Why sink strength is a key determinant of drought tolerance in common bean

Amber Nicole Hageman

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Reading Committee:

Elizabeth Van Volkenburgh, Chair

Richard G. Olmstead

Maria Reguera

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Abstract

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Amber Nicole Hageman

Chair of the Supervisory Committee:

Elizabeth Van Volkenburgh

Department of Biology

Throughout development, plants are constantly shifting resource allocation between their different tissue types; roots, stems, leaves, fruits and seeds. This allows them to balance resource gain, optimize growth, and increase reproductive output. While plants with different life history strategies have evolved very different patterns of allocation (ie. perennials conserve resources for the next year while annuals put all available resources into the next generation), the overall goal of increasing fitness underlies their behaviors. Still, it is not well understood what determines resource allocation into reproductive structures, specifically seeds. It's clear that carbon availability and developmental processes play a crucial role - flowers, fruits and seeds must be produced, but a priority of allocation towards these structures must also be established and maintained to ensure high reproductive output.

Drought is a significant factor which can impact allocation processes and seed filling. This can be due to changes in resource availability, as drought commonly decreases photosynthesis. However, allocation processes themselves are known to be affected by drought via signaling, particularly through the phytohormone abscisic acid. This means that even when resources themselves do not limit allocation and growth potential, other processes such as resource uptake into or metabolism within growing tissues may be slowed. Allocation processes therefore may be halted under drought stress, ultimately leading to decreased growth and/or seed filling. However, the degree to which different species and even different genotypes of a single species are impacted by drought differs strongly, resulting in some plants being better able to maintain allocation processes under drought. Lines which achieve this are deemed to be drought-tolerant.

What allows drought-tolerant genotypes to maintain allocation and seed filling under drought? We set out to answer this question using common bean (Phaseolus vulgaris), where drought-tolerant lines have been shown to maintain higher allocation rates. Our goal was to gain a better understanding of the physiological underpinnings which control differences in allocation between drought-tolerant and drought-sensitive common bean lines. To begin, we tested how allocation processes, generally, were affected in tolerant and sensitive lines. We wondered if allocation to seeds was impacted by drought, would allocation towards any sink tissue be interrupted by drought? We found that within a genotype, drought's impacts to growth and allocation acted in a consistent way across different sink tissues, such as leaves and seeds. That is, genotypes which had leaf growth strongly depressed by drought also had seed growth strongly depressed. This was not shown to be tightly correlated with resource availability or water status within the plant. Conversely, genotypes which maintained a high growth rate in one sink tissue,

retained higher growth in other sinks. Genotypes which achieved higher growth resulted in a faster maturation of seeds, effectively allowing these lines to avoid potentially worsening drought.

To better understand the differences in allocation between drought sensitive and drought tolerant lines, we quantified allocation across the different tissues of a plant over reproductive development. We found that drought-tolerant common bean lines (1) allocated biomass to seeds earlier, (2) allocated a larger percentage of total biomass to seeds than drought-sensitive lines, allowing the tolerant lines to achieve the same absolute yield as the sensitive line even with less total biomass, and (3) did not alter their seed filling profile under water stress, meaning the developmental program of seed filling was not altered by drought. In contrast, the sensitive line did have different seed filling profile under well-watered and water stressed. This suggests that the sensitive line alters development under drought whereas the tolerant does not.

We believe that maintenance of allocation and therefore growth in the drought-tolerant line is the direct result of maintaining what is known as ‘sink strength’. Sink strength is defined as the ability of a growing, sink tissue, to obtain resources for use within the sink. Many mechanisms are known to be involved in setting sink strength, including resource uptake via transporters, proton pump and enzyme levels and activity. Proton pumps work to establish gradients across the membrane to drive resource uptake via transporters, and enzymes are involved in the breakdown and metabolism of materials, allowing for their transport into and use within sinks.

We found that differences in these processes between genotypes determined differences in allocation and growth rate. When common bean seeds were floated in growth media containing sugar, uptake rates and growth were genotype and condition specific, rather than related to the concentration of sugar in the growth medium. This points to the seed itself as the point of regulation driving differences in seed filling.

What underlies differences in the drought-tolerant line and the drought-sensitive line may be related to drought sensitivity, particularly to the hormone mentioned above, abscisic acid (ABA). We found that the drought-tolerant lines did not change the seed filling profile, nor seed filling rate or acidification rates in in vitro assays when exposed to drought and ABA, respectively. The drought sensitive line however did change its seed filling profile, seed filling rate and acidification rate under drought. Therefore, we believe that tolerant lines may have impaired drought sensitivity. This is perhaps through a reduced ability to produce, sense or respond to ABA, or potentially ABA production or sensing is constitutively on. However this is achieved, reduced drought sensitivity results in plants which maintain allocating resources towards sinks, importantly seeds, under drought stress. This allows them to fill seeds faster and increase the percent of total biomass allocated to seeds, increasing yields under drought.

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Chapter 1: Introduction

1.1 Carbon capture or delivery: What limits seed growth?

Plants acquire resources throughout their lifespan. These resources are then moved around the plant, allocated, according to developmental and environmental conditions. Carbon, which makes up the majority of the dry mass of a plant ("some 90%" (Lalonde et al. 2003)), is the primary resource that is partitioned between different organs. The carbon that is fixed in photosynthetic tissues can be used, stored, or made into transportable compounds - usually sucrose (Zimmermann M.H. and H. 1975). These compounds are then translocated to other tissues in need, such as growing leaves, roots, and reproductive structures, to be used or stored.

Normal developmental programs (genetically encoded) play a large role in determining when and where carbon pools exist within a plant. This can vary widely depending on the species and the conditions in which it evolved. For example, plants with different life history strategies (such as deciduous perennials vs annuals) have very different patterns of allocation. In deciduous perennials, seasonality causes shifts in carbon allocation between roots and shoots in a yearly cycle. In the spring and summer, carbon resources are sent from roots to shoots to be used for growth and maintenance. From the newly acquired carbon, some is used for growth and reproduction, while another portion is transported to the roots to be stored over winter, allowing perennials to persist from year to year. Annuals, on the other hand, only grow for a single season. During this lifespan, certain cues trigger developmental transitions causing carbon allocation to switch from vegetative growth toward reproduction, exhausting all available resources each generation.

On top of genetic regulation, carbon allocation is also heavily influenced by environmental conditions. Under optimal conditions, a plant can allocate carbon according to developmental programs in order to increase resource gain and reproductive output. However, plants rarely experience ideal conditions and must be more discerning in order to optimize their fitness. To this end, they have evolved mechanisms which allow them to sense and respond to limiting environmental conditions, such as drought, nutrient deficiency or extreme temperature. One

adaptation to stress is the plant's ability to shift biomass partitioning between its different tissue types in order to balance resource use and gain. "Functional equilibrium" is the framework which describes this plasticity of biomass partitioning in response to changing conditions (Brouwer 1983). For example, plants experiencing stress such as drought or nitrogen deficiency may increase biomass allocation to their roots, increasing the surface area for water or nitrogen uptake (Rodrigues, Pacheco, and Chaves 1995). Likewise, if carbon is limiting, biomass will be sent towards shoots to increase stem length, leaf area or photosynthetic machinery for greater carbon capture (Wardlaw 1990).

Beyond growth and development, a plant's purpose for gathering resources is to be able to pass on its genes, often through the production of seeds. In annual plants, reproduction must happen during a single seasonal cycle, since annual plants die after the reproductive phase is complete. Depending on whether it has determinate-growth (with a genetically pre-determined end) or indeterminate-growth (non-terminating), two different strategies to deal with stress conditions occur in annuals. For *determinate* annuals, the switch from vegetative to reproductive is non-reversible. Therefore, if a stress is sensed during the reproductive phase in a determinate annual, the most adaptive response is to maintain seed filling and efficiently mobilize all available resources into the developing fruit and seeds. However, for *indeterminate* annuals, establishment and maintenance of reproduction is flexible. When conditions are good, reproduction can proceed normally. If conditions worsen, an indeterminate plant will switch to slower growth, halt reproductive development and await more favorable conditions to resume the reproductive program (Wardlaw 1990). This switching can be referred to as a "toggle" between faster and slower growth (Beebe et al. 2009).

As wild bean is an indeterminate annual in its native form, it is a species which toggles between fast and slow growth, pausing seed production under stress. Domestication of wild bean selected for determinate growth (Gentry 1969), yet these determinate lines do not act as natively determinate species do. Instead of allocating all available resources to reproduction once it has begun, domesticated bean inherited the trait of toggling from its wild predecessors and pause reproduction under stress. This results in the loss of reproductive structures even though the

ability to resume reproductive development which wild, indeterminate beans possess has been bred out of them.

Both determinate and indeterminate have evolved mechanisms which maximize production of seeds in nature, increasing fitness. Yet, the efficiency with which different species and lines are able to fill seeds under stress varies greatly, and the underlying mechanisms that establish and maintain efficient allocation of biomass to yield are not well understood. This efficiency, which can be quantified by harvest indices (HI) (weight of yield as a percentage of total plant biomass, strongly correlates with yield (Roger 1984). During the Green Revolution there were impressive increases in yield in many crops (corn, wheat, rice). While there were many reasons for this (increased use of fertilizers, pesticides, and herbicides), increases in yield were found to be largely due to increases in harvest indexes, rather than increased biomass production. It has also been shown that increased HI has the added benefit of increasing drought tolerance (Chang and Zhu 2017). Bean, however, has not seen these same increases in yield over time. This may be due to the indeterminate-like response under drought that determinate domesticated bean has unfortunately inherited. However, findings from the International Center for Tropical Agriculture (CIAT) have shown that harvest efficiencies, particularly PHI in bean, are the number one best predictor/correlator of high yield under stress in bean. This strengthens the need for a better understanding of the physiological mechanisms underlying carbon allocation, and how they are affected by abiotic stress. Therefore, a simple aim of my project (and the work at CIAT) is to close this gap between beans – a globally important food staple, particularly in areas where less meat is consumed, and beans are important for their nitrogen content – and other staple crops. As climate change worsens, crop yields are increasingly threatened by drought and heat stress, and the urgency of finding innovative solutions will only intensify.

1.2 Carbon use, storage, export & import

Plants capture CO_2 from the atmosphere and convert it into carbohydrates via photosynthesis. Tissues which perform photosynthesis, typically mature leaves, are called sources. Once it is fixed, photosynthate has one of three fates: 1) use in the source; 2) storage in the source; or 3) export for use or storage in other tissues. For use in sources, triose phosphates are made into

sugars. These sugars can then be used to produce ATP, or are building blocks for macromolecules such as cellulose, proteins, lipids or secondary metabolites (Stitt et al. 2010). Another important fate for newly fixed carbon is into storage pools. These stores of carbon are necessary for allowing processes to occur independently of photosynthesis. For example, respiration, growth and seed filling continue in the dark (Schnyder 1993). With no photosynthate being incorporated at this time, carbon reserves provide the resources to fuel these processes. To ensure this, a portion of the photosynthate assimilated during the day is stored in the chloroplast as starch or in the vacuole as sucrose which can be broken down when needed (Weber et al. 2000).

The remaining photosynthate that is not used or stored in the source tissue, up to 50 - 80% of fixed carbon (Ainsworth and Bush 2011; Lemoine et al. 2013), is transported to non-photosynthetic organs called sinks. Most land plants export photosynthate through a network of vascular connections called phloem in the form of sucrose (Zimmermann M.H. and H. 1975). Phloem tissue is made up of sieve-tube elements (SE) and specialized phloem cells called companion cells (CC). CCs are necessary for actively loading sucrose into the phloem, since SE are unnucleated and cannot actively take up sucrose (Julius et al. 2018). Movement throughout the plant is then driven by osmotically generated pressure differences that occur between source and sink tissues (Munch 1930). High pressure is generated in source phloem due to high sucrose concentrations, bringing water in via osmosis.

To ensure high sucrose concentrations in the source phloem, sucrose is actively loaded into phloem in most herbaceous species (Zhang and Turgeon 2018). As sucrose builds up in a mesophyll cell, it exits down its concentration gradient via transporters and enters the space between cells called the apoplast. Sucrose cannot flow directly from the mesophyll into the phloem because there are little to no plasmodesmatal connections between mesophyll and phloem. To cross the membrane, sucrose leaves via SWEETs – sucrose efflux transporters (Chen et al. 2012). From the apoplast, it is then actively taken up across the phloem companion cell membrane via SUTs – sucrose uptake co-transporters (Riesmeier, Willmitzer, and Frommer 1992). These transporters load sucrose against its concentration gradient via proton/sucrose co-transport, driven by a proton motive force. This driving force is established by pumping protons

into the cell wall via proton pumps (H+-ATPases) (Lalonde et al. 2003; Sauer 2007) and can concentrate sucrose in the source phloem to over 500 mM (Stitt 2013). Two other, less common loading mechanisms exist; polymer trapping and syplastic loading. In polymer trapping, sucrose is converted into raffinose or stachyose in special cells near phloem. These molecules are larger than sucrose and aren't able to move through plasmodesmata back towards the mesophyll. Therefore, these polymers are 'trapped' and can only move forward through larger, specialized plasmodesmata that connect to the phloem SE/CC complex cells. In contrast to the other two, symplastic loading is a passive process, with sucrose moving between the shared cytoplasm, made possible via plasmodesmata, down its concentration gradient from high to low. These three loading mechanisms are not mutually exclusive within one plant (Rennie and Turgeon 2009).

Once sucrose has been loaded into the phloem, it is translocated to sink tissues. Sinks are heterotrophic tissues with active growth or storage processes occurring such as roots, stems or developing leaves (Mason and Maskell 1928). While phloem loading is usually active in herbaceous species, unloading into sinks is often initially passive, with sucrose travelling through the symplast down its concentration gradient (Lemoine et al. 2013). Low concentrations of sucrose in the sink, maintained via growth, metabolic or storage processes, create a favorable gradient for sucrose to leave the phloem. This allows sucrose to diffuse out of the SE/CC complex through the symplast into the sink. Only once it reaches the maternal/filial tissue interface does sucrose have to leave the symplast, since no connections exist between these tissues (Gifford and Thorne 1985). Once in the apoplast, it must be taken up actively into filial tissue.

1.3 Control of carbon flux

There are many mechanisms that actively regulate carbon flux as it is partitioned or reallocated within a plant. These mechanisms are sensitive to developmental and environmental conditions and are essential in allowing a plant to allocate resources actively, rather than passively along concentration gradients. While having enough fixed carbon is essential for maintaining flux, its presence alone does not guarantee its transport through the phloem. This finding has become of great interest to breeders, since improvements of carbon utilization are associated with improved

yields (Wardlaw 1990). Processes discussed above, such as phloem loading, unloading and the transporters involved, have been found to be points of regulation which allow tunability of allocation processes. This section will explore how this plasticity is controlled and impacted by environmental variables.

An ample supply of fixed carbon is the first requirement for its flux. During the daytime, this supply can come from photosynthesis. However, sap flow and carbon gain in seeds has been shown to be fairly constant across day and night, despite daily fluctuations in carbon fixation (Mark E. Westgate and Grant 1989). This is possible due to transient starch storage. During the day, stores of starch accumulate in sources. At night, they are broken down at a constant rate, allowing carbon flux to continue. Amazingly, yet little understood, this rate of degradation has been shown to change, depending on the length of night, such that 95% of the store is used up by dawn (Graf et al. 2010). This guarantees carbon resources will be available for transport throughout the night while at the same time, efficiently using up all available resources to maximize growth .

However, even when photosynthate is available, this does not guarantee its flux into seeds (Thomas, Hetherington, and Patrick 2000). If carbon resources are not limiting, what then constrains carbon flux to the seeds? While a carbon supply is the first requirement, flux is ultimately determined by the concentration gradients between sources and sinks and the resistances along this path of flow.

Starting at the source, one of the first potential points of regulation is phloem loading. In apoplastic loaders, uptake is mediated by SUTs. By increasing or decreasing this uptake rate, plants can alter the resistance to flow which in turn impacts the concentrations of sucrose in the source phloem. Control over gradients and resistance is regulated by sucrose levels themselves, both directly and indirectly. High sucrose levels upregulate transcription of SUT mRNA (Vaughn, Harrington, and Bush 2002), increasing sucrose uptake into CCs. Sucrose concentrations also indirectly affect sucrose uptake due to impacts on turgor pressure. When sucrose loading exceeds unloading, sucrose concentrations at the source CC build up, increase osmotic potential and turgor pressure. H^+-ATPases, which establish the proton motive force

driving sucrose uptake, are inhibited by high turgor (J Daie 1987). Therefore, when sucrose builds up and turgor pressure increases, loading via SUT decreases. These two points of regulation act in opposition, with high sucrose levels both increasing SUT transcription and decreasing the proton motive force. Perhaps SUT levels establish primary regulation of sucrose flow with H^+-ATPase inhibition only superseding this under low sink demand. When sink uptake exceeds loading, pressure quickly decreases at the source (Lalonde et al. 2003). Opposite to their inhibition under high turgor, H^+-ATPases can be positively regulated by low turgor in sinks, such as seed coats of *P. vulgaris* (Patrick et al. 1986). Therefore, dynamic pressure changes impose large control over loading processes.

Unloading into sinks is another important point in regulation of carbon flux. Although export itself is less actively controlled, since no active transport is necessary for sucrose to leave the phloem, concentration gradients driving sucrose out of phloem can vary widely. Differences in these concentration gradients are set up by metabolism, growth, and compartmentalization. These processes are actively controlled. Metabolism and growth use up sugars, keeping levels low in the sink. This is controlled by enzymes which hydrolyze sucrose, such as invertases (INV, including cell-wall invertases (CWIN)) and sucrose synthase (Sus) or through the production of storage compounds such as starch via sucrose phosphate synthase (SPS) (Bihmidine et al. 2013; Herbers and Sonnewald 1998). Which enzyme is most relevant depends on the tissue and developmental stage (Stitt 2013). For example, INV play an important role early in fruit and seed development, since the products from INV cleavage - hexoses - are necessary regulators of cell division and expansion (Bihmidine et al. 2013). And in drought, INV levels have been found to decrease, contributing to embryo abortion (Cuellar-Ortiz et al. 2008). Sus and SPS become important later in seed development due to their involvement in storage processes that dominate the seed filling stage (Finkelstein and Gibson 2001). Evidence shows that when either INV or Sus is overexpressed, seed filling is improved (Braun, Wang, and Ruan 2014). This may be due to their impacts on lowering sucrose levels in sinks, maintaining a high concentration gradient between source and sink. Compartmentalization also helps to maintain favorable gradients to keep resources flowing into sinks. Again, transports may play an important role, with hexose- and sucrose-antiporters using a reversed proton motive force to drive sucrose uptake into the vacuole across the tonoplast membrane (Martinoia, Maeshima, and Neuhaus 2007).

1.4 Sink strength

If the rate of any of these processes at the sink are altered, this can affect what is referred to as "sink strength". Sink strength is defined here as the ability of a tissue to import resources under non-limiting substrate availability (Ho 1988; Minchin and Thorpe 1996). Enzymes and transporters control flux by changing sucrose concentrations (driving force) or conductance (inverse of resistance). In turn, sugar concentrations themselves control genes which contribute to sink strength according to source supply (Bihmidine et al. 2013). Sink strength exerts a major influence over where sucrose is partitioned throughout the plant. This is because the different organs of a plant vary in their sink sucrose concentrations, leading to differences in rates of carbon flux toward them and accumulation between them (Wardlaw 1990). Seeds are at the top of the hierarchy of sink strength, followed by fleshy fruits, shoot meristems and growing leaves, cambium, roots and lastly long-term storage (Minchin and Thorpe 1996). High sink strength in seeds is maintained throughout their development whereas accumulation rates in vegetative tissues are often variable and below their maximum potential (Minchin and Thorpe 1996).

