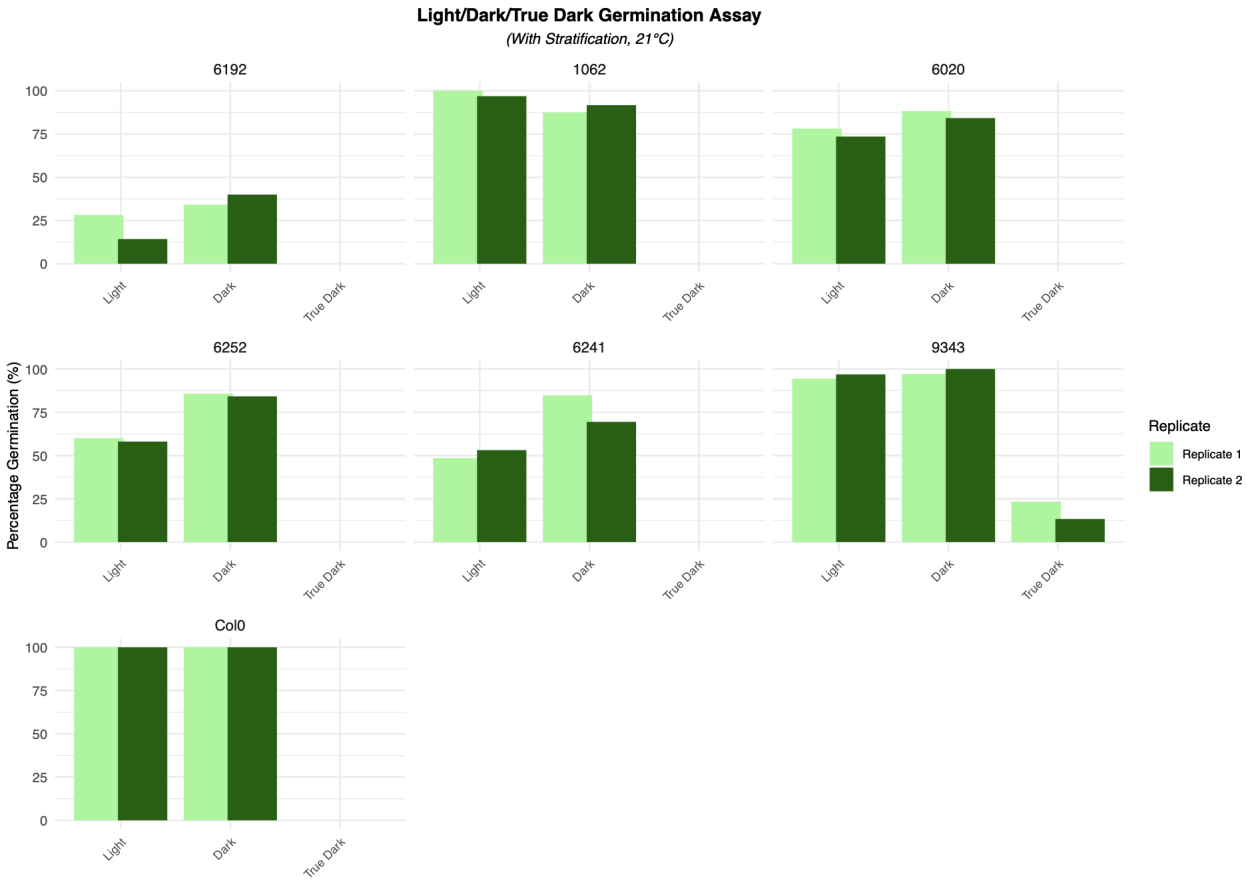


Figure 8: Germination percentages of *Arabidopsis thaliana* accessions under light, dark, and true dark conditions at 21°C following stratification.
Bar plots show maximum germination (%) for each accession in three conditions: continuous light, light-induced dark, and true dark. Each condition was tested in two biological replicates (light green = replicate 1; dark green = replicate 2). No consistent trend was observed between seed size classification and light dependency. Small seed accessions are shown in the top panel, big seed accessions in the second panel, Col-0 reference accession in the bottom panel.

3.3 Results of Hypocotyl Elongation Assays

3.3.1 Variation in hypocotyl elongation among *Arabidopsis thaliana* accessions and its potential correlation with seed size

Next, we aimed to characterize whether the seed size is associated with the earliest event during seedling development under the condition when the source of energy is the seed. This experiment was conducted to examine hypocotyl elongation in different *Arabidopsis thaliana* accessions under dark conditions during the skotomorphogenesis developmental phase. The hypocotyl, the embryonic stem of the seedling, is highly

influenced by light availability and temperature, making it a key trait for understanding early seedling development and potential adaptation differences between accessions (Jin & Zhu, 2017; Miyazaki et al., 2015; Koini et al., 2009).

By comparing the dark-grown hypocotyl length across accessions, we aimed to assess whether variations in elongation correlate with seed size and accession origin. The initial pilot experiments revealed noticeable visual differences in hypocotyl lengths among accessions. These early observations motivated us to expand the experiment with a larger set of accessions. Col-0 seeds were included in the experiment as a reference.

Seeds were plated on small petri dishes at a density of approximately 30 seeds per plate, with two replicate plates per accession. Plates were then stratified in complete darkness for 6 days at 4°C to break dormancy. Following stratification, all plates were exposed to 3 hours of light at 21°C before being transferred to continuous darkness for 6 days at 21°C.

