2017

The Impact Of Simulated Microgravity On The Growth Of The Model Legume Plant Medicago Truncatula

Gemma Elizabeth Lionheart
University of Mississippi

THE IMPACT OF SIMULATED MICROGRAVITY ON THE GROWTH OF THE MODEL LEGUME PLANT *MEDICAGO TRUNCATULA*

A Thesis

Presented for the

Master of Science

Degree

The University of Mississippi

Biology Department

Gemma Lionheart

December 2017

ABSTRACT

Simulated microgravity has been a useful tool to help understand plant development in altered gravity conditions. Thirty-one genotypes of the legume plant *Medicago truncatula* were grown in either simulated microgravity on a rotating clinostat, or a static, vertical environment. Twenty morphological features were measured and compared between these two gravity treatments. Within-species genotypic variation was a significant predictor of the phenotypic response to gravity treatment in 100% of the measured morphological and growth features. In addition, there was a genotype–environment interaction (G×E) for 45% of the response variables, including shoot relative growth rate ($p < 0.0005$), median number of roots ($p \sim 0.02$), and root dry mass ($p < 0.005$). These findings are discussed in the context of improving future studies in plants space biology by controlling for genotypic differences, and by connecting traits to their underlying genetic causes by using genome-wide association (GWA) mapping. In the long-term, manipulation of genotype effects, in combination with *M. truncatula*'s symbiotic relationships with rhizobacteria and arbuscular mycorrhizal fungi, will be important for optimizing legumes for cultivation on long-term space missions.

ACKNOWLEDGMENTS

Many thanks to my advisor, Dr. John Z Kiss, as well as other members of the Kiss lab – post-doctoral associate Dr. Joshua Vandenbrink, and M. Chase Caldwell – a fellow graduate student. Thanks to Erik Hom, Lucile McCook, and especially to Jason Hoeksema for all his efforts and invaluable statistical support. I would also like to thank the Noble Research Institute, OK for publishing The *Medicago truncatula* Handbook, and making it freely available from https://www.noble.org/medicago-handbook/, and also the *Medicago truncatula* Hapmap Project at http://www.medicagohapmap.org/ for all the germplasm used in this study. Financial support was provided by the University of Mississippi and the Mississippi EPSCOR program.

TABLE OF CONTENTS

LIST OF TABLES

FIGURE LEGENDS

INTRODUCTION

While the history of human space flight has focused primarily on the development of research facilities located in Lower Earth Orbit (LEO), such as Skylab, Salyut, Mir, and most recently the International Space Station (ISS) (Sherwood 2011), there has been a shift by the USA and the National Aeronautics Space Agency (NASA), as well as some international space agencies, to push farther afield – aiming at near-Earth asteroids, the Moon, and Mars (Ansdell et al. 2011). Food, oxygen, water, and waste disposal needs for LEO research facilities have thus far been met through resupply missions from Earth, which has been both costly and a circumscribing factor to the practical radius of human space flight (Ferl et al. 2002).

Extended-duration missions at farther distances will require a self-sustaining Advanced Life Support (ALS) system to recycle waste and provide for the nutritional needs of the crew (National Research Council 2015). Many approaches to utilizing ALS have been proposed, although plant-based solutions are an obvious primary contender, due to their natural and inherent properties of oxygen and food production, as well as CO_2 and grey water recycling (Monje et al. 2003; Lehto et al. 2006; Kiss et al. 2014; Vandenbrink and Kiss 2016). There are several difficulties with crop-farming in space, not least of which is the amount of physical space required for traditional cultivation. To make ALS feasible, we must optimize crops via selective breeding and/or genetic

engineering, for a high consumable yield to total biomass ratio (harvest index), robust growth under artificial light, dwarfed and fast growing cultivars, high CO_2 and water stress tolerance, and the ability to withstand low and/or fluctuating atmospheric pressure (Ferl et al. 2002). Increased nutrient uptake is also a key area of research for maximum ALS productivity (National Research Council 2015).

True microgravity can only be experienced either in space, such as via orbiting platforms including the ISS, or for shorter durations in Ground Based Facilities (GBF) such as drop towers, sounding rockets, or parabolic flights (Herranz et al. 2013). One of the major considerations when using GBFs for biological research is that they lack an accounting of space radiation (Ferl et al. 2002; Wolverton and Kiss 2009; Vandenbrink and Kiss 2016). There may be instances where this is of benefit, as radiation could be a confounding variable for the microgravity response. As a holistic space environment simulation, though, GBFs are limited in this way.

Flying an experiment on the ISS is extraordinarily costly (Ansdell et al. 2011; Kiss 2015), although commercial and academic endeavors over the last decade have made significant advances in the pursuit of more affordable conduits for space research, particularly with regard to the development of miniaturized satellites (CubeSats) (Ansdell et al. 2011; Babuscia et al. 2015; Ciaralli et al. 2015; Scholz and Juang 2015; Ciaralli et al. 2016; Escobar et al. 2016). There are also several privately-funded American spaceflight services aerospace companies, such as Blue Origin and SpaceX, who are working to develop and refine truly reusable rockets, to make space research much more accessible. However, all of these methods are expensive, to varying degrees, and troubleshooting on Earth is a prudent and critical step prior to flying experiments (National

Research Council 2015).

Clinorotation, which is the rotation of plants about an axis such that the gravity vector is constantly changing, is another GBF that has become a common tool for simulating microgravity in plants to develop and refine experiments prior to actual spaceflight (Kraft et al. 2000; Herranz et al. 2013; Brungs et al. 2016). Clinorotation can be an effective proxy for microgravity for many parameters, as the primary known plant gravitropic mechanism is the perception of settling starch-dense vesicles (statoliths) by columella cell membranes in root tips (Kiss 2000; Kraft et al. 2000). In a constantly rotating environment the statoliths are continuously "falling" inside of the cell, never settling at the bottom as it is ever changing (Herranz et al. 2013). Several studies have found that clinorotation at 1 rpm can be an effective simulation of microgravity for plant life, depending on the parameters considered (Kraft et al. 2000; Hou et al. 2003; Herranz et al. 2013; Dauzart et al. 2016).

Plants in the Fabaceae family, commonly known as *legumes*, are an agriculturally, nutritionally, and economically valuable group of crops that include peas, soy, alfalfa, lentils, peanuts, and many beans (Graham 2003; Massa and Mitchell 2012; Wang et al. 2012, Varshney and Kudapa 2013). Their importance on Earth is far reaching due to their high protein, carbohydrate, fat and fiber content, all of which make them an excellent source of nutrition for human beings and livestock, as well as a major source of vegetable oil (Song et al. 2017). Their high nutritional value makes them a good candidate for cultivation on long-term space missions.

Like all plants, legumes are unable to directly utilize the highly stable, triple-bonded gaseous form of nitrogen (N_2) found in air, which can be limiting as N_2 is an essential component for

building nucleic acids and proteins. However, many types of rhizobacteria are able to "fix" this atmospheric nitrogen, taking in N_2 and converting it into usable, more reactive, single bonded nitrogen in the form of ammonia (NH_3) (Toro et al. 2014). When NH_3 in the soil is low, leguminous roots secrete a class of metabolites known as flavonoids, which chemically signal rhizobacteria to colonize their roots and form small swellings. These swellings are symbiotic organs called nodules, inside of which the symbionts exchange bacterially-fixed nitrogen for photosynthetically-derived fixed carbon (Jones et al. 2007; Wang et al. 2012, Ahemad and Kibret 2014). Around 88% of legume species studied form these symbioses (Graham 2003).

Many rhizobacteria-legume symbioses have been shown to increase host plant nutrient uptake, increase tolerance of various environmental stresses such as drought, radiation, and high salinity, as well as enhancing plant growth (Miransari 2010; Ahemad and Kibret 2014). These rhizobacteria are known as Plant Growth Promoting Rhizobacteria (PGPR). Of particular relevance to our current studies is research showing that some *Medicago* species have shown to be less affected by water stress when inoculated with the PGPR *Sinorhizobium meliloti* (Nadeem et al. 2014). *M. truncatula* also forms symbioses with several arbuscular mycorrhizal fungi (AMF) species (Hogekamp and Küster 2013), which have been shown to alter plant growth response under simulated microgravity (Dauzart et al. 2016).

Medicago truncatula is an excellent model species for the Fabaceae family as it is a diminutive, fast-growing, nodulating legume with a relatively small (~500 MBP) diploid genome. Due to *M. truncatula* being a model system, there are number of large scale genetic projects regarding this organism. For example, the *Medicago* Hapmap Project (http://www.medicagohapmap.org/) is a

collaboration between the University of Minnesota, the National Center for Genome Resources (NCGR), Boyce Thompson Institute (BTI), J. Craig Venter Institute (JCVI) Hamline University, INRA-Montpellier, ENSAT-Toulouse, and the Noble Foundation. "Hapmap" refers to haplotype mapping, or the mapping of genomic segments with shared ancestry. This consortium has sequenced 384 inbred lines of *Medicago* – predominantly *M. truncatula*, using Illumina Next-Generation Sequencing (NGS) technology, and published the data online. As a component to their project, they have made available true-breeding seeds for each of these lines. Their goal is to create a free, accessible, genome-wide association (GWA) mapping resource for the plant research community. GWA studies (GWAS) are observational studies in which genetic variance between individuals is analyzed to see if it is associated with a phenotypic trait. The germplasm made available by the *Medicago* Hapmap Project is all true-breeding, minimizing heterozygosity. This allows for the collection of high-resolution single nucleotide polymorphisms (SNPs), insertions/ deletions (INDELs) and copy number variants (CNVs). These data can be compiled and used as a basis for haplotype identification, as well as a novel way to look at population structure.

