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Variation in *Theobroma cacao* populations in biomass growth and *Phytophthora spp*. resistance in relation to mycorrhizal inoculation

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Abstract

Cocoa (*Theobroma cacao*) is an economically important crop in many tropical developing regions, but production is generally below potential, due to low nutrient availability, lack of improved cocoa varieties, low adoption of production technologies, and large yield losses due to pests and disease. Arbuscular Mycorrhizal Fungi (AMF) provide a potential aid for improving cocoa production, among others providing the plant with nutrients and induced disease resistance. In recent years, plant breeding efforts have focused on finding a way to breed for improved plant-AMF interaction. The current project provides pioneering work to gain more knowledge on the possibilities to improve this interaction between cocoa and AMF with a plant breeding approach. To this end, progenies of selfings of three highly heterozygous cocoa clones were tested for growth performance, root characteristics and *Phytophthora capsici* resistance when inoculated with four different AMF-containing soils. Significant differences in growth performance and root characteristics were found between seedlings grown with different AMF inocula, but little difference was observed between the different progenies. Within-progeny variation was substantial, but it was unclear how much of this variation had a genetic basis. Root characteristics could not be correlated to aboveground growth performance. In the future, root colonization data will have to provide a more complete picture of the nature of the effect of AMF on the seedling performance. Moreover, another greenhouse experiment with cuttings of the progenies will provide more insight into the extent of the environmental and genetic variation, underlying variation in seedling performance. Lastly, the *Phytophthora* bioassays are in need of improvement before they can put to use to determine resistance of cocoa against *P. capsici*.

Preface

As a second-year Master student in Plant Sciences (Spec: Plant breeding) and Organic Agriculture (Spec: Agroecology), I am driven by an interest in the role of plant breeding in supporting a more sustainable agricultural system. That is what drew me to this project, which is laying the groundwork for breeding for improved plant-AMF interaction for more sustainable cocoa production. I had the honour of partaking in a project set up by Natalie Ferro Lozano in the beautiful Republic of Panama, at the Smithsonian Tropical Research Institute, based in Gamboa. The project turned out to be much more challenging than I expected beforehand, demanding me to be flexible and push my ways of thinking. After many struggles and unforseen circumstances, causing some delay in the process, I can finally say I can now deliver a piece of work that I am proud to put my name under.

List of Abbreviations

AMF – Arbuscular Mycorrhizal Fungi
CF – Cortex Fraction
HSD – Honest Significant Differences
IDIAP – Institute of Agricultural Research Panama
LDW – Leaf Dry Weight
MANOVA – Multivariate ANOVA
RCBD – Randomized Complete Block Design
RD – Root Diameter
RDW – Root Dry Weight
RTD – Root Tissue Density
SDW – Stem Dry Weight
SLA – Surface Leaf Area
sRDW – scanned Root Dry Weight
SRL – Specific Root Length
TDW – Total Dry Weight

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1 | Introduction

Cocoa (*Theobroma cacao*, 2n=2x=20) is a tree crop that is cultivated for its seeds, which are processed into various edible products. It has a generation time of three to five years. Although cocoa is self-compatible, it is typically allogamous, and suffers from inbreeding depression. Furthermore, seeds cannot be stored. These characteristics make breeding for improved cocoa varieties a challenging endeavour (Bekele & Phillips-Mora, 2019).

Originating from Latin American regions around the equator (Cuatrecasas, 1964), cocoa is best cultivated in tropical regions. Consequently, it is an economically important crop for many countries in South-east Asia, Sub-Saharan Africa, and Latin America. Cocoa is an especially important crop for smallholder farmers, which produce approximately 80 to 90% of all cocoa worldwide (World Cocoa Foundation, 2014). Being mainly grown by smallholders, cocoa yields are generally far below potential due to low nutrient availability, lack of improved varieties and low adoption of production technologies (Aneani & Ofori-Frimpong, 2013). Moreover, diseases like black pod disease – caused by *Phytophthora spp.* – can cause 30% yield losses in cocoa without pest and disease management (Adejumo, 2005; Helliwell et al., 2016; Nyassé et al., 1995). It is therefore important to find ways to ensure high productivity, and to reduce yield losses due to pests and diseases. At the same time, cocoa should be produced with a minimal amount of biocides and mineral fertilizers to prevent environmental pollution, and to ensure Fairtrade premium prices for farmers (Grizzetti et al., 2020; Sutton et al., 2013).

One potential aid for sustainably improving crop production is the utilization of arbuscular mycorrhizal fungi (AMF) (Basu et al., 2018). AMF are soil-borne fungi that can engage in symbiosis with many plant species. In return for carbohydrates and lipids (Bago et al., 2003), AMF facilitate (1) P uptake (2) enhanced growth of other beneficial soil microbiota, (3) direct competition with pathogens for infection sites and carbon, (4) induction of plant morphological changes, (5) abiotic stress reduction, and (6) induced disease resistance (Cortes et al., 2021; Linderman, 1994; Mosse, 1973; Tchameni et al., 2012; Vierheilig et al., 2008). AMF thereby support plant growth and resistance against pests and diseases. In cocoa, AMF were indeed shown to increase biomass production, plant height, stem diameter, leaf number, phosphorous content, and bean yield (Aggangan et al., 2019; Chulan Hashim & Ragu, 1986; Tuesta-Pinedo et al., 2017; Weisany et al., 2015). Moreover, AMF improve resistance against various cocoa pathogens, including *Phytophthora palmivora*, *P. megakarya* and *P. capsici* – the causal agents of black pod disease (Herre et al., 2007; Tchameni et al., 2011). It is therefore important to consider the cocoa-AMF interaction both in cocoa cultivation and variety improvement.

The extent to which AMF can successfully interact with the host plant is determined by biotic (e.g., genetics and physiology of the plant and AMF) (Bergmann et al., 2020; Koch et al., 2006) and abiotic factors (e.g., available P, soil pH, soil texture) (Alguacil et al., 2016; Amijee et al., 1993). According to Bergmann et al. (2020), an important plant physiological characteristic is the cortex fraction (CF) – the fraction of the root diameter that contains the cortex – which is closely related to the root

tissue density (RTD) and root diameter (RD). In general, plant species with a large CF and RD are more likely to outsource P acquisition to AMF. Moreover, since AMF reside in the root cortex, a large CF may be beneficial for the AMF-plant interaction (Bergmann et al., 2020). Another relevant root characteristic that links to AMF root colonization is the specific root length (SRL) – the root length per unit mass. Species with high SRL have a more elaborate and fibrous root system, enabling them to explore the soil for nutrients. Species with low SRL lack this ability and are therefore likely more dependent on nutrient acquisition by AMF (Bergmann et al., 2020). Azcón and Ocampo (1981) showed that mycorrhizal dependency differed for wheat cultivars with varying root traits and root:shoot ratio. However, little is known about the effect intraspecies variation in root characteristics on the mycorrhizal interaction in other species.

It is important to note that a successful interaction is not always beneficial for the host plant. In fact, costs of the symbiosis can in some cases exceed the benefits for the plant, hindering plant growth. Thereby, the symbiosis can be of parasitic, rather than mutualistic nature (Johnson et al., 1997; Klironomos, 2003). The cost-benefit ratio for the plant is partly determined by environmental factors (e.g., nutrient availability or irradiation) (Jacott et al., 2017), but can also be influenced by the ability of the plant to control the AMF colonization to maximize benefits and minimize costs (Johnson et al., 1997).

The dependence of plants on AMF and their ability to maximize benefits from AMF have a genetic basis (Bergmann et al., 2020; Hohmann & Messmer, 2017; Jacott et al., 2017; Johnson et al., 1997; Lehnert et al., 2017). Therefore, we can potentially breed for an improved interaction between plants and AMF. There is an ongoing debate on whether improving mycorrhizal interaction is a viable breeding goal, with opponents for instance pointing at the problematic nature of using mycorrhizal response indicators (Galván et al., 2011). Despite opposing views, in recent years, breeding efforts have increasingly focused on traits that facilitate improved partnership between the crop and beneficial microbes, like AMF, to support more sustainable agricultural practices (Cobb et al., 2021). Currently, research in this area has focused on crops like wheat, maize, various grain legumes, and cotton (Cobb et al., 2021; Duc et al., 2015; Foyer et al., 2018; Galván et al., 2011; Gruet et al., 2022; Li et al., 2021). However, no records were found of such efforts in cocoa breeding. Therefore, the current project serves as pioneering work towards gaining more knowledge in this field.

Li et al. (2021) highlighted the importance of the genetic diversity for improving plant-microbe partnerships. The progenies used in this study were derived from selfing three cocoa accessions grown in Bocas del Toro, Panama. Since cocoa originates from parts of Central and South America, including Panama (Cuatrecasas, 1964), these selfed progenies likely provide ample genetic diversity. We therefore hypothesized to find segregation within and between selfed progenies in terms of growth performance and black pod disease resistance. To test this, various above- and belowground growth characteristics were measured for sub-populations of the selfed progenies. These sub-populations were also tested for *Phytophthora* resistance.

Variation in AMF communities was shown to influence plant performance differently in different plant species (Klironomos, 2003; Koch et al., 2006). Since the four AMF communities in this study were taken from soils with different properties, the AMF species composition is expected to vary substantially between these communities (Alguacil et al., 2016). We therefore hypothesized to find differences between progenies in growth and resistance responses induced by different AMF communities. To test this hypothesis, the growth performance and *Phytophthora* resistance of selfed progenies were measured in the presence of different AMF communities.

2 | Materials and Methods

2.1 Plant material and growth conditions

Three wild cocoa clones from the experimental gardens of IDIAP in the province of Bocas del Toro were selected based on growth characteristics and disease resistance by IDIAP Breeders (Engr. Abiel Gutiérrez). Flowers of each clone were self-fertilized (November 15th, 2021), resulting in clone-derived selfed progenies AS-CP 26-59, AS-CP 26-60, and AS-CP 26-61 (hereafter respectively: Progenies 59, 60, 61). Since cocoa is predominantly allogamous, the selfed clones are likely highly heterozygous, meaning that the progenies resulting from selfing are segregating.

For the AMF treatments, we used two AMF-containing soils from plantations in Bocas del Toro (B4, B6), two AMF-containing soils from plantations in Herrera (H3, H7), and a non-mycorrhizal microbial wash as negative control (NC). The soil inocula were taken from the upper 20 cm of soil around a cocoa tree. To ensure much variation between AMF-inocula, soils were selected from plantations that differed most in pH and soil texture (Appendix Q), as these characteristics are the main factors determining mycorrhizal community composition (Alguacil et al., 2016; Jansa et al., 2014). A microbial wash of the four soils was used to minimize the differences between the mycorrhizal and non-mycorrhizal treatments (Wang et al., 2018). The microbial wash was made by separately soaking 250 mL of the four mycorrhizal soils in 2 L of filtered water for two hours. The solutions were then filtered by two-layer coffee filter paper and a 50 µm sieve. The resulting filtrates were then mixed to obtain a microbial wash of all soils combined. As mycorrhizal spores are generally larger than 50 µm, we assumed that nearly all spores were filtered out in this way.

Five months after fertilizing the cocoa flowers (April 15th, 2022), the resulting cocoa pods were harvested. Seeds were immediately prepared for germination by removing the mucilaginous layer with sterile sawdust. Seeds were then left to germinate for 21 days in sterilized substrate composed of one part river sand and two parts soil rich in organic matter. The substrate was sterilized by steaming at 80°C for two hours before cooling down overnight, turning it over and steaming again at 80°C for two hours. Plastic pots (h: 20 cm; ⌀: 22 cm) were filled with 4.5 L of this sterilized substrate. The next day, 500 mL of mycorrhizal soil was added on top of the sterile substrate in all pots for the AMF treatments. Then, germinated seeds were individually sown in the pots. All pots, including those inoculated with AMF, were then watered with 500 mL of tap water and 100 mL of microbial wash. To assure the establishment and prevent washing out of the AMF and other microbes, pots were left without watering for the next ten days.

Seedlings were grown on tables in a greenhouse at the Smithsonian Tropical Research Institute in Gamboa (9°07'10.8"N 79°42'06.0"W). The experiment was conducted with a randomized complete block design (RCBD), where the twenty-five tables represent the blocks (Appendix A). In every block, one of each selfed progeny-AMF combinations was present, adding up to fifteen plants per block, and twenty-five plants per progeny-AMF combination. The twenty-five plants were chosen to have a

large enough population to reveal genetic or phenotypic segregation, yet a small enough population to make the experiment workable. This enabled us to analyse average performance of selfed progenies with their respective AMF inoculum. Individual plants were randomly allocated to a position within the block, and the plants were randomly rearranged within the block every four weeks.

2.2 Seedling growth and root characterization

Stem height and leaf number were determined for every seedling, every week from the moment the seedlings lost their cotyledons until the seedlings were six months old. Only leaves that had fully expanded and at least started to lignify (stage D or E; Appendix B) were taken into account for the leaf count. Additionally, we recorded the number of leaves that had fallen off every week.

After six months, the seedlings were harvested by separating the roots, leaves and stems. Leaves of individual seedlings were photographed to analyse surface leaf area (SLA) using ImageJ (Appendix C). Then, leaves and stems were dried separately for three days at 60 degrees C, before weighing to determine the dry weight of leaves (LDW) and stems (SDW).

Roots were thoroughly washed to remove all soil. Then, lateral and side roots were separated from the main root (Appendix C). A subsample of side roots was wrapped in wet paper towel and stored at 5°C, until further use for root trait analysis. Within every treatment, we selected the five plants with the highest aboveground biomass and the five with the lowest for root architecture analysis. Roots from these plants were submerged in a shallow layer of water in a transparent tray and scanned (Epson Perfection V850 Pro) (Appendix C). Then, scanned roots and the rest of the roots were dried separately for three days at 60°C and subsequently weighed to determine the root dry weight of scanned roots (sRDW) and the total root dry weight (RDW). Root scans were analysed using WinRhizo software to determine the root diameter (D), specific root length (SRL), and root tissue density (RTD). WinRhizo automatically generated the root length (RL) and root diameter (RD). SRL was calculated as follows:

$$SRL = \frac{RL}{sRDW} \tag{1}$$

And RTD was calculated from the SRL as follows (Bergmann et al., 2020):

$$RTD = \frac{4}{\pi \times RD^2 \times SRL} \tag{2}$$

2.3 *Phytophthora* strains and propagation

Three *Phytophthora capsici* isolates (Lcm-1088, -1092, -1093) were taken from infected cocoa plants in Bocas del Toro, Panama. These isolates were grown on sterile V8 growth medium (20% V8 juice (v/v), 0.3% CaCO3 (w/v), 1.8% agar (w/v), 5 ppm rifampicin (w/v)). As a side effect of growing on artificial medium, *Phytophthora spp.* tends to lose its virulence after repeated subculturing (Bharath et al., 1999). Therefore, after every tenth subculture, the pathogens were passed through a susceptible host, to retain the original pathogenicity level.