Even though seeds have the highest sink strength capacity, they don't always fill. As discussed above, this can occur even when resources are available, since genetic constraints can prevent seeds from being maximally filled (Thomas, Hetherington, and Patrick 2000). For example in bean, partitioning indexes show that under favorable conditions, lines vary in their efficiency of C utilization (Polania et al. 2017). In tomato, increases in assimilate within the plant did not result in increased fruit growth, which agreed with earlier findings that differences between genotypes was due to active growth and metabolic properties of fruits themselves (Y.-L. Ruan, Patrick, and Brady 1997). Environmental impacts and stresses can compound from there, since many plants which are capable of high efficiencies under ideal conditions can be negatively impacted by stresses, such as drought (Polania et al. 2017). The hypothesis for *why* this is true for an indeterminate annual, evolutionarily, is that halting reproduction is adaptive under stressful conditions if it allows the plant to hold out long enough for conditions to improve such that reproduction and seed production to recommence.

Yet *how* these seemingly inherent sink strength capacities are differently established and regulated is not well understood. Many have suggested that it is simply due to substrate limitations, with sucrose production from photosynthesis or storage being halted or depleted as a result of the stress. And while it is true that drought can negatively impact yield via carbon starvation (eg. Zinselmeier, Lauer, and Boyer 1995), studies suggest phloem transport can continue during water deficits (Daie and Wyse 1985). Further, there is evidence that even when carbon resources are available, they are not always used to fill seeds (Bennett, Roberts, and Wagstaff 2012). This seed filling efficiency differs across and within species, suggesting that the limitation comes from differences in stress signaling and response between species, lines or genotypes, rather than simply through substrate limitation.

Additionally, drought stress can have a significant negative effect on sink growth. This could be through decreases to either source availability or sink strength. Drought is known to affect both by causing increases in the concentration of the phytohormone abscisic acid (ABA) (Vishwakarma et al. 2017). ABA has been shown to regulate many processes in response to drought stress, including reducing stomatal aperture and therefore decreasing photosynthesis (Wilkinson and Davies 2002), osmotic regulation (Tuteja 2007), CWIN inhibition (Yong-Ling Ruan 2014), as well as slowing rates of proton pumping and therefor sucrose uptake via SUCs (Bush 1993; Merlot et al. 2007). These, and other effects, result in decreases to seed set and seed size and therefore yields (Y. Ruan et al. 2010; M E Westgate, Passioura, and Munns 1996). ABA impacts vary however, as different tissues have been shown to have proton pumps which are differentially affected under drought (Michelet and Boutry 1995) and different tissues as well as different species can have opposite responses to ABA ((Humplík, Bergougnoux, and Volkenburgh 2017). Therefore, differences in response to drought between genotypes may stem from differences in ABA sensitivity or the effect that ABA has on developmental processes.

1.5 Research aims

Regardless of a plant's specific developmental program or the environment in which it grows, the overall goal of increasing fitness underlies its behaviors. To achieve this, plants carefully control how resources are allocated and adjust depending on genetics and environmental

conditions. While the general properties governing carbon flux are understood, precise regulation of these are still being pieced together and many questions remain to be answered. The work in this thesis aims to answer some of these questions in order to gain a deeper understanding of sink strength and drought resistance in common bean. To this end, the three chapters that make up this thesis detail: two bodies of experiments, one in the field, one in growth chambers, and a literature review summarizing what is known about the mechanisms which regulate sink strength. More specifically:

Chapter 2: Sensitivity of leaflet growth rate to drought predicts yield in common bean (*Phaseolus vulgaris*)

20 genotypes of *Phaseolus vulgaris* were grown under well-watered and droughted conditions in a field experiment, and impacts to sink growth, including leaf, pod and seed growth, were quantified. Results were compared to one another to determine whether all sink tissues were impacted by drought in a common way and whether the severity of impact helped predict yield under drought.

Chapter 3: Quantification of resource allocation over time between drought tolerant and drought sensitive common beans and the mechanisms which control differences in partitioning

Using one drought-tolerant and one drought-sensitive common bean genotype, biomass allocation was measured over reproductive development under both well-watered and droughted conditions in a growth chamber experiment. Differences in biomass allocation by timepoint and percent were compared between the two genotypes to determine potential bottlenecks to flow. Seed assays tested correlations between sucrose uptake, solution acidification rates, and biomass gains, as well as growth sensitivity to a drought-induced hormone, abscisic acid.

Chapter 4: Sink strength maintenance underlies drought tolerance in common bean

This literature review compiles what is known on the subject of sink strength and how it relates to drought tolerance. Mechanisms which help establish and maintain sink growth are detailed and commonalities in how they are regulated across tissues are explored.

While source-sink mobilization is still not understood, work which aims to better understand and ultimately manipulate these processes has the potential to improve yields. This is true for two reasons. First, recent increases in yield have been associated with increases in HI (Wardlaw 1990). Second, PHI has recently emerged as a strong predictor of yield under drought in crops, such as bean (Polania et al. 2016), and has been shown to improve stress tolerance generally (Chang and Zhu 2017). If we wish to alter source-sink relationships as a means to increase yields, breeding alone as the mechanism to increase this ability may not be enough, since breeders often struggle with low mobilization efficiencies (low HI)(Chang and Zhu 2017). Therefore, understanding control of these processes at the physiological and molecular scales and how they may be affected by drought may be necessary to achieve increased mobilization efficiencies and, in turn, higher yields (Julius et al. 2018).

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Chapter 2: Sensitivity of leaflet growth rate to drought predicts yield in common bean (*Phaseolus vulgaris*)

2.1 Abstract

Although drought limits yield by decreasing photosynthesis and therefore biomass accumulation, biomass is not the strongest predictor of yield under drought in common beans (*Phaseolus vulgaris* L.). Instead, resource partitioning from pod walls into seeds is a stronger correlate. Our aim was to determine whether growth rates of developing leaflets and pods, as independent indicators of sink strength, predict resource partitioning into seeds. Using 20 field-grown genotypes, we paired biomass, yield, and resource partitioning data with leaflet and pod growth rates under well-watered and droughted conditions. We hypothesised that genotypes with faster growing leaflets and pods under drought would fill seeds better. However, we found that leaflet and pod growth rates did not predict partitioning to seeds; rather, sensitivity of leaflet growth rate to drought was a good predictor of yield reduction. Further, plants with rapidly growing leaves under well-watered conditions were most vulnerable to decreases in leaflet growth rate under drought. This suggests that lines that inherited a conservative growth strategy were better able to maintain yield by allocating resources to seeds. Our findings indicate that inherent sensitivity of leaflet growth rate to drought may be used as a predictor of partitioning and yield in common beans.

2.2 Introduction

Among abiotic stresses, drought has the most detrimental impact on seed yield in the common bean (*Phaseolus vulgaris* L.) with over 60% of world production affected (Thung and Rao, 1999; Andrade *et al.*, 2016; Rao *et al.*, 2017). Although drought limits yield largely by its impact on photosynthesis and therefore canopy biomass accumulation, findings show that increasing canopy biomass alone does not necessarily lead to an increase in seed yield (Shibles and Weber, 1996). Instead, the ability to partition resources efficiently towards reproductive structures provides the stronger increase to seed yield under drought (Omae *et al.*, 2012; Polania *et al.*, 2016*a*). A particularly important correlate is pod harvest index (PHI), a measure of photosynthate remobilisation from pod walls into seeds (Assefa *et al.*, 2013). Plants with higher PHI values partition greater amounts of pod biomass into seeds, facilitating higher seed yield and

increasing resource-use efficiency. This means that although some plants can amass a large amount of canopy biomass under drought, only the ones most efficient at moving, or allocating, those resources into seeds obtain high yield. This raises the simple yet unanswered question: what makes genotypes differ in their ability to allocate resources towards seed production under drought?

Preliminary work on *P. vulgaris* lines varying in drought resistance conducted in greenhouse conditions showed good correlation between leaf growth rate and seed yield (Banan and Van Volkenburgh, 2012). While growth rates are not necessarily a measure of resource acquisition, especially under water deficit (Muller *et al.*, 2011), we hypothesized that they may approximate sink strength and drought sensitivity, such that genotypes whose leaves or pods maintain high growth rates under drought may also achieve higher seed yields and PHI values. In the present study, our objective was to test this hypothesis in field conditions by comparing leaflet growth rates (LGR), pod growth rates (PGR), seed yield and resource partitioning efficiency (PHI) to one another and determine drought's effects on these processes. Many studies have previously compared various lines' agronomy and phenology in the field (e. g. Beebe et al. 2013; Jose Polania et al. 2016; Rao et al. 2017; Smith 2018), but data on growth rates of pods and leaves collected alongside these measurements, with quantifications of the impact of drought on these traits, are lacking. This study aimed to fill that gap to: (1) determine whether relative decreases to growth rates and partitioning efficiency under drought relate to drought resistance (high seed yield); and (2) look at impact of drought on growth in leaflets and pods to better understand systemic drought responses.

2.3 Materials and methods

Plant material

We conducted a field study using 19 lines of common bean (*Phaseolus vulgaris* L.) and one line of tepary bean (*Phaseolus acutifolius* A. Gray). These 20 genotypes were chosen to represent a wide range of observed PHI values in field grown plants (see Table S1, available as Supplementary Material to this paper), providing variability for probing physiological responses. Sixteen of these genotypes were made up of two recombinant inbred line (RIL) populations, including the four parents and six RILs from each cross. These RIL populations were created

using parents (MD23-24 × SEA5 – MR RIL) and (BAT881 × G21212 – BH RIL), which differed in their response to abiotic stress, such that their offspring would mostly fall between them in traits related to stress resistance, including PHI (Polania *et al.*, 2017; Diaz *et al.*, 2018). The remaining lines, SEN56, INB841 and DOR390, were chosen as routine checks; DOR390 for drought sensitivity, INB841 for drought resistance, and SEN56 for high pod partitioning efficiency. Of these 19 lines, half had the reputation of drought resistance, half had not. *P. acutifolius* (G40001) was included since it is highly drought resistant and has very high PHI.

Growth environment

Field experiments were carried out at the International Centre for Tropical Agriculture (CIAT) in Palmira, Colombia (3°29′N, 76°21′W) at an altitude of 965 m from July–September of 2018. Characteristics of the field and rain-out shelter trial as well as soil characteristics were described previously (Polania *et al.*, 2016*a*). Climate data, including minimum and maximum temperature, rainfall, and pan evaporation during the field trails were collected at 15 min intervals. During the experiment, daily temperatures ranged between 14.1 and 37.2°C, with a daytime average of 25°C, average air relative humidity was 78%, average solar radiation was 500 W m^{-2} and average daylight PAR of 970 μmol m^{-2} s^{-1}). Total rainfall during the active crop growth period was 190 mm with potential pan evaporation 469 mm. Irrigation was maintained in the field for the control treatment, and two drought-stress treatment levels (see below) were managed under rain-out shelter conditions. The irrigated control treatment received six furrow irrigations (each 30 mm of water) together with two rains (55 and 30 mm) to ensure adequate soil moisture during the season. Soil volumetric water content was measured twice a day (08:00 and 15:00 hours) by 4 PR-2 probes in each block (1 m long, Decagon Devices). The experimental layout of all treatments was the same. For replication, all treatments (early drought, late drought and WW) were split into three separate randomised blocks each containing four internal subplots. In each block, two probe tubes were installed at the beginning of the experiment. Each of these subplots was made up of 20 rows, one for each genotype, which contained eight individual plants. Each row had a different, random order of the genotypes. In total, four rows of each genotype existed per each block, makes in total 12 replicates of each genotype per each treatment. A border *P. vulgaris* genotype ‘Amadeus’ was used at each exterior edge as well as between subplots and treatments. The soil is a Mollisol (fine-silty mixed, isohyperthermic Aquic Hapludoll) as

described by the USDA classification system, with no major fertility problems (pH = 7.7). For a more detailed description, see Beebe et al. (2008) and Rao et al. (2017). All other information concerning this field experiment was similar to Polania et al. (2016).

Fig. 1. Photograph of the field with rain-out shelter.

Drought treatment

Two different drought treatments were used to determine the independent effects of water stress on two different growth processes; leaflet growth rates (LGR) and pod growth rates (PGR). For determining impacts to leaflet growth rates, water was withheld 10 days before leaflet growth measurements began at BBCH stage 15–17 (5–7 true leaves unfolded; 27 days after sowing) (Feller *et al.*, 1995). After 5 consecutive days of leaflet measurements, this early droughted treatment (ED) was re-watered to 80% of field capacity using 30 mm of water by sprinklers.

After this re-watering, water was again withheld for the remainder of the experiment until the final harvest (69–77 days after sowing). For determining impacts to pod growth rates, a separate part of the rain-out shelter remained watered throughout canopy and flower development, similarly to the control field, and only had water withheld 5 days before pod measurements began at BBCH stage 69 (end of flowering, first pods visible, 37 days after sowing) (Feller *et al.*, 1995) referred to as the late drought treatment (LD). LD plants continued to have water withheld after pod measurements for the remainder of the experiment until the final harvest (69–77 days after sowing). To prevent any rainfall that might occur from disrupting either of the drought conditions, ED and LD fields were grown under a rain-out shelter – a transparent, rolling-roof structure that was positioned over the fields whenever rain threatened (Figure 1), otherwise remained open. Each drought treatment (ED and LD) was applied just before the specific developmental process being measured began (leaflet elongation and pod elongation respectively) in order to probe that specific process' response to water stress while limiting impacts to other processes, such as canopy development. A third field was maintained as the well-watered (WW) treatment, adjacent to the rain-out shelter but not under it. This field was watered to field capacity every two to three days to maintain relatively constant volumetric water content (Figure 2). Although the ED plants were only intended for LGR measurements initially, surprisingly high survival allowed for pod growth measurements to be taken on these plants, as well as yield, PHI and biomass dry weight. These data are included in some analyses as indicated.

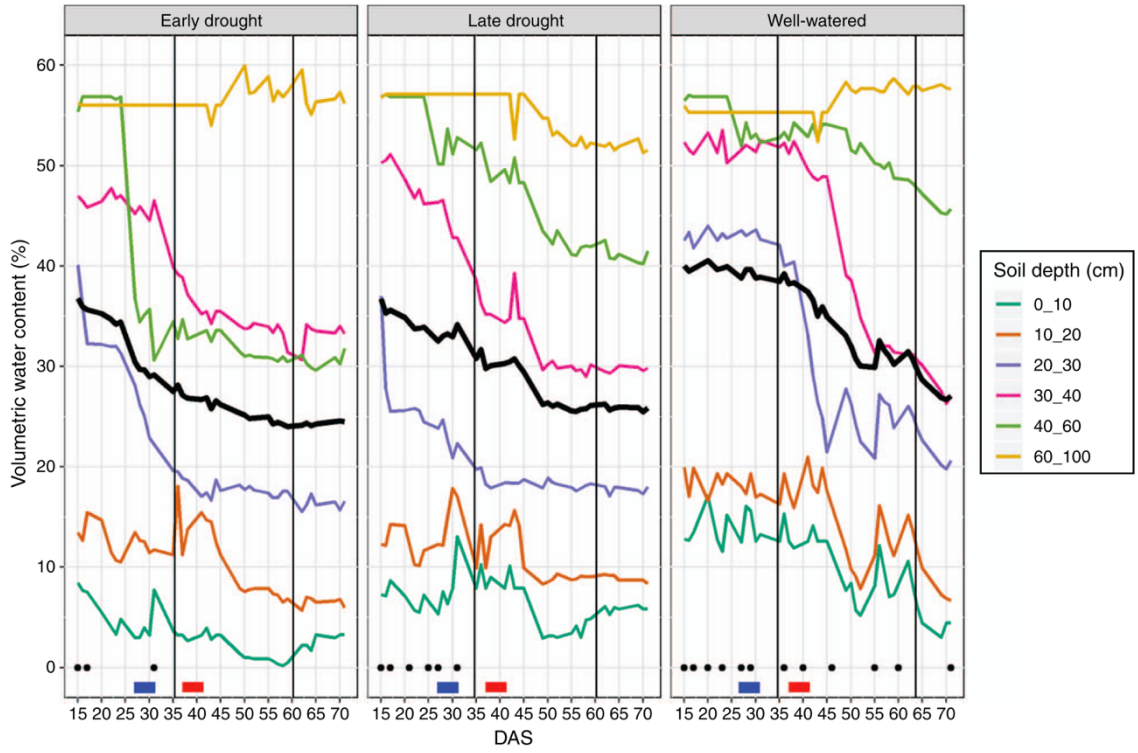

Fig. 2. Average volumetric soil water content at different depths across the three treatments; early drought, late drought and well watered. Volumetric water content at depths from 0–100 cm was monitored daily (DAS, days after sowing) across the three treatments, averaged, and plotted over the course of the experiment. The bold line inside graph is the average soil volumetric water content across the measured soil profile. The blue box shows the 5 days when LGR measurements were taken, red for PGR. Days to flowering and to physiological maturity, 35 DAS and 60 DAS in all treatments, respectively, are inserted as vertical lines.

Growth rates

The BBCH developmental stages were marked every second day as an average of all plant in the subplot. Four subplots were observed for each treatment. During canopy development, when the plants were 3.5 weeks old, LGR were measured in both WW and ED treatments. It is important to mention that at this stage, the individual developmental differences between genotypes were minimal. Within each randomised block, three rows in different subplots and three individual plants were selected for a total of nine replicates per treatment. For each replicate, a terminal leaflet between the length of 40–70 mm, around 30–50% fully expanded (corresponding to the

start of linear elongation phase, data not shown), was tagged and blade length from base to tip was measured using a ruler and recorded. Length measurements of the same leaflet continued for a total of five consecutive days, each measurement taken 24 h apart.

The sampling layout for PGR was similar. In all three treatments, three individual plants were chosen per each subplot, for a total of nine replicates per treatment, and the very first and second pods that developed on each individual plant were marked to ensure uniform developmental starting point. Pod lengths from base to tip were measured with a ruler daily. Measurements began when pod lengths were between 10–20 mm and continued for 3–6 consecutive days, each measurement taken 24 h apart.

Water potential

During the week of leaflet length measurements, leaf water potential only for plants in ED and WW conditions was measured (since the LD condition had not yet entered its drought condition). During the week of pod elongation measurements, leaf water potential for ED, LD and WW were all measured. An individual leaf per plot was measured for each genotype, and this was repeated across two blocks. This resulted in two measurements per genotype per treatment. These replicates came from different plants than those marked for growth rate measurements. Near fully-expanded terminal leaflets near the top of the canopy were selected and cut at the furthest end of their petiolule from the leaflet blade using a razor blade. The leaflet was quickly put into a humid plastic bag and stored in the dark on ice until the measurement was determined, no longer than 10 min after cutting. This method was corroborated without significant negative effect. Leaf water potential was taken using established protocols for a Scholander pressure chamber with a compression gasket system (model 615, PMS Instrument Co.). For midday measurements, leaves were collected between 14:00 and 16:00 hours. Pre-dawn measurements were made with the same procedure between 05:30 and 08:00 hours.

Solute potential

After each leaflet's water potential was measured, leaflets were individually placed into a 2 mL Eppendorf tube and stored on ice until placed into a –20°C freezer (no effect on the readings in comparison to immediate sample freezing, data not shown). Later, solute potential was determined using established protocols with a vapour pressure osmometer (model 5100B,

Wescor Inc.). Samples were thawed for 20 min, exposed to remove condensation, and slightly pressed between two microscope glass slides to release sap, which was then measured. Three different measurements of each replicate sample were taken and averaged.

Yield, PHI and biomass

Upon physiological maturity, three uniform plants were destructively harvested from each of 12 replicate plots. All dry seeds from these three plants were weighed together then divided by three to give dry seed yield per plant. All tissues were oven-dried for a minimum 5 days at 70°C in electric screen-type oven with active open-system air circulation. Whole pod dry weight (including seeds) was also determined for the same three plants together in each plot and PHI was calculated per each replicate plot using Eqn 1:

$$\text{PHI} = \frac{\text{seed biomass dry weight at harvest}}{\text{whole pod biomass dry weight at harvest}} \times 100. \quad (1)$$

Whole canopy (aboveground upon physiological maturity) dry biomass was averaged for the same three plants weighed together from each plot.

Statistical analyses

Linear regression correlations were made between traits that were then tested for statistical significance via the Student's *t*-test using Excel (one-tailed, unpaired, Microsoft Corp.) $\alpha = 0.05$ level of significance. Graphs represent mean values ± s.d.