Association mapping is being used widely across biological disciplines (Stapley et al. 2010), including human studies (Yang et al. 2010; Bossdorf and Zhang 2011; Herrera and Bazaga 2013; Choudhury et al. 2014), animal studies (Pritchard et al. 2000), bacterial studies (Epstein et al. 2012), and increasingly for plants (Rafalski 2002; Zhu et al. 2008; Ganal et al. 2009; Myles et al. 2009; Branca et al. 2011; Young et al. 2011) and even non-model species (Ekblom and Galindo 2010). SNPs, INDELs, and CNVs occur at such frequency and specificity as to enable extremely fine-scale resolution of quantitatively inherited traits, allowing scientists to perform whole-genome scans and identify closely-linked alleles that are significantly correlated with quantitative

trait variation (Brachi et al 2011). There is also research showing how these genetic motifs can be used to tentatively draw conclusions more broadly, for example, it has been demonstrated that SNPs in nature are population-specific, and non-randomly distributed (Choudhury et al. 2014).

RESEARCH QUESTIONS AND HYPOTHESES

This study was designed to characterize the morphological plasticity among *M. truncatula* genotypes, and investigate whether or not the growth of each genotype responds to simulated microgravity in a similar manner and with the same magnitude. The *Medicago* Hapmap project is mapping haplotype associations in symbiosis-related phenotypes between *Medicago* individuals, with a view to uncovering genotype:phenotype associations related to symbiotic success. Similarly, we intend to create a GWA mapping framework but with the goal of uncovering genotype:phenotype associations related to microgravity success. Additionally, we were interested in studying the genetic mechanisms at play in phenotypic plasticity in *M. truncatula*. In the long term, this could lead to more detailed and accurate genome annotation, not only of the *Medicago* genomes, but potentially as a guide to identifying homologous (or perhaps even analogous) allele effects from other taxa.

We hypothesized that, overall, clinorotated plants would exhibit phenotypic differences, in terms of growth parameters compared to plants grown vertically at 1-*g*. This hypothesis is supported by a large body of research, and would also be a confirmation of our own findings (Miyamoto et al. 1999; Kraft et al. 2000; Aarrouf et al. 2003; Hou et al. 2003; Kern et al. 2005;

Sobol et al. 2005; Braun and Limbach 2006; Hoshino et al. 2007; Blancaflor 2013; Herranz et al. 2013; Soh et al. 2015; Dauzart et al. 2016). Furthermore, we hypothesized that genotypic variation correlates with plastic and varied responses to gravity. This concept is important because, in the long term, we want to study more closely how various combinations of symbioses and genotypes will affect gravity response. The *absence* of genotype-specific morphological variation in response to different gravity conditions would not eliminate the possibility of interaction effects manifesting when symbioses are considered. However, we suspect that the more variation we observe here across genotypes, the more likely it will be a confounding factor in subsequent experiments, especially those including symbioses as an additional variable. Previous research has shown the limitations of using only a few genotypes in most space biology studies (Vandenbrink and Kiss 2016), and the broader scope of this work is to reassess how we perform plant space biology experiments, and how much we can extrapolate from results gleaned from only one or two genotypes.

This research also considers the mechanisms behind phenotypic plasticity, which is pertinent to all plant studies, as plasticity is especially adaptive in sessile organisms (Van Kleunen and Fischer 2005). It is well documented, and should be noted, that epigenetics is thought to be a strong component in the mechanism of phenotypic plasticity (Johannes et al. 2008; Bossdorf and Zhang 2011; Herrera and Bazaga 2013; Duncan et al. 2014; Kooke et al. 2015). However, there are also studies suggesting that genetic differences within populations, between individuals of the same species, can also play a significant role in plastic responses (Bergelson and Roux 2010). How particular genotypes change their phenotype in different (in this case gravitational) environments is known as a genotype–environment interaction (G×E). In this report we studied whether

genotypes within the *Medicago truncatula* species behave differently from one another under clinorotation, and if some variants are more plastic than others, exhibiting not just a different response, but a more or less extreme response.

MATERIALS AND METHODS

PLANT MATERIAL AND GENOTYPE SELECTION

We studied *Medicago truncatula,* which is considered a model legume system. Genotype selection was based primarily on the 262 *M. truncatula* accessions from the Mt 4.0 SNP GWAS dataset, the latest available from the *Medicago* Hapmap project at the time our experiment began (Table 1, http://www.medicagohapmap.org/downloads/mt40). Each accession is designated an alias beginning with "HM" and followed by a 3-digit number. Twenty-six accessions (HM001 - HM016, HM019, HM021, HM023 - HM028, and HM101) had been sequenced to 15X average aligned depth. The remaining accessions were sequenced to an average aligned depth of ~6X (Branca et al. 2011; Stanton-Geddes et al. 2013). In addition, some germplasm from outside of this dataset were cultivated, due to availability and cultivation success (Table 1). In general, germination rates were low (~40%), and mortality during the first 10 days was high (~60%). However, once seedlings reached 15-days old they were extremely robust, and very few individuals were lost for the remainder of the experiment.

Table 1. Accession designations of *M. truncatula* in Mt 4.0 SNP GWAS dataset (*Medicago* Hapmap ID).

* Accessions which were successfully cultivated to full term, with at least one duplicate.
** Accessions with at least one duplicate for both gravity treatments.
In italics: HMID accessions from outside the Mt 4.0 SNP GWAS dataset that were also included in this study.

HM001 **	HM044 *	HM082	HM130	HM177	HM217	HM270	*HM103* *
HM002 **	HM045	HM083	HM131	HM178	HM218	HM271 *	*HM140* *
HM003 **	HM046 **	HM084	HM133	HM179	HM219	HM276	*HM204* *
HM004	HM047 *	HM085	HM134	HM180	HM220	HM277	*HM255* *
HM005 **	HM048 *	HM086	HM135	HM181	HM221	HM278	*HM257**
HM006 **	HM049 *	HM087	HM138 *	HM182 **	HM222	HM279	*HM258* *
HM007 **	HM050 *	HM088	HM139	HM183 **	HM223	HM280	*HM263* *
HM008 **	HM051 *	HM089	HM141	HM184 *	HM224	HM287	*HM264* *
HM009 **	HM052 **	HM091 *	HM143	HM185	HM225	HM288 *	*HM272* *
HM010 **	HM053	HM092	HM145 *	HM186	HM226	HM289	*HM274* *
HM011 *	HM054	HM093	HM146 *	HM187	HM227	HM290 *	*HM318**
HM012 *	HM055 *	HM095	HM147	HM188	HM228	HM293 *	*HM324**
HM013 *	HM056 **	HM096	HM148	HM189	HM229	HM294	*HM325**
HM014 **	HM057 *	HM097	HM149	HM190	HM230	HM295 *	*HM326**
HM015 **	HM058 **	HM098	HM150 *	HM191	HM231	HM296 *	*HM330**
HM016 **	HM059	HM099 *	HM151	HM192	HM232	HM297 *	*HM331**
HM019 *	HM060 **	HM101	HM152 **	HM193	HM233	HM298 *	*HM333**
HM020-I	HM061 *	HM105 **	HM153	HM194	HM234	HM299 *	*HM334**
HM021	HM062	HM106	HM154 *	HM195	HM235	HM300	*HM337**
HM023	HM063	HM107	HM155	HM196	HM236	HM301 *	*HM338**
HM024	HM064	HM108 *	HM156	HM197 *	HM237	HM302 *	
HM025	HM065 **	HM109	HM157	HM198	HM238	HM304	
HM026 *	HM066 *	HM111	HM159	HM199	HM239	HM305 *	
HM027 **	HM067	HM112	HM160	HM200	HM240	HM306 *	
HM028 **	HM068	HM114	HM161	HM201 *	HM241	HM307	
HM031	HM069	HM115	HM162	HM202	HM242	HM308 *	
HM032 **	HM070	HM117	HM163	HM203 *	HM243	HM309 *	
HM033 **	HM071 *	HM118	HM164	HM205	HM244	HM310	
HM034 **	HM072	HM119	HM165	HM206 *	HM245	HM311 *	
HM035 **	HM073	HM120	HM166	HM207	HM253	HM312	
HM036 **	HM074	HM121	HM167	HM208	HM256	HM313	
HM037 *	HM075	HM122	HM168	HM209	HM259 *	HM314	
HM038 *	HM076	HM124	HM169	HM210	HM260 *	HM315	
HM039 **	HM077	HM125	HM170 *	HM211	HM262	HM316	
HM040 **	HM078	HM126	HM172	HM212	HM266 *		
HM041 *	HM079	HM127	HM173	HM213	HM267 *		
HM042 **	HM080	HM128	HM175	HM214	HM268 *		
HM043 *	HM081	HM129	HM176	HM215	HM269 *		

GERMINATION OF SEEDS

Seeds of each genotype were scarified using > 99.7% (v/v) sulfuric acid for 15 min, vortexed briefly, then rinsed 4X with deionized (DI) water using 5 inversions between each decanting. Seeds were then surface-sterilized in 30% (v/v) bleach for 10 min and again rinsed 4X in DI water. Surface-sterilized seeds were placed in ~7 mL fresh DI water and shaken at 1000 rpm for 4 hours, then germinated in upside down, sterile, Parafilm-sealed 10-cm Petri dishes for 36-h. Seedlings were sown into containers termed "Cone-tainers" (Stuewe and Sons, Oregon, USA) plugged with cotton wool, and half filled with an autoclaved sand:pebble 2:1 mixture, saturated with DI water, and topped with autoclaved sand (Fig. 1). Sown seedlings were sprayed 10 times with a 1/8 strength Hoagland's nutrient solution (Table 2) then grown under a 16:8 light-dark cycle at 20-22°C with a light intensity of ~150 μmol·m^{-2}. Seedlings were watered as needed for 15-days via a spray bottle of 1/8 strength Hoagland's nutrient solution. The *Medicago truncatula* Handbook (Barker et al. 2006; Garcia et al. 2006) was used in the development of these procedures.