2.4 Detached leaf bioassays

To determine the effects of the selfed progeny and AMF inoculum on black pod disease resistance, detached leaf bioassays were performed, as these bioassays were shown to be a reliable proxy for actual Phytophthora resistance (Iwaro et al., 1997; Nyassé et al., 1995). Prior to the bioassays, the virulence of the *Phytophthora* isolates was assessed to establish which isolate was most suitable. To this end, detached leaf bioassays were performed on leaves of a small number of seedlings of the

studied progenies that would not be used for further characterization.

The zoospore solution used for the bioassay was obtained as explained by Bharath et al. (1999). Sterile petri dishes (⌀: 90 mm) were filled with 50 mL of the previously described V8 growth medium and left to set. Then an agar plug with active *P. capsici* mycelium – i.e., from the edge of a growing culture – were placed in the middle of the growth medium and incubated for fifteen days at 25°C in the dark. After incubation, the colony was flooded with 15 mL of pre-cooled, sterile, distilled water before incubating for 30 minutes at 5°C and then for 30 minutes at 25°C. The spores were then counted in a hemacytometer to determine the concentration, after which the spores were diluted to a concentration of approximately 1×10^6 spores mL^{-1}.

Following Mejía et al. (2012), stage C leaves (Appendix B) were used to perform the bioassays, since these are unlignified and can be readily infected by *Phytophthora*. One or two leaves of every plant were cut off, and both ends were removed to fit the middle part of the leaf inside a petri dish (⌀: 140 mm). The middle part of the leaf was put into the petri dish on a wet filter paper, to ensure high humidity. Leaves were then inoculated on one side of the middle nerve with three 10 µL drops of *Phytophthora* zoospore solution. On the other side of the nerve, we placed three drops of sterile water as a negative control (Mejía et al., 2012). Petri dishes were then closed, sealed, and left for incubation in a growth chamber for three days at 25°C and with a 12:12 hour light-dark cycle. Afterwards, inoculated leaves were photographed, and lesion diameter was measured using ImageJ software (imagej.nih.gov).

2.5 Statistical analysis

The experimental units were the individual seedlings. The twenty-five seedlings used in all progeny-AMF combinations were considered as a random subpopulation of the selfed progenies. Therefore, we assumed that their average performance is equal to the population average. Consequently, comparing these averages allowed us to draw conclusions on the effects of parental clone and AMF inoculum on plant performance. All data analyses were performed with R in RStudio (r-project.org).

During the experiment, it was observed that the table on which a seedling was grown had a substantial influence on growth performance – i.e. there was a substantial blocking effect. To be able to get an idea of the extent of this blocking effect, tables were divided into three categories (low, moderate and high performance) based on average total dry weight (TDW) on the given table. Variation in TDW was then visualized in violin plots, separated by progeny-AMF combinations and table performance category.

To check the validity of height increment (HI) and leaf number increment (LNI) as proxies for seedling performance, correlation analyses were performed between HI and LNI versus TDW, SDW, LDW, RDW and SLA. Pairwise correlation plots were produced and Pearson's correlation coefficient (r) were calculated using the R-package GGally. This was done for all data combined and separated by AMF treatment. Likewise, the correlation between root characteristics and aboveground growth performance was analysed with a correlation analysis between RD, RTD and SRL versus TDW, SDW, LDW, RDW and SLA.

Differences in HI and LNI increase over the entire growth period were analysed between progeny-AMF combinations. To this end, a type III two-way ANOVA was performed with the HI as response variable and progeny, AMF inoculum and table as the independent variables. A type II two-way ANOVA was performed for LNI with the above-mentioned independent variables. Type II and III ANOVAs and Tukey-Kramer's HSD test were chosen because of the unbalanced data set.

To get a more detailed image of the course of the height increment over time, the height increment in four four-week

intervals was calculated for all individuals. These were first analysed with a type III three-way mixed-measures ANOVA with the height increment as response variable, progeny and AMF inoculum as independent between-subject variables and the time as independent within-subject variable. Then, type III two-way ANOVAs were performed on all separate time intervals with height increment as dependent variable and progeny, AMF inoculum and table as independent variables. Subsequently, pairwise comparisons were performed by means of Tukey-Kramer's Honest Significant Differences (HSD) test ($\alpha = 0.05$).

Response variables TDW, SDW, LDW, RDW and SLA were log-transformed and analysed with a multivariate type II two-way ANOVA (MANOVA), followed by separate univariate type II two-way ANOVAs with progeny, AMF and table as independent variables. Univariate ANOVAs were followed by Tukey-Kramer's HSD test ($\alpha = 0.05$) on the main effects of progeny and AMF inoculum. Variation in performance within progenies was analysed visually by means of violin plots and variation coefficients. This allowed the comparison of distributions of plant performance between the progenies. The same process was repeated for RD, RTD and SRL, albeit without transforming the data, and using type I two-way ANOVA and Tukey's HSD test, because of the balanced data set.

3 | Results

3.1 Growth of non-AMF seedlings

In general, the non-AMF seedlings (NC) grew as tall as the AMF seedlings, but had thinner stems (Appendix E). Moreover, NC seedling leaves often showed yellow and brown spots, and were remarkably smaller than the leaves of AMF seedlings (Appendix D). Overall, this gave the NC seedlings an unhealthy appearance. Moreover, it was observed that after twenty weeks post germination (wpg), NC seedlings started losing many leaves. By 22 wpg, NC seedlings were on average losing more leaves than new leaves were produced, shown by a decline in the leaf number (Appendix E). These observations were true across all three selfed progenies and tables. The growth deficiency of NC seedlings was also reflected in the total dry weight at the end of the experiment, which was much lower for NC seedlings than for AMF seeldings (Appendix E).

These observations showed that cocoa seedlings have many growth abnormalities when grown in the absence of AMF. Moreover, in practice, cocoa is never grown in the absence of AMF, meaning that the NC treatment has no practical relevance. Including the NC seedlings in the analyses, would therefore likely introduce unwanted and unnecessary variation in the data, possibly leading to wrong conclusions. The NC treatment was therefore excluded from all further analyses.

3.2 Distribution in the greenhouse

Throughout the greenhouse, there seemed to be a gradient of biomass production. Especially on the tables in the north-west corner (Appendix F), seedlings produced substantially more biomass than in other parts of the greenhouse. This was possibly caused by a hole in the shading cloth covering the greenhouse, allowing more sunlight to reach these tables.

Upon dividing the tables into groups with low, moderate and high performance, it becomes clear that individual seedlings of a specific progeny-AMF combination that grow on tables with lower performance do not necessarily perform worse than those grown on higher performance tables (fig. 1). This indicates that variation in seedling performance caused by the progeny-AMF combination exceeds the variation caused by the table.

3.3 Correlations between seedling growth performance measurements

Correlation analyses showed that stem, leaf, root and total dry weight (respectively SDW, LDW, RDW and TDW) and surface leaf area (SLA) are all positively correlated to one another (fig. 2). However, when doing the correlation analyses separately for all AMF treatments, the correlation between root DW and leaf DW weakens or vanishes. The same holds for the correlation between SLA versus SDW and LDW (Appendix G).

Visual assessment of the seedlings throughout the experiment indicated that seedling height increment (HI) was not indicative of seedling vigour. For example, seedlings with large HI did not necessarily have thicker stems or more or larger leaves (Appendix D). However, as can be seen in the correlation plots (fig. 2), a weak, but significant correlation was present between

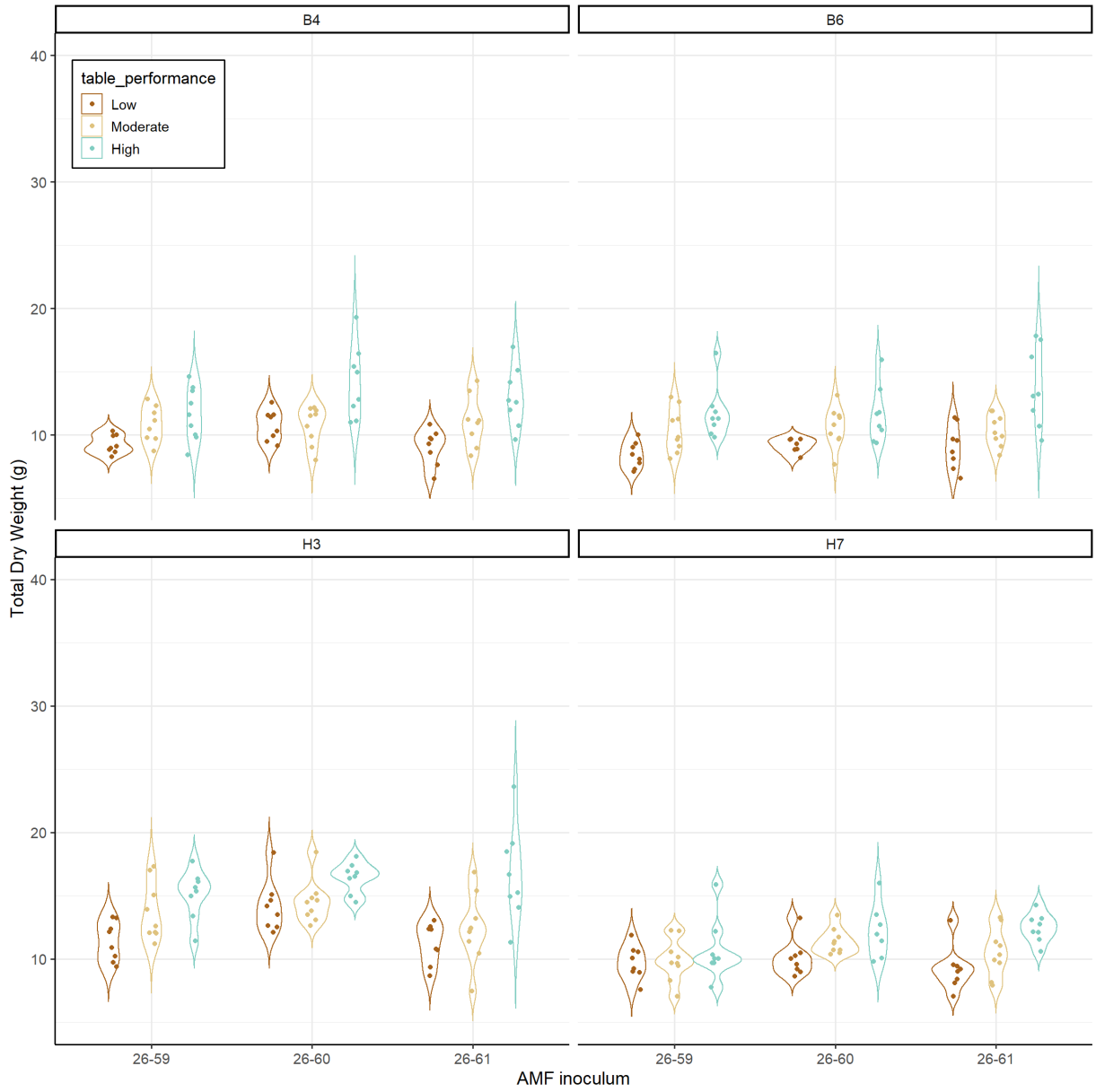


Figure 1: Violin plots showing the variation in total dry weight production for low-, moderate- and high-performance tables, facetted AMF inoculum and progeny (x-axis). Every point within the violins represents an individual seedling.

HI and TDW, SDW, LDW and SLA (Pearson's r: 0.338, 0.369, 0.257 and 0.203, respectively). HI is also positively correlated with TDW and SDW for all AMF treatments (Appendix G).

Leaf number (LN) is positively correlated to TDW, SDW, LDW, RDW and SLA when doing the analysis for all data combined (fig. 2). However, upon separating the analysis by AMF treatments, LN only showed a correlation with SLA for AMF treatment B6, H3 and H7, while all other correlations vanished (Appendix G). This lack of correlation indicates that the correlations observed between LN and the other growth parameters in the overall data merely reflects the effect that AMF have on seedling growth. Closer assessment of the correlation plots indeed shows that AMF treatments with higher LN also have higher TDW, SDW, LDW and RDW, but within the AMF treatments, these correlations are weak (Appendix H).