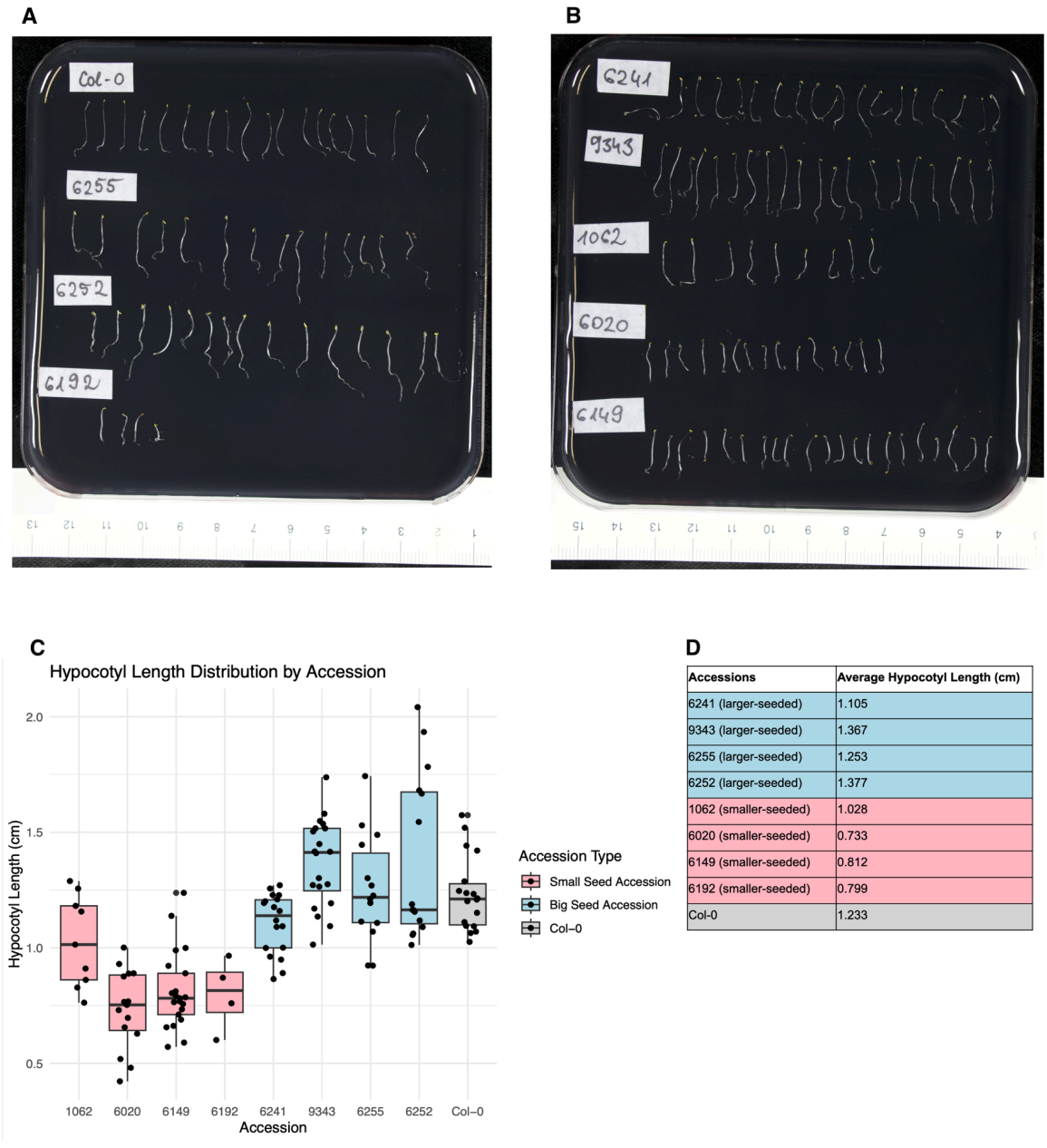
The results (Figure 9) show variation in hypocotyl length among the set of *Arabidopsis thaliana* accessions, with bigger-seeded accessions consistently exhibiting longer hypocotyls than smaller-seeded accessions. This trend suggests a possible relationship between seed size and hypocotyl elongation, where bigger seeds may support more rapid or extended growth in the early seedling stage.

Among the bigger-seeded accessions, 6252 displayed the longest average hypocotyl length (1.377 cm), followed by 9343 (1.367 cm) and 6255 (1.253 cm). Accession 6241 had a slightly lower average hypocotyl length (1.105 cm) but still fell within the expected range for bigger-seeded accessions. Col-0, included as a reference, exhibited an intermediate hypocotyl length (1.233 cm), overlapping with the bigger-seeded accessions.

In contrast, all smaller-seeded accessions had consistently shorter hypocotyls. 1062 had the longest hypocotyls among the small-seeded group (1.028 cm), while the other

small-seeded accessions, 6020, 6149, and 6192, all had hypocotyl lengths below 0.9 cm, with 6020 being the shortest at 0.733 cm.

Since all bigger-seeded accessions exhibited longer hypocotyls than all smaller-seeded accessions, these results may indicate a clear size-dependent trend in hypocotyl elongation. However, while this trend is consistent, further investigation is needed to determine whether seed size is the direct cause of these differences or if underlying genetic factors contribute to this pattern.



Accessions	Average Hypocotyl Length (cm)
6241 (larger-seeded)	1.105
9343 (larger-seeded)	1.367
6255 (larger-seeded)	1.253
6252 (larger-seeded)	1.377
1062 (smaller-seeded)	1.028
6020 (smaller-seeded)	0.733
6149 (smaller-seeded)	0.812
6192 (smaller-seeded)	0.799
Col-0	1.233

Figure 9: Hypocotyl elongation across *Arabidopsis thaliana* accessions after 6 days in darkness at 21°C. (A, B) Representative images of seedlings from different accessions, with a ruler included for scale. (C) Box plot showing the distribution of hypocotyl lengths, with bigger-seeded accessions (blue), smaller-seeded accessions (pink), and Col-0 (gray) plotted separately. (D) Table displaying the average hypocotyl length (cm) for each accession, highlighting variation in elongation across seed size categories.

3.3.2 Differences in hypocotyl elongation among more *Arabidopsis thaliana* accessions

To get a better understanding of hypocotyl elongation patterns, we tested another set of *Arabidopsis thaliana* accessions. Seeds underwent the same treatment as before. Although more accessions were initially included, some failed to germinate and were left out of the final analysis.

The accessions that successfully germinated included bigger-seeded accessions 9343, 6255, 6252, 6241, and 9382, as well as smaller-seeded accessions 6020, 6202, 1062, 6149, and Col-0 (reference accession). Hypocotyl length was measured using Fiji software to assess whether the previously observed trends in elongation persisted in this expanded dataset.

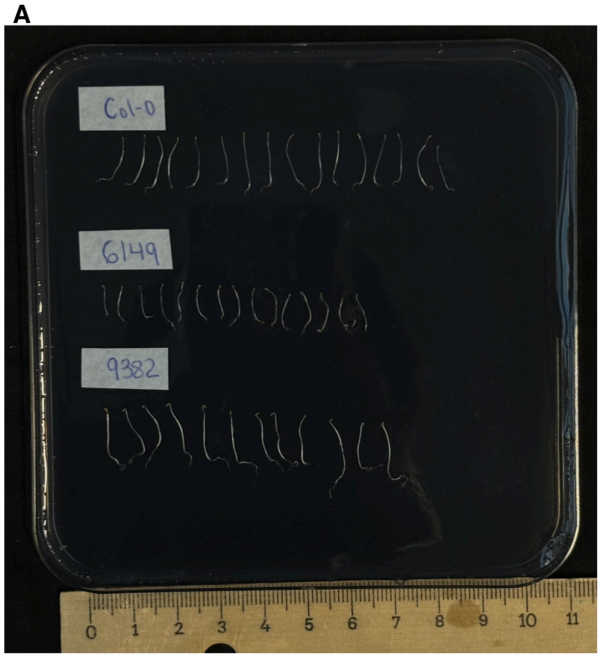
The results (Figure 10) show a consistent trend of bigger-seeded accessions exhibiting longer hypocotyls than smaller-seeded accessions, reinforcing previous findings. However, variation within each group suggests that factors beyond seed size contribute to elongation differences.