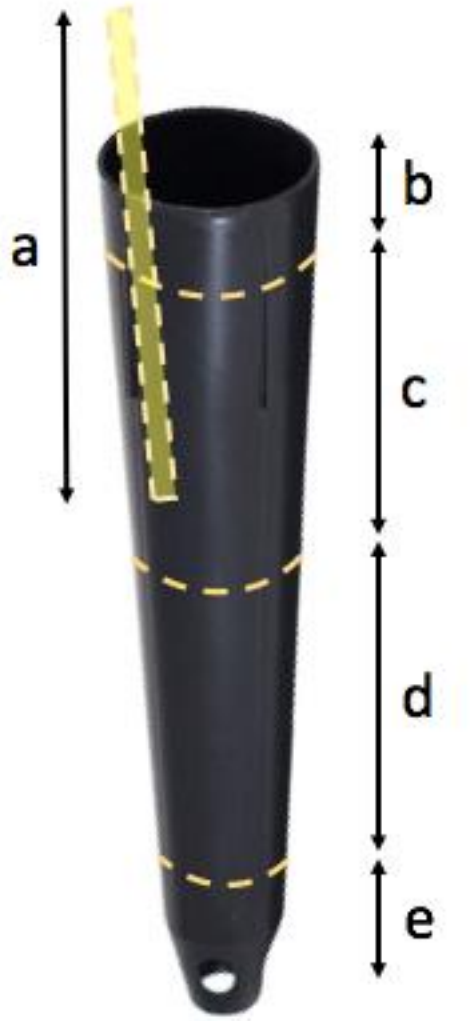


Figure 1. a) 6.35 mm diameter plastic tubing for watering b) Foam plug c) Sand d) Sand:pebbles 2:1 ratio e) Cotton wool.

Table 2. Hoagland's nutrient solution used in our studies. A concentrated stock solution was made for each component and sterilized via autoclave [with the exception of $Ca(NO_3)_2$ which was sterile filtered]. From these stocks, an aliquot was added to a container and brought to volume at 1L with DI water to make a 1X Hoagland's nutrient solution. This 1X solution was diluted further (1/8 strength) for use in our experiments.

Component	Individual stock solution concentration	Volume of stock solution aliquot to add to 1 L to make 1X Hoagland's nutrient solution
KNO_3	2 M	2.5 mL
$Ca(NO_3)_2$	0.5 M	10 mL
EDTA-Fe^{+2}	0.04 M	1.5 mL
$MgSO_4$	2 M	1 mL
KH_2PO_4 (pH to 6.0)	1 M	0.5 mL
H_3BO_3	46 µM	1 mL
$MnCl_2$	9.1 µM	1 mL
$ZnSO_4$	0.7 µM	1 mL
$CuSO_4$	2 µM	1 mL
Na_2MoO_4	0.5 µM	1 mL
1M NH_4NO_3	1 M	1 mL

TRANSPLANTING OF SEEDLINGS

At day 15, the healthy seedlings were removed from the Cone-tainers for initial growth parameter measurements and transplanting. During transplanting, seedlings were rinsed in DI water, photographed, measured for root and shoot length, weighed, then transplanted back into Cone-tainers and watered thoroughly with DI water. A 9-cm long plastic tube (6.35 mm diameter) was planted with them, covered with a thin layer of dry sand, then secured in place by wrapping the shoot and tubing with a foam plug (Fly Plugs 89140-960, VWR) (Fig. 1). At this point, each plant was transferred to its gravity treatment – either returned to a vertical stand, or placed horizontally onto a ~1 rpm clinostat (Dauzart et al. 2016) (Fig. 2). Clinostats rotated 24-hours a day for the duration of the experiment. All plants were watered via a 10-mL syringe through their tube every other day with either 8 mL DI water or 8 mL of 1/8 strength Hoagland's nutrient solution, alternately.

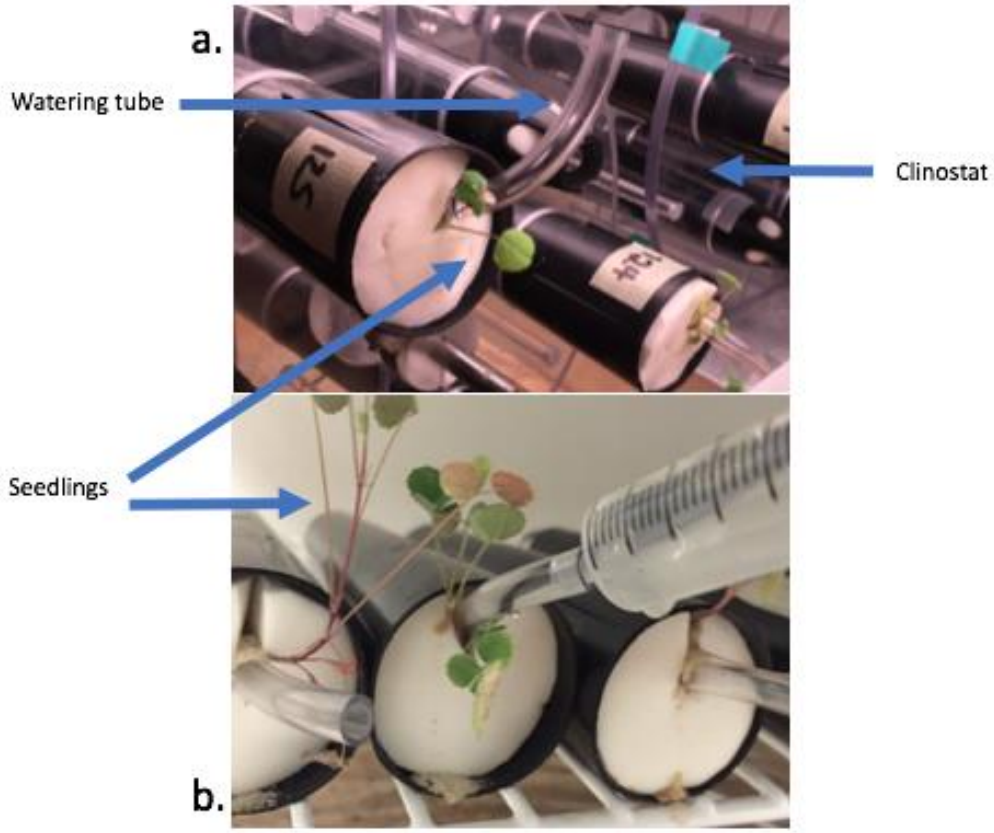


Figure 2. Photographs of *Medicago truncatula* seedlings growing in Cone-tainers, sealed with foam plugs and watering tubes.

a) Seedlings rotating on the clinostat.
b) Seedlings being watered in Cone-tainers via a syringe.

HARVEST AND DATA COLLECTION

Twenty-one days after transplanting (36-days post germination), the plants were removed from their Cone-tainers, rinsed with DI water, and photographed. They were each measured for root and shoot growth, as well as total fresh biomass accumulation. Image analysis was performed using GiA Roots (Galkovskyi et al. 2012) on all root network images. All images were taken from a fixed distance, and included a 10-mm reference for scale. After assessing accuracy of scale in seedling images, they were cropped or edited to remove the scale, as well as any visible shoots and any obvious background noise (Fig. 3), allowing Gia Roots to assess only the root network, with a minimum of interference. Outputs from image analyses were in units of pixels. Using the average conversion factor collected from ten images at random, all pixel units were converted to mm. Parameters measured and calculated are noted in Table 3. Relative growth rates (RGRs) are measurements of growth rate relative to size, sometimes called the exponential or continuous growth rate. RGRs were calculated using the logarithmic equation RGR= (ln W2 - ln W1)/(t2 - t1), with t1 the date of transplant, and t2 the harvest/data collection date. W denotes the measurement of growth recorded at either time point (t1 or t2). Shoot and root RGRs are based on length, and the simple RGR response is based on mass.

The goal of this study was to examine how *M. truncatula* growth varied among genotypes and in response to clinorotation – a proxy to simulate microgravity conditions for plants, as compared to plants grown vertically at 1-*g*. Two-way ANOVAs were performed for all measured morphological response variables listed and defined in Table 3, using gravity treatment (vertically grown or clinorotated), genotype (HapMap ID or HMID), and the gravity x genotype interaction.

All of the p-values for these tests are shown in Table 4. All calculations and statistical analyses were computed using R (R Core Team) (See Appendix). Any data point for which there was not at least one duplicate (from both the same genotype and the same gravity treatment) was discarded, so that all the data points used for statistical analyses were averages across replicates. The number of individuals that passed this threshold was n=451.