The impact of treatments was calculated as relative percent decrease between the droughted and WW values for a trait (Eqn 2):

$$\text{percent decrease in growth rate} = \frac{\text{WW growth rate} - \text{WS growth rate}}{\text{WW growth rate}} \times 100. \quad (2)$$

The genotypes that decreased by a larger percentage were considered to be more impacted and drought sensitive.

2.4 Results

Leaf water and solute potential

To quantify internal water status within the plants, leaf water potential (LWP) was measured predawn and at midday. Measurements were taken over multiple days and values from 2–3 days

of measurements were averaged by treatment and graphed by week (Figure 3*a*). As intended, when all genotypes were averaged together, water potential values were significantly lower in water-stressed conditions compared with WW, showing the drought treatments resulted in water deficit compared with WW. This was true for all pre-dawn and midday WW to water-stressed comparisons.

When each genotype was looked at individually, grouped by treatment and time point, difference among genotypes within a treatment/timepoint ranged from –1.1 to –0.74 MPa for WW/week of leaflet elongation, which had the most similar values between genotypes, to –1.3 to –0.78 MPa for LD/week of pod elongation, which had the most different values between genotypes. Even though variation existed in leaf water potential among genotypes, leaf water potential value by genotype did not correlate with the other physiological or agronomic traits measured for that genotype, such as leaflet and pod growth rates (LGR, PGR), biomass and PHI. This suggests that maintaining higher (closer to zero MPa) leaf water potential values did not result in faster growing leaflets or pods or in higher seed yield, PHI or canopy biomass. Among the individual genotypes, there was also a range in relative percent decrease in water potential under drought. When values were compared between ED and WW during the week of leaflet elongation, some genotypes had little to no difference in water potential between these two conditions, whereas others had a relative percent decrease in the ED treatment close to 50% of the WW values. However, as with the water potential values, genotypes whose water potential had the highest relative decrease under drought were not the same genotypes whose growth rates, PHI, yield, or biomass had the highest relative decreases under drought (data not shown).

Solute potential trends were similar to water potential, with values typically decreasing between droughted and WW (ED for leaflet measurement, LD for pod measurement) (Figure 3*b*). One notable difference is that the solute potentials of LD vs WW during the week of pod measurements were not different from each other. Lastly, as with water potential values, solute potential values and relative percent decreases to solute potential values did not correlate with growth or other agronomic traits nor with the relative percent decrease of drought on growth rates or agronomic traits respectively.

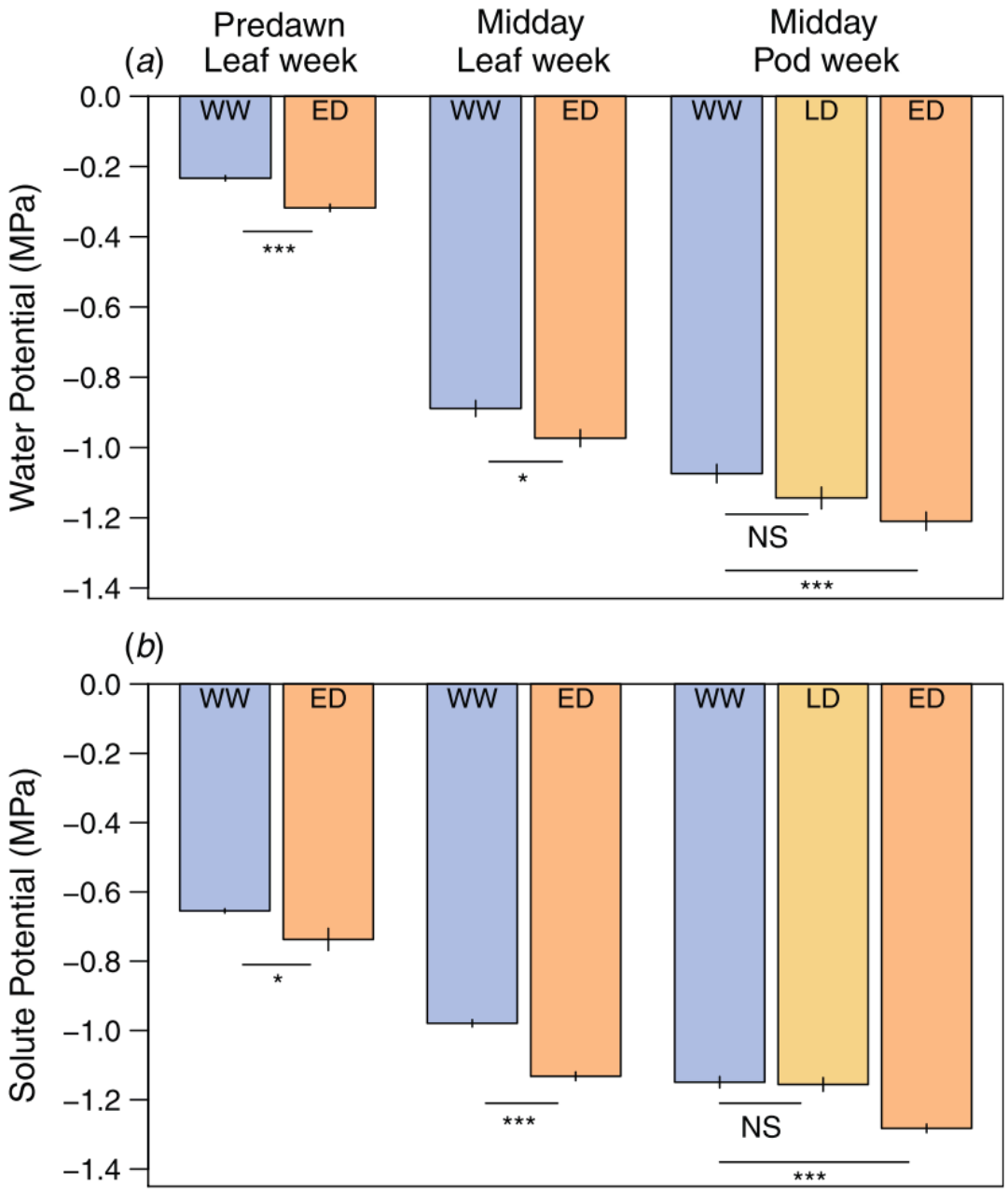

Fig. 3. Average leaf water potential (*a*) and solute potential (*b*) at predawn and midday during the 2 weeks of growth measurements. 'Leaf week' refers to when leaflet growth rate measurements were taken, 'Pod week' when pod growth rates were taken. All bars represent the average across all 20 genotypes, 2–3 measurements per genotype (60 or 90 total measurements). Error bars show s.e. Significance levels indicated: ***, $P < 0.001$; **, $P < 0.01$; * $P < 0.05$.

Leaflet and pod growth rates

As expected, most LGR (measured in the ED condition) were significantly reduced by drought; however, fewer PGR (measured in the LD condition) were significantly reduced. LGR always decreased between ED and WW, but the amount of decrease varied widely by genotype, ranging from 3 to 46% (Figure 4*a*). All decreases of 20% or more were significant, which was the case for 16 of the 20 genotypes. PGR also typically decreased between LD and WW treatments, by around 2 to 45%, although for three genotypes (MR116, INB841 and BH50), PGR increased under LD (Figure 4*b*). Of those three, only increases in BH50 were significant. Relative percent decreases to PGR were only significant for 7 of the 20 genotypes. However, because both LGR and PGR were decreased to similar extents, we hypothesised that genotypes whose LGR had the highest relative decreases under drought would also have the highest relative decrease in PGR. This would suggest that drought impacts growth processes in different tissues in a common or conserved way. However, the degree to which these different tissue types were affected was not consistent across genotypes; genotypes whose LGR decreased the most under drought did not have pods whose growth rates decreased most. Instead, these two growth rates seemed to be independently impacted by drought stress within a genotype suggesting different sensitivity to drought (Figure 4 inset).

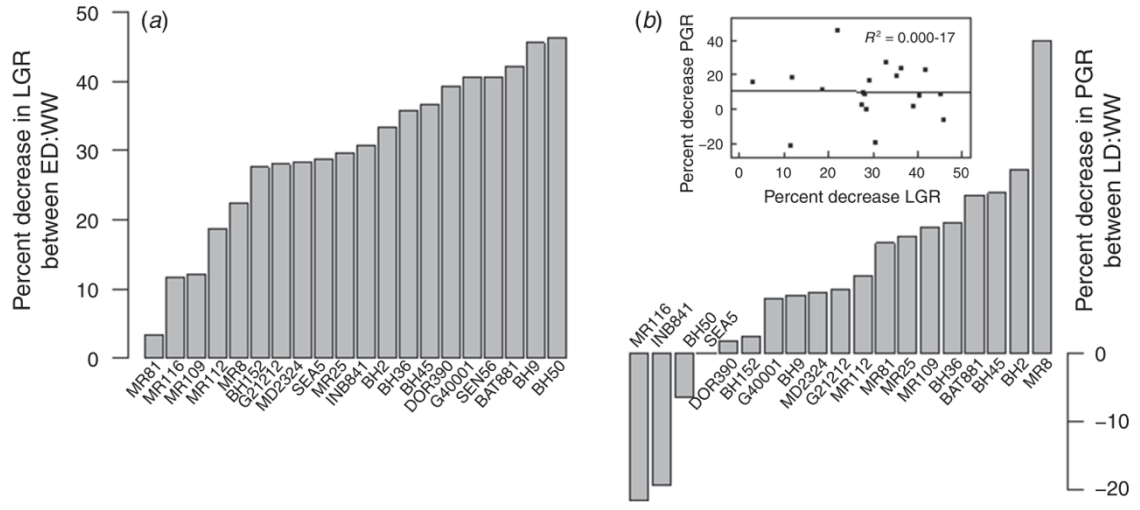

Fig. 4. Relative percent decrease under drought on leaflet growth rates (LGR) (*a*) and pod growth rates (PGR) (*b*) by genotype. Percentage decrease in rate is calculated between well-watered (WW) and droughted; early drought (ED) for LGR comparison (*a*) and late drought

(LD) for PGR comparison (*b*). A negative percent decrease, as occurred for four genotypes in (*b*), indicates an increase in rate under LD compared with WW. Inset shows correlation between percent decreases under drought of LGR and PGR.

Growth rates and seed yield

LGR had a weak but significant correlation with yield under WW. However, neither LGR nor PGR were correlated with yield under drought conditions, nor was there correlation between PGR and yield under WW conditions (Figure 5*a, b*). Instead, we found that the genotypes whose LGR were most relatively decreased under drought (compared between ED and WW) also tended to have seed yield most decreased as well (compared between LD and WW). This was true among the 19 *P. vulgaris* genotypes (Figure 5*c*). Further, although yield of *P. acutifolius* was hardly decreased by drought (which was as we had expected), LGR was highly reduced under drought, showing that the impacts of drought on LGR and yield in this species are decoupled. Unlike LGR, relative decreases to PGR under drought did not correlate with relative yield decreases under drought across all lines (Figure 5*d*).

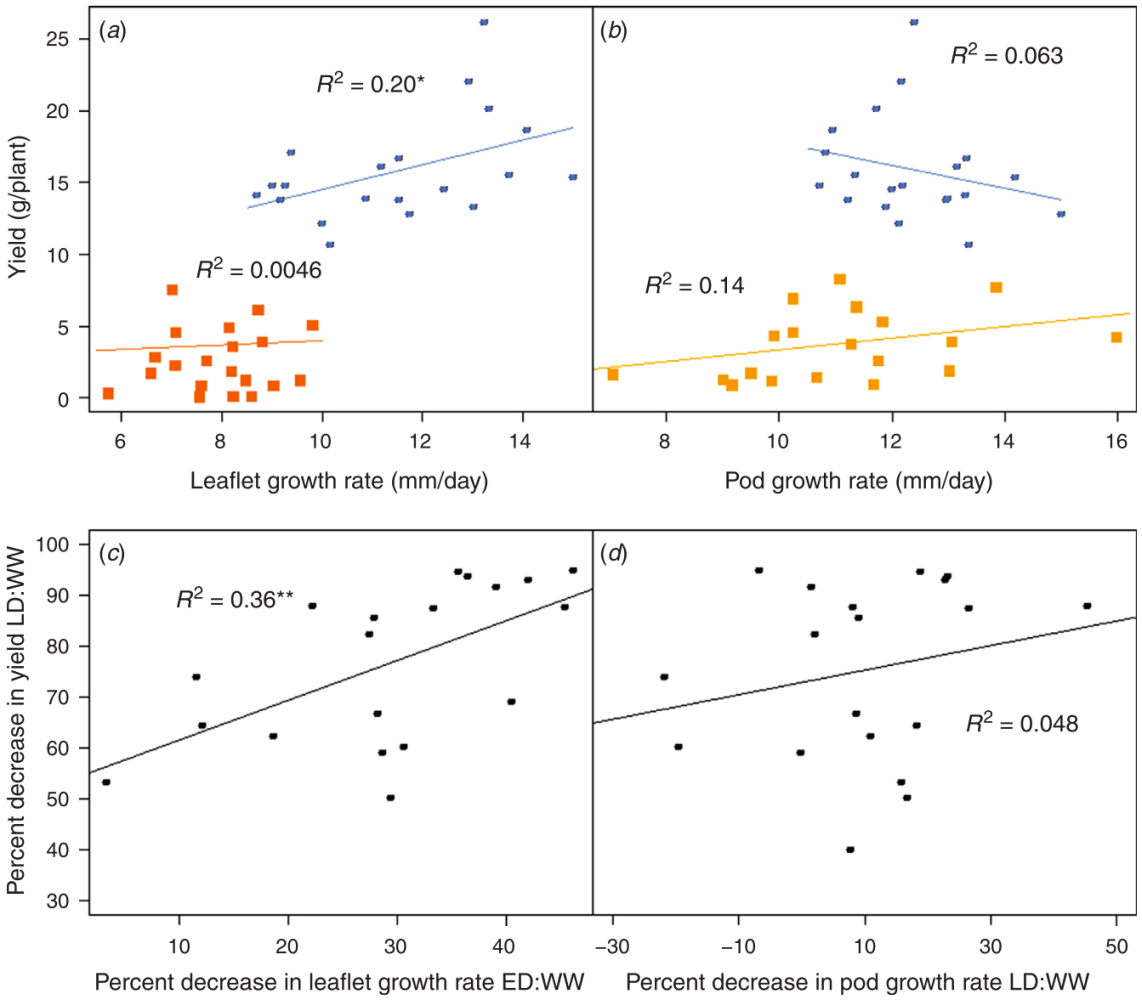

Fig. 5. Correlations between yield and growth rates. (*a*) Correlations were tested between average well-watered (WW) leaflet growth rates (LGR) and WW yield (blue circles) and average early drought (ED) LGR and late drought (LD) yield (orange squares). (*b*) As for (*a*) but for pod growth rates (PGR) and yield under WW (blue circles) and LD (yellow squares). (*a*, *b*) Circles denote WW and squares represent water droughted. (*c*, *d*) Tested correlations between yield reductions (relative percent decrease between LD and WW) and LGR reductions (relative percent decrease between ED and WW) (*c*) or PGR reductions (relative percent decrease between LD and WW) (*d*). Note in (*a*, *c*) drought leaflet growth was compared with late drought yield, whereas in (*b*, *d*), late drought pod growth was compared with late drought yield.

The weak correlation between LGR and yield under WW, and the stronger correlation between the relative percent decrease under drought on these two traits suggests there might be a positive relationship between maximum LGR (observed in WW plants) and the reduction of LGR under drought. This correlation (Figure 6) shows that genotypes with the highest LGR under WW conditions (BH50, BH9, and BAT881) had LGR most decreased under ED (Figure 6). This result, in combination with the fact that these genotypes also experienced strong decreases in yield under LD (95, 88 and 93% respectively), indicates that genotypes exhibiting rapid leaflet expansion under WW conditions are at high risk of reductions to both LGR and seed yield under drought. However, this is not true in the case of pods. Genotypes with the fastest growing pods under WW conditions did not have PGR that were most relatively decreased under LD (data not shown – $R^2 = 0.08$).

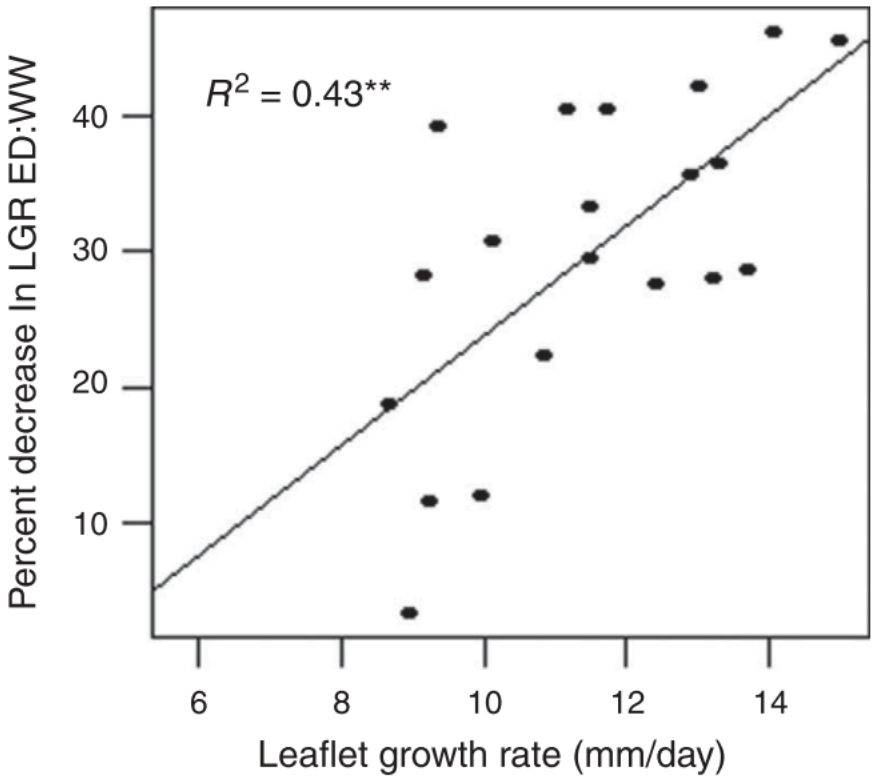

Fig. 6. Correlation between well-watered (WW) leaflet growth rates (LGR) and relative percent decrease in LGR under drought.

PHI

Under WW, average PHI values ranged from 0.74 to 0.83, with few significant differences among genotypes. However, under LD, average PHI values ranged from 0.47 to 0.78 and under ED even wider, from 0.24 to 0.77 (Figure 7). Although differences amongst WW genotypes were small and rarely significant, larger differences existed amongst both the ED and the LD genotypes individually, as well as differences between each drought condition compared with WW for most genotypes. Although significant decreases in PHI between WW to ED and LD existed for most genotypes, the size of the difference between droughted and WW values varied widely by genotype, from 0.02 to 0.31 under LD and 0.03 to 0.54 under ED. The genotype with one of the highest PHI values under WW also had one of the smallest relative reductions to PHI under both drought conditions was G40001, *P. acutifolius*, which was included because it was known to maintain high PHI under drought.

When PHI values were compared against LGR and PGR, no significant correlations were found in either WW or WS conditions, nor were relative percent decreases to these values correlated (data not shown).

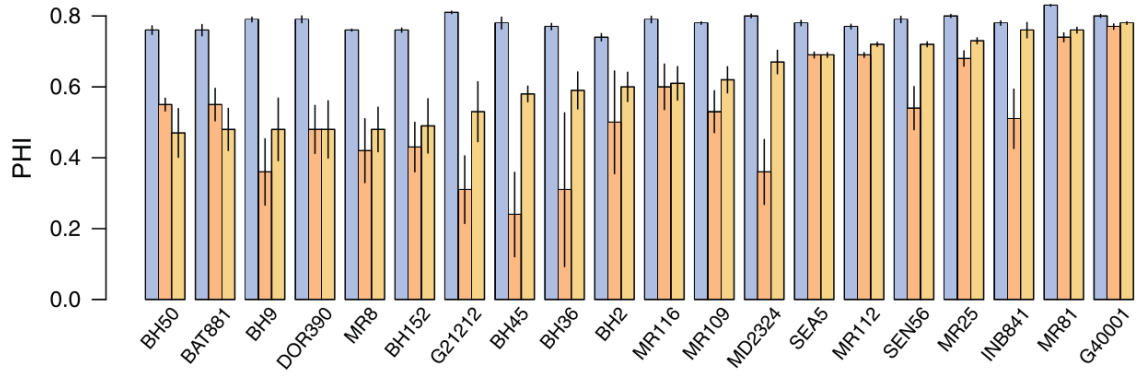

Fig. 7. Pod harvest index (PHI) values for all 20 genotypes under well-watered (WW, blue), early drought (ED, orange), and late drought (LD, yellow) conditions, respectively. Genotypes are in ascending order based on late drought PHI values. Error bars show s.e.