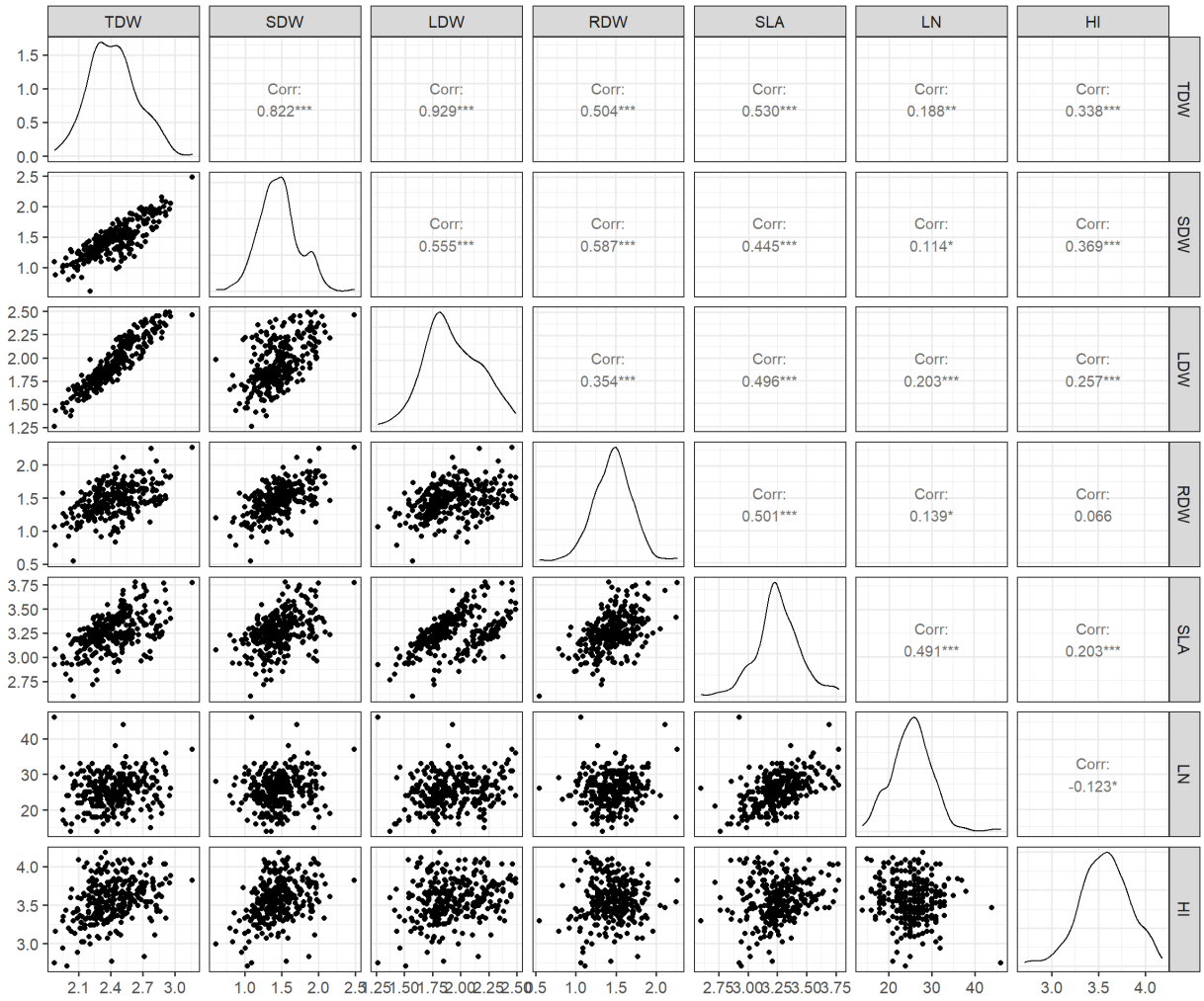


Figure 2: Dot plots (bottom-left of matrices) showing correlations between measured growth traits for all individual seedlings. Labels on top indicate the parameter represented by the x-axes in the columns. Labels on the right indicate the parameter represented by the y-axes in the rows. Values of all parameters, except leaf number (LN) were log-transformed. Line graphs on the diagonal axis of the matrix indicate the distribution of values for its corresponding parameter. Values in the top-right half of the matrices indicate Pearson's r (Corr) and the asterisks behind the values indicate significance levels of the correlation; $p < 0.05$ = *; $p < 0.01$ = **; $p < 0.001$ = ***.

3.4 Effects of AMF and progeny on stem height increment

The two-way ANOVA of the effect of progeny, AMF and their interaction indicated that there is a strong interaction effect ($p < 0.001$) (Appendix I). Height increment of seedlings from progeny 60 was significantly higher than both other progenies with any of the AMF inocula. No significant differences were observed between progenies 59 and 61 for height increment for any of the four AMF inocula (figure 3a).

When looking at the effect of AMF inocula within progenies, some differences are observed. AMF inoculum H3 causes a larger height increment than inocula H7, B4 and B6 in progenies 59 and 61. In contrast, seedlings from progeny 60 inoculated with H3 have significantly smaller height increment than those inoculated with H7. These contrasting results between progenies illustrate an interaction between the progeny and AMF inoculum, represented by the

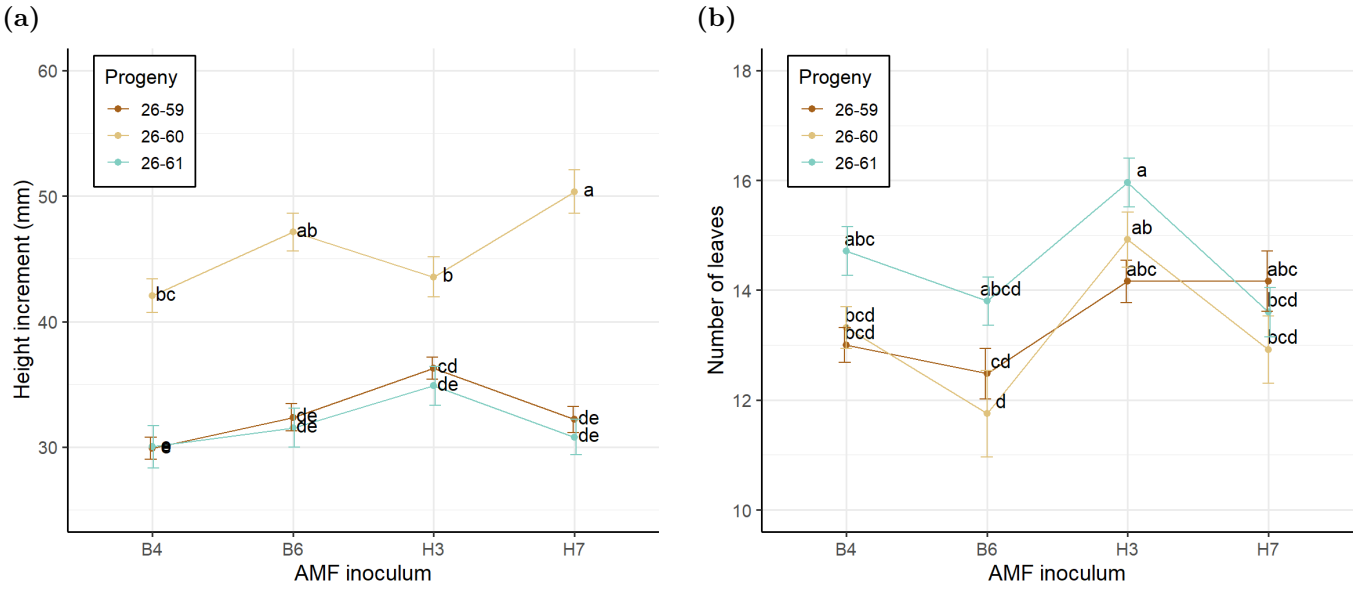


Figure 3: Graphs showing (a) the total stem height increment and (b) leaf number increase in the time between the first and last measurements (9 and 27 wpg, respectively). Groups that do not share a letter are significantly different from one another, according to Tukey-Kramer's HSD test ($p < 0.05$).

Table 1: Results of multivariate ANOVA (mAOV) for main, interaction and blocking effects (cursive), and results of univariate ANOVAs for separate above- and belowground response variables. Values represent p-values and significance levels are represented by *** ($p < 0.001$), ** ($p < 0.01$), * ($p < 0.05$), ns ($p > 0.05$). Full ANOVA tables can be found in Appendix L

Factor	*Aboveground*						*Belowground*			
	mAOV	**TDW**	**SDW**	**LDW**	**RDW**	**SLA**	***mAOV***	**SRL**	**RD**	**RTD**
Progeny	***	***	***	**	ns	*	**	ns	**	***
AMF	***	***	***	***	***	***	**	***	ns	ns
P×M	*ns*	ns	ns	*	ns	ns	*ns*	ns	*	ns
Table	***	***	***	***	***	***	*	*	***	***

Table 2: Results of Tukey-Kramer HSD for AMF main effect. Groups with different letters are significantly different from each other ($p < 0.05$).

AMF	*Aboveground*					*Belowground*		
	TDW	**SDW**	**LDW**	**RDW**	**SLA**	**SRL**	**RD**	**RTD**
B4	b	b	b	b	b	a	a	a
B6	b	b	b	c	b	ab	a	a
H3	a	a	a	a	a	b	a	a
H7	b	b	b	bc	b	b	a	a

Table 3: Results of Tukey-Kramer HSD for AMF main effect. Groups with different letters are significantly different from each other ($p < 0.05$).

Progeny	*Aboveground*					*Belowground*		
	TDW	**SDW**	**LDW**	**RDW**	**SLA**	**SRL**	**RD**	**RTD**
26-59	b	b	b	a	b	a	a	b
26-60	a	a	a	a	ab	a	b	a
26-61	b	b	ab	a	a	a	a	b

diverging lines between H3 and H7 in figure 3a.

Upon doing the same analysis with four-week time intervals (11-15, 15-19, 19-23 and 23-27 wpg), one can see that the height increment is not steady (Appendix J). For instance, the previously described interaction effect is very clearly visible in interval 19-23 wpg. Even though a similar trend remains visible in the intervals 15-19 wpg and 23-27 wpg, the interaction effect lacks significance.

3.5 Effects of AMF and progeny on increase in leaf count

The type II two-way ANOVA for the effect of progeny, AMF and the interaction showed that there is no interaction effect, but the AMF and progeny main effects are highly significant ($p < 0.001$) (Appendix K). Indeed, no interaction becomes apparent in figure 3b. Whereas the largest height increment was shown by progeny 60, the number of leaves of seedlings from progeny 61 was significantly higher than those of progenies 59 and 60 (Appendix I). Seedlings inoculated with AMF H3 showed a significantly higher increase in number of leaves than those inoculated with any of the other AMF inocula (Appendix I).

3.6 Effects of AMF and progeny on dry weight and leaf area

A type II two-way MANOVA showed that there is no interaction effect between the progeny and AMF inoculum for aboveground growth ($p = 0.285$). The main effects of progeny and AMF inoculation are however highly significant ($p < 0.001$) (table 1).

Univariate type II two-way ANOVAs indeed showed that there is no significant interaction effect in any of the growth parameters ($p > 0.05$), except for leaf DW ($p = 0.036$) (table 1). However, further analysis with a Tukey-Kramer HSD showed no apparent interaction between the progeny and AMF inoculum for leaf DW (Appendix M). The AMF main effect was highly significant for all growth parameters, while the progeny effect was significant for all parameters but the root DW (table 1).

On average, seedlings inoculated with AMF H3 had the highest performance for all growth characteristics (table 2). No significant differences were observed between AMF inocula B4, B6 and H7 for SDW, LDW and SLA. Seedlings inoculated with B4 did on average show significantly larger TDW and RDW than those inoculated with B6.

No significant differences were observed between progenies for RDW (table 3). Progeny 60 showed the highest average TDW, SDW and LDW. SDW of progeny 60 was significantly higher than that of both progenies 59 and 61, whereas the TDW and LDW were only significantly higher than that of progeny 59. In contrast, progeny 61 had the largest average SLA, which was significantly higher than that of progeny 59 (table 3).

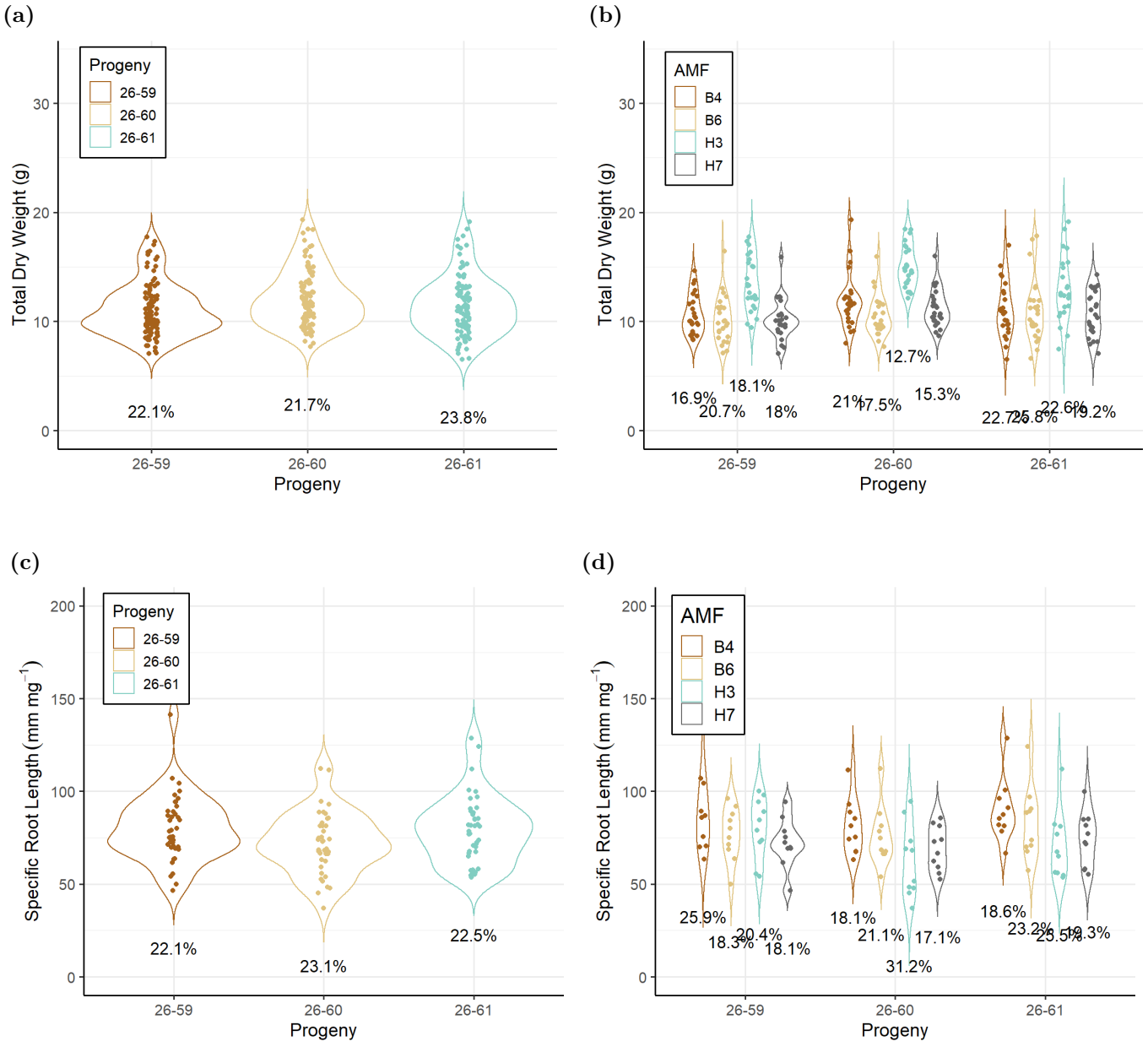


Figure 4: Violin plot showing the variation in total dry weight and specific root length within the progenies (a and c, respectively) and within progeny-AMF combinations (b and d, respectively). Every point represents an individual of the corresponding progeny. Numbers beneath the violin plot represent the variation coefficient.