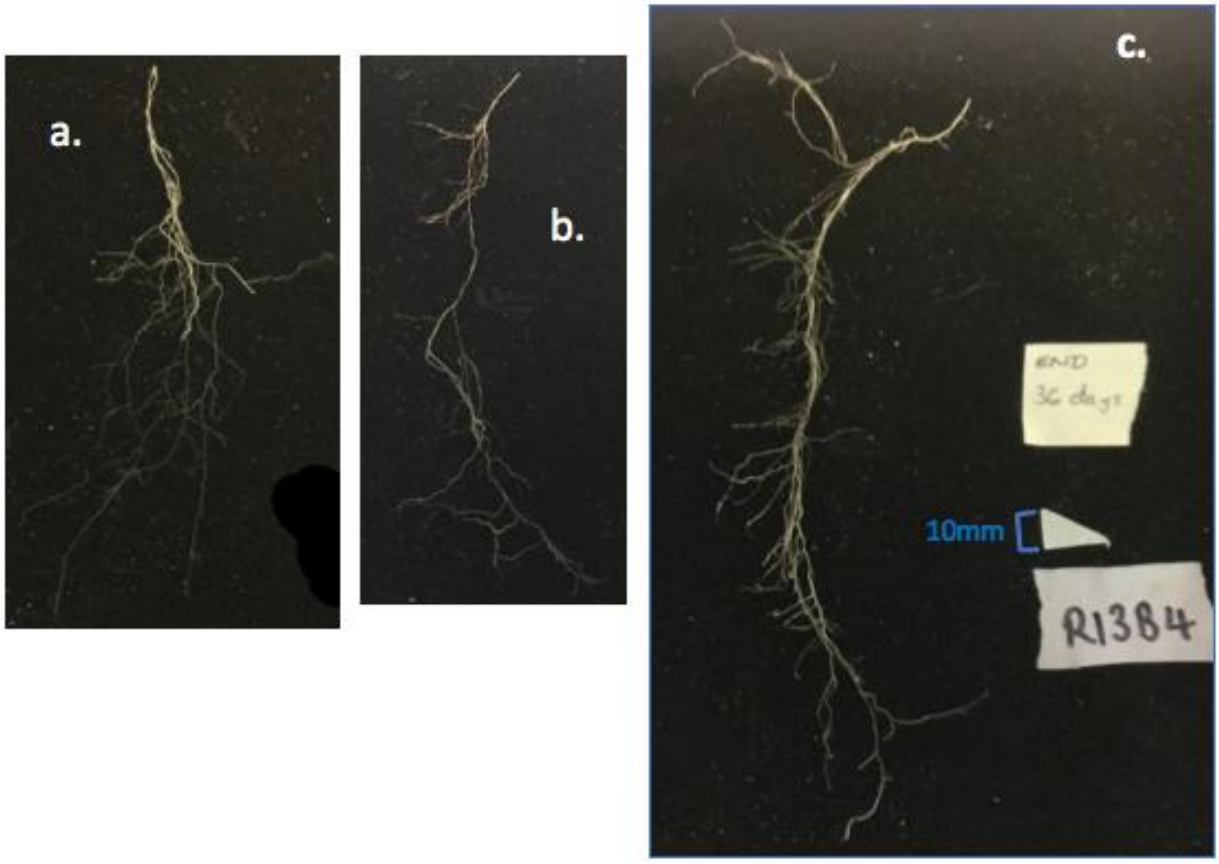


Figure 3.
Photographs taken at harvest (36-days old) a) Clinorotated HM001 ready for image analysis b) Vertically grown HM001 ready for image analysis c) A raw image of a different plant, before clean-up for analysis. All images are the same scale. After a mm to pixel ratio was established for all images, extraneous labeling was removed from images so as to derive the most accurate results from GiA root image analysis software.

Table 3. Definitions of all the growth and development parameters used in these studies.

Response Variable	Definition
Root Length (mm)	Length from farthest root tip to origin of primary root.
Shoot Length (mm)	Length of aerial tissue from origin to tip.
Root Fresh Biomass (g)	Mass of the roots taken immediately at harvest. Dabbed dry with a Kim wipe, then weighed.
Shoot Fresh Biomass (g)	Mass of the aerial tissue taken immediately at harvest. Dabbed dry with a Kim wipe, then weighed.
Root Dry Mass (g)	Mass of the roots after tissue had been desiccated.
Shoot Dry Mass (g)	Desiccated aerial tissue mass.
Total Fresh Biomass (g)	Sum of Root Fresh Biomass and Shoot Fresh Biomass
RGR (g/g/day)	Relative growth rate from the time seedling was transplanted on its gravity treatment, to the time of its harvest (based on fresh mass of plant).
Shoot RGR (mm/mm/day)	Relative growth rate of only the aerial shoot tissue from the time seedling was transplanted on its gravity treatment, to the time of its harvest (based on length of shoots).
Root RGR (mm/mm/day)	Relative growth rate of only the root tissue from the time seedling was transplanted on its gravity treatment, to the time of its harvest (based on length of roots).
SRL (mm/g)	Specific Root Length: the ratio of root length to dry root mass.
Average Root Width (mm)	The mean value of the root width estimation computed for all pixels of the medial axis of the entire root system.
Network Bushiness	The ratio of the maximum to the median number of roots.
Maximum Number of Roots	After sorting the number of roots crossing a horizontal line from smallest to largest, the maximum number is considered to be the 84th-percentile value (one standard deviation).
Median Number of Roots	Result of a vertical line sweep in which the number of roots that crossed a horizontal line was estimated, and then the median of all values for the extent of the network was calculated.
Network Area (mm^2)	Number of network pixels in the image of the root system.
Network Perimeter (mm)	Total number of pixels connected to a background pixel (using an 8-nearest neighbor neighborhood).
Network Surface Area (mm^2)	The sum of the local surface area at each pixel of the network skeleton, as approximated by a tubular shape whose radius was estimated from the image.
Network Length (mm)	Total number of pixels in the network skeleton.
Network Volume (mm^3)	Sum of the local volume at each pixel of the network skeleton, as approximated by a tubular shape whose radius was estimated from the image.

Table 4. P-values from 2-way ANOVAs used to analyze the growth and development data. P-values denote degree of correlation between the response variable and either the genotype or gravity treatment, with the final column showing p-values for interaction effects between genotype and gravity for each growth parameter. Shading intensity refers to degree of statistical significance, with a p-value threshold of 0.05. Please refer to legend.

Growth parameters (response variables)	p-values		
	Genotype	Gravity	Interaction
Root length (mm)	1.97E-05	2.03E-02	2.05E-01
Shoot length (mm)	6.27E-41	1.52E-07	1.38E-05
Root Fresh Biomass (ln(g))	1.07E-41	8.43E-02	8.13E-01
Shoot Fresh Biomass (g)	7.98E-34	6.67E-07	1.77E-01
Root Dry Mass (g)	5.15E-10	1.01E-02	6.20E-01
Shoot Dry Mass (g)	6.13E-35	3.82E-03	4.05E-03
Total Fresh Biomass (g)	2.37E-49	8.17E-01	7.08E-01
RGR (g/g/day)	4.44E-33	4.82E-01	3.22E-01
Shoot RGR (mm/mm/day)	1.38E-36	2.27E-06	1.96E-05
Root RGR (mm/mm/day)	4.18E-26	8.27E-01	1.22E-01
SRL (mm/g)	4.52E-14	3.03E-02	4.69E-01
Average Root Width (mm)	1.68E-14	2.51E-01	5.54E-02
Network Bushiness	3.17E-08	6.67E-02	6.49E-02
Maximum Number of Roots	1.06E-12	6.02E-03	4.18E-02
Median Number of Roots	1.84E-14	6.74E-03	2.09E-02
Network Area (mm^2)	1.05E-09	1.32E-03	4.46E-02
Network Perimeter (mm)	8.36E-11	1.96E-03	3.57E-02
Network Surface Area (mm^2)	5.13E-10	1.57E-03	4.67E-02
Network Length (mm)	2.47E-11	2.53E-03	3.88E-02
Network Volume (mm^3)	2.52E-09	1.93E-03	7.93E-02

Legend:	$p > 0.05$	$p < 0.05$	$p < 0.005$	$p < 0.0005$

RESULTS

In the first part of our study, we considered the effects of gravity on growth parameters across the entire population of *M. truncatula* (temporarily disregarding genotype). Statistically significant and clear differential responses to simulated microgravity were seen in terms of the following parameters: the fresh biomass of shoots (Fig. 4a, $p < 0.0005$), the dry mass of roots (Fig. 4b, $p \sim 0.01$), and the Specific Root Length (SRL) (Fig. 4c, $p \sim 0.03$) (Table 4). Differences in gravity response also occurred in plant root length ($p \sim 0.02$), but the standard error of each gravity group overlapped, causing us to disregard this ambiguous result in spite of its statistical significance in the ANOVA.