Among the bigger-seeded accessions, 9382 had the longest average hypocotyl length (1.332 cm), followed by 9343 (1.264 cm), 6241 (1.231 cm), and 6252 (1.196 cm). The shortest large-seeded accession was 6255 (1.138 cm), though it still had a longer average hypocotyl length than all smaller-seeded accessions.

The smaller-seeded accessions consistently exhibited shorter hypocotyls, with 1062 showing the longest hypocotyls in this group (1.020 cm), while 6149 had the shortest (0.792 cm). 6020 and 6202 fell in between, measuring 0.949 cm and 0.832 cm, respectively.

Despite some variation, the bigger-seeded accessions all exhibited longer hypocotyls than the smaller-seeded ones, with no overlap between the two groups. This strengthens the observed possible correlation between seed size and hypocotyl elongation. However, differences among accessions within the same seed-size category suggest that other factors may also influence hypocotyl elongation.

A



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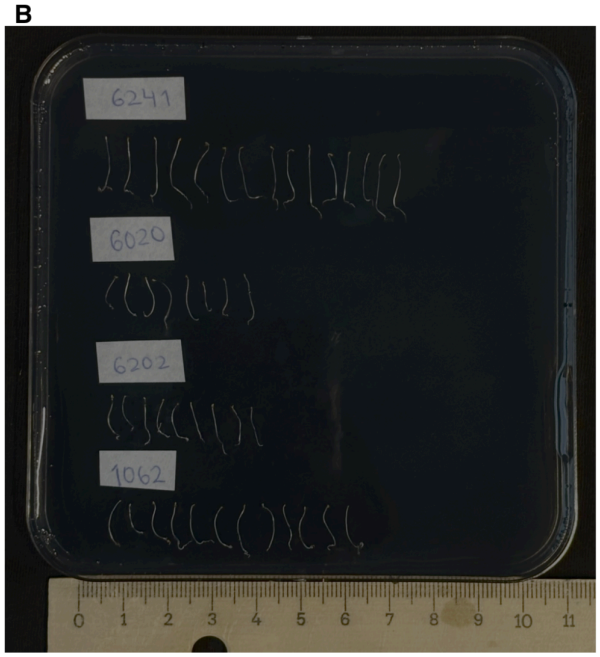

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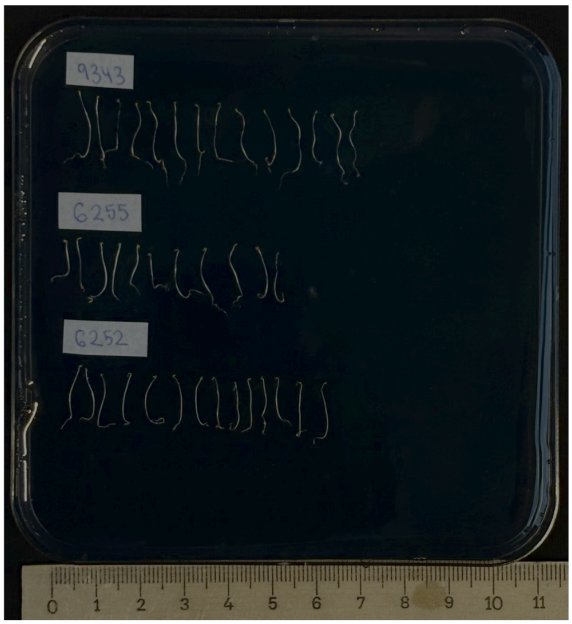


D

Accessions	Average Hypocotyl Length (cm)
9343 (larger-seeded)	1.264
6255 (larger-seeded)	1.138
6252 (larger-seeded)	1.196
6241 (larger-seeded)	1.231
9382 (larger-seeded)	1.332
6020 (smaller-seeded)	0.949
6202 (smaller-seeded)	0.832
1062 (smaller-seeded)	1.020
6149 (smaller-seeded)	0.792
Col-0	1.113