3.7 Effects of AMF and progeny on belowground growth characteristics

The type I two-way MANOVA showed that there is no significant effect of the progeny-AMF interaction on root tissue density (RTD), root diameter (RD) and specific root length (SRL), whereas the main effects are significant. Univariate ANOVAs pointed out that AMF inoculum has a significant effect on SRL, whereas progeny has a significant effect on RD and RTD (table 1). Seedlings inoculated with

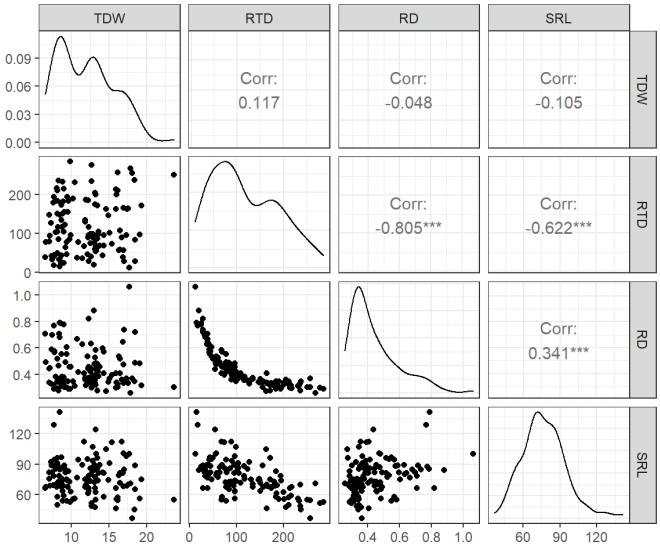


Figure 5: Dot plots (bottom-left of matrix) showing correlations between measured growth traits for all individual plants (a). Labels on top indicate the parameter represented by the x-axes in the columns. Labels on the right indicate the parameter represented by the y-axes in the rows. TDW data were log-transformed. Line graphs on the diagonal axis of the matrix indicate the distribution of values for its corresponding parameter. Values in the top-right half of the matrices indicate Pearson's r (Corr) and the asterisks behind the values indicate significance levels of the correlation; $p < 0.05$ = *; $p < 0.01$ = **; $p < 0.001$ = ***.

AMF B4 had the highest RD; significantly higher than those inoculated with AMF H3 or H7, but not significantly higher than those inoculated with AMF B6 (table 2). The ANOVA also suggested that there is a significant effect of the progeny-AMF interaction. However, further analysis with a Tukey HSD test showed no apparent interaction effect (Appendix M). Seedlings from progeny 60 on average showed significantly higher RD and significantly lower RTD, than seedlings from progeny 59 and 61 (table 3).

When including the NC seedlings in this analysis, one can see that the NC seedlings on average have the highest SRL (Appendix N). Interestingly, RD is also significantly higher for NC compared to any of the other AMF treatments. In contrast, for RTD NC seedlings show the lowest values on average.

3.8 Variation in growth characteristics

No remarkable differences were found between populations for within-population variation. The distributions of TDW and SRL, and variation coefficients of the different progenies are strikingly similar (figures 4a and 4c). The same holds for the other above- and belowground growth parameters (Appendix O). Likewise, looking at the variation within progeny-AMF combinations did not show any remarkable differences in variation between treatments (figures 4b and 4d).

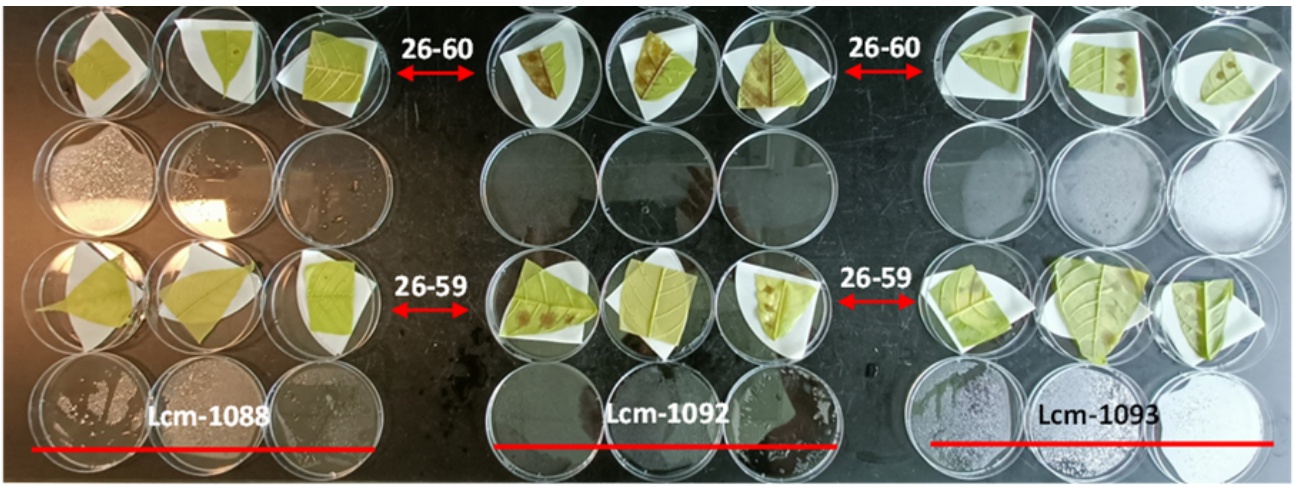
3.9 Linking root traits to biomass production

No correlation was found between root characteristics (RD, RTD and SRL) and TDW (fig. 5). Moreover, upon analysing the correlations between the root characteristics and all other growth parameters, no significant correlations were found, except between SLA and RD (Pearson's r = 0.244) (Appendix P). This correlation is however rather weak and as there are no other correlations present, there is no reason to think that RTD, RD or SRL give any indication of aboveground growth performance.

3.10 *Phytophthora* resistance

Of the three candidate P. capsici strains, only spore solutions from 15-day-old colonies of Lcm-1092 and Lcm-1093 showed considerable virulence (fig. 6). Lcm-1092 showed some very severe infections in leaves from progeny 60, indicating high virulence. This complicated the lesion size analysis, since it was not always clear where a lesion started and ended. Therefore,

Figure 6: Picture of results of Phytophthora bioassay trials for progenies 59 (bottom) and 60 (top), infected with Lcm-1088 (left), Lcm-1092 (middle) and Lcm-1093 (right).



Lcm-1093 was used for the following bioassays, rather than Lcm-1092.

However, response rates were very low during the actual experiment. Of the 179 leaves that were tested, only 93 leaves showed lesions (52%). Moreover, of the 537 inoculations, only 183 (34%) induced a lesion. We also observed a large variation in lesion sizes. Some lesions covered several hundreds of mm^2, whereas other lesions were contained to a single speck, oftentimes no larger than 1 mm^2. These large differences in lesion size could not be explained by progeny or AMF inoculum.

Leaves of plants grown in sterilized soil (i.e., those used for the trials) were also tested during the experiment and also showed very low and inconsistent response. Furthermore, a small number of bioassays were performed without diluting the zoospore solution ($\sim 2 \times 10^7$ zoospores/ml). Again, the response was inconsistent or non-existent.

4 | Discussion

Cocoa breeding is a challenging endeavour, partly because of the lack of knowledge about it (Bekele & Phillips-Mora, 2019). This study attempted to provide cornerstone information on breeding cocoa while taking into account the interaction with AMF. It shed light on the variation in above- and belowground growth within and between progenies of three selfed cocoa clones. Moreover it attempted to gain insight into how growth of these progenies is influenced by different AMF communities.

4.1 Between- and within-progeny variation in seedling growth

According to the ANOVA and Tukey-Kramer tests, seedlings from progeny 60 on average performed slightly better in terms of biomass production than progenies 59 and 61. However, distribution of values of biomass production showed no clear superiority of progeny 60, but rather all progenies seemed to perform equally well. A possible explanation for this lack of between-progeny variation is that clones were already selected for high performance, prior to selfing.

Seeing as the progenies used in this experiment were derived from selfings of wild cocoa clones, it was expected that the three progenies showed large within-progeny variation (Cuatrecasas, 1964). There was indeed considerable variation in growth performance within all three progenies. However, large variation in average performance was also observed between tables. Since seedlings were randomly distributed over the tables, it is highly unlikely that certain tables got seedlings with more vigorous genotypes than other tables. Therefore, this indicates a potentially large environmental effect on seedling growth. This variation caused by environmental factors may have overshadowed the genetic variation (Wright, 1968). Moreover, because every plant was genetically unique, and there were no genetically identical controls present on each table, we had no indication of the extent of the environmental effect on plant performance. On the other hand, it was shown that the variation can at least in part be explained by the genetics, because some seedlings that were grown on tables with low average performance outgrew plants grown on tables with high average performance.

Root diameter differed significantly among progenies; progeny 60 showed significantly smaller RD than progenies 59 and 61. This may indicate lower suitability of seedlings from progeny 60 to harbour AMF in their roots (Bergmann et al., 2020). However, mycorrhizal staining will have to shed more light on the extent to which AMF managed to infect the seedling roots. Within-progeny variation was present, but as explained before, we could not know whether this has an environmental or genetic basis.

In future experiments, genetically identical clones (e.g., cuttings of the parental clone) should be added to every block to be able to estimate the environmental effect (Wagoire et al., 1999; Wright, 1968). In that case, it is also advisable to use cuttings of seedlings, rather than seedlings itself, in order to minimize the differences between parental controls and progeny. It should then also be taken into account that root morphology of adventitious roots differs significantly from that

of primary or lateral roots (Mulatya et al., 2002). Moreover, adventitious roots respond differently to environmental stresses and are prone to different regulatory mechanisms than lateral roots (Bellini et al., 2014; Hattori et al., 2013; Steffens & Rasmussen, 2016). Since there is no clear difference in variation or average performance between the three progenies, it seems not to matter which progeny is used in future experiments. For the same reason, it is recommended to use the progeny of just one selfed clone.

4.2 Effect of AMF inocula on seedling growth

Cocoa has previously been shown to be highly dependent on AMF (Aggangan et al., 2019; Baon, 2015; Oladele, 2015; Tchameni et al., 2011). Likewise, the current experiment showed the importance of AMF for growth and development of cocoa. Although non-AMF seedlings grew as tall as AMF seedlings, their overall appearance was unhealthy, and biomass production and leaf area were substantially lower than for AMF seedlings.

Koch et al. (2006) found that AMF communities with varying species composition cause different growth responses in carrots. Likewise, our experiment showed significantly different biomass production for seedlings inoculated with different AMF communities. Seedlings grown with AMF inoculum H3 generally had the highest performance in terms of biomass production, compared to those inoculated with other AMF inocula. As was shown by Jomao-as et al. (2023), growth benefits of AMF for cocoa are partly determined by the concentration of AMF spores in the inoculum. In the current experiment, it was unknown how high AMF spore concentrations were. The AMF species composition is expected to vary between inocula, because they were isolated from soils with varying pH and texture (Alguacil et al., 2016; Jansa et al., 2014). However, analysis will still be done to determine the AMF species composition, and AMF colonization is yet to be quantified by mycorrhizal staining. It should also be determined what the initial AMF spore concentration was in the soil inocula. This will give more clarity about the nature of the improved growth performance.

Klironomos (2003) found that various AMF species had different effects on biomass production of different plant species – i.e. an interaction between AMF and plant species. No such interaction was found for biomass production or leaf area in the current experiment where we compared progenies from different clones of the same species, but it was found for height increment. It is possible that no interaction was observed because all individual seedlings within one progeny were very different. The different AMF inocula might have had varying effects on seedlings with various genotypes, but since performance was averaged over heterogenous progenies, this interaction could not be detected.

Previous research showed that AMF can induce root architectural changes, like reduced specific root length (SRL) and increased root diameter (RD) (Atkinson et al., 1994; Hooker & Atkinson, 1996). Accordingly, we found significantly higher RD for seedlings inoculated with AMF H3 and H7, compared to non-AMF (NC) seedlings. However, we found no differences in SRL. Due to discrepancies in the root scans, broad edges of the scans could not be analysed. This meant that large parts of the scans were left out, thereby excluding part of the roots from the analysis. Consequently, total root volume could not be measured accurately. Seeing as SRL is calculated based on root volume (Bergmann et al., 2020), SRL values may have been off.

4.3 Correlation between aboveground and belowground growth

According to Bergmann et al. (2020), plant species with higher RD and lower SRL can better harbour AMF. Besides, Azcón and Ocampo (1981) showed the same relationship for different wheat cultivars. We therefore expected higher AMF colonization and increased biomass production in plants with higher RD and lower SRL. We found no evidence for correlation between TDW versus RD and SRL. These results are therefore more in accordance with Maherali (2014), who showed that higher RD and lower SRL did not necessarily lead to increased mycorrhizal response. It is however noted that species with high mycorrhizal dependency, like cocoa (Baon, 2015), are still expected to show substantial growth benefits from AMF (Maherali, 2014).

As soon as AMF colonization data is available, it would be interesting to see whether it shows a correlation with plant performance. It would also be interesting to see whether root colonization rates can be related to root characteristics. However, in the current experimental setup, it is not possible to determine whether the AMF colonization causes root architectural changes, or whether root architecture influences root colonization as proposed by Bergmann et al. (2020). In order to find this out, future experiments should be carried out with two genetically identical clones; one AMF-inoculated and one non-inoculated.

4.4 Performance indicators for growth over time

Leaf number was seemingly positively correlated to seedling dry weight and surface leaf area. However, redoing the correlation analysis separately for AMF treatments revealed that this correlation was merely a reflection of the effect of AMF, as the correlation disappeared. Leaf number should therefore not be used as an indicator for plant performance.

In contrast, the stem height increment (HI) showed stronger correlation with other growth parameters, like total dry weight. However, there is reason to critically look at HI as a seedling performance indicator. Aggangan et al. (2019) showed that cocoa seedlings grown in AMF soils grew taller than those grown in sterile soil. Moreover, taller plants also had thicker stems in that experiment. In contrast, the current experiment showed that cocoa seedlings grown in absence of AMF generally had higher stem elongation rates, but had thin stems and appeared very unhealthy. The seedlings in our experiment grew in a greenhouse covered with a black cloth for shade, whereas the plants in Aggangan et al. (2019) grew in non-shaded conditions. Although cocoa generally grows under shade trees in natural conditions, it has been shown that cocoa exerts shade avoidance responses, like stem elongation, in shaded conditions (Inada & Nishiyama, 1987). Thus, the seedlings in the current experiment were possibly experiencing light stress. Since AMF can alleviate host plants from abiotic stress, including light stress, the shade-avoidance response was possibly reduced in the AMF-inoculated seedlings (Israel et al., 2022; Kyllo et al., 2003). Consequently, it is unclear whether HI reflects plant performance, or if it just represents the ability of AMF to relieve the plants from shade stress.

As was shown by Martial et al. (2019), HI over time can be used as a reliable proxy for growth in shaded conditions. However, there was no AMF or other abiotic stress relieving factor present. In the conditions of the current experiment, HI should not be used as a reliable proxy for plant performance.