PHI and yield

Studies from CIAT consistently show PHI correlates more strongly with seed yield under drought and WW conditions than biomass, with these correlations typically strongest in droughted plants over those growing in WW conditions (Rao *et al.*, 2017). In our study, PHI did not correlate with yield under WW conditions; however, under drought the relationship between PHI and yield was high, as expected, with significant positive correlation under LD conditions (Figure 8*a*). Likewise, decreases in PHI and yield under LD have significant positive correlation (Figure 8*b*).

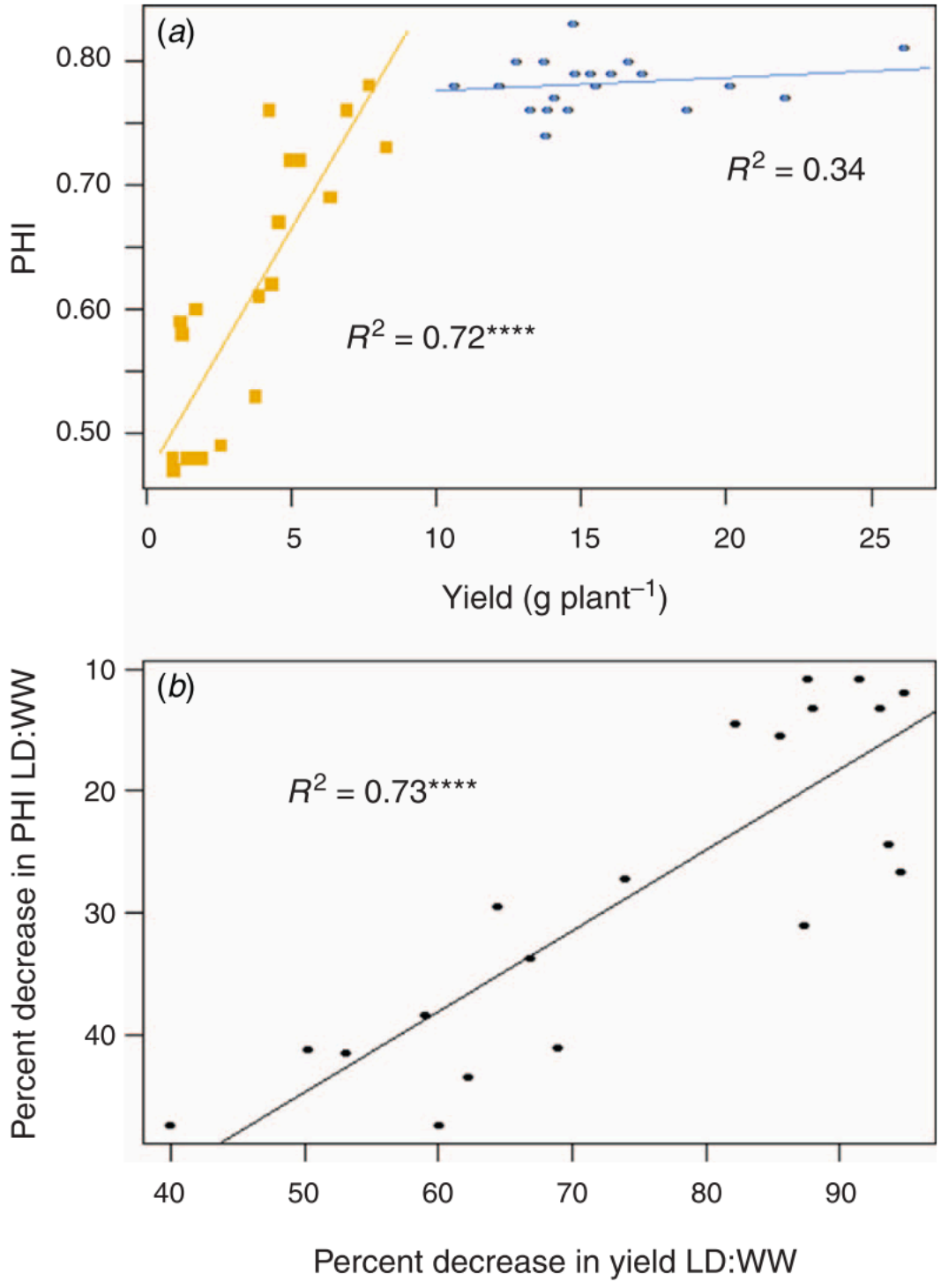

Fig. 8. Correlations between yield and pod harvest index (PHI). (*a*) Correlations were tested between yield and PHI under well-watered (WW, blue circles) and late drought (LD, yellow

squares) conditions for the 20 genotypes. (*b*) Correlations between relative percent decreases on these two traits comparing LD to WW.

Canopy biomass and seed yield

Although canopy biomass was correlated with seed yield under LD conditions (data not shown – $R^2 = 0.59$), the correlations between the relative percent decrease under drought on these two traits were even higher. Specifically, the relative decreases of canopy biomass under LD correlated more strongly with seed yield than any other trait (Figure 9). Those plants with the largest decreases in canopy biomass also had the largest decreases in seed yield. This suggests that although biomass itself is a large contributor to seed yield potential, the relative sensitivity of a plant's biomass accumulation to drought was a better predictor of yield over values of biomass itself.

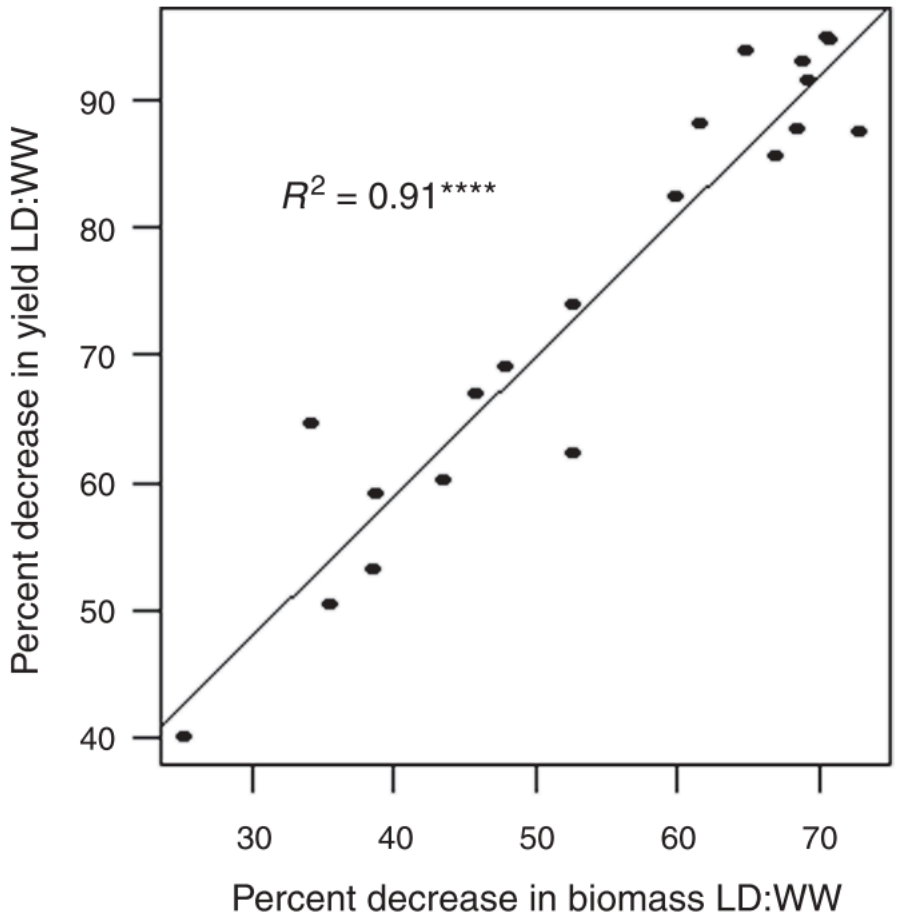

Fig. 9. Correlation between relative percent decreases on yield and biomass between late drought (LD) and well-watered (WW) conditions.

Phenology

Analysis of phenology – both time to flowering and time to physiological maturity – provided two notable results. Time to flowering varied across the 20 lines (30–40 days) but neither ED nor LD treatment altered the average number of days to flowering compared with WW (see Fig. S1, available as Supplementary material to this paper). Time to physiological maturity was reduced for all lines under both drought treatments. This was as expected and is commonly observed under drought (Fig. S2). As a result, yield of LD plants is correlated with days to flowering (R^2 = 0.66) showing that phenology plays a significant role in determining yield in the LD plants.

2.5 Discussion

This study was designed to test a hypothesis that leaf growth rate could be a reliable indicator of yield in common bean. The idea arose from data collected in a greenhouse study of well-watered and droughted bean lines, which found a strong correlation between leaflet growth rate and seed dry weight at harvest, under both conditions (Banan and Van Volkenburgh, 2012). This suggested that each plant had an inherent sink strength (plants with rapidly growing leaves would also have rapidly growing seeds), and that drought would affect both organs similarly. If this were true, the relative percent decreases of drought on LGR would be a good indicator of how much drought would reduce seed yield.

The plants chosen for this study include 19 lines of *P. vulgaris*; 16 represent 2 inbred lines developed for variation in drought resistance and pathogen resistance, their parents, and 3 are unrelated but commonly grown lines of *P. vulgaris. P. acutifolius* was included because it is known to be highly drought-resistant. By testing a range of genotypes, we hoped that correlations of traits would indicate either support for our hypothesis or suggest other mechanisms relevant to drought resistance in common bean.

This study was conducted in the field during one season (summer 2018) in research plots maintained at CIAT, the CGIAR centre charged with bean breeding for the tropics and site of the world's largest collection of common bean seeds. At CIAT there is one research field fitted with

a moveable rain-out shelter allowing experiments to be conducted side-by-side, WW and drought. We designed two drought treatments; early drought (ED), to assess the effect of drought on canopy development (leaflet growth), and late drought (LD), to do the same during reproductive development (pod and seed growth). As intended, both ED and LD water-stress treatments resulted in water deficits within the plants when compared with WW. Surprisingly, leaf water status did not seem to play a direct role in limiting growth and yield, since neither leaf water potential nor solute potential values correlated with growth rates, canopy biomass, PHI or yield. Nor were genotypes that had water or solute potential values most decreased under drought compared to WW conditions the same genotypes that had growth rates, canopy biomass or PHI most decreased under drought. Thus, genotypes with the largest drops in leaf water potential under drought did not have largest drops in the above-mentioned traits, suggesting that leaflet and pod elongation, biomass accumulation and resource partitioning are not limited directly by more negative leaf water potential.

In common bean, leaf growth rate determines canopy biomass at canopy closure, a predictor of yield (Watson, 1952). The rate of leaf growth is determined both by light-driven proton efflux (Van Volkenburgh and Cleland, 1980) and a photosynthetic contribution from the growing leaves themselves (Blum *et al.*, 1992; Van Volkenburgh, 1999). Leaf growth rate and leaf area are reduced under water-deficit conditions (Davies and Van Volkenburgh, 1983). This is not due to a reduction of turgor in the growing leaves (Van Volkenburgh, 1999), but more likely is due to accumulation of abscisic acid, inhibition of proton efflux, and a reduction of cell wall loosening. This mechanism controlling leaf cell growth rate is similar to that regulating seed growth (Tegeder *et al.*, 2000) and is likely a target of regulation of sink strength.

We predicted that leaflet growth rate (LGR) and pod growth rate (PGR) would correlate with seed yield and partitioning efficiency (PHI). However, what we found was that effects of drought on LGR and PGR within each genotype were uncoupled, meaning genotypes whose LGR decreased the most (under ED – Figure 4*a*) were not the same as those whose PGR decreased most (under LD – Figure 4*b*). The impact of drought on LGR did not predict the relative percent decrease of PGR under drought either (Figure 4 inset). This could be due in part to the fact that under drought, tissue types become water stressed at different rates, with pods and seeds being the last impacted (Westgate and Grant, 1989). Seeds in droughted plants have also been shown to be capable of buffering the impacts of drought by maintaining nutrient concentrations similar to

those of irrigated plants (Smith et al. 2019) and studies show that seed filling in *P. vulgaris* is at most only partially coupled with photosynthesis (Smith, 2018). Pods and seeds may have evolved to be buffered from this stress because of the critical role they play in survival, although the mechanisms underlying this are not known. Additionally, genetic differences in time to physiological maturity were more pronounced under drought due to shortening of plant life span. This resulted in a strong correlation between phenology and yield in LD conditions, and may have contributed to the observed decoupling of leaf responses to drought from pod and seed responses. And although plants that flowered sooner were likely further along in their pod and seed development when LD stress was imposed, we cannot say whether this imparted the drought resistance or rather that plants that have higher sink strength (and therefore better ability to fill seeds and grow leaves) mature faster and that this trait of high sink strength actually imparts the drought resistance.

Leaflet and pod growth rates themselves did not correlate with partitioning or yield under water deficit, but the sensitivity of LGR to drought did predict the sensitivity of yield to drought. In other words, genotypes whose LGR were most decreased under drought compared to WW had yields most decreased under drought as well. Our hypothesis, that LGR would predict PGR and seed filling, was based in part on published results showing that terminal drought resistance was explained by higher efficiency of carbon mobilisation from leaves to pods and seeds (Cuellar-Ortiz *et al.*, 2008; Rosales *et al.*, 2012). This hypothesis does not appear to be true when looking at growth rates and yield, as some high yielders had lower leaflet and pod growth rates (Figure 5*a, b*). Yet it does appear true in terms of sink strength sensitivity, where drought's relative decreases on LGR (during early drought) and yield (during late drought) seem to be linked by a conserved mechanism affecting processes related to sink strength (Figure 5*c*). These data suggest that LGR sensitivity to drought may act as a good predictor of overall drought resistance.

Unsurprisingly, given that LGR and PGR within a genotype were not similarly decreased by drought, relative PGR reductions under LD (drought during pod elongation) were not a good predictor of LD yield (Figure 5*d*). This could again be because the pod may be buffered from water stress, separating it from other sink tissues due to the evolutionary priority of seed production. However, for the ED treatment, PGR and yield did have a positive correlation (although not significant, Figure 5*b*). Since we only found this to be true under ED, we believe this could mean that more severe stress (especially when it leads to large relative decreases to

biomass, as it did in the ED treatment) significantly impacts PGR due to lack of accumulated resources, whereas under the LD treatment, genotypes that slowed most did so due to a stronger response on drought signalling or status, rather than a lack of substrate. This would fit with previous findings showing that *P. vulgaris* seeds among different genotypes take up sucrose at differing rates, even when there were no differences in available sucrose in the sap surrounding the seed (Tegeder *et al.*, 2000).

Unlike LGR and PGR, canopy biomass values did correlate strongly with seed yield under both WW and LD conditions. Yet, stronger still were correlations between relative reductions to biomass and relative reduction to yield under LD (Figure 9). This again supports the above-mentioned hypothesis that sensitivity of canopy development (leaflet growth) to drought may be the strongest predictor of yield under drought. Particularly, under LD conditions, genotypes in the present study that achieved a similar biomass displayed a range of yields, with some genotypes differing even up to 80%. Therefore, although the correlations show that higher biomass is necessary to achieving higher seed yield under drought stress, as has been shown previously, (Polania *et al.*, 2017; Rao *et al.*, 2017), reductions in canopy biomass alone do not fully answer the question as to what results in reduction of yield under drought.

Therefore, based on previous findings that PHI is the best predictor of yield, we assessed whether PHI values help to account for differences in yield when biomass could not. First, we tested correlations between PHI and yield under the three conditions. We found that PHI did not correlate with yield under WW conditions. However, we did find correlations under early drought conditions and even higher under late drought conditions (Figure 8*a*). We also found that reductions to yield and PHI between LD and WW correlated significantly (Figure 8*b*). Beyond testing how PHI alone is related to yield, we wanted to know whether differences in PHI could be used to better understand how genotypes can acquire the same canopy biomass yet achieve different yields. Indeed, we found they could. For example, under LD, average canopy biomass ranged from 20–55 g plant^{-1}, depending on genotype. As mentioned above, within that range, genotypes whose canopy biomass was the same could vary by 80% in their yield. Specifically, genotypes MR81 and BH45 had very similar high average canopy biomass under LD – around 43 g. Yet although MR81 yielded second highest of all the genotypes under LD, with 6.72 g dry seed weight, BH45 was on the lower end of the yield spectrum with only 1.25 g. If 6.72 g is considered 100% of the potential yield possible for this canopy biomass, a yield of

1.25 g is only 19% of that potential. When we paired this finding with PHI values for these genotypes, the differences in yield between the two could be explained by differences in their partitioning efficiency, with MR81 maintaining a high PHI of 0.76 while BH45 had a much-reduced value of 0.58 (Figure 7).

Our results help to tease apart systemic and tissue-specific responses to drought and to understand how impacts to different growth or partitioning processes under drought relate to yield. Although our results did not support the hypothesis that leaflet and pod growth rates *per se* predict seed yield, our findings together suggest that among domesticated bean lines, inherent differences in partitioning efficiencies and drought sensitivity may point to a mechanism underlying drought resistance shared across stages of plant development. Domestication has involved development of fast-growing plants, most likely by selection of plants lacking mechanisms for downregulation growth rate. Slow-growers are conservative plants well adapted to the myriad stresses in a natural environment. It is possible that genetic regulatory mechanisms exist in the wild progenitors of *P. vulgaris* breeding lines enabling rapid growth rates in leaves, but these are not expressed until breeding breaks or removes the regulation. If this is so, what we saw in the array of lines tested in this study was a range of plants from fast-growers to slow-growers. The fast-growers were more susceptible to drought, which reduced leaf growth rate, canopy biomass and seed yield. Perhaps the development of fast-growing bean plants by domestication shifted internal regulation of allocation such that substrates are preferentially shunted towards canopy growth, even during drought.

Many crops whose yield has improved during the green revolution did so not by increasing their total production, but instead by partitioning a greater amount of resources to yield (Wardlaw, 1990). For example, rice and wheat yield went from having 30% of total biomass in yield at maturity to 50% in the 1960s (Khush, 1999). *P. vulgaris* yield partitioning efficiency on the other hand, has yet to see similar improvements as common bean maintains the ancestral trait of delayed seed production under drought (Beebe *et al.*, 2008, 2013). The observation that partitioning appears to limit yield under drought might fuel work to attain further improvements to partitioning in *P. vulgaris*. Gaining a deeper understanding of how partitioning is affected by drought may allow for larger genetic gains in efficiency in this trait. Discoveries on sink strength in common bean may also help to uncover common mechanisms shared by other crops,

increasing the impact of our findings. An earlier submitted version of a portion of this work is available as a preprint (Hageman *et al.*, 2019).

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Chapter 3: Quantification of resource allocation over time between drought tolerant and drought sensitive common beans and the mechanisms which control differences in partitioning

3.1 Abstract

Drought limits plant growth rate, accumulation of biomass and yield in crops, including common bean (*Phaseolus vulgaris* L.). However, even with less biomass, drought-tolerant bean lines allocate a greater proportion of existing biomass to seeds than drought-sensitive lines, increasing yields. To address possible mechanisms underlying this response, a drought-tolerant and a drought-sensitive genotype were grown in pots in controlled-environment chambers under two treatments, well-watered and water-deficit. Destructive harvests were carried out over the period of reproductive development to understand how biomass allocation shifts over time, and how this differed between the two genotypes. Excised seed assays were conducted to measure sucrose uptake and proton efflux, physiological processes driving seed growth. The tolerant line filled seeds earlier than the sensitive line and ended up with a higher proportion of total biomass contained in seeds by allocating more biomass from the pod wall into the seeds. Sucrose uptake into seeds, and pH of the incubation medium both correlated with weight gain rates in seed assays. Addition of the drought-induced phytohormone abscisic acid to the seed-incubation media reduced weight gain and sucrose uptake rates only for the sensitive genotype. The result suggests that sensitivity to this hormone may play a role in determining seed filling rates under drought stress in common bean.