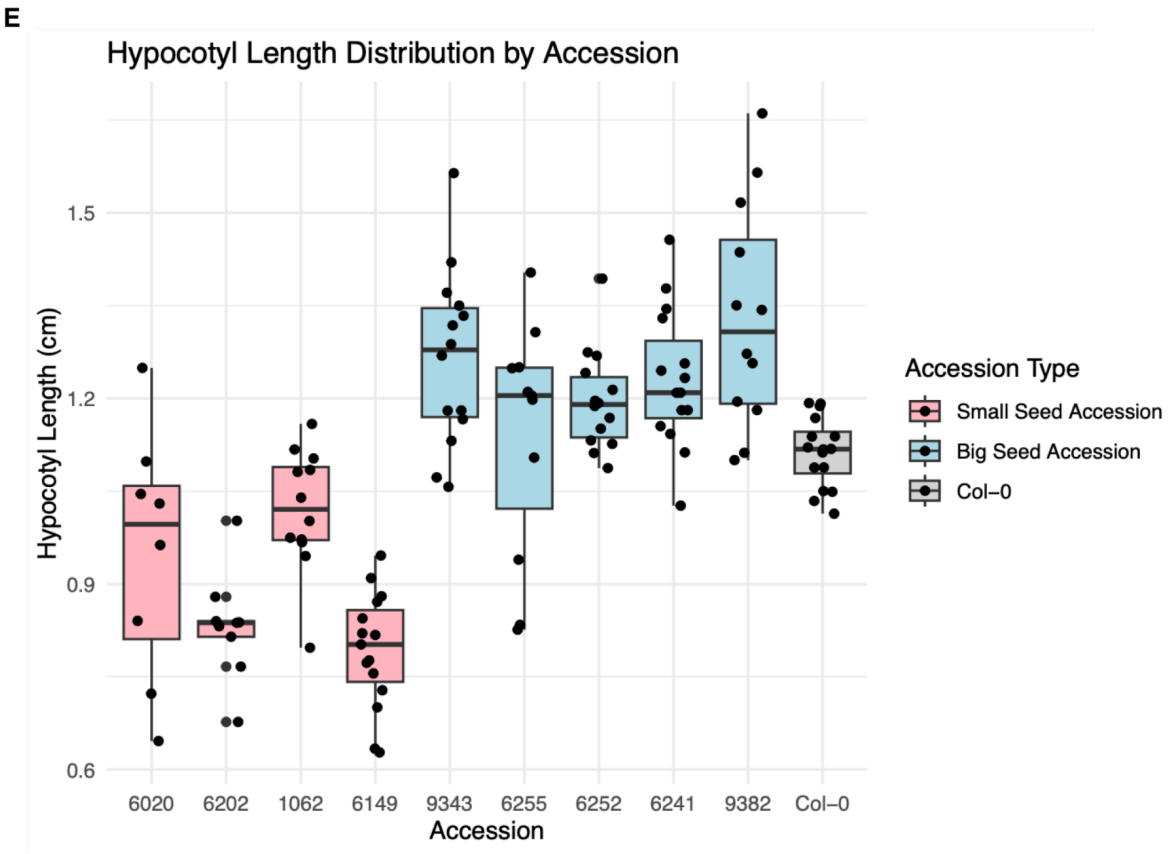


Figure 10: Hypocotyl elongation across *Arabidopsis thaliana* accessions after 6 days in darkness at 21°C. (A, B,C) Representative images of seedlings from different accessions, with a ruler included for scale. (D) Table displaying the average hypocotyl length (cm) for each accession, highlighting variation in elongation across seed size categories. (E) Box plot showing the distribution of hypocotyl lengths, with bigger-seeded accessions (blue), smaller-seeded accessions (pink), and Col-0 (gray) plotted separately.

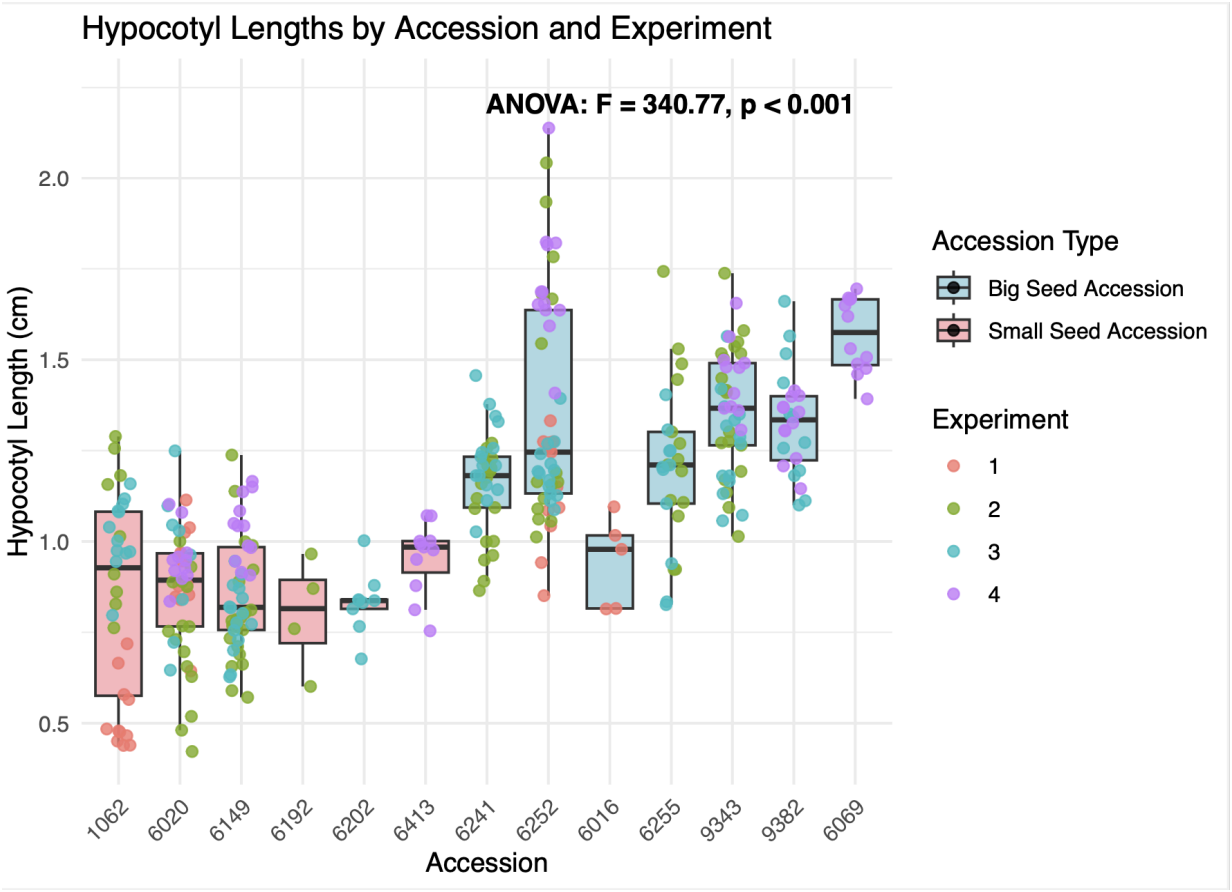


Figure 11: Hypocotyl length distribution across Arabidopsis accessions of varying seed size. Boxplot shows hypocotyl lengths (in cm) for 13 accessions classified as either small-seeded (light pink) or big-seeded (light blue). Each dot represents an individual seedling, color-coded by experiment. ANOVA revealed a significant difference in hypocotyl length between small and big seed accessions ($F = 340.77$, $p < 0.001$), suggesting that seed size contributes to early seedling growth potential under the tested conditions.