4.5 *Phytophthora* resistance

The trial experiments were done with seedlings grown on sterilized soil and with an undiluted spore solution. Plants that were initially used

for the bioassays were inoculated with AMF or microbial wash. We hypothesized that this might have caused the difference in response, as the microbiome in general is known to induce disease resistance (Liu et al., 2019). However, after adding the same plants from the trials to the experiment, again no or very low responses were observed. Also, on the day where we used an undiluted spore solution (approximately 20 million spores per mL, 20 to 100 times the recommended concentration (Bharath et al., 1999; Nyassé et al., 1995)), hardly any response was observed. The large difference in outcome between the trial bioassays and the actual bioassays illustrates the difficulty of *Phytophthora* detached leaf bioassays. It also indicates the importance of controlling experimental conditions. Bharath et al. (1999) noted that it is wise to use multiple representative strains of *Phytophthora* pathogens, because of the variability of *Phytophthora spp.* strains. Thus, in future experiments, it might be advisable to perform the bioassay with multiple strains of the same *Phytophthora* species.

4.6 Critical notes

It is important to keep in mind that breeding for improved AMF interaction is meant to restore the ability of plants to interact with AMF that has been lost in previous breeding efforts (Nerva et al., 2022). For instance, Li et al. (2021) proposed that modern maize cultivars have lost genes that facilitate AMF interaction. In contrast, cocoa has hardly been bred before and the majority of cocoa is produced with landraces by smallholder farmers (World Cocoa Foundation, 2014). It is therefore unlikely that earlier breeding efforts have deteriorated cocoa's ability to properly interact with AMF. Focus should therefore be on retaining and strengthening this ability, rather than restoring it.

Another issue to keep in mind is the fact that the low adoption rates of improved cocoa varieties is not just caused by a lack of improved varieties, but rather by a lack of financial resources by farmers (Aneani & Ofori-Frimpong, 2013; Kosoe & Ahmed, 2022). Consequently, improving cocoa varieties will only be effective if there is financial support from e.g. governments (Adebayo et al., 2022; Kosoe & Ahmed, 2022).

4.7 Concluding remarks

In conclusion, substantial variation in growth performance and root traits was found within progenies, indicating segregation for these traits. Although it is unclear to what extent this variation was caused by environmental and genetic factors, there are indications that genetic variation was present. Although there were difference in seedling growth between AMF communities, different progenies did not show differences in response to these AMF communities. We did therefore not find proof for an interaction effect between different progenies and AMF communities.

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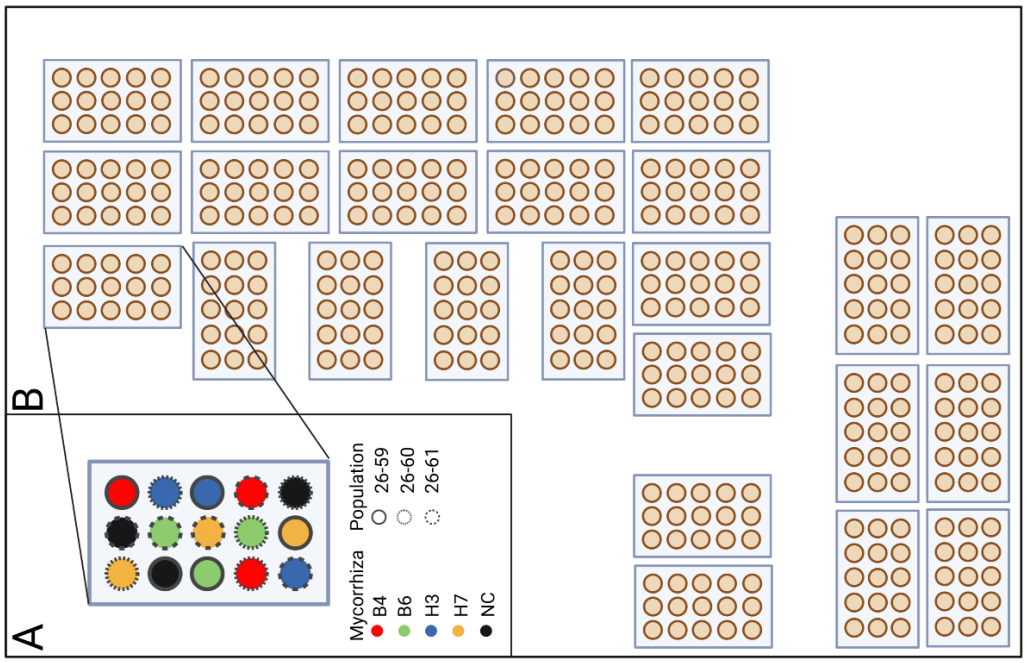
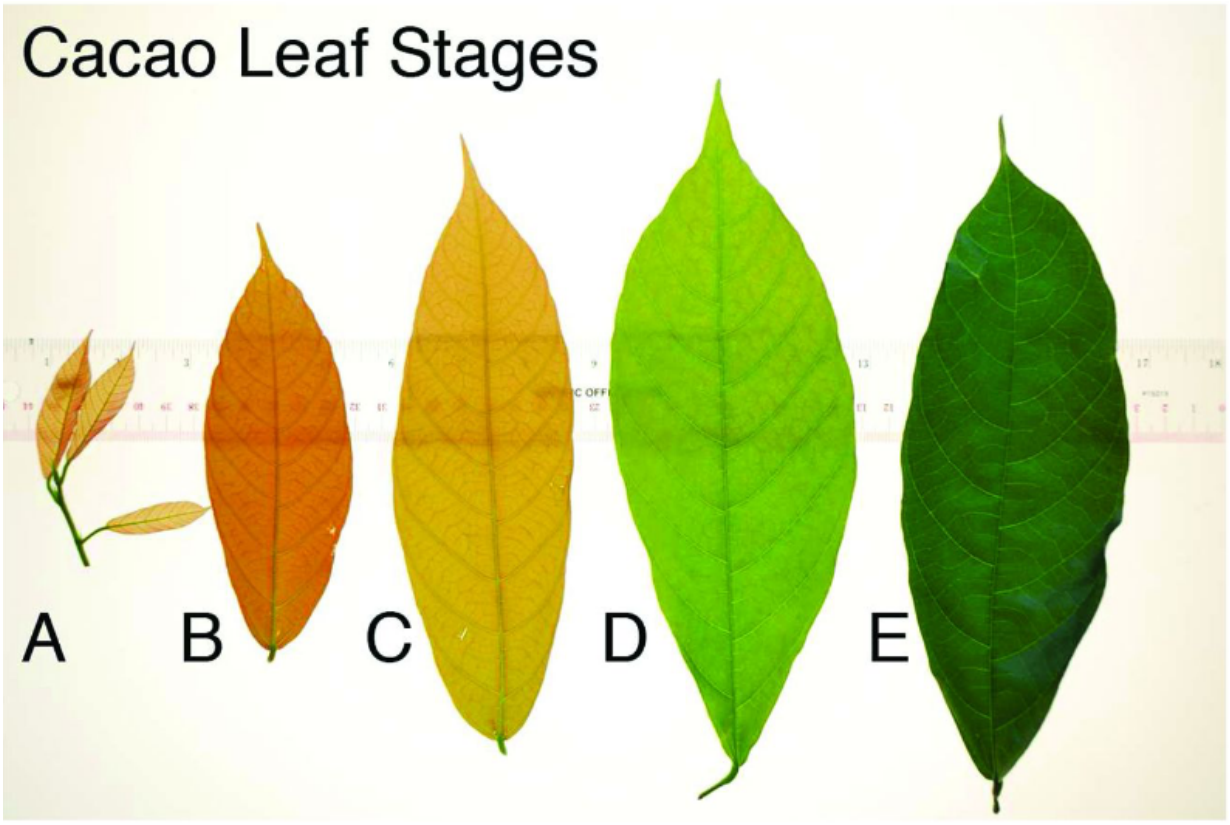
Appendix A

Visualization of the randomised complete block design (RCBD). (A) Light blue rectangle represents a table. Circles within the rectangle represent the fifteen potted seedlings. Colours indicate the mycorrhizal treatment and the outline of the circles represent the plant populations. The randomization shown in this table is an example; in reality, the setup of the tables may look very different. (B) Overview of the distribution of the twenty-five tables over the greenhouse.



Appendix B

Five stages of cocoa leaf development. Stage A represents emerging leaves, which are thin and often have a distinctive red colour. In stage B and C, leaves are still rather thin, but are already undergoing expansion. In stage D, the leaf begins to turn green and becomes more rigid. Stage E represents a fully matured leaf that is leathery and dark-green. Ruler: top is in inches, bottom is in cm. From: Mejía et al. (2012)



Appendix C

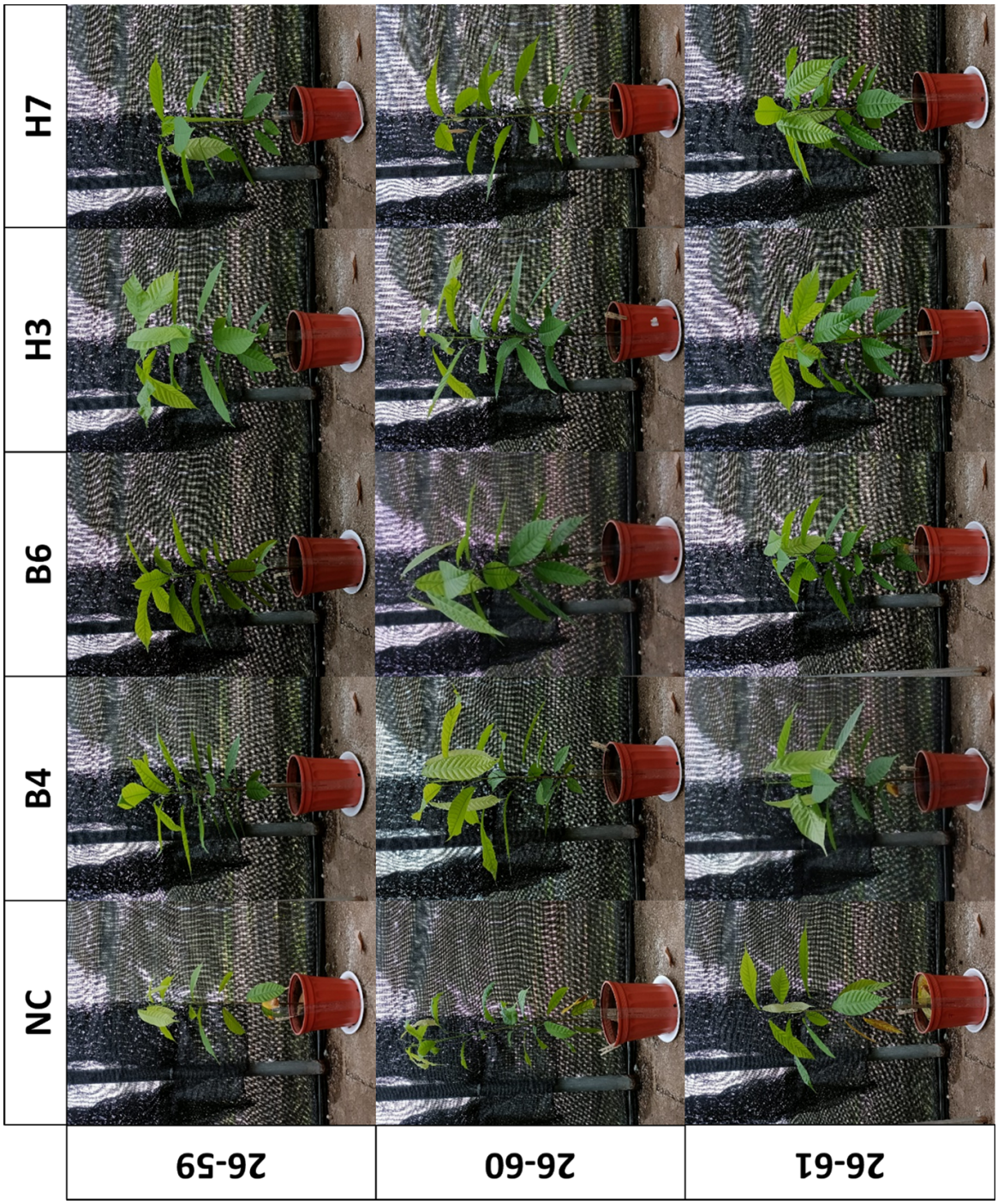
Picture of the leaves spread out on a white acrylic with a ruler at the top for setting the correct scale.

Lateral and side roots (top) were separated from the main root (bottom-right).

Example of a root scan. This particular scan was taken from part of the roots of the seedling from population 26-60, inoculated with AMF B6, and grown on table 1.

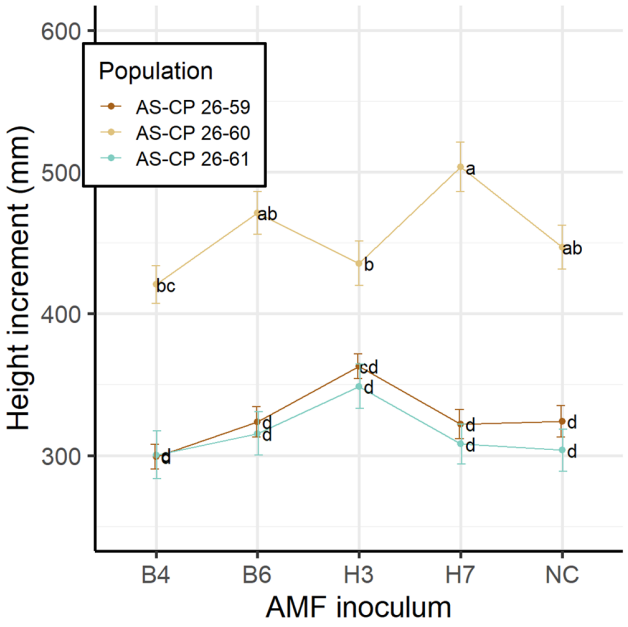
Appendix D

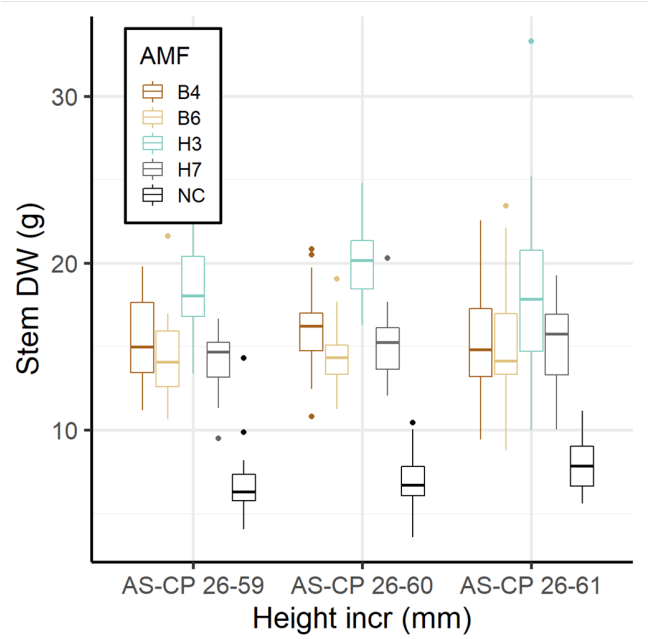
Overview of the general appearance of the plants in the greenhouse. Every plant represents the 'average' plant within a population-mycorrhizal treatment combination. Pictures were taken twenty-four weeks post germination.