However, much more prevalent than a clear main gravity response for the entire population was the presence of interaction effects i.e., the effect of gravity on an individual's growth morphology *depended* on genotype. These interaction effects are illustrated in reaction norm graphs in Figure 5, which show how for some genotypes the effect of gravity was to increase the response phenotype, while for others there was a clear decrease. The strongest interaction effects

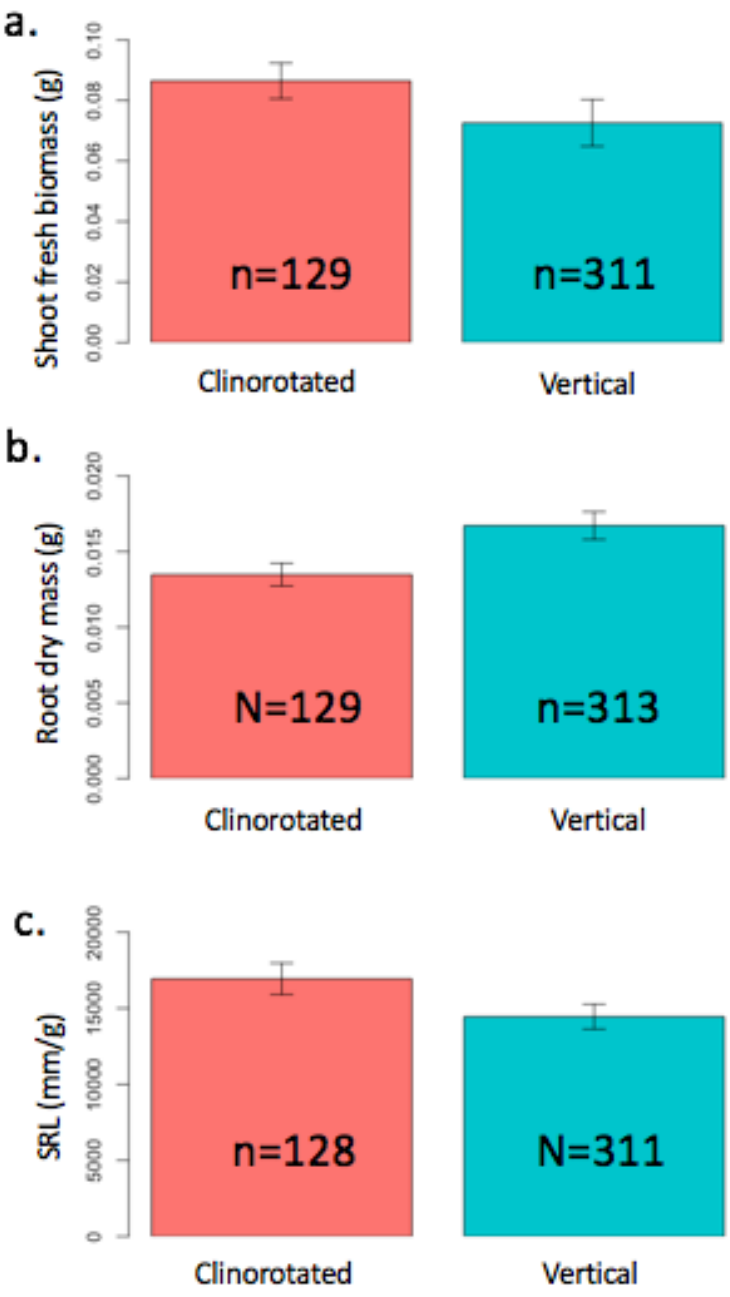


Figure 4. The effects of gravity treatment on growth parameters of the entire population of *Medicago truncatula* examined. Error bars signify +/- 1 SE.

a) biomass of fresh shoots
b) dried mass of the roots
c) SRL (Specific Root Length)

were seen in some of the shoot-related phenotypes. Shoot length and shoot RGR were both extremely affected ($p < 0.0005$, Fig. 5a,b), and shoot dry mass was also affected strongly by the interaction between gravity and genotype (Fig. 5c, $p < 0.005$). Other less potent, though still significant, interaction effects between genotype and gravity treatment played a role in several of the root phenotypes, including network area, network perimeter, maximum number of roots, and network length, all with $p \sim 0.04$ (Fig. 5d,e,f,g), as well as network surface area (Fig. 5h, $p < 0.05$), and median number of roots ($p \sim 0.02$).

Figure 5. Interaction effects of gravity treatment with genotype on growth parameters of *M. truncatula*. Replicates of each genotype for each gravity treatment ranged from 2-6 individuals.

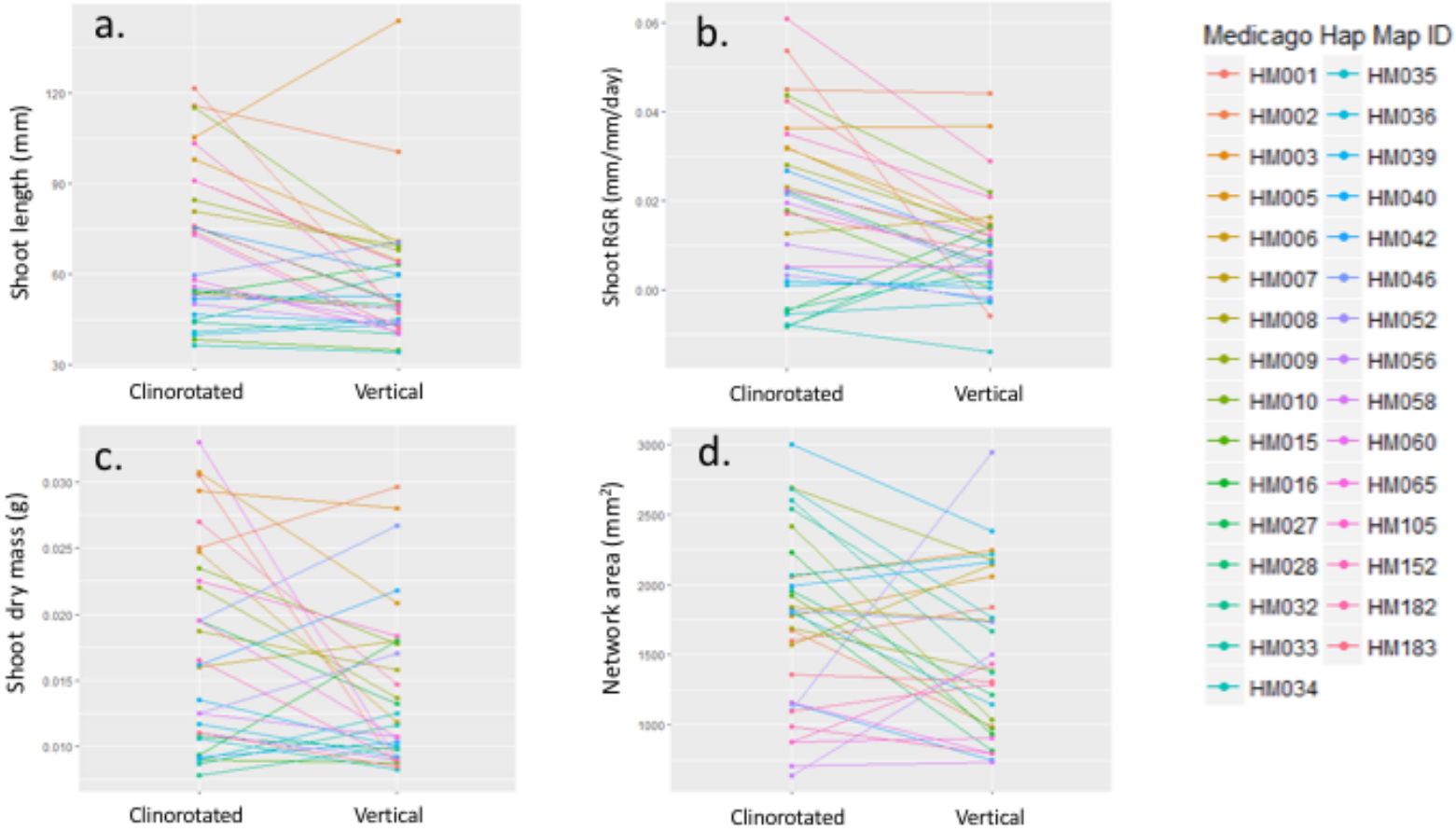


Figure 5. Interaction effects of gravity treatment with genotype on growth parameters of *M. truncatula*. Replicates of each genotype for each gravity treatment ranged from 2-6 individuals.