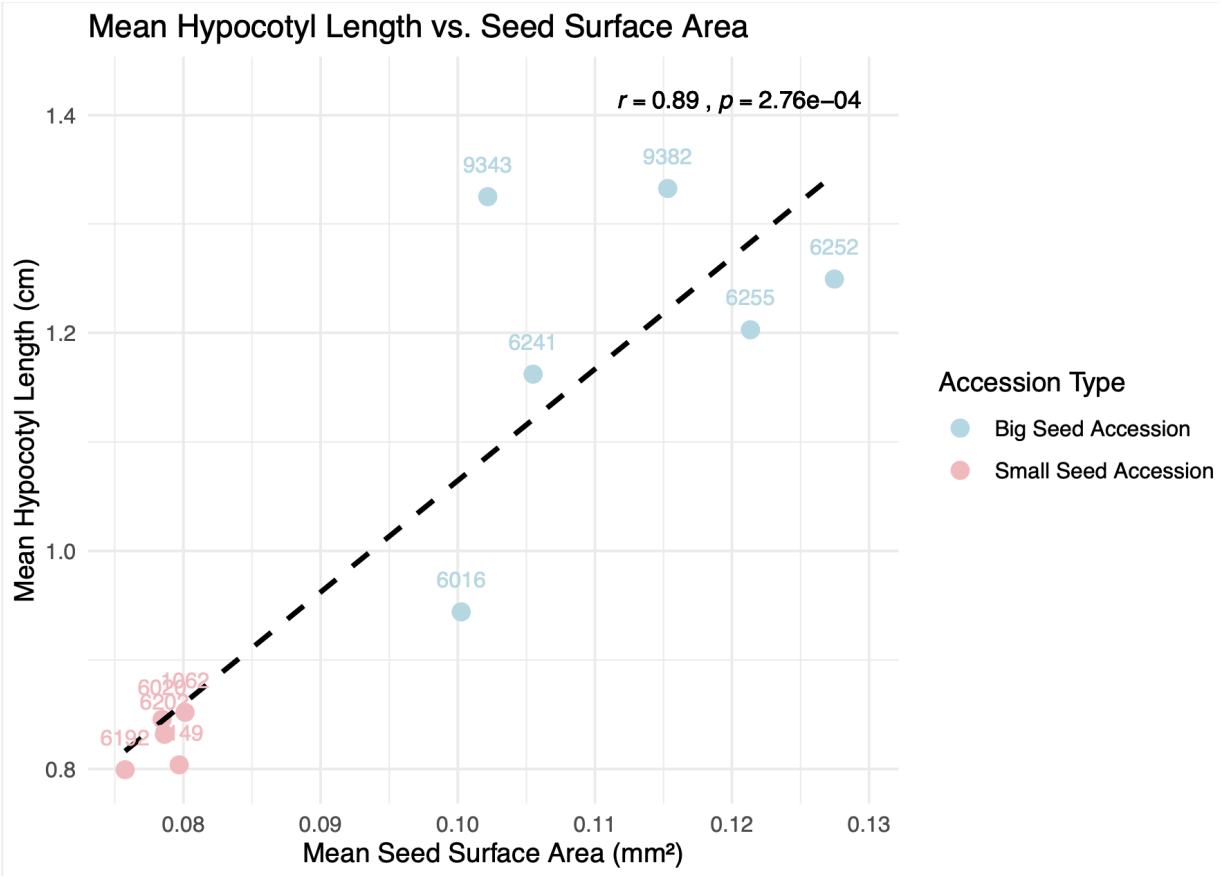


Figure 12: Correlation between seed surface area and mean hypocotyl length across accessions. Each point represents the mean seed surface area (mm²) and mean hypocotyl length (cm) for one accession. Accessions are colored by seed size classification as either small-seeded (light pink) or big-seeded (light blue). A strong positive correlation was observed between seed surface area and hypocotyl length (Pearson's r = 0.89, p = 2.76 × 10^{-4}), suggesting that bigger seeds tend to produce seedlings with longer hypocotyls.

3.3.3 Differences in hypocotyl elongation using size-sorted seeds

For this experiment, a subset of bigger-seeded and smaller-seeded accessions was selected to investigate whether seed size itself influences hypocotyl elongation. The experiment followed the same procedure as the previous hypocotyl elongation assays, with seeds stratified for one week at 4°C, exposed to 3 hours of light at 21°C, and then grown in continuous darkness for 6 days at 21°C. However, unlike the previous experiments, seeds were first sorted using the Boxeed Robot (Labdeers, Czech Republic) to ensure that only seeds of the exact same size were used for all accessions

(Figure 13). This approach allowed for a direct comparison of elongation patterns across accessions while controlling for initial seed size as a variable.

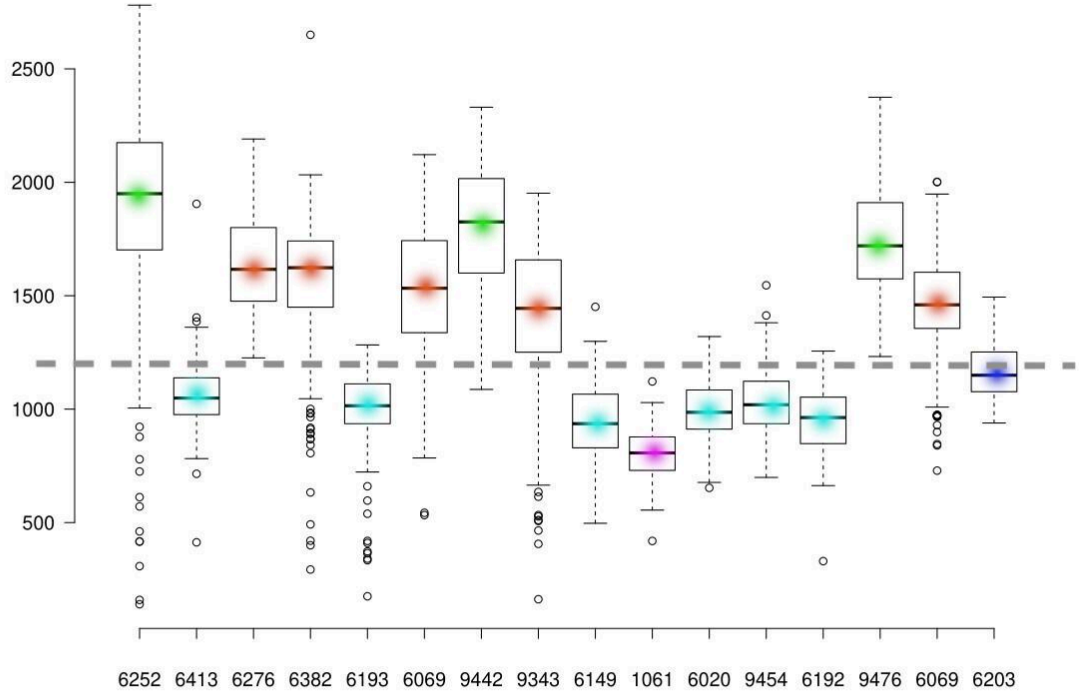


Figure 13: Box plot shows the distribution of seed area (px) for each accession as measured using the Boxeed Robot (Labdeers, Czech Republic). The dashed grey line represents the average seed area (~1200 px) around which seeds were selected for the size-sorted hypocotyl elongation experiment (±100 px).