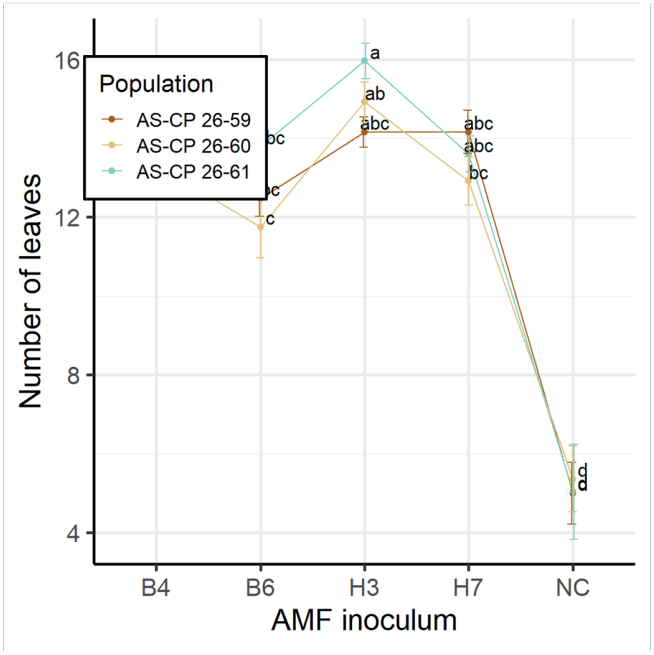


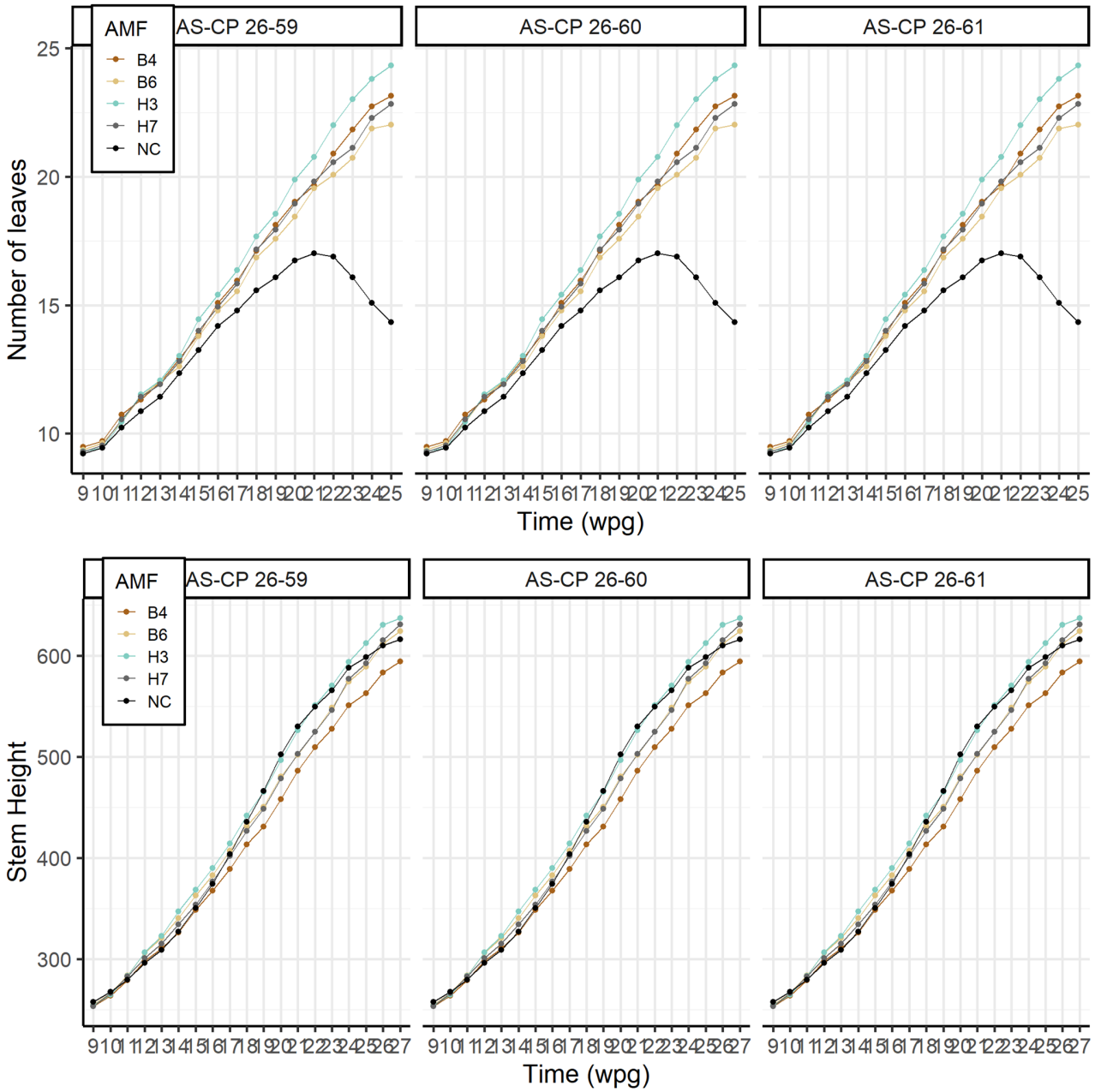
Appendix E

Some figures showing the differences in growth between AMF-inoculated and non-AMF seedlings.



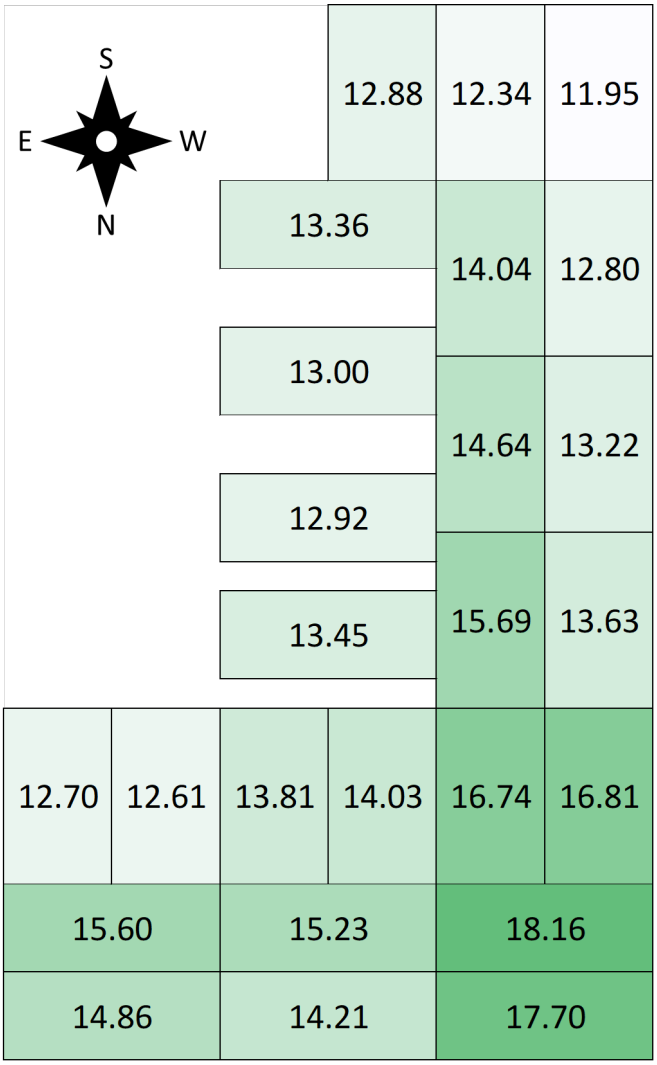





AMF
B4
B6
H3
H7
NC
AS-CP 26-59
AS-CP 26-60
AS-CP 26-61
Number of leaves
Time (wpg)
Stem Height
Time (wpg)

Appendix F

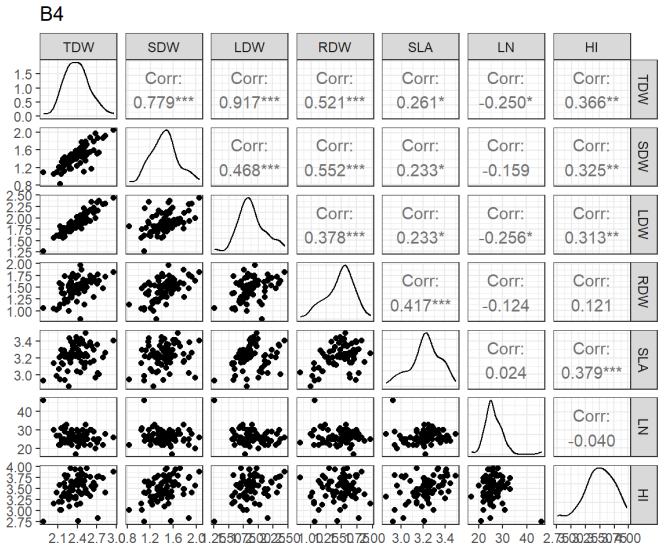
Average total dry weight per table. Compass rose provides orientation.



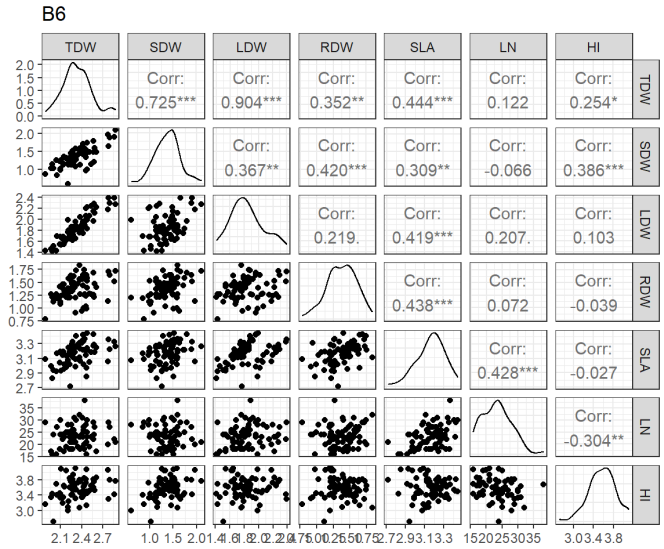
Appendix G

Correlation plots separated by AMF treatment.

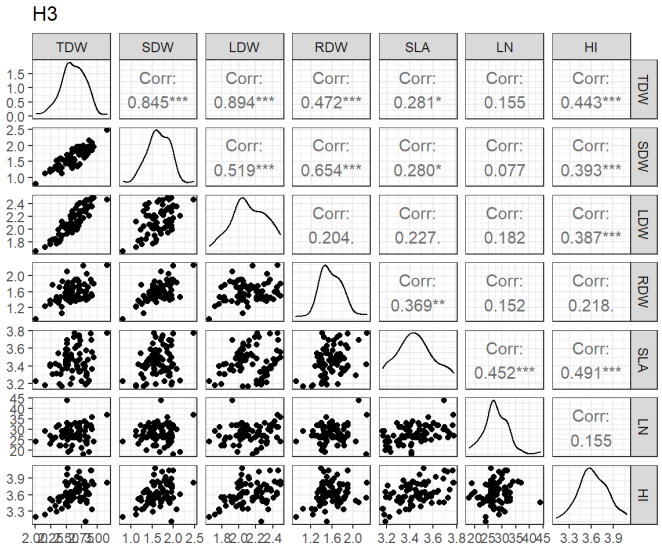
(a)



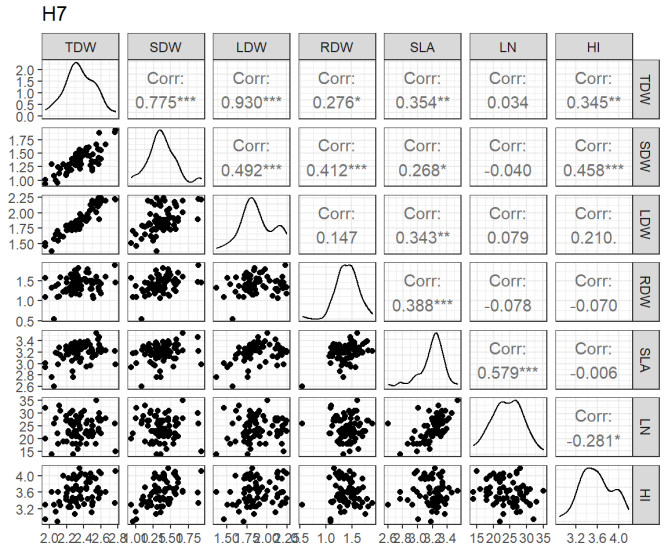
(b)



(c)



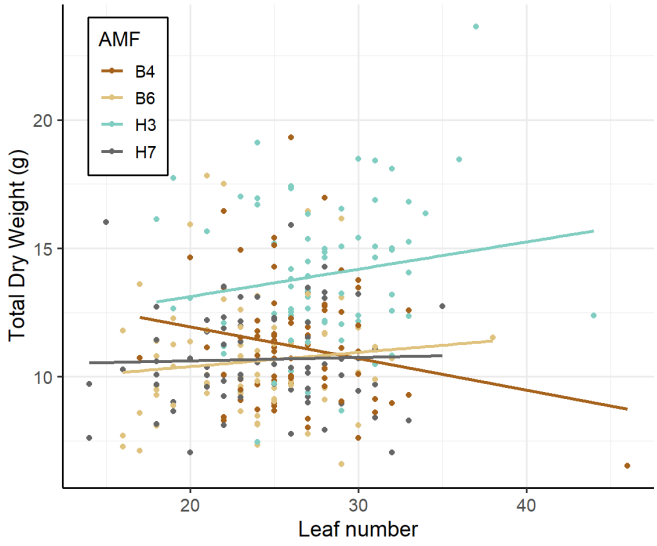
(d)



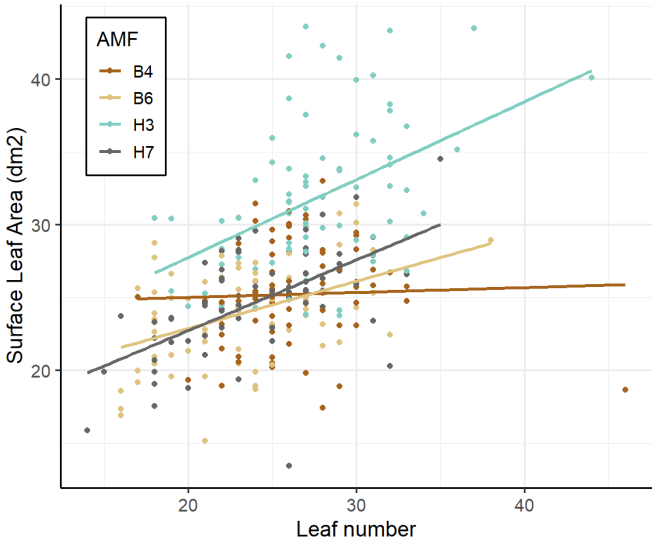
Appendix H

Correlation plots of leaf number against various other growth parameters, divided into AMF treatments by colouring. Lines indicate linear relation per AMF treatment.