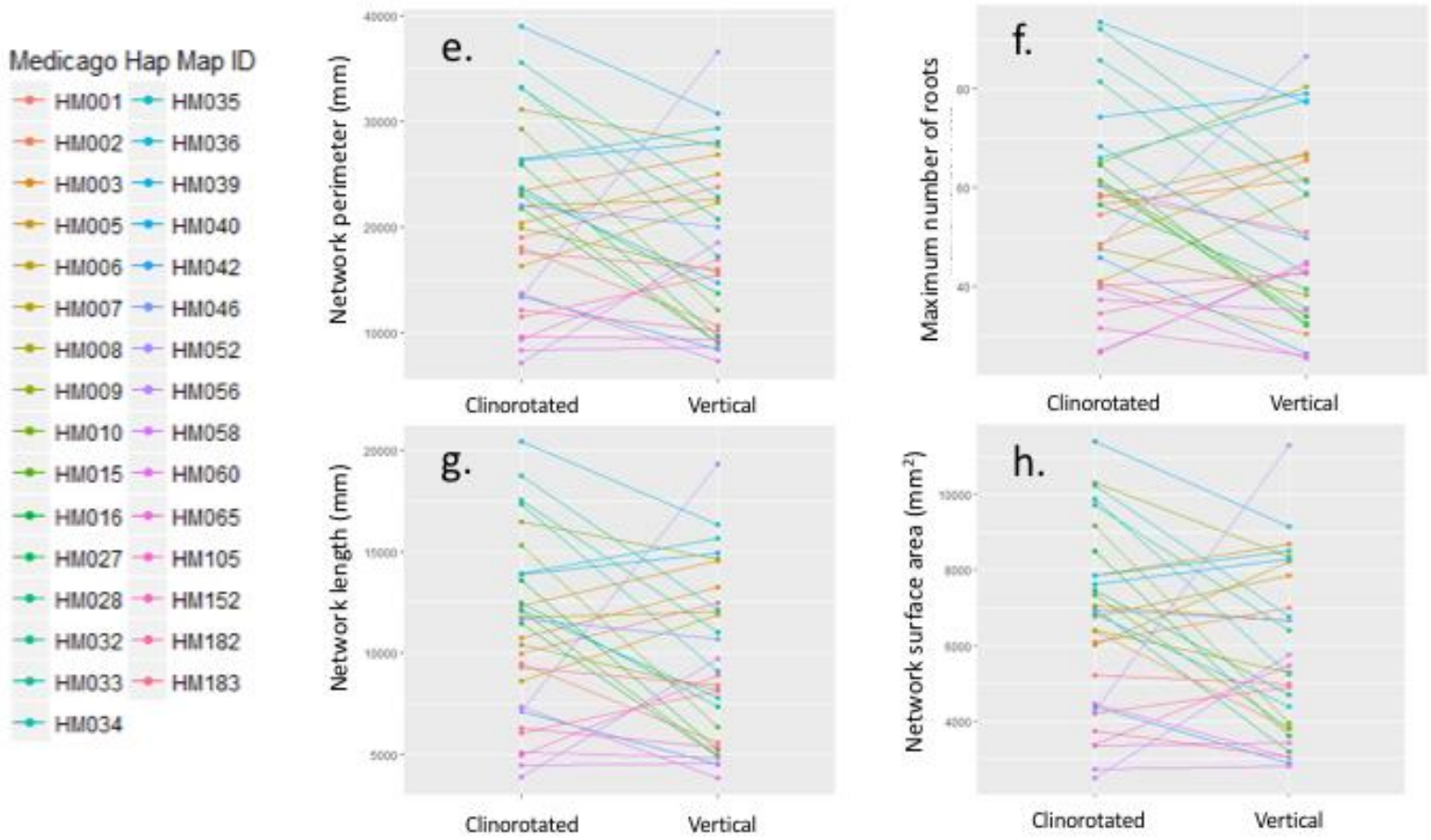
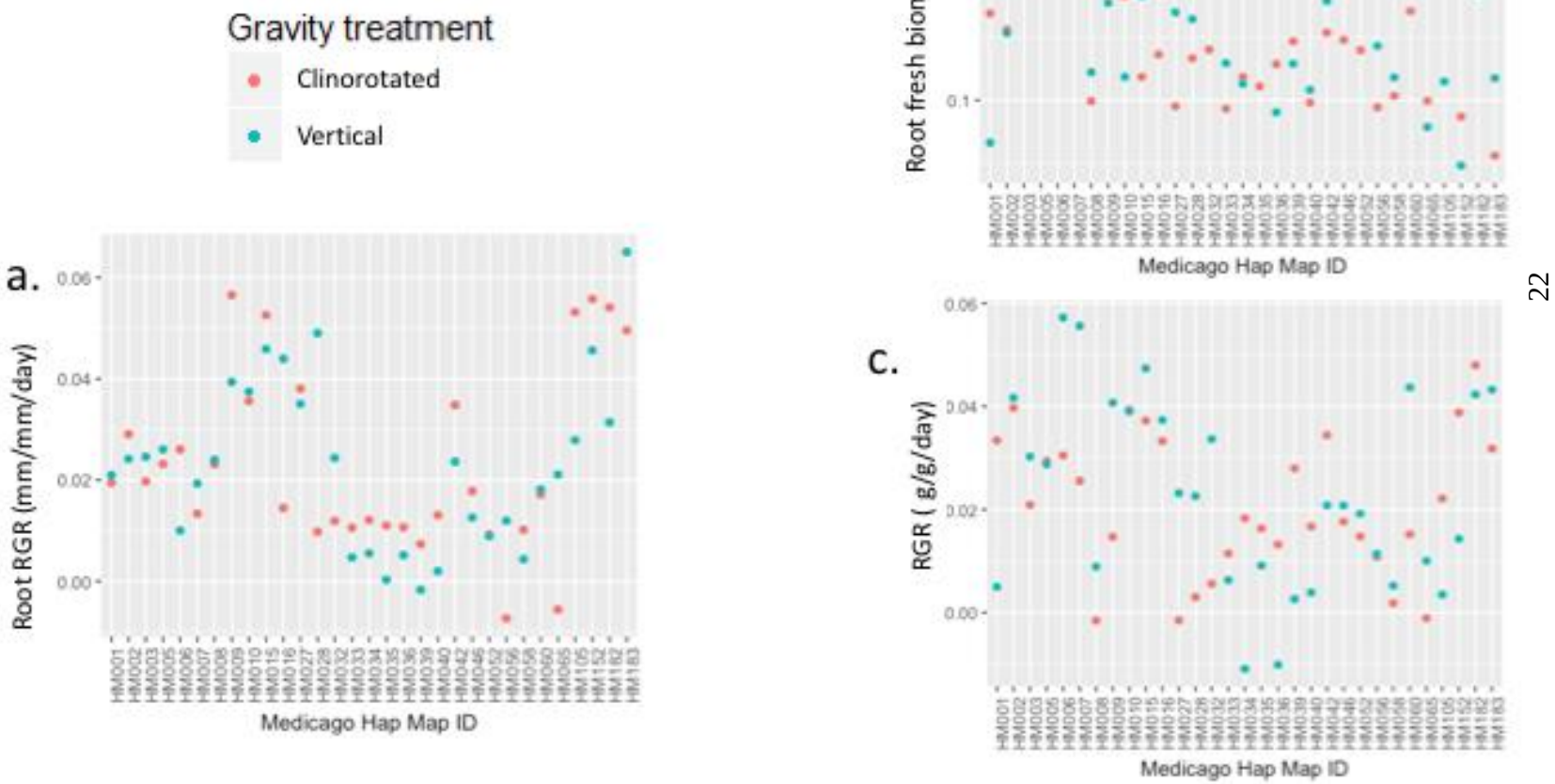


Figure 6. Phenotypic responses that were not significantly affected by gravity, but were affected by genotypic variation. Replicates of each genotype for each gravity treatment ranged from 2-6 individuals.



Genotype affected all measured response variables (Table 3). Figure 6 shows some examples of morphological traits that exhibited *only* this main effect. These examples showed no differential responses to gravity treatment when looked at as a whole, but strong correlations between growth and genotype. Several root-related phenotypes fell into this category, including root RGR and root fresh biomass (Fig. 6a,b, $p <<< 0.0005$), along with network bushiness ($p < 0.0005$) and average root width ($p << 0.0005$). Additionally, both RGR based on mass (Fig. 6c), and the total fresh biomass, showed a morphological response to genotype alone ($p <<< 0.0005$) and not to the gravity treatment.

Collectively, these results show that not only does genotype play a significant role in *M. truncatula* morphology, it frequently affects the plant's response to gravity treatment, influencing *both* the magnitude and direction of the gravity response.

DISCUSSION

Based on these studies, we make two overarching conclusions. First, that genotypic variation in *M. truncatula* significantly affected all measured response variables (Table 3). Second, that within-species genotype variation caused a plastic (G×E) interaction with the gravity treatment, making the phenotypic response to simulated microgravity differ among genotypes. These results suggest that we must be cautious in our interpretations of gravity-based experiments that do not take genotype into account (Vandenbrink and Kiss 2016).

A vast literature of plant space biology research has been published, and considered in a number of review articles (Ferl et al. 2002; Kiss 2013; Wolverton and Kiss 2009). We must begin to assess how those findings can be extrapolated and utilized for future scientific inquiry and space exploration. It is also critical that we acknowledge the limitations of our research and fill in any gaps. For example, when we perform reduced gravity experiments on *Arabidopsis thalania*, what exactly does that mean? There are many genotypes of *A. thalania* – over 1100 ecotypes sequenced already (http://1001genomes.org/), and researchers are already working on how to interpret and use these data (Kiss 2000, Gan et al. 2011). While occasional studies are genotype-specific, many

more are not. There are inherent difficulties involved in ameliorating these past approaches. For example, it would be untenable to use hundreds or thousands of genetic variants within a species for all experiments! A more practical approach to addressing this issue would be to gather data from a large foundation of ground-based experiments delineating which loci are correlated with different phenotypic outcomes, within and across species, and use those data along with NGS analyses of the variants being tested as a lens through which to interpret subsequent results. GWAS seeks to reconnect traits back to their underlying genetics (Korte and Farlow 2013), and the more we elucidate those connections, the better we can statistically control for them in future studies.

We acknowledge the scale of this task. Beyond aligning morphological outcomes with SNP, INDEL, and CNV data, any serious model used to assess future plant space biology data would also have to consider epigenetics, and even epistatic effects. This prospect sounds daunting, but there may well be patterns and themes in these data. As plants have all evolved under an unchanging gravity vector since the origins of plant life on this planet, it seems reasonable to assume that they have not evolved specific plastic mechanisms for tolerating gravity stress. However, we know that plants do, in spite of this, exhibit gravitropic responses to varying extents, and we know some of the underlying mechanisms involved (Kiss 2000). It could be that the loci strongly correlated with gravitropic responses are random, but it seems more likely that they will be linked in some fashion. This knowledge, along with the ever-increasing speed and accuracy of NGS platforms and bioinformatics as a whole, should enable us to account for plasticity in phenotypic responses across genotypes in the future. These data are the next step in precise and effective genetic engineering of plants, for optimal vigor and productivity in future space travel.

CONCLUSION

Further studies are needed to expand and confirm our results. The next step would be to use the *Medicago* Hapmap resources to perform a Genome-Wide Association Study (GWAS). This would enable us to map trait loci and begin to understand how individual haplotypes correlate to phenotypic plasticity and responses to altered gravity states. These studies will also have to be replicated, in some form, in true microgravity, as clinorotation is a useful but limited microgravity simulator for plant experiments.