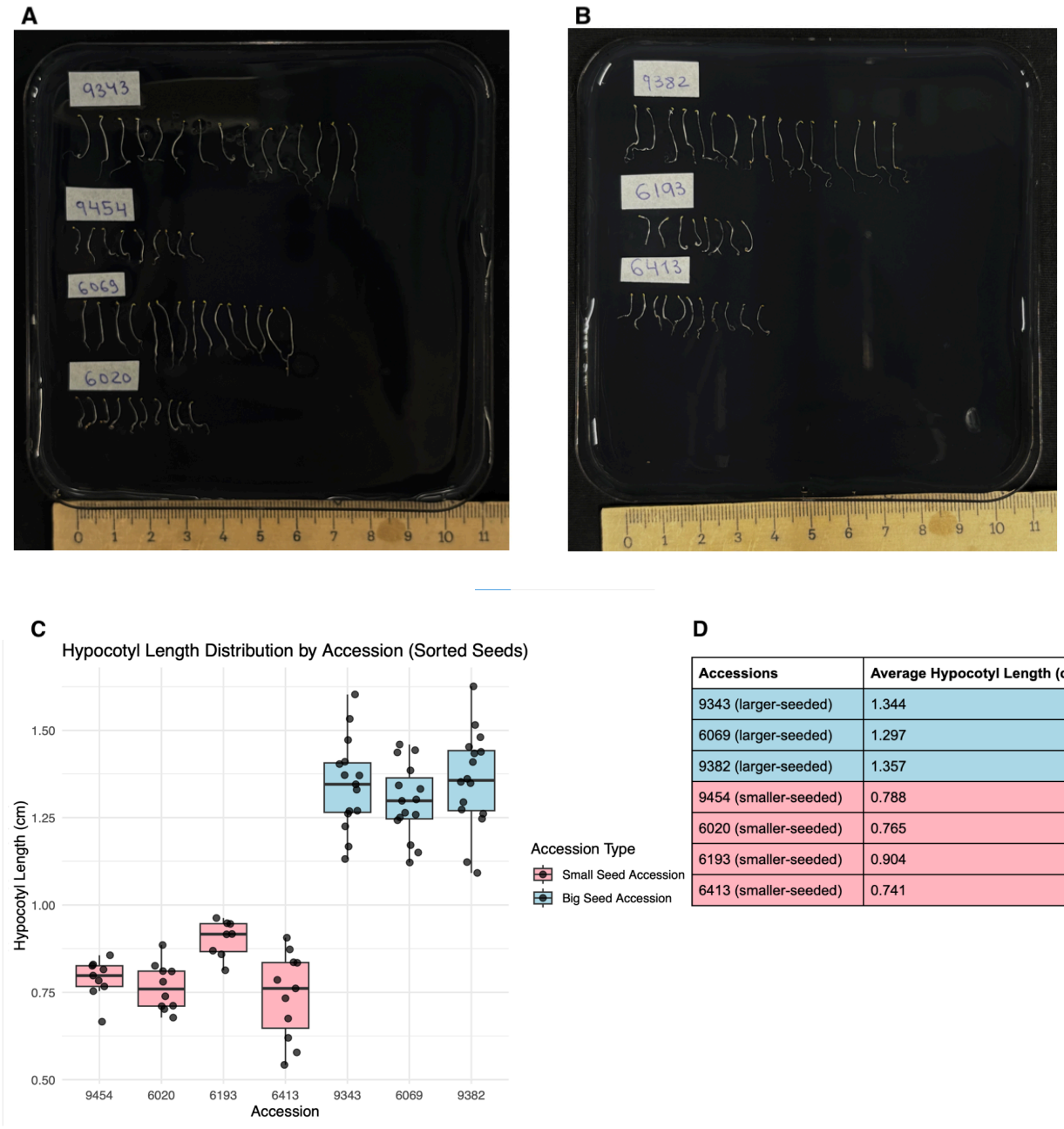
It is important to note that accession 6252, the largest of the bigger-seeded accessions, did not germinate, likely because the seeds of the selected size were too small to be viable. Out of the many seeds sent for sorting, only four seeds of this size were available for testing, which may have contributed to the lack of germination.

The results (Figure 14) show that even when seed size was controlled, bigger-seeded accessions still exhibited consistently longer hypocotyls than smaller-seeded accessions. This suggests that the previously observed trend of bigger-seeded accessions having longer hypocotyls was not solely due to differences in initial seed size.

Among the accessions tested, 9343 and 9382 exhibited the longest hypocotyls, reaching lengths close to 1.4 cm. In contrast, 6020 and 6193 had the shortest hypocotyls, with average lengths below 1 cm.

Since all accessions in this experiment started with seeds of the same size, these results indicate that seed size alone does not fully explain differences in hypocotyl elongation. The fact that bigger-seeded accessions still showed longer hypocotyls despite size control suggests that genetic background or other physiological factors are likely contributing to these differences.

These findings reinforce the idea that while there may be a correlation between seed size and hypocotyl elongation, it is not purely due to seed size itself. Instead, inherent genetic differences among accessions likely drive variation in elongation, with bigger-seeded accessions consistently exhibiting longer hypocotyls regardless of initial seed size.



Accessions	Average Hypocotyl Length (cm)
9343 (larger-seeded)	1.344
6069 (larger-seeded)	1.297
9382 (larger-seeded)	1.357
9454 (smaller-seeded)	0.788
6020 (smaller-seeded)	0.765
6193 (smaller-seeded)	0.904
6413 (smaller-seeded)	0.741

Figure 14: Hypocotyl elongation across same seed-sized *Arabidopsis thaliana* accessions after 6 days in darkness at 21°C. (A, B) Representative images of seedlings from different accessions, with a ruler included for scale. (C) Table displaying the average hypocotyl length (cm) for each accession, highlighting variation in elongation across seed size categories. (D) Box plot showing the distribution of hypocotyl lengths, with bigger-seeded accessions (blue) and smaller-seeded accessions (pink).

3.3.4 Differences in hypocotyl elongation using more size-sorted seeds

Previous data showed that the hypocotyl size correlates with seed size, however, seeds of different accessions sorted at the same size range also resulted in different hypocotyl length among accessions. Therefore, we compared the hypocotyl length from unsorted seeds and sorted seeds of the same accessions.