3.2 Introduction

Drought is a major limiter of grain yield (Boyer and Westgate 2004), and in common bean (*Phaseolus vulgaris*), drought is the most detrimental abiotic stress, reducing yields from moderately to completely (Villordo-pineda *et al.* 2015; Polania *et al.* 2016). In South America and Southern and Southeastern Africa, 60% of bean production is grown under intermittent or terminal drought conditions on small-holder farms which typically have less access to inputs

such as irrigation (Broughton *et al.* 2003; Omae *et al.* 2012). This has significant implications for food security, since common bean is an important source of protein for ~300 million people in these regions (Cavalieri *et al.* 2011). Therefore, breeding drought tolerant varieties of common bean remains an important priority.

At the International Center for Tropical Agriculture (CIAT), new varieties of common bean are produced through conventional breeding methods and screened for drought-tolerance among other traits valued in field-grown beans (Beebe *et al.* 2008). Breeders screen for and classify drought-tolerance and drought-sensitivity based on large-scale agronomic traits which have been correlated with high or low yield under drought, respectively. However, less is known about the physiological processes underlying these traits which cause these lines to perform better or worse under drought. The goal of this project was to develop laboratory-based growing conditions and experimental methods to test hypotheses about mechanisms of drought tolerance in a drought-tolerant and a drought-sensitive genotype of common bean produced at CIAT.

Although production of sufficient canopy biomass is necessary for seed production, high biomass does not always translate into yield (Rai *et al.* 2020). Rather, traits related to allocation efficiency of available resources towards seed production act as stronger indicators of yield, especially under drought (Asfaw *et al.* 2017; Karimzadeh Soureshjani *et al.* 2020). In particular, a trait called pod harvest index (PHI) has been identified by CIAT as the strongest predictor of yield under drought in common bean (Assefa *et al.* 2013). This trait quantifies the proportion of dry pod biomass allocated to the seeds compared to what remains left in the pod wall. This proportion of seed dry weight to total pod dry weight correlates most strongly with higher yield under drought, even over traits that may seem to be more strongly linked such as total biomass and pod or seed number. While this finding alone is very useful for breeding purposes, it begs the question of why; what makes the efficiency of moving this small amount of dry biomass from the pod wall to the seed the single best indicator of overall drought tolerance?

To address these questions, we designed a series of destructive harvests to track movement of dry biomass from the canopy to pods and seeds over time during reproductive development. This allowed us to quantify biomass pools over time to discover possible bottlenecks to carbon flow.

From these data, important tissues and timepoints were pinpointed and tissue-level physiological assays were performed to look into mechanisms involved in the transport processes. An *in vitro* seed assay was performed. Excised cotyledon halves were placed in a liquid growth medium containing sugar and was incubated for 24 hours. After incubation, fresh weight gains were measured as well as changes in sugar concentration of the incubation media. Additionally, the effects of abscisic acid (ABA), a drought induced phytohormone which induces many stress responses (Vishwakarma *et al.* 2017), were quantified by adding ABA to the aqueous growth media and measuring effects on biomass gains and solution sugar concentrations.

We found that while seed biomass (yield) did not differ between the drought-sensitive and drought-tolerant lines under water stress in the growth-chamber conditions, the tolerant line did achieve higher resource allocation to seeds, resulting in higher PHI. Additionally, tracking biomass by tissue over time showed that the tolerant line fills seeds earlier in development than the sensitive, and that in the sensitive line, the pod wall does appear to be the bottleneck to carbon flow. Assays of sucrose and proton fluxes captured differences in seed filling rates. Further experimentation is needed to determine whether these fluxes establish early seed-filling in tolerant lines and confirm hormone sensitivity or lack thereof in regulation of these fluxes.

3.3 Materials and methods

Plant materials

A drought-sensitive genotype MR8, and MR81, a drought-tolerant genotype, were used in this study. Twenty four 1-liter pots of each genotype were sown in premoistened soil (Sunshine Mix #4, SunGro Horticulture) with two seeds each and thinned to one seedling per pot two weeks after planting. Plants were grown in a 40 ft^2 Conviron PGW40 walk-in growth room (Controlled Environments Ltd (Conviron), Winnipeg, MB, Canada) under 14 hours a day of light at 1200 $\mu molm^{-2}s^{-1}$ and 25°C, and 10 hours dark at 18°C. Chambers were kept at ambient humidity all day and fresh air flow was 55 CFM. All pots were watered daily to saturation until flowering, determined for each genotype by recording the date when 50% of all 24 individual plants from each genotype had opened at least one flower (Andrade *et al.* 2016). After the flowering date had been determined for each genotype, all pots continued being watered daily until 6 days after

flowering, after which point half the individuals of each genotype were randomly assigned to the water-stress (WS) treatment while the other half remained watered to saturation daily, well-watered (WW). For the WS treatment, plants were not watered again until they began to wilt, at which point, that specific pot would be watered to saturation. Watering for the WS treatment continued in this way, with water being withheld until first signs of wilting, for the remainder of the experiment. Therefore, watering typically occurring ever 4-6 days for each WS treatment pot. Genotype-treatment pairs (WW tolerant, WS tolerant, WW sensitive, WS sensitive) were evenly yet randomly spread throughout the chamber to account for chamber effects, with three blocks of 16 pots (4 pots of each of the 4 genotype-treatment pairs). Within each block of 16, the 4 pots from each genotype-treatment pair were grouped together and the order in which these were arranged was randomized in each of the three blocks (all different from one another). Two chambers had this exact experimental design and set up – one for determining changes in biomass over time via destructive harvests, the other for spot harvesting of pods and seeds for more in depth physiological measurements.

Destructive harvests for biomass determination over time

Destructive harvests were carried out on plants from one of the chambers at four developmental stages over the course of seed development. The first harvest was done at 20 days after flowering (DAF), which corresponded to the start of linear seed filling stage. The following three were at 28, 36 and 43 DAF, corresponding to mid seed filling stage, maximum seed fill, and harvest maturity (when the seeds reach their minimum seed water content). At each harvest, three plants of each genotype-treatment pair were selected - one randomly from each of the three blocks. Plant were then broken down by tissue type: leaves, pods, seeds, and stems. Roots were not harvested. Each tissue type was weighed individually at harvest to obtain fresh weights then placed into a paper bag and dried in a 70° oven for 48 hours. Each tissue was then reweighed per plant for obtaining dry weight. Water weight was calculated as the difference between fresh and dry weights. Percent weights for each tissue type were calculated by dividing each tissue's dry weight by the total canopy dry weight from that plant.

Destructive harvests for seed assays

From the second chamber, pods of roughly equal developmental stage were harvested over the course of 2 weeks. This stage corresponded roughly to between timepoints 2 and 3 from above, mid seed filling and maximum seed filling. One pod from each of the three individual plants of all four genotype-treatment pairs were harvested for each experiment. Seeds were excised from pods, then the embryo (made up of the cotyledons and the radicle) was further dissected out of the seed coat. Last, the embryos were broken apart into the two cotyledon halves. These dissections were done as quickly as possible after the pods had been harvested and were kept under moist paper towels to prevent water loss. Six cotyledon halves, two from each of the three individual plants per genotype-treatment pair, were then pooled together and placed into a single well of an assay plate containing 5mL of growth media.

Fresh weight gain and sucrose uptake measurements

Aqueous growth media containing 10mM KCl and either 50, 100, or 200 mM sucrose were adjusted to pH 5.5 using NaOH and HCl, and osmotically adjusted to 300 mOsm using D-Sorbitol (adapted from Tegeder *et al.* 1999). 10^{-4} ABA was subsequently added to specific wells when necessary for a specific assay, 1:1000, resulting in 10^{-7} ABA experimental concentration.

Using a Brix meter, we measured changes in sucrose concentration in the aqueous solution over time. Initial °Brix readings were taken for each solution before excised cotyledon halves were added, then after a 24-hour incubation at room temperature, the solution was homogenized by mixing and a second measurement was taken. After creating a calibration of sucrose concentrations to °Brix, we converted initial and final °Brix measurements to sucrose concentrations to determine the amount of sucrose depleted from solution over the 24-hour incubation. Depletion rates were then calculated per gram of starting fresh weight.

In addition to the Brix meter measurements, fresh weight gains were also measured over this 24-hour period. Initial fresh weight of the six cotyledon halves per well were taken just before placing them into the assay solution. After 24 hours, the cotyledon halves were removed from the solution, blotted on tissue paper to removed surface water, then weighed for final fresh weight. Finally, they were dried in a 70° oven for 48 hours and dry weight was recorded for normalizing both sucrose uptake rates and fresh weight gains by grams of dry weight.

pH assays

Assays were performed using the dissected cotyledon halves and floating them in wells of the 200 mM sucrose aqueous growth media described above and covered. The plates were then placed onto a shaker table to maintain even mixing. Measurements of pH were taken using a handheld pH meter before cotyledons were added to the media, and again after 24 hours. The cotyledons were then placed into a drying oven to obtain dry weight. The difference in pH was divided by cotyledon dry weight and net proton uptake over the 24-hour period was calculated.

Data analysis

Comparisons for results will always be between either; (1) genotypes or (2) treatments. If comparing across genotypes, comparisons will be between WW tolerant to WW sensitive or WS tolerant to WS sensitive. When comparing across treatments, comparisons will be within the same genotype, either the WW to WS tolerant pair or WW to WS sensitive pair. All graphs show averaged values with standard error calculated for error bars.

3.4 Results

Canopy biomass

At the first harvest timepoint, both genotypes in the WW treatment had amassed more total dry canopy biomass than their corresponding WS treatment (Figure 1). Looking over time, all four genotype-treatment pairs (WW tolerant, WS tolerant, WW sensitive, WS sensitive) increased dry canopy biomass over the first three timepoints, from the start of seed fill to maximum seed fill. Between the third and fourth timepoints, maximum seed fill and harvest maturity, there was no change in dry canopy biomass for any of four genotype-treatment pairs as expected. Both the WW tolerant and the WW sensitive plants increased their dry canopy biomass at similar rates over the first three timepoints, gaining on average 9.5 and 10.0 grams between each timepoint, respectively. Likewise, both the WS tolerant and WS sensitive increased their dry canopy biomass at similar rates to one another over the first three timepoints, however at slower rates than their corresponding WW treatment. Under WS, tolerant and sensitive plants' dry canopy

biomass increased on average by 6.4 and 7.0 grams, respectively, between each of the first three timepoints.

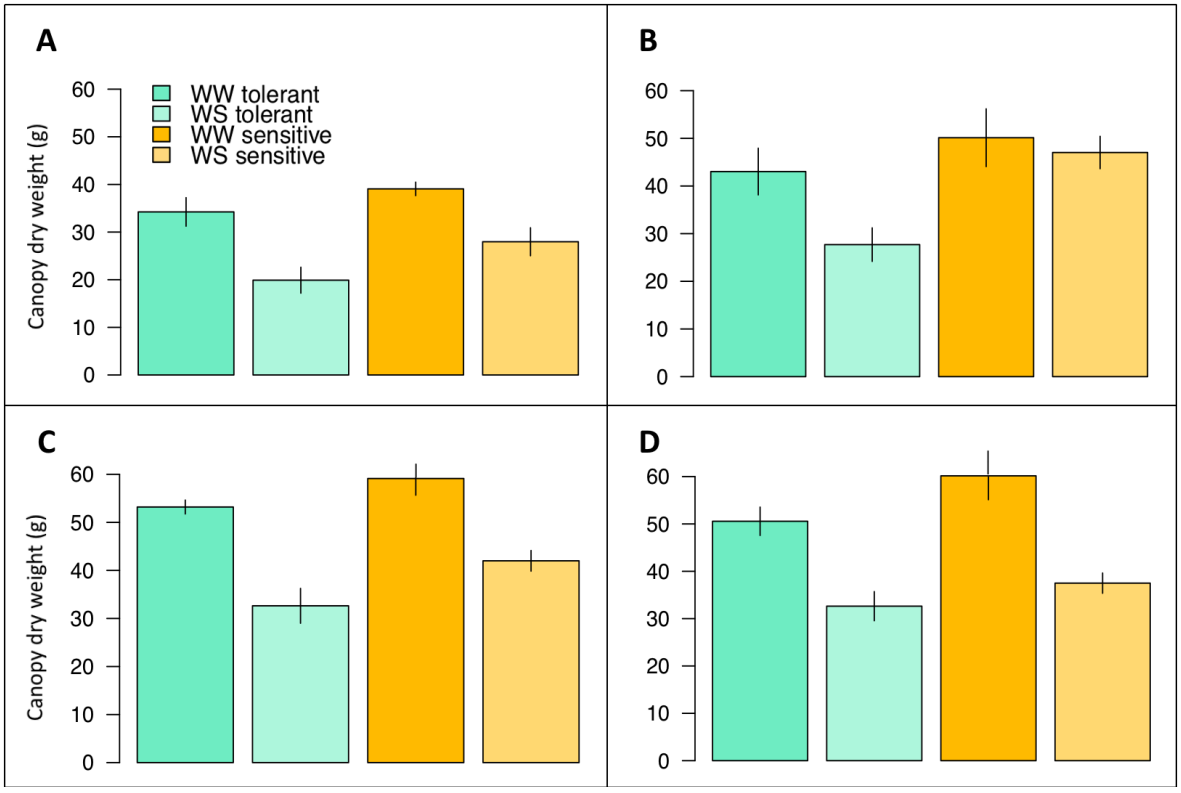

Figure 1. Whole plant dry canopy biomass (g) for the four genotype-treatment pairs over time. (A) shows plants harvested at start of seed fill, (B) mid seed fill, (C) end of seed fill and (D) harvest maturity. Graph shows total canopy biomass in grams for both genotypes well-watered treatment (WW) (darker bars) and water-stressed treatment (WS) (lighter bars). Bars for the tolerant are in shades of teal, bars for sensitive are in shades of orange. Destructive harvests were done at 20, 28, 36, and 43 days after flowering, corresponding to the start of seed fill, mid seed fill, end of seed fill and harvest maturity, respectively. Three replicates per measurement were taken and averaged. Error bars show standard error.

Looking at dry canopy biomass broken down by tissue type; stems, leaves, pod walls and seeds, we can see more detail of how canopy biomass is distributed and how this changed over time (Figure 2). Under WW conditions, the tolerant line allocated biomass towards seeds earlier than the sensitive line. This can be seen by comparing seed dry biomass between WW tolerant (dark teal) and WW sensitive (dark orange) across timepoints one, two and three. Later in development, the WW sensitive line did eventually reach the same dry biomass of seeds as the WW tolerant and even surpassed it, sometime between timepoints 2 and 3. Under WS conditions though, the sensitive and tolerant lines did not differ from one another in either the timing or amount of biomass they put towards seeds – rather, they put a similar amount of biomass at

similar rates towards seed production, with dry seed biomass not differing significantly from one another at any of the four timepoints. This resulted in seed dry biomass that is not different than each other at harvest maturity, 43 days after flowering. Dry biomass of stems, leaves and pod walls was almost always lower in the tolerant line compared to the sensitive line, for both the WW and the WS comparisons. This fits with findings above that the tolerant line accumulated less total dry canopy biomass than the corresponding sensitive line. However, although stems, leaves and pod walls of the sensitive genotype (both WW and WS) had more biomass than their corresponding tolerant comparison, the same is not true for seed biomass. In fact, the tolerant line sometimes had more dry biomass in seeds compared to sensitive line (comparing WW-WW and WS-WS), even though total canopy dry biomass was lower. This shows the tolerant line, under both WW and WS conditions, allocated more of its available biomass to seeds than did the sensitive line.

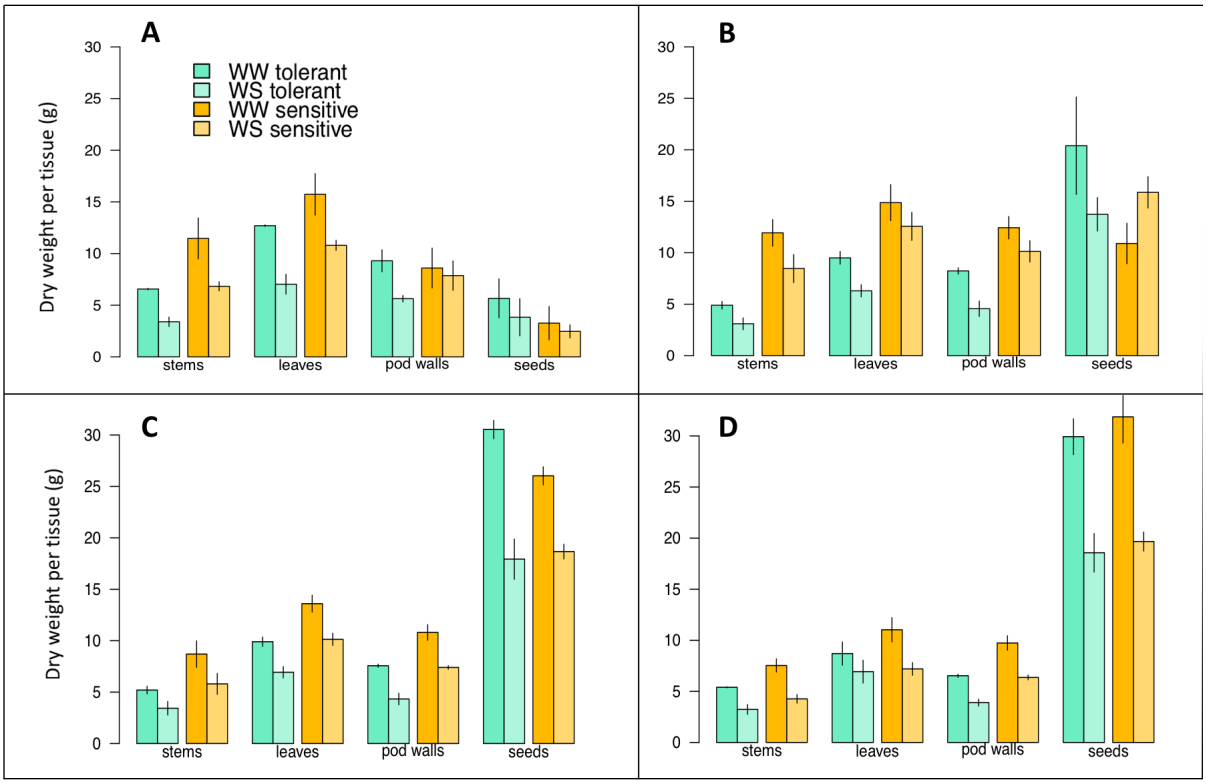

Figure 2. Dry canopy biomass (g) by tissue type for the four genotype-treatment pairs over time. (A) shows plants harvested at start of seed fill, (B) mid seed fill, (C) end of seed fill and (D) harvest maturity. For both genotypes well-watered treatment (WW) (darker bars) and water-stressed treatment (WS) (lighter bars), graph shows dry biomass in grams separated by tissue type; stems, leaves, pod walls, and seeds. Bars for the tolerant are in shades of teal, bars for the sensitive line are in shades of orange. Destructive harvests were done at 20,28, 36, and 43 days after flowering, corresponding to the start of seed fill, mid seed fill, end

of seed fill and harvest maturity, respectively. Three replicates per measurement were taken and averaged. Error bars show standard error.