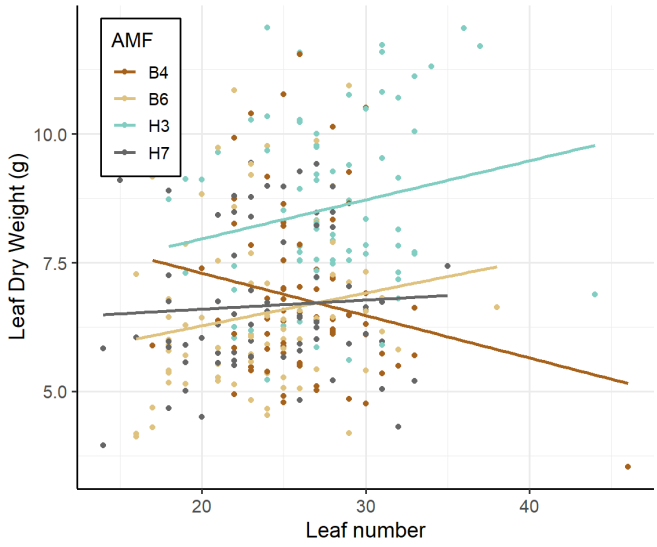
(a)



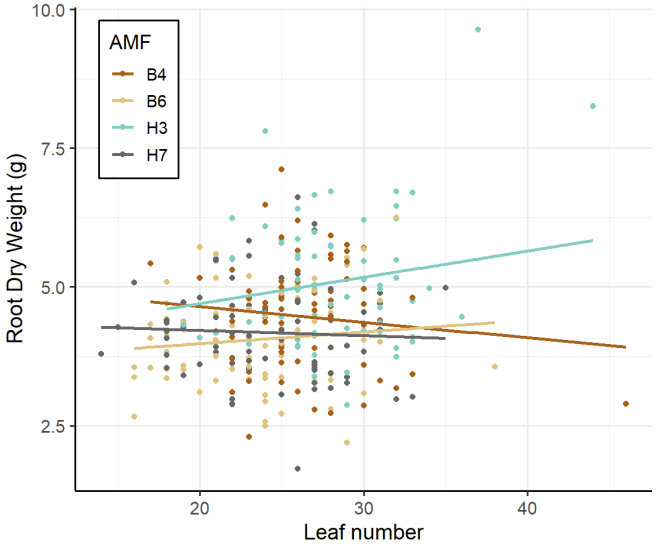
(b)



(c)

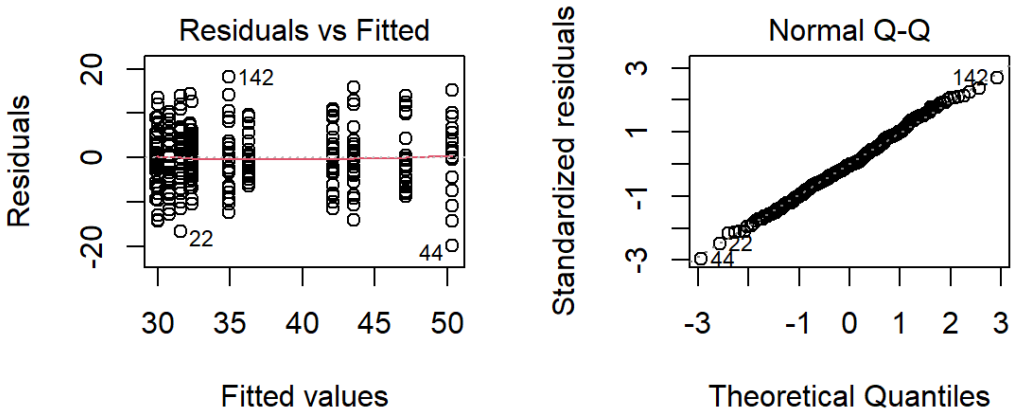


(d)



Appendix I

Residuals vs. Fitted plot (left) and QQ-plot for normality of residuals (right) for height increment over the entire period of measuring, showing that the assumptions of homoscedasticity and normality are not violated.



Type III two-way ANOVA table showing significance levels of the main effects (Progeny, AMF, table) and the interaction effect (Progeny:AMF).

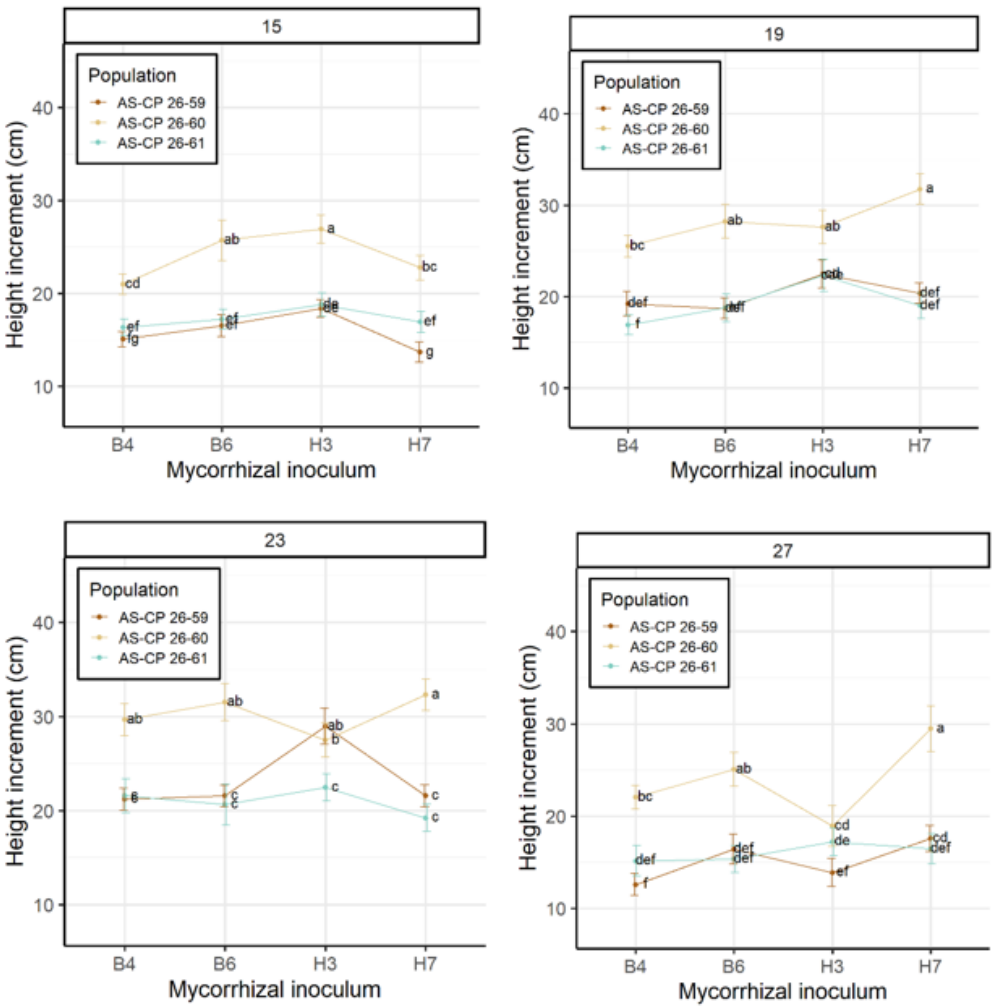
```
## Anova Table (Type III tests)
##
## Response: HI
##              Sum Sq  Df  F value    Pr(>F)
## (Intercept)  8033.1   1 188.2226 < 2.2e-16 ***
## Progeny      2402.0   2  28.1399 8.655e-12 ***
## AMF           529.1   3   4.1326 0.0069361 **
## table        2153.4  24   2.1023 0.0025097 **
## Progeny:AMF  1101.6   6   4.3019 0.0003724 ***
## Residuals   11096.5 260
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

Appendix J

Results of the mixed measures ANOVA test and separate two-way ANOVAs per time interval. $p < 0.05$ = *; $p < 0.01$ = **; $p < 0.001$ = ***.

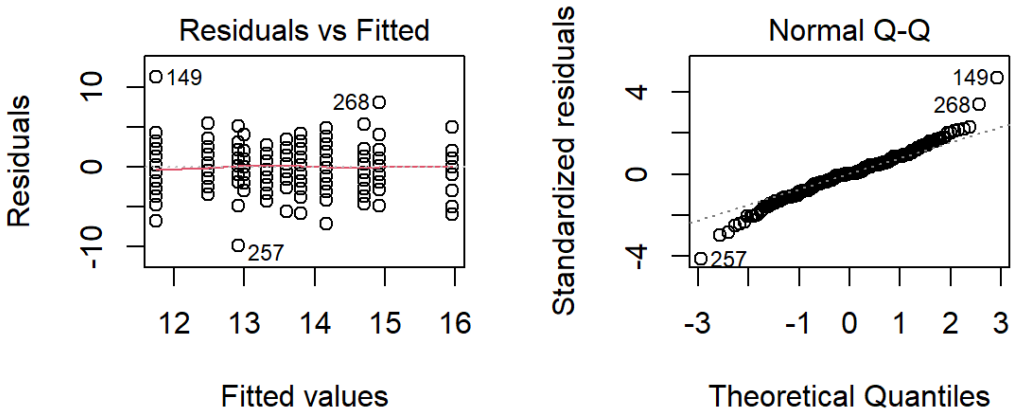
	Mixed-measures ANOVA	Two-way ANOVA for 11-15 wpg	Two-way ANOVA for 15-19 wpg	Two-way ANOVA for 19-23 wpg	Two-way ANOVA for 23-27 wpg
Progeny	***	***	***	***	***
AMF	**	**	ns	**	ns
PxM	**	ns	ns	**	ns

Line plots showing eventual interaction between AMF and progeny for different time intervals. Groups that do not share a letter are significantly different from one another, according to Tukey-Kramer's HSD test ($p < 0.05$).



Appendix K

Residuals vs. Fitted plot (left) and QQ-plot for normality of residuals (right) for leaf number increase over the entire period of measuring, showing that the assumptions of homoscedasticity and normality are not violated.



Type II two-way ANOVA table showing significance levels of the main effects (Progeny, AMF, table) and the interaction effect (Progeny:AMF).

```
## Anova Table (Type II tests)
##
## Response: LN_incr
##              Sum Sq  Df F value    Pr(>F)
## Progeny       91.56   2  7.8432 0.0004922 ***
## AMF          209.39   3 11.9571  2.32e-07 ***
## table        186.98  24  1.3347 0.1408114
## Progeny:AMF   60.53   6  1.7282 0.1146679
## Residuals   1523.51 261
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

Outcome of Tukey-Kramer HSD test. Letters indicate significance groups and the parenthesized numbers behind the letters indicate mean leaf number increase.

Progeny	Significance group	AMF	Significance group
AS-CP 26-59	b (13.5)	B4	b (13.7)
AS-CP 26-60	b (13.2)	B6	b (12.9)
AS-CP 26-61	a (14.5)	H3	a (15.0)
		H7	b (13.6)

Appendix L

```
##
## Type II MANOVA Tests: Pillai test statistic
##             Df test stat approx F num Df den Df    Pr(>F)
## Progeny      2   0.24160   9.0681     10    660 4.201e-14 ***
## AMF          4   1.03123  23.0646     20   1328 < 2.2e-16 ***
## table       24   1.74782   7.4568    120   1665 < 2.2e-16 ***
## Progeny:AMF  8   0.13369   1.1435     40   1665    0.2493
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
## Anova Table (Type II tests)
##
## Response: TDW
##             Sum Sq  Df  F value    Pr(>F)
## AMF         53.516   4 474.4979 < 2.2e-16 ***
## Progeny      0.506   2   8.9730 0.0001603 ***
## table        6.518  24   9.6316 < 2.2e-16 ***
## AMF:Progeny  0.566   8   2.5099 0.0116631 *
## Residuals    9.333 331
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
## Anova Table (Type II tests)
##
## Response: SDW
##              Sum Sq  Df  F value    Pr(>F)
## AMF         22.7857   4 137.1571 < 2.2e-16 ***
## Progeny      0.7623   2   9.1774 0.0001322 ***
## table        6.8103  24   6.8323 < 2.2e-16 ***
## AMF:Progeny  0.5044   8   1.5181 0.1496571
## Residuals   13.7056 330
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
## Anova Table (Type II tests)
##
## Response: LDW
##             Sum Sq  Df  F value    Pr(>F)
## AMF         94.033   4 596.0128 < 2.2e-16 ***
## Progeny      0.399   2   5.0538  0.006883 **
## table       10.745  24  11.3504 < 2.2e-16 ***
## AMF:Progeny  0.828   8   2.6231  0.008499 **
## Residuals   13.134 333
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
## Anova Table (Type II tests)
##
## Response: RDW
##              Sum Sq  Df  F value    Pr(>F)
## AMF         21.5770   4 140.0541 < 2.2e-16 ***
## Progeny      0.2009   2   2.6078   0.07520 .
## table        5.1689  24   5.5918 4.376e-14 ***
## AMF:Progeny  0.6936   8   2.2511   0.02364 *
## Residuals   12.7871 332
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
## Anova Table (Type II tests)
##
## Response: SLA
##              Sum Sq  Df  F value    Pr(>F)
## AMF         100.870   4 654.4186 < 2.2e-16 ***
## Progeny       0.508   2   6.5902 0.0015599 **
## table         2.180  24   2.3568 0.0004348 ***
## AMF:Progeny   0.640   8   2.0774 0.0374658 *
## Residuals    12.832 333
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
##              Df  Pillai approx F num Df den Df    Pr(>F)
## Progeny       2 0.13837   2.7253      6    220  0.014220 *
## AMF           4 0.64045   7.5321     12    333 2.502e-12 ***
## table        24 0.79695   1.6731     72    333  0.001407 **
## Progeny:AMF   8 0.21272   1.0589     24    333  0.390129
## Residuals   111
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
##              Df Sum Sq Mean Sq F value   Pr(>F)
## AMF           4  49010   12253  32.888  < 2e-16 ***
## Progeny       2    616     308   0.826 0.440265
## table        24  22099     921   2.472 0.000787 ***
## AMF:Progeny   8   1296     162   0.435 0.897812
## Residuals   111  41353     373
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
## 222 observations deleted due to missingness
```

```
##              Df  Sum Sq  Mean Sq F value   Pr(>F)
## AMF           4 0.02325 0.005814  13.519 5.21e-09 ***
## Progeny       2 0.00248 0.001238   2.879   0.0604 .
## table        24 0.01848 0.000770   1.791   0.0226 *
## AMF:Progeny   8 0.00432 0.000540   1.255   0.2744
## Residuals   111 0.04773 0.000430
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
## 222 observations deleted due to missingness
```

```
##              Df Sum Sq Mean Sq F value   Pr(>F)
## AMF           4 166691   41673  13.908 3.15e-09 ***
## Progeny       2  50738   25369   8.467 0.000378 ***
## table        24 186895    7787   2.599 0.000409 ***
## AMF:Progeny   8  52694    6587   2.198 0.032737 *
## Residuals   111 332589    2996
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
## 222 observations deleted due to missingness
```