The results show that sorting seeds by size did not lead to notable differences in hypocotyl elongation within any of the four accessions tested (Figure 15). For both big- and small-seeded accessions, seedlings from sorted and unsorted seed populations showed similar elongation patterns as before.

These results suggest that the variation in hypocotyl length observed between accessions is not primarily driven by within-accession variation in seed size. Instead, the consistent elongation trends across both sorted and unsorted groups may imply that factors such as genetic background between accessions play a larger role. In this context, seed sorting did not substantially alter hypocotyl growth outcomes, reinforcing the idea that inter-accession differences, rather than intra-accession seed size variation, may account for observed elongation differences.

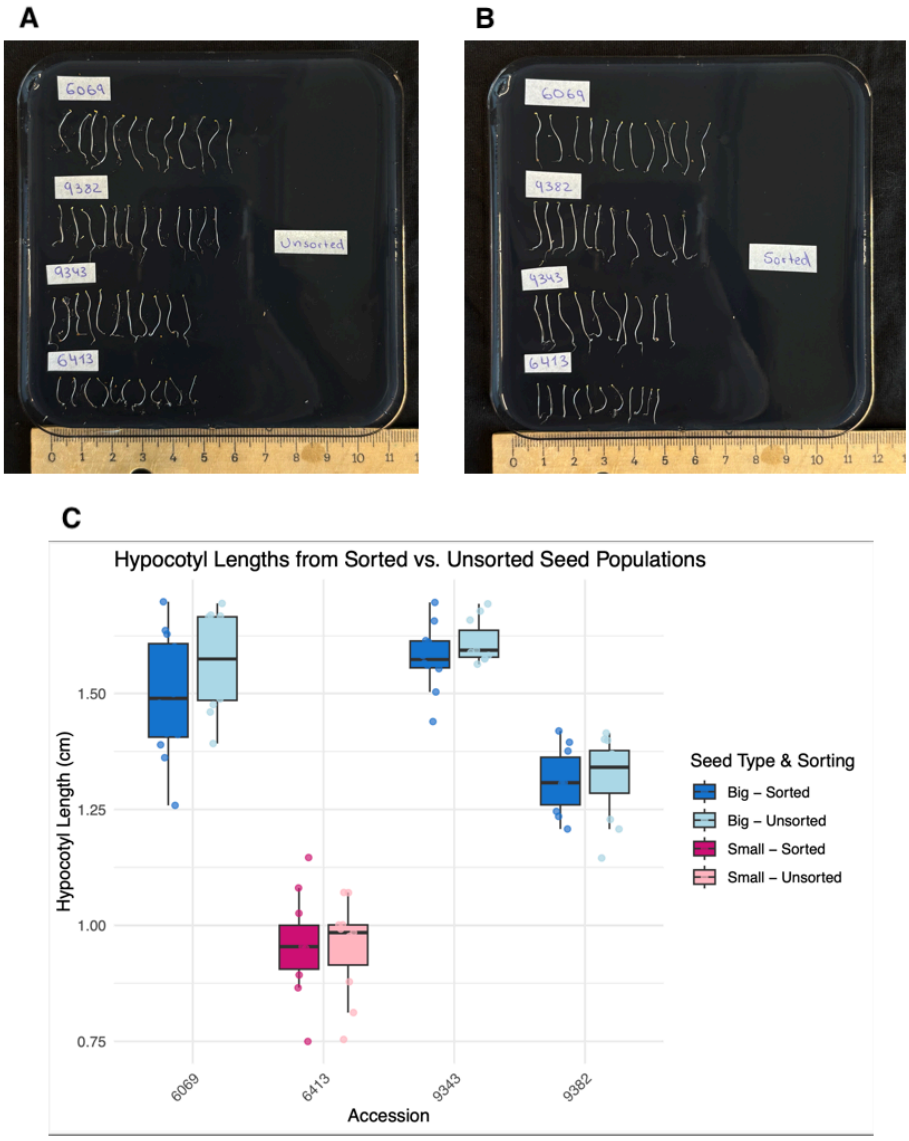


Figure 15: Effect of seed size sorting on hypocotyl elongation in four *A. thaliana* accessions.

(A–B) Representative images of seedlings from different accessions, with a ruler included for scale. Panel A shows seedlings grown from unsorted seed populations, while panel B shows seedlings grown from seeds that were pre-sorted by size using the Boxeed robot. Accessions 6069, 9343, and 9382 are classified as big seed accessions, and 6413 is classified as a small seed accession. (C) Quantification of hypocotyl lengths (cm) from sorted and unsorted seed populations. Boxplots represent the distribution of hypocotyl lengths for each accession and treatment. Unpaired two-sample t-tests showed no significant differences between sorted and unsorted seed populations in any accession: 6069 (p = 0.138), 6413 (p = 0.937), 9343 (p = 0.228), and 9382 (p = 0.873), indicating that seed sorting had no major effect on hypocotyl elongation outcomes.

4. Discussion

This thesis explored how natural variation in seed size among Swedish *Arabidopsis thaliana* accessions relates to seed germination and early seedling traits, with a particular focus on germination behavior and hypocotyl elongation. While germination responses to temperature and light were tested across accessions, no consistent pattern clearly separated big- and small-seeded accessions. Germination was generally highest at 15°C and 21°C, while no accession reached full germination at 25°C, suggesting that excessively high temperatures may inhibit germination. Similarly, although most accessions showed reduced germination in darkness compared to light, a few accessions germinated well in both conditions, indicating that light sensitivity may vary independently of seed size.

In contrast, the hypocotyl elongation assays revealed a clearer pattern: accessions classified as large-seeded consistently produced longer hypocotyls than small-seeded ones. This trend persisted even when seed size was controlled by sorting, indicating that elongation potential is not solely a function of initial seed mass but is likely influenced by underlying genetic differences. These results show that seed size may signal developmental strategies encoded at the genomic level. Measuring hypocotyl elongation under nutrient-free conditions provided a robust, resource-independent readout of early seedling vigor, making it a valuable trait for understanding early-stage performance and potential fitness advantages.

Future work could build on these findings by further investigating the genetic basis of seed size and its downstream developmental effects. Candidate gene expression profiling could show whether certain alleles contribute to enhanced elongation. Additionally, expanding phenotyping to include a root vs. shoot trade-off assay would help determine how different accessions allocate early resources. Measuring root length and shoot width or mass in controlled conditions could reveal whether bigger seeds prioritize shoot growth over root development or balance both.