Since the four genotype-treatment pairs differed in their total canopy biomass, we compared dry canopy biomass by tissue on a percent basis of the total weight (Figure 3). That allowed us to compare across the genotype-treatment pairs relatively, to see if they differed in how they allocated biomass. Across all four timepoints, both the WW and WS tolerant plants allocated more percentage of biomass to seeds than the sensitive comparison (when looking at seed DW, dark teal bars are always higher than dark orange bars, and light teal are always higher than light orange). The WW sensitive line followed a similar pattern as seen above, where its rate of seed filling lagged behind the WW tolerant in time. However, it ultimately reached a similar percentage by the end, although did not surpass it in this case. We also saw both WS treatments filled seeds faster than their WW comparison, which was most pronounced in the sensitive line at timepoint two. Since the tolerant line allocated more percent biomass to seeds than the sensitive line, we know that there must be a smaller percent biomass in either the leaves, stems, and/or pod walls of the tolerant line. Indeed, we see that at timepoint four, the tolerant line allocated had a smaller percentage of biomass in pod walls, whereas the sensitive line contains a larger proportion of canopy biomass in pod walls at maturity. Stem and leaf biomass did not significantly differ between the two genotypes at maturity, which points to the difference in these two genotypes allocation being something at the pod wall/seed interface.

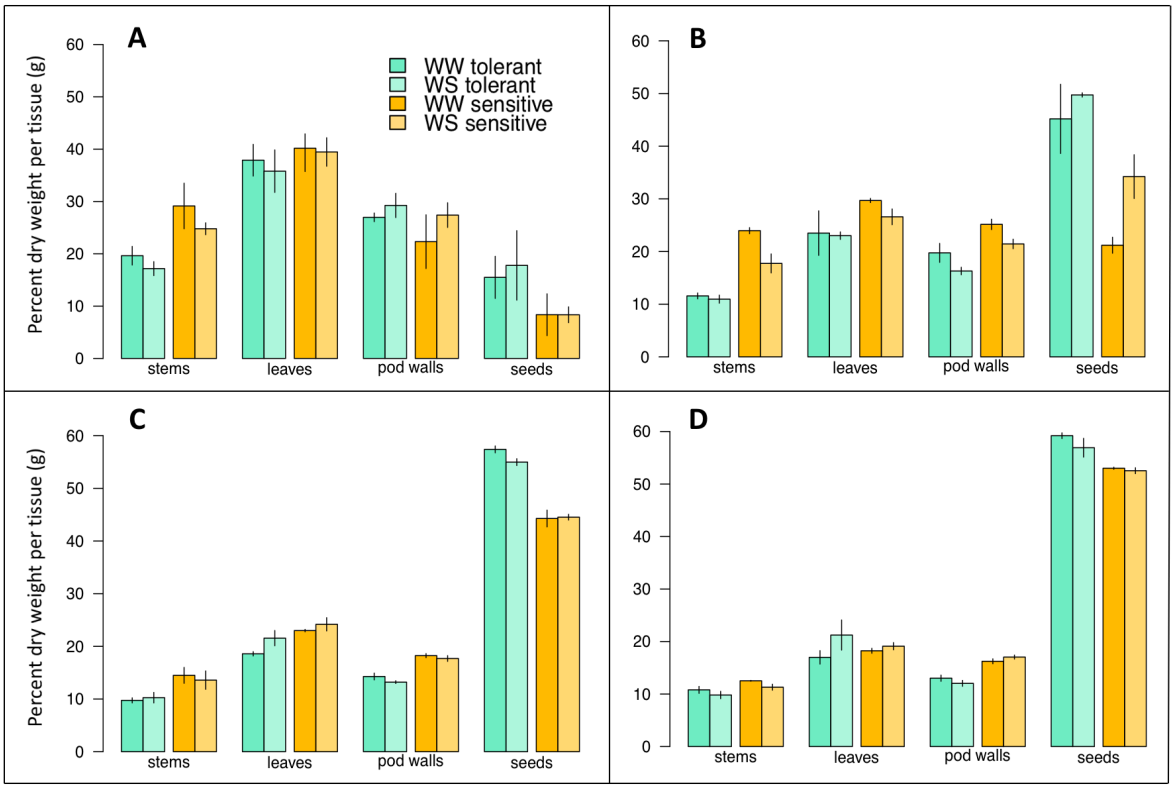

Figure 3. Percent dry canopy biomass per tissue for the four genotype-treatment pairs over time. (A) shows plants harvested at start of seed fill, (B) mid seed fill, (C) end of seed fill and (D) harvest maturity. Graph shows the percent of total dry biomass contained within each tissue type; stems, leaves, pod walls, and seeds. Both genotypes well-watered treatment (WW) are shown with darker bars, the water-stressed treatments (WS) is shown in lighter bars. Bars for the tolerant are in shades of teal, bars for the sensitive line are in shades of orange. Destructive harvests were done at 20, 28, 36, and 43 days after flowering, corresponded to the start of seed fill, mid seed fill, end of seed fill and harvest maturity, respectively. Three replicates per measurement were taken and averaged. Error bars show standard error.

Pod Harvest Index

Although seed biomass did not differ between the tolerant and sensitive genotypes under WS, the biomass of the pod walls was always significantly lower for the WS tolerant line when compared to the WS sensitive line (Figure 2 and 3). Therefore, Pod Harvest Index (PHI), the ratio of seed dry biomass to whole pod dry biomass, was always higher in the WS tolerant line compared to the WS sensitive line. Likewise, the WW tolerant line also had a higher PHI at all four timepoints compared to the WW sensitive, even when the WW sensitive line increased its seed dry biomass over the WW tolerant line at timepoint four.

POD HARVEST INDEX		
	Timepoint 4	CIAT 2018 - Field
WW TOL	0.82	0.83
WS TOL	0.83	0.76
WW SEN	0.77	0.76
WS SEN	0.76	0.48

Table 1. Pod Harvest Index values. Pod Harvest Index was calculated by dividing seed dry weight by total pod dry weight (including seeds). Values shown are from timepoint four, harvest maturity, across the 4 genotype-treatment pairs, as well as values at harvest maturity from a 2018 field study from the same genotypes in WW and a water deficit treatment. Values represent averages taken from three replicates for Timepoint 4 and 12 replicates for the field data.

Water content

Subtracting dry canopy weight from fresh canopy weight, we calculated water weight. The pattern seen with total canopy water weight (Figure 4) mirrored total dry canopy biomass (Figure 1), with the sensitive line containing more water relative to the tolerant line when comparing WW-WW and WS-WS. When broken down on a tissue-specific basis (Figure 5), we saw that each tissue type for all lines decreases over time, however, pod wall water weights went down faster in both the WW and WS tolerant line treatments when compared to the corresponding sensitive line treatments, seen most easily between the first two timepoints.

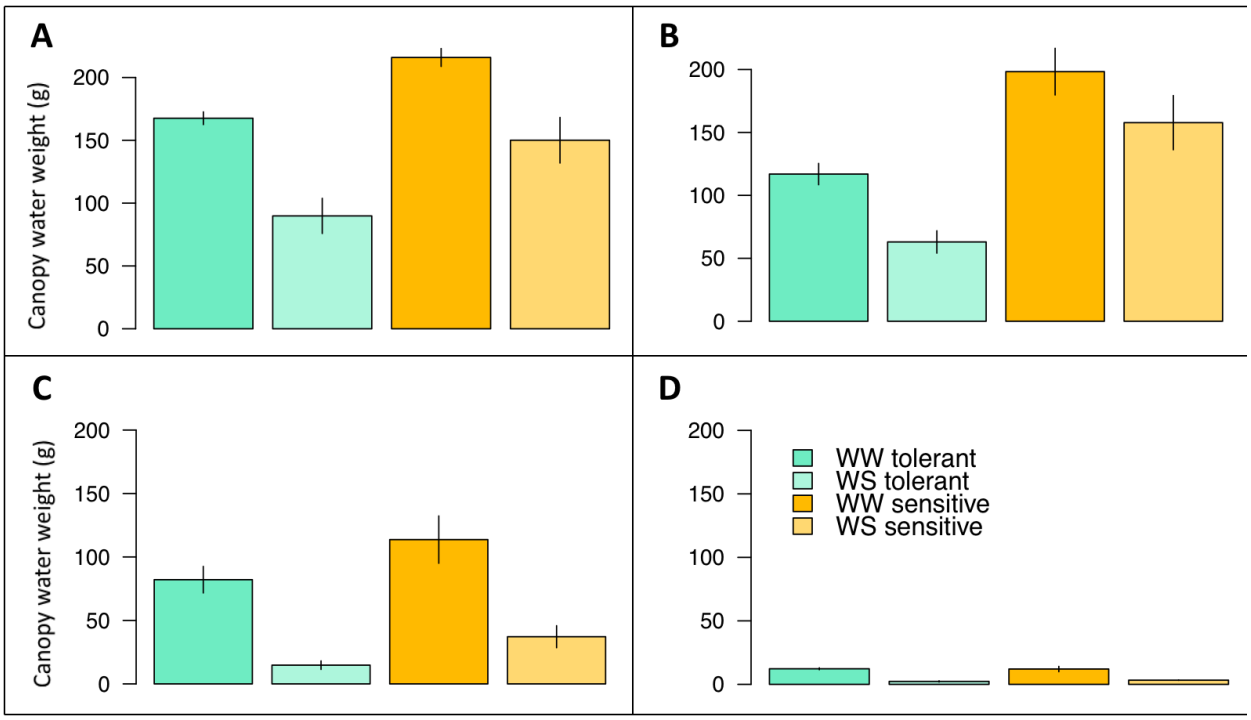

Figure 4. Canopy water weight (g) for the four genotype-treatment pairs over time. (A) shows plants harvested at start of seed fill, (B) mid seed fill, (C) end of seed fill and (D) harvest maturity. Graph shows total water weight in grams contained in each genotype-treatment pair, calculated by subtracting canopy dry weight from fresh weight. Tolerant and sensitive well-watered treatments (WW) are the darker bars, tolerant and sensitive water-stressed treatments (WS) are shown as lighter bars (teal and orange, respectively). Destructive harvests were done at 20, 28, 36, and 43 days after flowering, corresponded to the start of seed fill, mid seed fill, end of seed fill and harvest maturity, respectively. Three replicates per measurement were taken and averaged. Error bars show standard error.

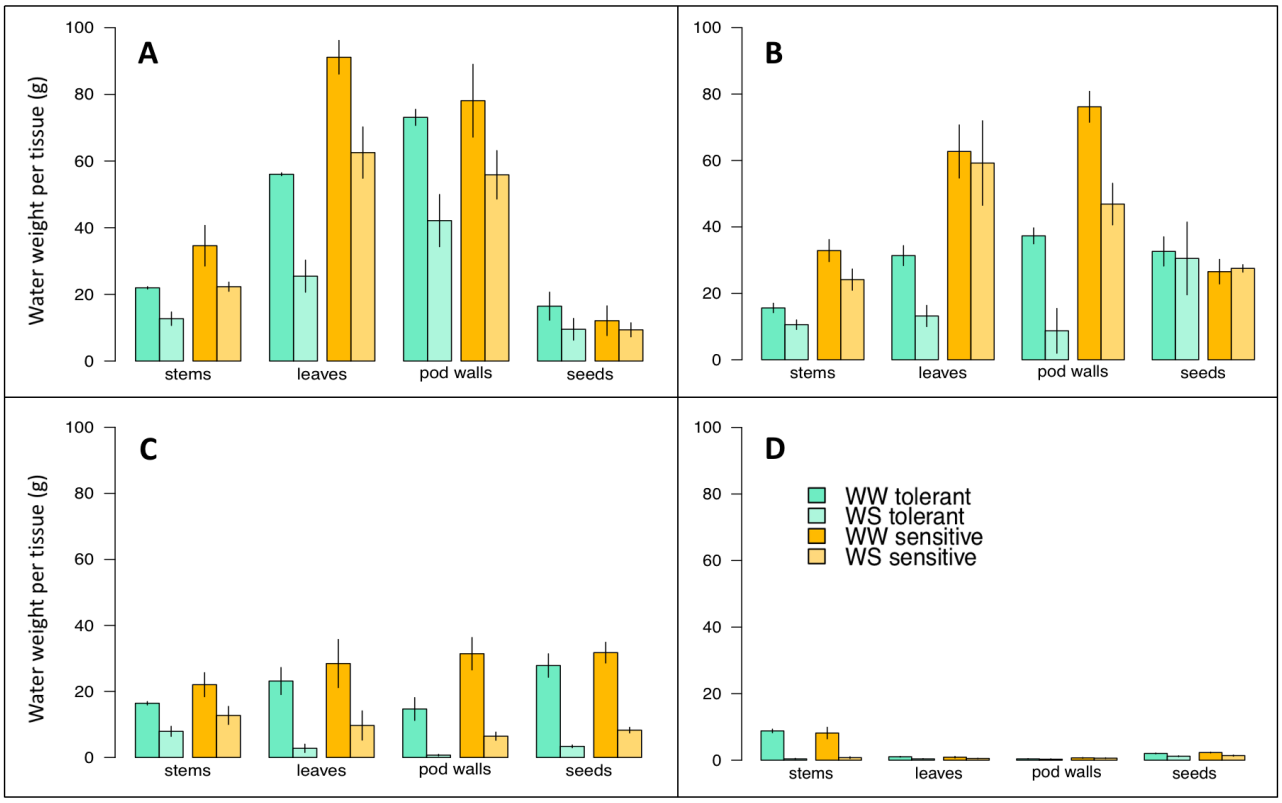

Figure 5. Canopy water weight (g) per tissue for the four genotype-treatment pairs over time. (A) shows plants harvested at start of seed fill, (B) mid seed fill, (C) end of seed fill and (D) harvest maturity. Graph shows the water weight in grams contained within each tissue type; stems, leaves, pod walls, and seeds, across the seed development. Both genotypes well-watered treatment (WW) are shown with darker bars, the water stressed treatments (WS) is shown in lighter bars. Bars for the tolerant are in shades of teal, bars for the sensitive line are in shades of orange. Destructive harvests were done at 20, 28, 36, and 43 days after flowering, corresponded to the start of seed fill, mid seed fill, end of seed fill and harvest maturity, respectively. Three replicates per measurement were taken and averaged. Error bars show standard error.

Maturation profile

By graphing seed water content (seed water weight divided by seed fresh weight X 100) and yield (seed dry weight) changes over time, we can see differences in timing and rates of seed filling and dry down (Figure 6). Most notably, the WW sensitive has a different seed filling profile than the three other genotype-treatment pairs. While the other three had highest rates of seed filling between timepoints one and two, which decreased steadily from there (logarithmic curves), the WW sensitive line had a slow filling rate between timepoints one and two, increased from there between two and three and then slowed again between three and four (sigmoidal curve). Seed water content decreased earlier in both WS conditions compared to their WW

comparison as expected. The WS tolerant line had a much quicker dry down (and therefore maturation) than the WS sensitive, particularly between timepoints two and three.

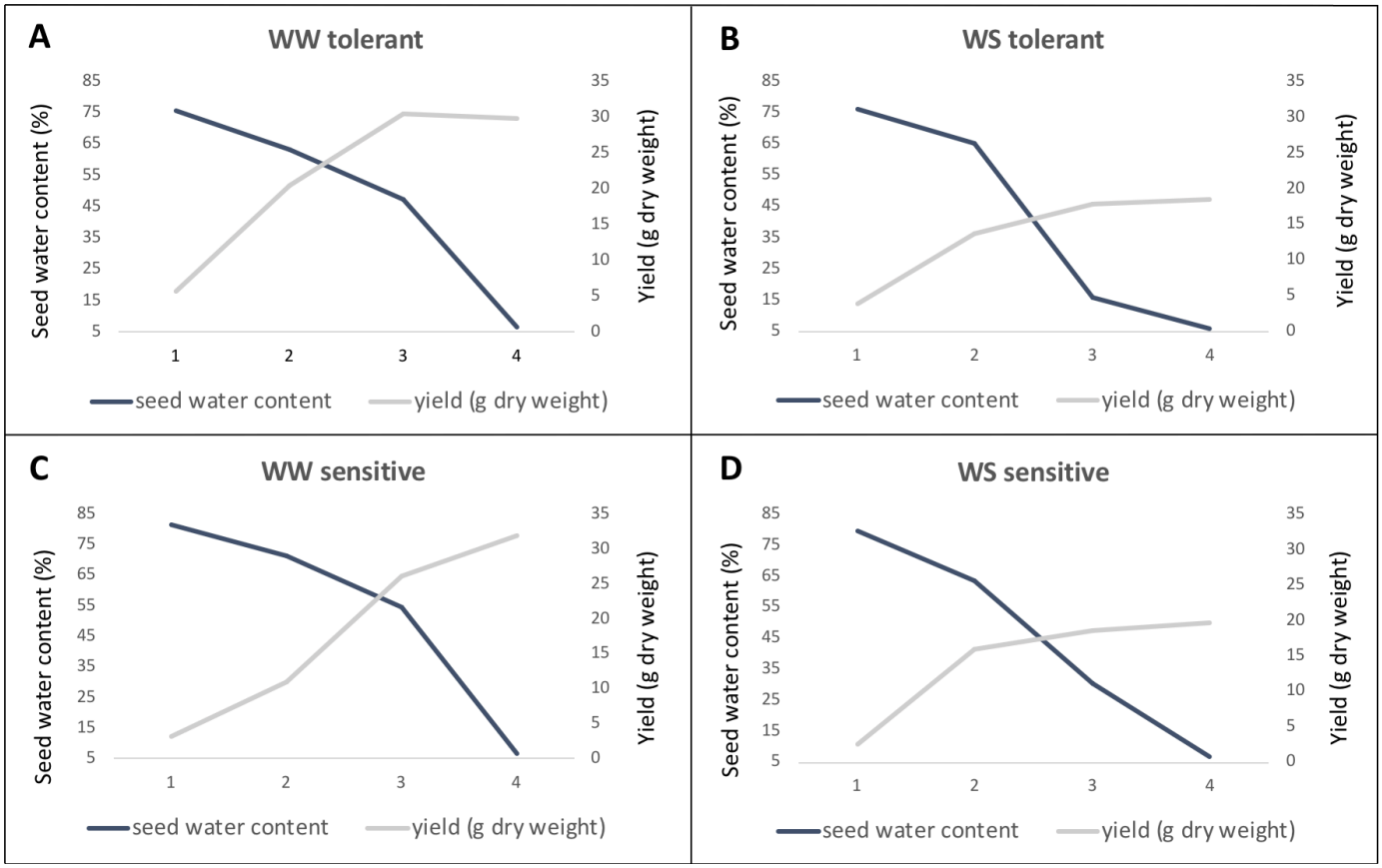

Figure 6. Seed water content and dry seed biomass profiles over time. (A,B) show profiles for the tolerant line under the WW (A) and WS (B) treatments, (C,D) show profiles for the sensitive line under the WW (C) and WS (D) treatments. For each genotype-treatment pair, seed water content (%) was calculated from fresh and dry seed weights collected across the four destructive harvests, shown as dark gray line. The light gray line shows dry seed biomass (g) gains over time.

Sucrose uptake assays/ fresh weight biomass gains

When seeds were removed from pods and floated in solution, biomass gain rates were different than what we see *in planta*. While WS seeds contained within pods on plants from both genotypes had slower rates of biomass gain than their corresponding WW comparison between timepoints two and three (Figure 6), excised seeds floated in solution had WS fresh weigh biomass gains higher than their WW comparison for both genotypes (figure 7). Rate of weight gain in the sensitive line was higher than the comparative rate of the tolerant line (WW-WW and WS-WS). Sucrose uptake rates closely matched weight gain rates, with rates of the sensitive line being higher than comparative rates of the tolerant line and both WS rates higher than

comparative WW rates. When 10^{-7} ABA was added to the assay medium, rates of weight gain and sucrose uptake for both the WW and WS sensitive line decreased largely and significantly compared to rates without ABA. Additionally, both sucrose uptake rates for the tolerant line (WW and WS) were also lowered due to the addition of ABA, however to a much smaller degree than the sensitive line.