Appendix M

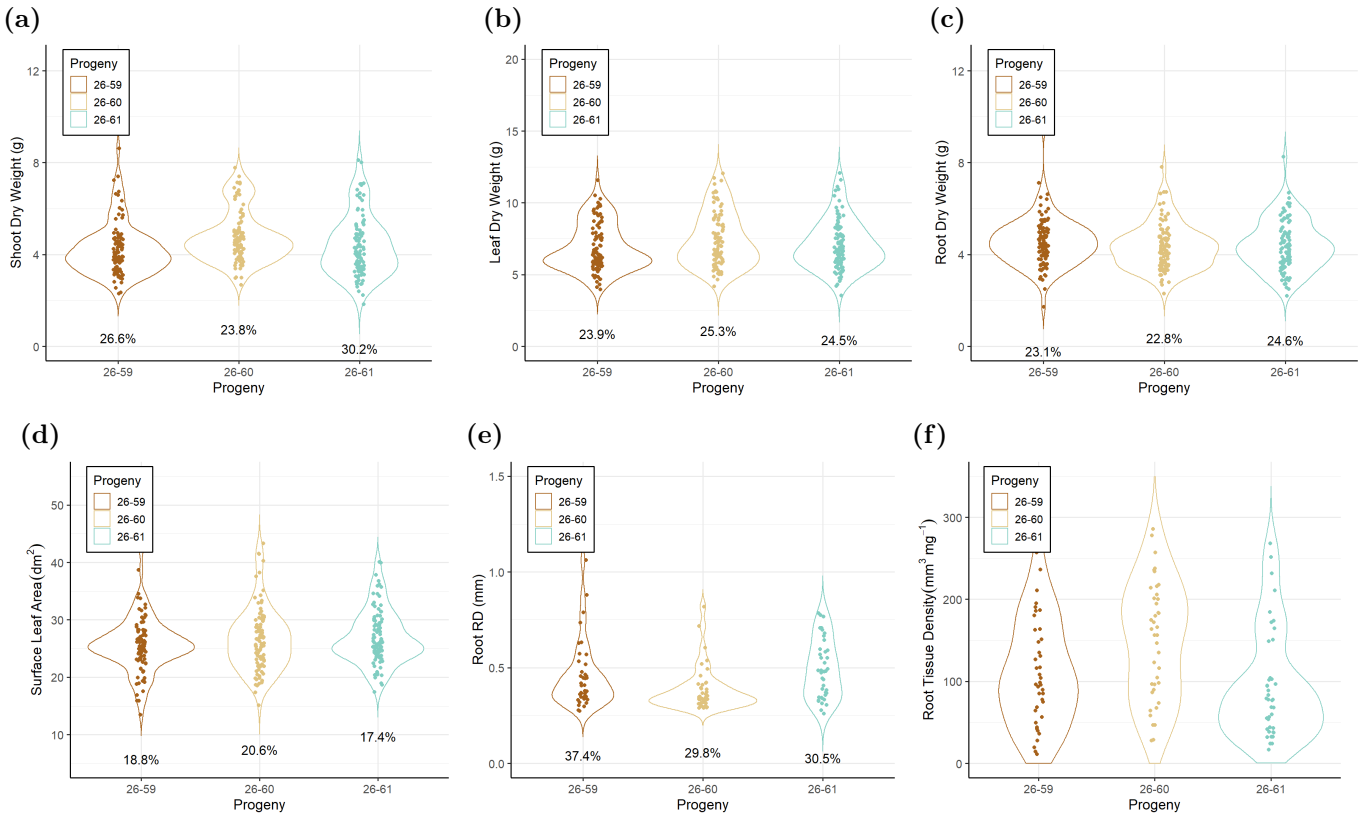
```
##               LDW groups
## H3:26-60 2.195538      a
## H3:26-59 2.102894      a
## H3:26-61 2.079533      a
## B4:26-60 1.948364      b
## H7:26-60 1.912229      b
## H7:26-61 1.901371      b
## B6:26-61 1.873626      b
## B4:26-61 1.863492      b
## B4:26-59 1.855124      b
## B6:26-59 1.826197      b
## B6:26-60 1.824322      b
## H7:26-59 1.820546      b
```

```
##                 RD groups
## B4:26-61 0.05866657      a
## H3:26-59 0.05147327     ab
## B6:26-59 0.04948192     ab
## H7:26-61 0.04832521     ab
## H3:26-61 0.04830886     ab
## B4:26-59 0.04176175     ab
## B6:26-61 0.04084056     ab
## H3:26-60 0.04048949     ab
## H7:26-60 0.04012741     ab
## H7:26-59 0.03939384      b
## B4:26-60 0.03710207      b
## B6:26-60 0.03683466      b
```

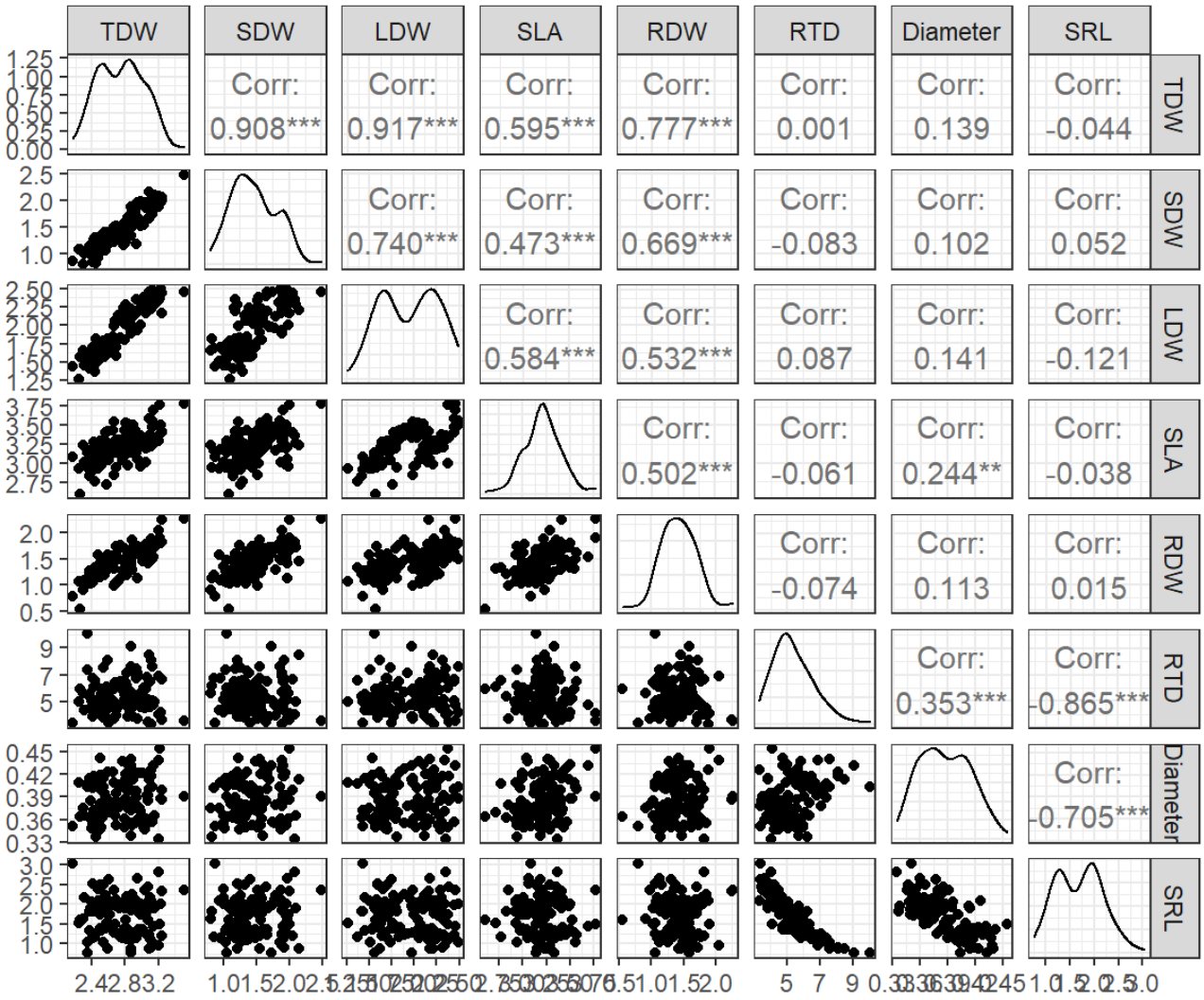
Appendix N

	Belowground		
	SRL	RD	RTD
B4	b	b	a
B6	bc	b	a
H3	c	b	a
H7	c	b	a
NC	a	a	b

Appendix O


(a)
Shoot Dry Weight (g)
Progeny
26-59
26-60
26-61
26.6%
23.8%
30.2%
(b)
Leaf Dry Weight (g)
23.9%
25.3%
24.5%
(c)
Root Dry Weight (g)
23.1%
22.8%
24.6%
(d)
Surface Leaf Area (dm2)
18.8%
20.6%
17.4%
(e)
Root RD (mm)
37.4%
29.8%
30.5%
(f)
Root Tissue Density(mm3 mg-1)

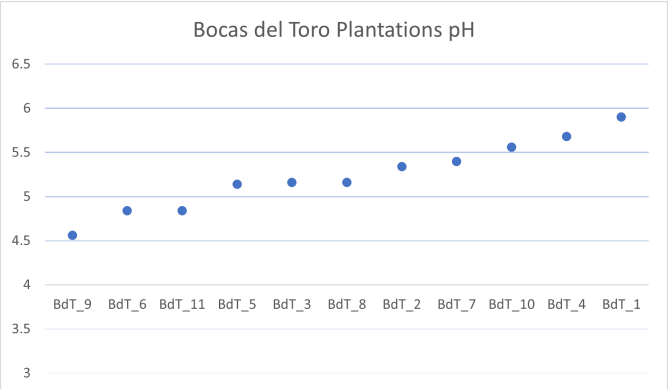
Appendix P


TDW
SDW
LDW
SLA
RDW
RTD
Diameter
SRL
Corr: 0.908***
Corr: 0.917***
Corr: 0.595***
Corr: 0.777***
Corr: 0.001
Corr: 0.139
Corr: -0.044
Corr: 0.740***
Corr: 0.473***
Corr: 0.669***
Corr: -0.083
Corr: 0.102
Corr: 0.052
Corr: 0.584***
Corr: 0.532***
Corr: 0.087
Corr: 0.141
Corr: -0.121
Corr: 0.502***
Corr: -0.061
Corr: 0.244**
Corr: -0.038
Corr: -0.074
Corr: 0.113
Corr: 0.015
Corr: 0.353***
Corr: -0.865***
Corr: -0.705***

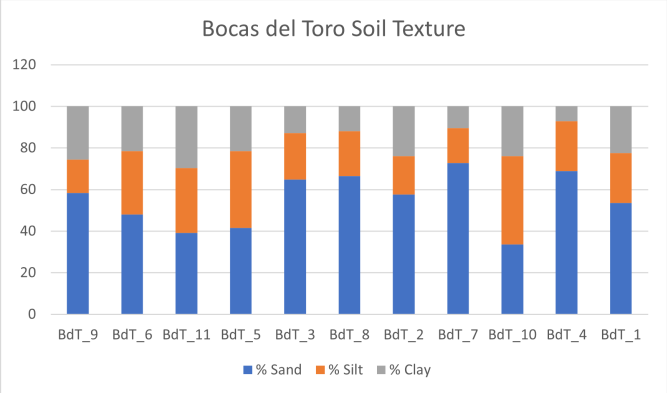
Appendix Q

Soil texture an pH of different AMF-containing soils, showing that BdT_4 (B4) and BdT_6 (B6) differ greatly in pH and soil texture (fig a,b). Her_16 (H7) and Her_12 (H3) differ mostly in pH (fig c,d).

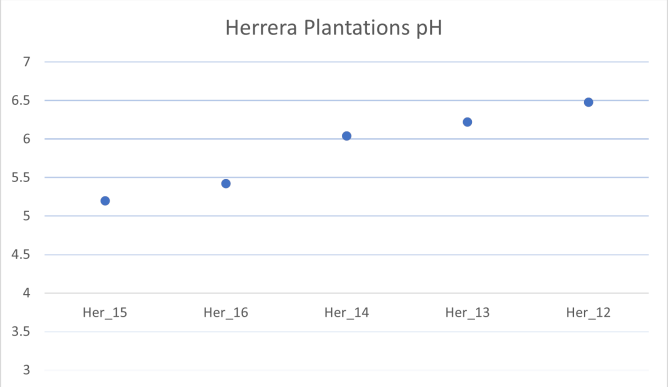
(a)



(b)



(c)



(d)