Appendix

Accession	Mean Surface (mm²)	Median Surface (mm²)	Classification
9454	0.0668	0.0661	Smaller-seeded
6188	0.0753	0.0752	Smaller-seeded
6192	0.0758	0.0757	Smaller-seeded
6020	0.0785	0.0811	Smaller-seeded
6202	0.0786	0.0788	Smaller-seeded
6149	0.0797	0.0819	Smaller-seeded
1062	0.0801	0.0798	Smaller-seeded
6193	0.0850	0.0844	Smaller-seeded
6413	0.0933	0.0929	Smaller-seeded
6016	0.1003	0.1011	Bigger-seeded
9343	0.1022	0.1030	Bigger-seeded
6241	0.1055	0.1048	Bigger-seeded
9382	0.1153	0.1181	Bigger-seeded
6255	0.1214	0.1235	Bigger-seeded
6069	0.1217	0.1227	Bigger-seeded
6252	0.1275	0.1293	Bigger-seeded

Table 1: Seed size classification of *Arabidopsis thaliana* accessions based on average seed surface area. Seed surface area (mm²) was measured using the Boxeed platform. The table lists the mean and median seed surface area for each accession, with accessions classified as "bigger-seeded" or "smaller-seeded". This classification was used throughout the thesis to group accessions in germination and hypocotyl elongation assays.

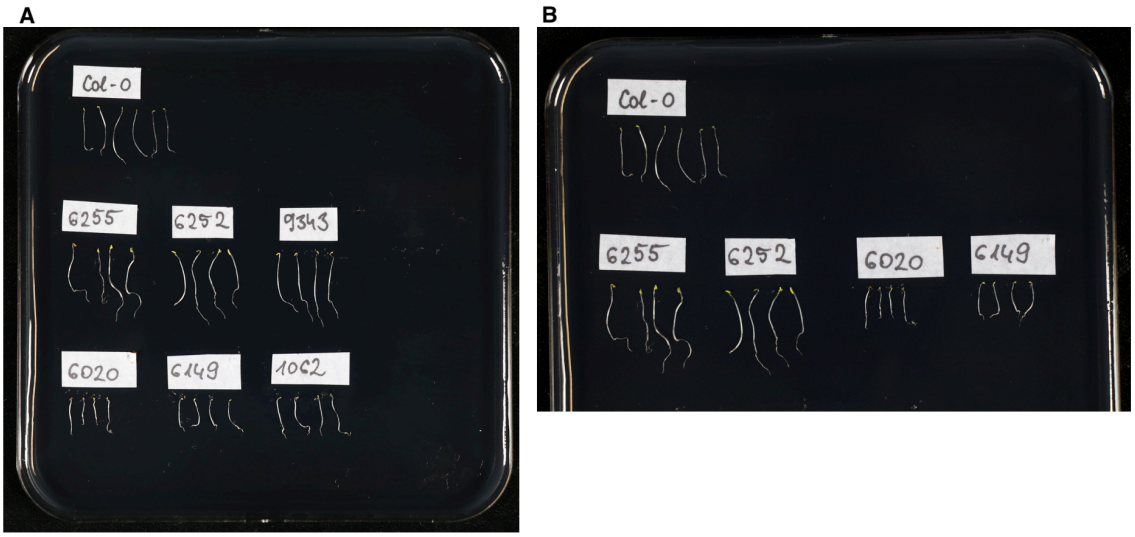


Figure 16: Reorganized seedlings for visual comparison of hypocotyl elongation in bigger- and smaller-seeded *Arabidopsis thaliana* accessions. (A, B) Seedlings were rearranged on plates for direct visual comparison of hypocotyl length between bigger-seeded accessions (6255, 6252, 9343) and smaller-seeded accessions (6020, 6149, 1062). Col-0 is included as a reference. Differences in elongation among accessions can be observed under identical growth conditions.

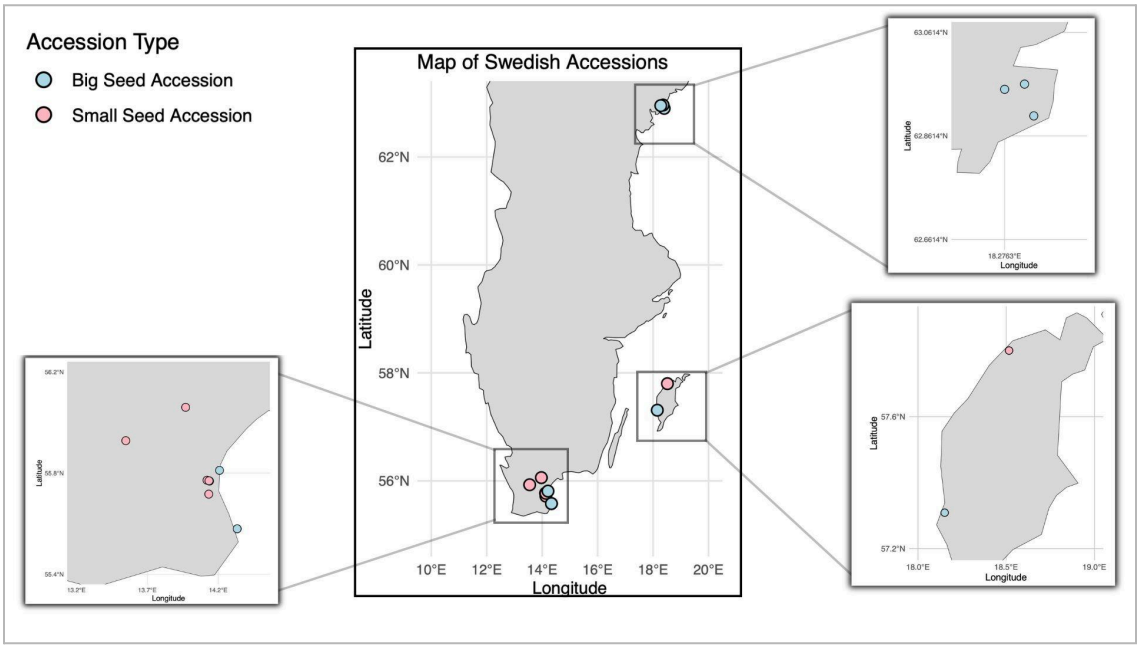


Figure 17: Geographic distribution of Swedish *Arabidopsis thaliana* accessions used in this thesis. Map showing the collection locations of both bigger-seeded (blue) and smaller-seeded (pink) accessions across Sweden. Insets highlight specific regions with multiple accessions to provide a clearer view.

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