Of particular interest to space biology would be to then explore how *M. truncatula* symbioses with Plant Growth Promoting Rhizobia (PGPR) and Arbuscular Mycorrhizal Fungi (AMF) affect plant genotypes differently, with a view to deeply understanding these connections and, ultimately, using these interactions to improve and refine crop cultivars and growing conditions for space explorations. Extended-duration space travel and manned-missions beyond Lower Earth Orbit (LEO) will require reliable and sustainable Advanced Life Support (ALS) systems. Manipulation of genotype, in combination with *M. truncatula*'s symbiotic relationships with rhizobacteria and AMF, will be important for optimizing legume productivity for cultivation on long-term space missions.

LITERATURE CITED

Aarrouf, J., Demandre, C., Darbelley, N., Villard, C., Perbal, G.: Development of the primary root and mobilisation of reserves in etiolated seedlings of *Brassica napus* grown on a slowly rotating clinostat. J. Plant. Physiol. **160**(4), 409–413 (2003).

Ahemad, M., Kibret, M.: Mechanisms and applications of plant growth promoting rhizobacteria: Current perspective. J. King Saud Univ. Sci. **26**(1), 1–20 (2014).

Ansdell, M., Ehrenfreund, P., McKay, C.: Stepping stones toward global space exploration. Acta Astronaut. **68**(11-12), 2098–2113 (2011).

Babuscia, A., Cheung, K.-M., Divsalar, D., Lee, C.: Development of cooperative communication techniques for a network of small satellites and CubeSats in deep space: The SOLARA/SARA test case. Acta Astronaut. **115**(C), 349–355 (2015).

Barker, David G., Pfaff, T., Moreau, D.: Choice of substrates and growth conditions. In: Medicago truncatula Handbook. pp. 1–26 (2006).

Bergelson, J., Roux, F.: Towards identifying genes underlying ecologically relevant traits in *Arabidopsis thaliana*. Nat. Rev. Genet. **11**(12), 867–879 (2010).

Blancaflor, E.B.: Regulation of plant gravity sensing and signaling by the actin cytoskeleton. Am. J. Bot. **100**(1), 143–152 (2013).

Bossdorf, O., Zhang, Y.: A truly ecological epigenetics study. Mol. Ecol. **20**(8), 1572–1574 (2011).

Brachi, B., Morris, G.P., Borevity, J.O.: Genome-wide association studies in plants: the missing heritability is in the field. Genome Biol. **12**(232), 1-8 (2011).

Branca, A., Paape, T.D., Zhou, P., Briskine, R., Farmer, A.D., Mudge, J., Bharti, A.K., Woodward, J.E., May, G.D., Gentzbittel, L., Ben, C., Denny, R., Sadowsky, M.J., Ronfort, J., Bataillon,

T., Young, N.D., Tiffin, P.: Whole-genome nucleotide diversity, recombination, and linkage disequilibrium in the model legume *Medicago truncatula*. Proc. Natl. Acad. Sci. U.S.A. **108**(42), E864–70 (2011).

Braun, M., Limbach, C.: Rhizoids and protonemata of characean algae: model cells for research on polarized growth and plant gravity sensing. Protoplasma. **229**(2-4), 133–142 (2006).

Brungs, S., Petrat, G., Wiesche, von, M., Anken, R., Kolanus, W., Hemmersbach, R.: Simulating parabolic flight like *g*-profiles on ground - a combination of centrifuge and clinostat. Microgravity Sci. Tech. 28(3). 231-235 (2016).

Choudhury, A., Hazelhurst, S., Meintjes, A., Achinike-Oduaran, O., Aron, S., Gamieldien, J., Jalali Sefid Dashti, M., Mulder, N., Tiffin, N., Ramsay, M.: Population-specific common SNPs reflect demographic histories and highlight regions of genomic plasticity with functional relevance. BMC Genomics. **15**(1), 437–42 (2014).

Ciaralli, S., Coletti, M., Gabriel, S.B.: Performance and lifetime testing of a pulsed plasma thruster for Cubesat applications. Aerosp. Sci. Technol. **47**(C), 291–298 (2015).

Ciaralli, S., Coletti, M., Gabriel, S.B.: Results of the qualification test campaign of a Pulsed Plasma Thruster for Cubesat Propulsion (PPTCUP). Acta Astronaut. **121**(C), 314–322 (2016).

Dauzart, A.J.C., Vandenbrink, J.P., Kiss, J.Z.: The effects of clinorotation on the host plant, *Medicago truncatula*, and its microbial symbionts. Front. Astron. Space Sci. **3**(3), 1–10 (2016).

Duncan, E.J., Gluckman, P.D., Dearden, P.K.: Epigenetics, plasticity, and evolution: How do we link epigenetic change to phenotype? J. Exp. Zool. (Mol. Dev. Evol.). **322**(B), 208–220 (2014).

Ekblom, R., Galindo, J.: Applications of next generation sequencing in molecular ecology of non-model organisms. Heredity. **107**(1), 1–15 (2010).

Epstein, B., Branca, A., Mudge, J., Bharti, A.K., Briskine, R., Farmer, A.D., Sugawara, M., Young, N.D., Sadowsky, M.J., Tiffin, P.: Population genomics of the facultatively mutualistic bacteria *Sinorhizobium meliloti* and *S. medicae.* PLoS Genet. **8**(8), e1002868–10 (2012).

Escobar, E., Diaz, M., Zagal, J.C.: Evolutionary design of a satellite thermal control system: Real experiments for a CubeSat mission. Appl. Therm. Eng. 1–11 (2016). htttp://dx.doi.org/10.1016/j.applthermaleng.2016.03.024

Ferl, R., Wheeler, R., Levine, H.G., Paul, A.L.: Plants in space. Curr. Opin. Plant Biol. **5,** 258-263 (2002).

Galkovskyi, T., Mileyko, Y., Bucksch, A., Moore, B., Symonova, O., Price, C.A., Topp, C.N., Iyer-Pascuzzi, A.S., Zurek, P.R., Fang, S., Harer, J., Benfey, P.N., Weitz, J.S.: GiA Roots: software for the high throughput analysis of plant root system architecture. BMC Plant Biol. **12**(1), 1–12 (2012).

Gan, X., Stegle, O., Behr, J., Steffen, J.G., Drewe, P., Hildebrand, K.L., Lyngsoe, R., Schultheiss, S.J., Osborne, E.J., Sreedharan, V.T., Kahles, A., Bohnert, R., Jean, G., Derwent, P., Kersey, P., Belfield, E.J., Harberd, N.P., Kemen, E., Toomajian, C., Kover, P.X., Clark, R.M., Rätsch, G., Mott, R.: Multiple reference genomes and transcriptomes for *Arabidopsis thaliana.* Nature. **477**, 419–423 (2011).

Ganal, M.W., Altmann, T., Röder, M.S.: SNP identification in crop plants. Curr. Opin. Plant Biol. **12**(2), 211–217 (2009).

Garcia, J., Barker, D.G., Journet, E.-P.: Seed storage and germination. In: *Medicago truncatula*

Handbook. pp. 1–9 (2006).

Graham, P.H.: Legumes: importance and constraints to greater use. Plant Physiol. **131**(3), 872–877 (2003).

Herranz, R., Anken, R., Boonstra, J., Braun, M., Christianen, P.C.M., de Geest, M., Hauslage, J., Hilbig, R., Hill, R.J.A., Lebert, M., Medina, F.J., Vagt, N., Ullrich, O., van Loon, J.J.W.A., Hemmersbach, R.: Ground-based facilities for simulation of microgravity: organism-specific recommendations for their use, and recommended terminology. Astrobiology. **13**(1), 1–17 (2013).

Herrera, C.M., Bazaga, P.: Epigenetic correlates of plant phenotypic plasticity: DNA methylation differs between prickly and nonprickly leaves in heterophyllous *Ilex aquifolium* (Aquifoliaceae) trees. Bot. J. Linn. Soc. **171**, 441–452 (2013).

Hogekamp, C., Küster, H.: A roadmap of cell-type specific gene expression during sequential stages of the arbuscular mycorrhiza symbiosis. BMC Genomics. **14**(306), 1-18 (2013).

Hoshino, T., Miyamoto, K., Ueda, J.: Gravity-controlled asymmetrical transport of auxin regulates a gravitropic response in the early growth stage of etiolated pea (*Pisum sativum*) epicotyls: studies using simulated microgravity conditions on a three-dimensional clinostat and using an agravitropic mutant, *ageotropum*. J. Plant Res. **120**(5), 619–628 (2007).

Hou, G., Mohamalawari, D.R., Blancaflor, E.B.: Enhanced gravitropism of roots with a disrupted cap actin cytoskeleton. Plant Physiol. **131**(3), 1360–1373 (2003).

Johannes, F., Colot, V., Jansen, R.C.: Epigenome dynamics: a quantitative genetics perspective. Nat. Rev. Genet. **9**(11), 883–890 (2008).

Jones, K.M., Kobayashi, H., Davies, B.W., Taga, M.E., Walker, G.C.: How rhizobial symbionts

invade plants: the *Sinorhizobium– Medicago* model. Nat. Rev. Microbiol. **5**(8), 619–633 (2007).

Kern, V.D., Schwuchow, J.M., Reed, D.W., Nadeau, J.A., Lucas, J., Skripnikov, A., Sack, F.D.: Gravitropic moss cells default to spiral growth on the clinostat and in microgravity during spaceflight. Planta. **221**(1), 149–157 (2005).

Kiss, J.Z.: Mechanisms of the early phases of plant gravitropism. CRC Crit. Rev. Plant Sci. **19**(6), 551–573 (2000).

Kiss, J.Z.: Plant biology in reduced gravity on the Moon and Mars. Plant Biol J. **16**(1), 12–17 (2013).