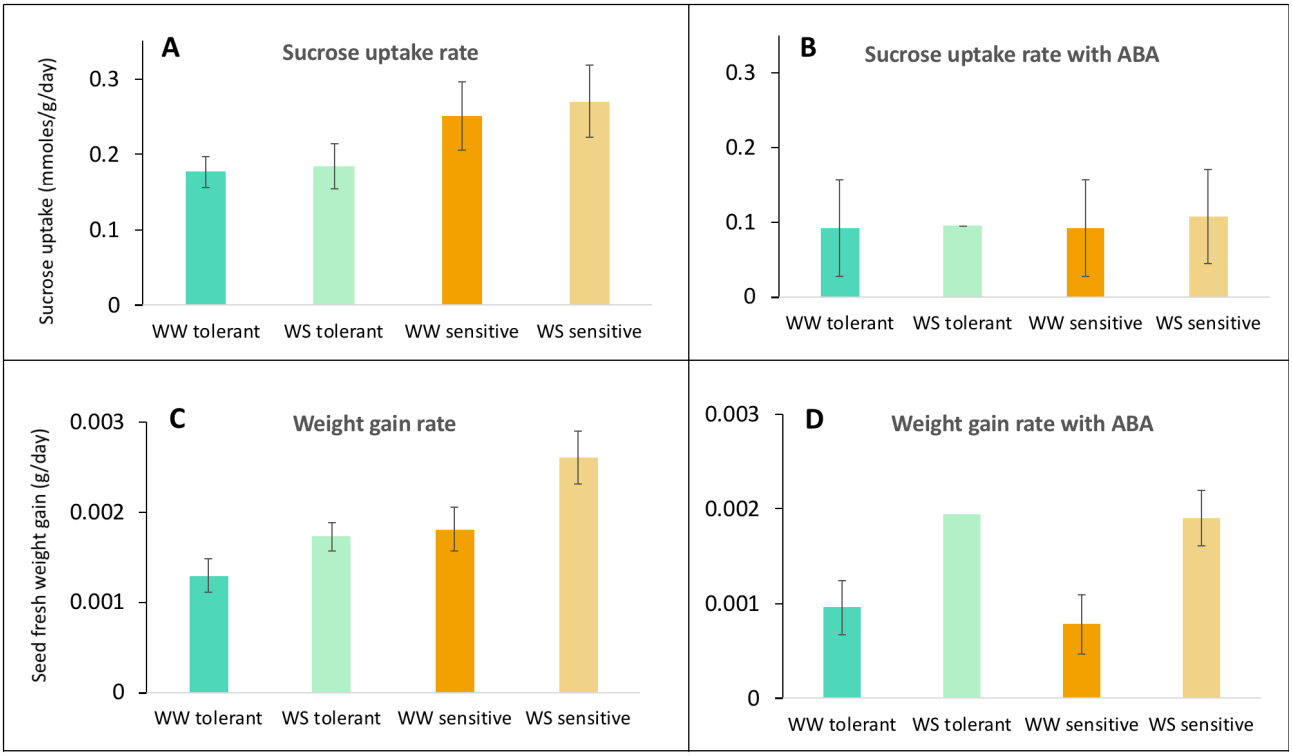

Figure 7. Rates up sucrose uptake and biomass gains in seeds. Seed sucrose uptake assays were performed by dissecting cotyledons out of pods and seed coats and floating them in a solution of 10mM KCl and 100mM, sucrose adjusted to pH 5.5 and 300 mOsm. (A,B) Brix meter was used to measure changes in the sucrose concentration in the growth solution over 24 hours. (A) shows changes in sucrose concentration (mM) per day normalized by seed starting fresh. (B) is the same as (A) except it had 10^{-7} mM ABA added to the growth media to measure its effect on sucrose depletion rate. (C,D) Initial and final fresh weights of the cotyledons were taken for quantifying biomass gains over the course of the assay. (C) shows weight gain normalized by initial fresh weight of seeds per day. (D) is the same as (C) except it had 10^{-7} mM ABA added to the growth media to measure its effect on weight gain.

pH assays

Changes to pH of the incubation medium in the floating assays showed increases, alkalization, for all 4 genotype-treatment pairs over 24 hours (Figure 8). This indicates that more protons were taken up into the cotyledons than were pumped out of the cotyledons. Higher rates of alkalization over the incubation period indicate larger net uptake of protons into cotyledons during this time.

In the sensitive line (MR8), there was a large difference in the rate of net proton uptake between WW and WS. Under WW, net proton uptake into MR8 cotyledons was moderate (comparatively), while the net proton uptake into WS MR8 cotyledons was higher. However, both the WW and WS tolerant line (MR81) had moderate net proton uptake and were no different from one another. This mirrors the response seen in both the BRIX and weight gain measurements.

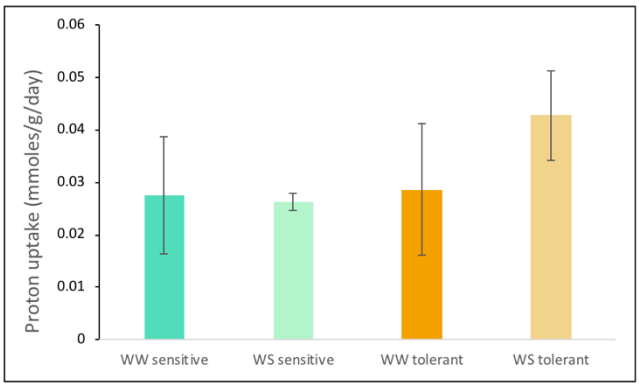

Figure 8. Net proton uptake into cotyledon halves measured via pH change of seed assay solution over 24 hours. Cotyledon halves were dissected out of each of the 4 genotype-treatment pairs (WW tolerant, WS tolerant, WW sensitive, WS sensitive) and placed into aqueous growth media containing 200 mM sucrose at pH 5.5. Cotyledons were left, covered, to incubate on a shaker table for 24 hours. pH was measured after the 24-hour incubation, cotyledon dry weights were obtained, and graphed values are the difference in pH over the 24 hours divided by the grams dry weight of the cotyledons which induced the change, n=6 averaged together.

3.5 Discussion

Biomass data (Figure 1) verified that the four timepoints we chose for harvests corresponded to the intended developmental timepoints: start of seed fill, mid seed fill, end of seed fill, and harvest maturity. This conclusion is supported by observed linear increases in seed dry biomass from timepoints one to three and no change between three and four (Figure 2) as well as seed moisture content steadily decreasing, approaching zero by timepoint four (Figure 6).

Both genotypes had higher canopy biomass under WW conditions than under WS (Figure 1). Differences between treatments began prior to timepoint one, as the WS treatments had been under water deficit for 13 days at that point. The trend widened over time, as both sensitive and tolerant WW treatments gained weight at a faster rate than the corresponding WS treatments for the remainder of the experiment. Comparison of genotypes show that the tolerant line had lower

canopy biomass than the sensitive line under both WW and WS at all timepoints. This fits with previous findings that drought tolerant common bean genotypes grew more slowly than sensitive genotypes (Hageman *et al.* 2020). Under WW conditions, the tolerant line allocated biomass towards seed production earlier than the sensitive line (Figures 2 & 6). However, by timepoint four, the sensitive line ultimately allocated more total biomass to seeds, resulting in higher yield in the sensitive line. This points to a difference in timing and rate of seed filling between these two genotypes under WW conditions. However, under WS, we were surprised to find that at all timepoints, sensitive and tolerant had no significant difference in the amount of biomass in seeds, meaning their yields under water-stress did not differ. We had expected that under WS, the tolerant line would have higher yield than the sensitive line by harvest maturity (timepoint 4), since these two lines had large differences in yield under drought in a field study (Hageman *et al.* 2020).

While Figure 1 shows the tolerant line had lower total dry biomass than the sensitive line, Figure 2 shows that even with less total biomass, the tolerant line achieved the same or higher seed biomass compared to the sensitive line. This is true under both WW and WS conditions. Figure 3 shows that the tolerant line achieves the same or higher total seed yield through allocating a higher percentage of total biomass to seeds by timepoint 4. This increased seed biomass percentage comes directly from allocating more biomass out of the pod walls into seeds, as pod wall percentages were lower in the tolerant line, while stem and leaf percentages were the same between the WW and WS comparisons (WW tolerant to WW sensitive and WS tolerant to WS sensitive). This led to higher PHI values for the tolerant line for all timepoints and treatments (Table 1). Higher PHI for the tolerant line points towards a higher efficiency of resource use for seed production, which likely contributes to this genotype's drought tolerance as seen in the field. Even in this study, while the tolerant line ended up producing less canopy biomass as a whole, it was still able to achieve the same yield in grams as the sensitive. This finding fits with what has been observed in field studies, that the tolerant line has higher PHI values than the sensitive. However, while in the field, higher PHI is the trait most linked to higher yield under drought in the field, we did not find PHI correlated with yield here (data not shown).

Total canopy water content decreased earlier in the tolerant line than in the sensitive line (Figure 4). When broken down by tissue type (Figure 5) we see that earlier dry-down of leaf and pod wall drives this, since stem dry-down is similar between sensitive and tolerant lines, and seed water content actually increases between timepoints 1 and 2 in both lines. This fits with our finding that tolerant lines allocate biomass towards seeds earlier. Since the tolerant line acts more quickly to fill seeds, mature and dry down under water-stress (Figure 6), this may contribute to a drought tolerance mechanism referred to as avoidance ((Beebe *et al.* 2013)). This is achieved by accelerating reproductive development and maturation before too much drought is experienced. Since the line matures more slowly, this may lead to it being more impacted ultimately since it continues development under intensifying drought. Perhaps we did not find as strong an impact on our WS treatment as compared to the field trial because our drought stayed at a modest level throughout seed development and did not intensify over time.

Differences between allocation in the tolerant and sensitive genotypes began even before timepoint 1 (Figures 3 & 6). For example, tolerant lines had allocated a much larger proportion of their available biomass towards seed production than sensitive plants already by timepoint 1. We believe that difference between the genotypes early in seed filling may be key to understanding drought tolerance since differences between the tolerant and sensitive lines are accentuated early on. The lines become more similar to one another as the plants mature. Under a harsher drought, those differences early on might be maintained or even exacerbated if the sensitive genotype is not able to catch up. Under our drought regime, all WS plants continued receiving water into the WS treatment, albeit less water than in WW. In the field study, to which we are comparing our data (Hageman et al. 2020), the drought regime was complete withholding of irrigation 6 days after flowering, resulting in deepening stress over the course of seed filling. We have good reason to believe our drought regime in the growth chambers resulted in a much less severe stress within the plants than that of the field drought, since we found only 38% yield reduction under drought for both genotypes in this study, while in the field, the rates dropped much more; 63% in the tolerant line and 86% in the sensitive line. Likewise, there was no change to PHI in our chamber experiment while in the field, tolerant PHI decreased 8% and sensitive decreased 37%.

The data discussed above lead to a hypothesis that seeds of the tolerant line are better able to extract resources from pods than are seeds of the sensitive line. This was tested with in vitro seed growth assays (Figures 7 & 8). We expected weight gain rates and sucrose uptake rates to correspond to one another, as weight gains were predicted to be driven primarily by sucrose uptake rates. Indeed, results did show a similar trend between the two measurements (Figure 7). However, rates of weight gain and sugar uptake from the solution were higher for both WW and WS sensitive lines compared to WW and WS tolerant samples. Additionally, both WS rates were higher than their corresponding WW rates. This was not the result we expected initially, as we predicted uptake rate in the tolerant line would be higher, leading to higher PHI. This discrepancy can be understood by the discrepancy of phenology between lines.

The *in vitro* assays represent only one developmental timepoint. Since the assays were done on cotyledons which were developmentally between timepoints 2 and 3, that is the only appropriate comparison to make to the *in planta* data. When we make that comparison, the findings are consistent between the *in vitro* seed assays and the biomass harvest measurements – sensitive biomass uptake rates are higher than tolerant in both. Therefore, when fitting this into the context of the maturation profile data from the biomass harvest experiment (Figure 6), we see that initially, the tolerant line started filling seeds faster, earlier than the sensitive line. Over time, that trend reversed as the tolerant line began to slow filling as it reaches maturity, yet the sensitive line continued filling. Therefore, between timepoints 2 and 3, the sensitive line reached a higher filling rate than the tolerant.

To gain insight into the mechanisms underlying differences in drought response between the two genotypes, first, the effect of the drought-associated phytohormone abscisic acid (ABA) was tested in the seed assays. ABA is a known signal of drought which initiates a cascade of water-stress responses within plants (Vishwakarma *et al.* 2017). One of the responses known to be impacted by ABA is the activity of the plasma membrane-localized proton pump, which shows decreased rates when ABA is present, although the mechanism linking the two is not yet known (Duby and Boutry 2009). Proton pumps set up a proton gradient across the membrane, providing the key driving force for sucrose uptake into seeds via sucrose proton co-transport into the seed across the membrane (Weber *et al.* 1997; Tegeder *et al.* 2000). Therefore, we suspected ABA

would slow biomass gains and sugar depletion rates. We found that when ABA was added to the seed assay solution, both WW and WS sensitive samples showed large, significant decreases in both weight gain rates as well as sucrose uptake rates. However, ABA had a much smaller affect on the WW and WS tolerant lines. Both decreases to sucrose uptake rates and biomass gains were much less than the sensitive line in response to additions of ABA in the assay solution – (Figure 7). This suggests that the tolerant line may either be less responsive or sensitive to ABA than the sensitive line or that the tolerant line always has elevated concentrations of ABA (even under WW conditions). This latter possibility is supported by findings that the tolerant line shows slower weight gain rates and sucrose uptake rates than the sensitive line under WS as well as WW conditions. In addition to the ABA assays, we also measured changes to solution pH in the in vitro seed assays. Increased pH of the apoplast drives sucrose uptake, so changes in pH may reflect differences in rates of sucrose uptake. We found that pH changes showed a similar pattern to weight gain and sucrose uptake rates (Figure 8). While the net proton uptake was moderate in the tolerant line, for both WW and WS, there was a large difference between WW and WS for the sensitive line, with the WS treatment showing a larger net proton uptake. This again suggests that the sensitive line changes physiological processes under WS while the tolerant does not, suggesting the sensitivity to drought differs between these two genotypes. A loss of sensitivity to ABA could also describe why the tolerant line does not change its seed filling strategy between WW and WS, while the sensitive line does (Figure 6). Both genotypes under the WS condition filled seeds fastest early in seed development and slowed over time (logarithmic curve). The tolerant line also fills seeds following the same pattern. However, the WW sensitive line has a different profile of seed filling. It starts at a much slower rate (compared to WW tolerant) and only speeds up later in development (sigmoidal curve). These findings fit with what is known about these lines being tolerant or sensitive. While the tolerant line does not change its pattern under WS (does not change developmental pattern), the sensitive line does adopt a different developmental program under WS, moving to fill seeds quicker. Although in this study, this change in pattern allows the WS sensitive line to achieve the same yield as the WS tolerant line, perhaps this change in seed filling strategy is less optimal for the WS sensitive line under more severe water-stress observed in the field. The profile of the WS tolerant line, drying seeds earlier and at faster rates than the WS sensitive line also points to higher capacity to maintain seed filling under drier conditions.

While the drought-tolerant line did not ultimately produce a higher yield under WS than the drought-sensitive line, we did find differences in traits which likely contribute to differences in yield as seen under more extreme field droughts. As in the field, the tolerant line in this study achieved a higher PHI than the sensitive line. In addition, the tolerant line allocated biomass from leaves, stems and pods into seeds earlier than the sensitive line, allowing the seeds to mature and dry down sooner. We believe that under more severe drought conditions, this avoidance mechanism greatly benefits the tolerant line over the slower maturation of the sensitive line.

Faster seed filling early in development reflects stronger sink strength ability. Sink strength quantifies resource uptake in growing, sink tissues, which in turn affects growth and metabolism within those tissues. If the seeds of tolerant lines are able to demand and draw resources to themselves more quickly, that would allow them to fill, mature and dry down sooner. This is what we observed both in the growth chamber study reported here, and in the field study (Hageman et al. 2020). We hypothesized that increased expression or activity of sucrose uptake transporters (SUC) and/or H+-ATPase underlies this ability, which would have led to increased weight gain rates, increased sucrose uptake rates, and/or net proton uptake in the tolerant line vs the sensitive. However, the *in vitro* seed assays did not allow us to support this prediction, as instead, weight gain, sucrose uptake and net proton uptake all were higher in the sensitive line. This is likely because the seed assays were performed too late in development to catch the important timepoint which contributes to earlier seed filling in the tolerant line (likely between time points 1-2 and even earlier). Additionally, we showed that ABA decreased sink strength in the sensitive line and not the tolerant line, suggesting a mechanism by which sink strength regulation may differ between the two lines.

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Chapter 4: Sink Strength Maintenance Underlies Drought Tolerance in Common Bean

4.1 Abstract: Drought is a major limiter of yield in common bean, decreasing food security for those who rely on it as an important source of protein. While drought can have large impacts on yield by reducing photosynthesis and therefore resources availability, source strength is not a reliable indicator of yield. One reason resource availability does not always translate to yield in common bean is because of a trait inherited from wild ancestors. Wild common bean halts growth and seed filling under drought and awaits better conditions to resume its developmental program. This trait has been carried into domesticated lines, where it can result in strong losses of yield in plants already producing pods and seeds, especially since many domesticated lines were bred to have a determinate growth habit. This limits the plants ability to produce another flush of flowers, even if the first set is aborted. However, some bred lines are able to maintain higher yields under drought through maintaining growth and seed filling rates even under water limitations, unlike their wild predecessors. We believe that maintenance of sink strength underlies this ability, since plants which fill seeds under drought maintain growth of sinks generally, and growth of sinks correlates strongly with yield. Sink strength is determined by a tissue's ability to acquire resources, which in turn relies on resource uptake and metabolism in that tissue. Lines which achieve higher yields maintain higher resource uptake rates into seeds and overall higher partitioning efficiencies of total biomass to yield. Drought limits metabolism and resource uptake through the signaling molecule abscisic acid (ABA) and its downstream affects. Perhaps lines which maintain higher sink strength and therefore higher yields do so through decreased sensitivity to or production of ABA.

Keywords: SUT; proton pump; yield; Pod Harvest Index; Harvest Index; partitioning; allocation

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4.2 Introduction

Common bean (*Phaseolus vulgaris* L.) is grown around the world as a staple crop and is an important source of dietary protein for more than 300 million people [1]. However, each year, more than 60% of bean crop production is affected by water deficits [2]. Those reductions of yields range from minor to complete loss. Much of common bean production is on small-holder farms in

areas where droughts are common and access to irrigation is limited [3]. This can have large impacts on food security in these regions. Droughts are only expected to increase in much of these growing areas [4], underlining the importance of better understanding drought tolerance in common bean.

The severity of yield losses depends on many factors, including genotype, developmental timepoint at onset of drought [5], soil type and other climatic conditions [6]. Drought has an impact on plants by limiting photosynthesis and therefore depriving the plant of building blocks necessary for growth and reproduction. However, correlations between photosynthesis and yield are not strong [7] particularly under suboptimal conditions [8], and plants may fail to set and fill seeds, even when not limited by photosynthetic resources [9]. While this phenomenon is much less understood, one important reason it may occur in common bean is the particular natural history and domestication path of bean.

As humans bred wild bean plants into domesticated forms, we altered both their genetics and growth environment in ways which made their native physiological responses to stress less important and even detrimental to yield [6]. For example, common beans toggle between a faster growth phase when conditions are favorable and a slower phase when they are not. While this trait is beneficial for wild, indeterminate bean plants, the persistence of this trait in domesticated species can prevent us from maximizing crop yields. The reason for this is that domestication of common bean has selected for determinate growth [10]. This bush growth habit resulted in more synchronized flowering and fruit set which uses up all reproductive potential at once. However, domestication did not eliminate the tendency for determinate bean plants which are producing reproductive structures to toggle back to slow growth during stress conditions [4]. As a result, allocation of resources to developing flowers, pods, and seeds may be interrupted during a drought episode leading to flower and pod abortion, or failure to fill seeds fully. Coupled with the inability to produce new flowers that came with determinacy, this tendency makes it so their one opportunity at reproduction may be largely diminished [4].

With the goal of increasing yield, researchers discovered that leaf area index, which quantifies leaf area per unit of land, and canopy biomass accumulation stand out as a strong indicator of yield [11–14], even above net assimilation rates [15]. Similarly, direct relationships between yield and rates of leaf production and expansion have also been seen in crops [16] and leaf size correlates with seed size in common bean [17]. Further, studies show harvest indexes (HI), which measure

the efficiency in which a plant turns biomass into seeds, are an even stronger predictor of yield [2,12]. Increased HI has been increasingly selected for in breeding of crops, including common bean, for drought tolerance [18]. In common bean, a trait that quantifies how much of the total pod biomass is partitioned to seeds at maturity, called Pod Harvest Index (PHI), is consistently the strongest predictor of yield under both well-watered and droughted conditions in common bean [11]. Therefore, genotypes which continue filling seed under drought, rather than toggling back into to slower growth as wild beans do, are the lines that fare best under drought. Together, these findings show a tight linkage between growth, allocation and yield.