Kiss, J.Z.: Conducting plant experiments in space. In: plant gravitropism. pp. 255–283. Springer New York, New York, NY (2015).

Kiss, J.Z., Aanes, G., Schiefloe, M., Coelho, L.H.F., Millar, K.D.L., Edelmann, R.E.: Changes in operational procedures to improve spaceflight experiments in plant biology in the European Modular Cultivation System. Adv. Space Res. **53**(5), 818–827 (2014).

Kooke, R., Johannes, F., Wardenaar, R., Becker, F., Etcheverry, M., Colot, V., Vreugdenhil, D., Keurentjes, J.J.B.: Epigenetic basis of morphological variation and phenotypic plasticity in *Arabidopsis thaliana*. Plant Cell. **27**(2), 337–348 (2015).

Korte, A., Farlow, A.: The advantages and limitations of trait analysis with GWAS: a review. Plant Methods. **9**(29), 1-9 (2013).

Kraft, T., van Loon, J., Kiss, J.Z.: Plastid position in *Arabidopsis* columella cells is similar in microgravity and on a random-positioning machine. Planta. **211**, 415–422 (2000).

Lehto, K.M., Lehto, H.J., Kanervo, E.A.: Suitability of different photosynthetic organisms for an

extraterrestrial biological life support system. Res. Microbiol. **157**(1), 69–76 (2006).

Massa, G.D., Mitchell, C.A.: Sweetpotato vine management for confined food production in a space life-support system. Adv. Space Res. **49**(2), 262–270 (2012).

Miransari, M.: Interactions between arbuscular mycorrhizal fungi and soil bacteria. Appl Microbiol Biot. **89**(4), 917–930 (2010).

Miyamoto, K., Yamamoto, R., Fujii, S., Soga, K., Hoson, T., Shimazu, T., Masuda, Y., Kamisaka, S., Ueda, J.: Growth and development in *Arabidopsis thaliana* through an entire life cycle under simulated microgravity conditions on a clinostat. J. Plant Res. **112**(1108), 413–418 (1999).

Monje, O., Stutte, G.W., Goins, G.D., Porterfield, D.M.: Farming in space: environmental and biophysical concerns. Adv. Space Res. **31**(1), 151-167 (2003).

Myles, S., Peiffer, J., Brown, P.J., Ersoz, E.S., Zhang, Z., Costich, D.E., Buckler, E.S.: Association mapping: critical considerations shift from genotyping to experimental design. Plant Cell. **21**(8), 2194–2202 (2009).

Nadeem, S.M., Ahmad, M., Zahir, Z.A., Javaid, A., Ashraf, M.: The role of mycorrhizae and plant growth promoting rhizobacteria (PGPR) in improving crop productivity under stressful environments. Biotechnol. Adv. **32**(2), 429–448 (2014).

National Research Council: Recapturing a future for space exploration. National Academies Press (2015).

Pritchard, J.K., Stephens, M., Donnelly, P.: Inference of population structure using multilocus genotype data. Genetics. **155**(2), 945-59 (2000).

R Core Team (2016). R: A language and environment for statistical computing. R Foundation for

Statistical Computing, Vienna, Austria. URL https://www.R-project.org/.

Rafalski, A.: Applications of single nucleotide polymorphisms in crop genetics. Curr. Opin. Plant Biol. **5**(2), 94–100 (2002).

Scholz, A., Juang, J.-N.: Toward open source CubeSat design. Acta Astronaut. **115**(C), 384–392 (2015).

Sherwood, B.: Comparing future options for human space flight. Acta Astronaut. **69**(5-6), 346–353 (2011).

Sobol, M.A., González-Camacho, F., Rodríguez-Vilariño, V., Kordyum, E.L., Medina, F.J.: Clinorotation influences rDNA and NopA100 localization in nucleoli. Adv. Space Res. **36**(7), 1254–1262 (2005).

Soh, H., Srikanth, K., Whang, S.S., Lee, S.: Morphological and gene expression pattern changes in wrky46mutant *Arabidopsis thaliana* under altered gravity conditions. Botany. **93**(6), 333–343 (2015).

Song, Y., Wang, X.-D., Rose, R.J.: Oil body biogenesis and biotechnology in legume seeds. Plant Cell Rep. **36**(10), 1519–1532 (2017).

Stanton-Geddes, J., Paape, T., Epstein, B., Briskine, R., Yoder, J., Mudge, J., Bharti, A.K., Farmer, A.D., Zhou, P., Denny, R., May, G.D., Erlandson, S., Yakub, M., Sugawara, M., Sadowsky, M.J., Young, N.D., Tiffin, P.: Candidate Genes and Genetic Architecture of Symbiotic and Agronomic Traits Revealed by Whole-Genome, Sequence-Based Association Genetics in *Medicago truncatula*. PLoS ONE. **8**(5), e65688 (2013).

Stapley, J., Reger, J., Feulner, P.G.D., Smadja, C., Galindo, J., Ekblom, R., Bennison, C., Ball, A.D., Beckerman, A.P., Slate, J.: Adaptation genomics: the next generation. Trends Ecol.

Evol. **25**(12), 705–712 (2010).

Toro, N., Martínez-Rodríguez, L., Martínez-Abarca, F.: Insights into the history of a bacterial group II intron remnant from the genomes of the nitrogen-fixing symbionts *Sinorhizobium meliloti* and *Sinorhizobium medicae*. Heredity. **113**(4), 306–315 (2014).

Van Kleunen, M. and Fischer, M.: Constraints on the evolution of adaptive phenotypic plasticity in plants. New Phytol. **166**(1) 49–60. (2005)

Vandenbrink, J.P., Kiss, J.Z.: Space, the final frontier: A critical review of recent experiments performed in microgravity. Plant Sci. **243**, 115–119 (2016).

Varshney, R.K., Kudapa, H.: Legume biology: the basis for crop improvement. Funct. Plant Biol. **40**(12), v–viii (2013).

Wang, D., Yang, S., Tang, F., Zhu, H.: Symbiosis specificity in the legume - rhizobial mutualism. Cell. Microbiol. **14**(3), 334–342 (2012).

Wolverton, C., Kiss, J.Z.: An update on plant space biology. Grav. Space Biol. **22**(2), 13–20 (2009).

Yang, J., Benyamin, B., McEvoy, B.P., Gordon, S., Henders, A.K., Nyholt, D.R., Madden, P.A., Heath, A.C., Martin, N.G., Montgomery, G.W., Goddard, M.E., Visscher, P.M.: Common SNPs explain a large proportion of the heritability for human height. Nat Genet. **42**(7), 565–569 (2010).

Young, N.D., Debellé, F., Oldroyd, G.E.D., Geurts, R., Cannon, S.B., Udvardi, M.K., Benedito, V.A., Mayer, K.F.X., Gouzy, J., Schoof, H., Van de Peer, Y., Proost, S., Cook, D.R., Meyers, B.C., Spannagl, M., Cheung, F., De Mita, S., Krishnakumar, V., Gundlach, H., Zhou, S., Mudge, J., Bharti, A.K., Murray, J.D., Naoumkina, M.A., Rosen, B., Silverstein, K.A.T.,

Tang, H., Rombauts, S., Zhao, P.X., Zhou, P., Barbe, V., Bardou, P., Bechner, M., Bellec, A., Berger, A., Bergès, H., Bidwell, S., Bisseling, T., Choisne, N., Couloux, A., Denny, R., Deshpande, S., Dai, X., Doyle, J.J., Dudez, A.-M., Farmer, A.D., Fouteau, S., Franken, C., Gibelin, C., Gish, J., Goldstein, S., González, A.J., Green, P.J., Hallab, A., Hartog, M., Hua, A., Humphray, S.J., Jeong, D.-H., Jing, Y., Jöcker, A., Kenton, S.M., Kim, D.-J., Klee, K., Lai, H., Lang, C., Lin, S., Macmil, S.L., Magdelenat, G., Matthews, L., McCorrison, J., Monaghan, E.L., Mun, J.-H., Najar, F.Z., Nicholson, C., Noirot, C., O'Bleness, M., Paule, C.R., Poulain, J., Prion, F., Qin, B., Qu, C., Retzel, E.F., Riddle, C., Sallet, E., Samain, S., Samson, N., Sanders, I., Saurat, O., Scarpelli, C., Schiex, T., Segurens, B., Severin, A.J., Sherrier, D.J., Shi, R., Sims, S., Singer, S.R., Sinharoy, S., Sterck, L., Viollet, A., Wang, B.-B., Wang, K., Wang, M., Wang, X., Warfsmann, J., Weissenbach, J., White, D.D., White, J.D., Wiley, G.B., Wincker, P., Xing, Y., Yang, L., Yao, Z., Ying, F., Zhai, J., Zhou, L., Zuber, A., Dénarié, J., Dixon, R.A., May, G.D., Schwartz, D.C., Rogers, J., Quétier, F., Town, C.D., Roe, B.A.: The *Medicago* genome provides insight into the evolution of rhizobial symbioses. Nature. **480**, 520-524 (2011).

Zhu, C., Gore, M., Buckler, E.S., Yu, J.: Status and prospects of association mapping in plants. Plant Genome-US. **1**(1), 5–16 (2008).

VITA

Associate of Science degree in Biotechnology, Biomanufacturing, and Molecular Techniques.
Montgomery County Community College, Blue Bell, PA. 2014.

Bachelor of Science degree in Cell Biology and Biochemistry.
Bucknell University, Lewisburg, PA. 2014.

Research Technician at the Johns Hopkins University School of Medicine.
Dr. Laura Wood Lab - Pathology. 2014-2015, 2017-current.