Focusing in on these findings, a new study measured and tested correlations between leaf growth rates and PHI across 20 common bean genotypes [19]. It showed that leaf growth rates and harvest indices are both similarly affected by drought within a genotype—that is, genotypes that slow their leaf growth strongly under water deficit also slow their allocation of biomass towards yield. Conversely, genotypes which maintained leaf growth under drought also maintained allocation towards seeds. That these two processes, leaf growth and biomass allocation to yield, are similarly affected by drought, and that both are strong predictors of yield under drought, led us to ask what common mechanisms control growth and allocation processes and how does drought affect those commonalities. Understanding how these responses differ within drought-tolerant and drought-sensitive lines could allow breeders to find novel solutions to help beans overcome their native drought response.

4.3 Limitations on Growth

The ability to continue growing leaves and seeds in common bean and other crops could be hindered by one of two big picture limitations, or both: source limitations or sink limitations [20,21]. Sources are any tissue that provides resources for export to the rest of the plant—photosynthetically active leaves, as well as storage tissues such as stems or roots. Sinks on the other hand are tissues that take in resources for growth, maintenance or storage, such as immature leaves, flowers and seeds. Resources flow between sources and sinks through the phloem according to concentration gradients (traveling from high to low) and resistances (such as membranes). The ability for sources to export and sinks to competitively import resources is quantified as source and sink strength, respectively [20]. Growth rates of sinks are a good reflection

of sink strength [22], even though some resources are used up in respiration and therefore do not add to dry weight gain [20].

Sources can limit growth by not having enough resources to export, or by not exporting them even if they are available. The best example of source limitation is caused by the inhibition of photosynthesis. One reasons this can occur is when levels of abscisic acid (ABA), a well-studied stress-related phytohormone [23], increase under drought. ABA acts as a stress signal, increasing under a number of stressors but most strongly under drought, and helps plants sense their environment and adjust their physiology accordingly [24]. The mechanism of ABA action includes inhibition of the plasma membrane proton pump, leading to loss of cellular K^+ to the apoplast, resulting in a decrease of turgor of guard cells and stomatal closure. Stomatal resistance slows the rate of photosynthesis by reducing carbon dioxide diffusion to the chloroplasts. This in turn reduces the amount of carbohydrate fixed in the source tissues, and thus source strength [25]. Source strength may also decrease under drought, since enzymes which are involved in sucrose synthesis are inhibited which can lead to less sucrose available for export [26].

However, even though sources can limit sink development, there are many examples that show sink growth and development are not directly or exclusively linked to source strength. One simple demonstration of this independence is that once seed filling has begun, dry matter accumulation remains linear even when source:sink ratios change [8]. Many crops are able to remobilize stored resources under drought to compensate for reduced photosynthetic fixation [27]. Instead, a decrease in available resources will decrease seed filling duration rather than rate [28]. Other examples show that in common bean, apoplastic sucrose levels surrounding the seed do not correspond to sucrose uptake rates within those seeds [29]. Therefore, even though seeds may have access to resources, they do not necessarily take them up. Likewise, in grain legumes, defoliation rates (33%, 66% and 99%) do not proportionally predict yield losses (20%, 32% and 35%, respectively) [30]. Additionally, “stay-green” genotypes, whose leaves senesce later than other genotypes and do more photosynthesis as a result, do not necessarily have increased yields—indeed, yields are actually lower in many stay-green genotypes under drought when compared to non-stay-green genotype [31,32]. When considering whether leaf growth rates of immature sink leaves are also decoupled from source strength, evidence suggests sink leaves also exert control over their growth independent from source availability. Leaf disk studies in common bean indicate that light, decoupled from photosynthesis, acts as a stronger stimulator of growth than does

sucrose, pointing to regulatory mechanisms playing a larger role than simply substrate availability [33]. Studies also show that partial defoliation in common bean leads to increased photosynthetic rates of the remaining leaves in order to meet the demand of the sink leaves [34]. Since source strength was able to increase, this indicates sink limited growth in immature leaves [35]. Additionally, in another study, partial defoliation of common bean actually lead to increased growth rates of remaining leaves [36]. Lastly, in two common bean genotypes whose growth rates are high and low, comparatively, photosynthetic rates do not differ significantly on a per area basis (Banerjee and Van Volkenburgh, unpublished).

Therefore, it is clear that sinks exert fine control over their ability to acquire resources and grow when source limitations are not hindering sink growth, while sources exert a more course control [37]. Sinks exert this control by either changing uptake into the sink or metabolism within the sink; both result in reducing the gradient that acts as the driving force for continued resource delivery from sources. As mentioned above, in common bean lines which are filling seeds, two different lines can have the same supply of sugar available to the seeds yet different rates at which they take up the sugar [29]. Growth rates of those lines' cotyledons, cultured in vitro, retain differences between genotypes, suggesting filial tissues themselves (the seeds) have control over filling rates (sink strength). These differences, which cannot be fully explained by differences in seed surface area, may be controlled genetically within the seed and play an important role in determining yield. This conclusion has been reached by others, who find that yield, seed growth rates, and general sink strength are typically sink limited [38,39]. What then controls sink strength and how is this affected by drought?

4.4 Control of Sink Strength

Since at least the late nineties [40], it has been speculated that the growth of a sink leaf may be most directly regulated by the ability of its individual cells to expand, rather than the carbohydrate supply available to the sink. This expansion is necessary for cell division in early development, setting up a strong gradient to continue drawing resources to that organ. Cell expansion in leaves is thought to be controlled by the acid-growth mechanism in which an acidic environment in the cell wall results in increased loosening of the wall via expansins, which in turn allows it to be extended by turgor pressure within the cell [41,42]. Cell wall acidification is set up via H^+-ATPases actively pumping protons out of the cell and into the apoplastic space. The pH

gradient resulting from acidification of the cell wall set up by the proton pumps also acts as the driving force bringing resources in (carbon, nitrogen) via secondary active transport. This then affects metabolism and turgor pressure through changes to solute and ion concentrations within the cell.

We suggest that all sink growth, whether that sink is a leaf, fruit or seed, is primarily controlled by cell expansion. Indeed, recent work continues to highlight the significance of cell wall loosening and expansion for regulating sink strength in tissues beyond leaves [43,44]. Acidification, which fuels loosening and expansion, also increases uptake rates of sucrose and nitrogen (and therefore metabolism) as well as other solutes (and therefore turgor). If all sinks grow via the same mechanism, it seems likely that all sink tissue growth within a single plant would relate to one another. This would explain the findings mentioned above that leaf size is positively correlated with seed size [45] and more generally, that different tissues' growth, size and weight gain all positively correlate within a genotype [17]. Additionally, breeders consistently find that traits which measure carbohydrate allocation to seeds, harvest index and pod harvest index in bean, have the strongest correlation with yield across all conditions and genepools [2]. Therefore, when differences exist in yield between different genotypes that is not described by differences in photosynthate availability, this may be due to differences in sink strength between genotypes. Control could stem from differences in the ability to regulate (1) pH of the cell wall via proton pumping, (2) carbohydrate and ion uptake via transporters and channels, and/or (3) metabolism via enzymes. These three mechanisms all offer important points of regulation that could originate from within sink cells themselves to control leaf sink strength via cell expansion.

However, while all three of these mechanisms are vital for cell expansion, we believe resource uptake rates, particularly carbohydrate uptake rates, exert the largest control over a cell's ability to grow. Although regulation of uptake, turgor and pH shows many interconnections, the simple result that differences in *Phaseolus vulgaris* seed growth rates were accounted for by dry matter weight gain and size [46], which in turn were found to be determined by sucrose uptake, suggest that regulation of uptake is at the core. In bean and many other crops, this is accomplished via sucrose uptake co-transporters, SUCs (sometimes called SUTs), which actively co-transport sucrose with protons into the cell [47]. Genotypes with the highest growth rates had highest maximum SUC rate, highest SUC protein levels, and the highest proton pump protein concentrations [29]. Carbon uptake itself exerts control over pH since sucrose is symported into

the cell with protons. This means increased uptake of carbon also leads to increased wall pH. Expressing more proton pumps could counteract this affect and keep the wall acidic however. Additionally, both of these mechanisms affect turgor through changing osmolytes in the cell as well as cell wall loosening via pH and therefore expansion. How then is carbohydrate uptake regulated?

Carbon, which makes up 90% of both a plant's overall mass and bean seed dry weight [48,49], reaches the sink apoplasm through the phloem. At the apoplast/cell interface, uptake of carbohydrates, often in the form of sucrose, must occur across the plasma membrane. SUCs rely on a proton gradient across the membrane to fuel this movement, set up by a proton pump on the seed membrane. Sucrose may also enter a sink cell via an unsaturable, passive mechanism however, probably the sucrose gradient is not sufficient to drive this transport mechanism. Instead, secondary active transport predominates in determining the total rate of uptake. Differences in sucrose uptake rates between genotypes likely arises from differences in densities of SUCs or proton pumps. Strong correlations ($r^2 = 0.95$) between maximum SUC rates and sucrose uptake, normalized by size, support this hypothesis [29]. Further, sucrose uptake correlates strongly with SUT1 protein levels, suggesting that when the protein is present, it is actively working to take up sucrose [29].

Densities of SUCs and proton pumps are regulated within sink tissues themselves. Increasing sucrose import into seeds leads to an increased metabolism of both C and N within seeds, resulting in a positive feedback loop which maintains continued uptake of both. When sucrose concentrations decrease within the sink, sugar sensing mechanisms detect this drop and a signaling cascade results in increased SUC transcription [50] (Figure 1). With more SUCs comes an increase in C uptake into the seed, which lowers the sucrose concentration in the apoplast and increases water potential. This leads to a loss of water from the apoplast to surrounding tissues, the seed coat in bean, increasing turgor pressure within the seed coat. Increased turgor upregulates sucrose facilitators, which allow sucrose release from maternal tissues into apoplast [51,49]. Therefore, more C enters the apoplast and can be taken up into the seed, leading to the increases of C and N metabolism again. Increased phloem and seed loading of nitrogen in the form of amino acids results in increased SUC concentrations as well as root nitrate transporters [52], showing nitrogen and carbon metabolisms are highly linked. Additionally, import of carbohydrate is increased via upregulation of starch biosynthesis, which fits with the sugar sensing feedback loop described

above [43]. Conversely, increased sucrose concentration correlates highly with decreases SUT1 expression [50].

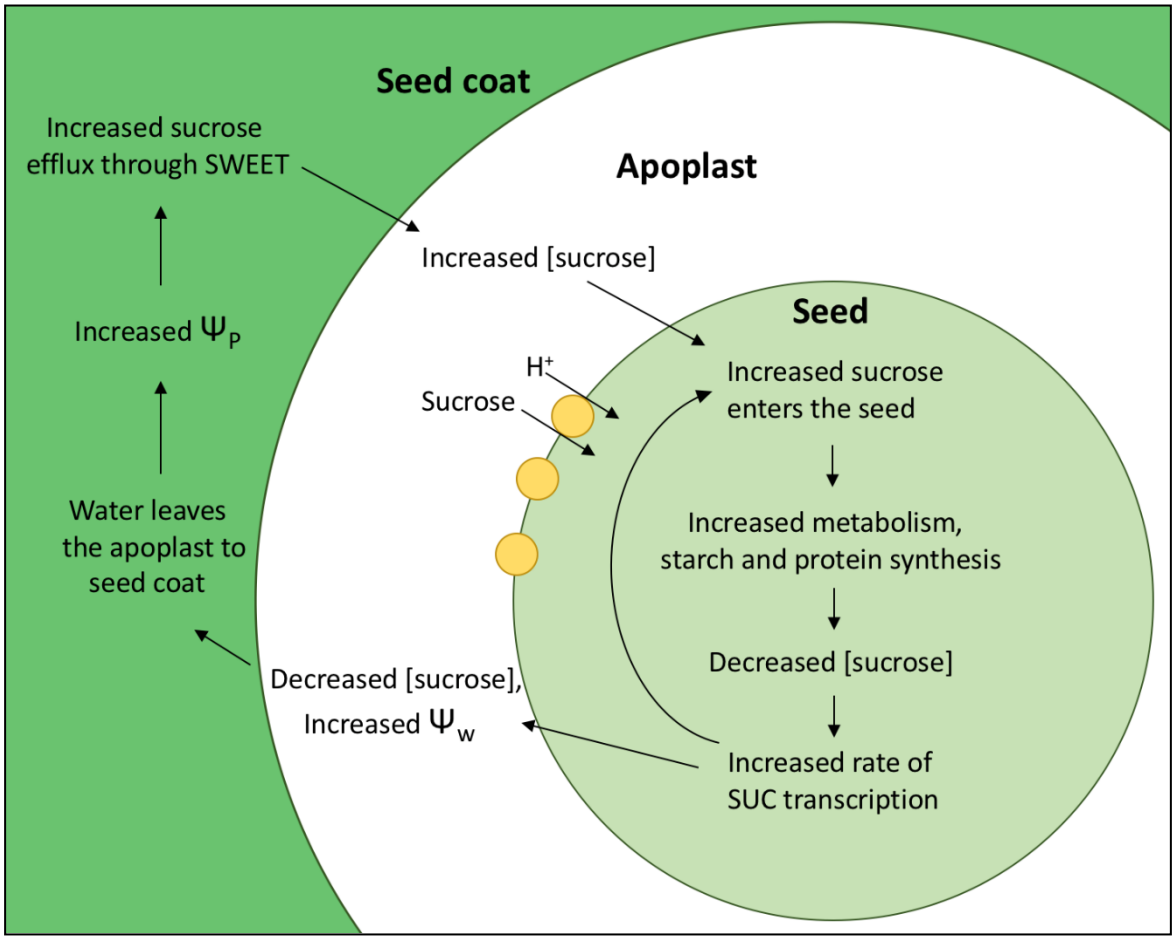

Figure 1. Regulation of sucrose uptake rates via sugar sensing feedback. SUC transcription is upregulated when low sucrose concentrations are sensed within the seed. This leads to increased levels of resources within the seed for metabolism and synthesis. When sucrose uptake rates increase, this leads to decreased levels of sucrose in the apoplast, increasing solute potential and therefore water potential (Ψ_w). Water flows into the seed coat, down the water potential gradient. The resulting increase in turgor (Ψ_p) in the seed coat increases sucrose efflux into the apoplast via channels called SWEETS. Increased sucrose in the apoplast leads to increased uptake via SUC, starting the cycle again.

4.5 Impacts of Drought on Sink Strength Via ABA

Water deficits in the soil lead to decreases in water content within plants. This drop in water availability has the potential, depending on severity and developmental timepoint, to affect nearly every aspect of growth and development, including growth rates and cell turgor (loss of turgor leads to wilting). Interestingly, growth rates actually slow before loss of turgor in cells, showing that decreased growth is not a direct result of decreased turgor [53–56]. Additionally, drought does not result in evenly distributed water deficit within a plant. For example, after 6 days of water withholding, soy bean leaf water potential dropped steeply, however, seed water potential was buffered from the effects of water deficit, having not changed from well-watered conditions [57]. In addition, under drought conditions, older source tissues generally are the first to wilt and abscise. This allows resources to be remobilized out of these older tissues into newer growth, which either has more potential for future resource gathering or for reproductive output. Sink tissues are generally protected from the impacts of drought over other tissues [28], yet their growth still slows even when buffered against water and resource limitations. Therefore, with resource limitations and water availability not acting as primary regulators of sink growth under drought, what effects changes in physiology?

Physiological responses to drought have been largely attributed to the signaling hormone abscisic acid, ABA. Although ABA response is most classically associated with decreases in stomatal aperture, and therefore reduction to photosynthetic rates, ABA has also been shown to impact growth and development through other signaling pathways [57. Of note, sometimes ABA stimulates growth, like it does in roots and in seedlings, while other times it inhibits growth, complicating how we understand both ABA action and the regulation of growth-related processes [58]. This phenomenon is not unique to ABA signaling, since drought generally has an opposite effect on root vs. shoot metabolic responses [59]. However, when we narrow our focus to above ground sinks, such as growing leaves or seeds, ABA usually acts as a growth inhibitor.

ABA inhibits growth by affecting multiple mechanisms related to resource uptake. In droughted wheat plants, ABA accumulates and correlates with decreases in seed set [60]. This may be due to ABA's known impact on increasing cell-wall invertase (CWIN) inhibitors, since CWIN mutants have been shown to have small seeds [61] and seed size correlates with CWIN levels in seed coats [62]. CWIN hydrolysis of sucrose is essential for maintaining the sucrose gradient which drives normal carbon flux, therefore decreasing its activity would reduce carbon flow. ABA is also known to reduce cytokinins, phytohormones which promote growth through activating

CWIN and cell cycle genes [63]. During the seed cell division and expansion phase, reduced cytokinins can result in full abortion of the seed [28]. ABA also regulates processes such as plant water balance and osmotic regulation in response to stress [64]. This may be through affects to water hydraulics via changes to water permeability through regulation of aquaporins [65].

ABA may also be an important regulator of resource uptake through its ability to inactivate some H^+-ATPases [66]. In *Arabidopsis*, there are proton pumps unique only to the seed tissue, allowing for the possibility that control may stem from regulation of tissue specific proton pumps under drought [67]. These may be differentially impacted by ABA, changing proton motive forces and therefore carbon uptake into different tissues. Magnitudes of proton motive forces in general have been shown to estimate accumulation rates of sucrose in sinks [68], further supporting this theory. Proton pumps also differ in their activity depending on the developmental stage of the tissue. In mature leaves, activity is largely restricted to the guard cells and phloem [69], while activity is maintained throughout development in seed tissues [67].

Even in examples where ABA does not inhibit proton pumping, ABA plays an essential role in translating changes in pH into action. Drought causes apoplastic pH to increase and this in turn reduces wall extensibility and growth [55]. This can result in increased turgor, which helps cells maintain structure. Yet, in barley leaves, drought still induced apoplastic pH increases in ABA mutants. This shows ABA is not necessary to increase pH of the apoplast. However, leaf growth did not slow unless ABA was suppled [53]. This suggests ABA did not affect pH directly by inhibiting proton pumping, but somehow exerted control over cell wall loosening via some other mechanism. If growth can still occur under more alkaline conditions when ABA is not present, perhaps acidic pH causes an increase in ABA, which then leads to decreased cell wall loosening, perhaps through ABA regulation of the activity of expansins or xyloglucans. Another hypothesis is that perhaps ABA directly inhibits the uptake of sucrose and potassium via uptake co-transport mechanisms, rather than inhibiting proton pumps, affecting osmolarity and turgor.

Sensitivity of flowers, immature fruits and seeds to drought also plays a vital role in determining yield. The health and development of these tissues are essential for ensuring yield production, since they are what give rise to mature fruits and seeds. Yet, these tissues are highly susceptible to abortion when exposed to water deficits. As with other sinks, development of flowers and young fruits/seeds is slowed or halted under drought even when resources are not limiting. In common bean, this has been largely attributed to decreased cytokinin levels in flower

and developing pods [70] which we know can be down-regulated by ABA. Abortion of pods is much less likely to occur after seed cell expansion and filling phase begin, because seed filling is not dependent on cytokinin activity. However, even though abortion of flowers and pods can be strongly increased under drought, high remobilization efficiency remains the strongest predictor of yield under drought in common bean, more so than pod or seed number. This may be due to two factors. First, common bean consistently produces many more flowers than will ever become mature fruits with seeds [71]. In fact, seed yield was only slightly lowered, or even increased, in common bean plants which had all their flowers removed for the first 15 days following anthesis. Therefore, there is a large buffer, such that many flowers can abort without affecting yield. This is true in both well-watered and water-limited conditions [72]. Therefore, even when abortion does occur, yields are not impacted as long as the number aborted does not go above a certain point. Second, imperfect synchronization of flowering, fruit and seed development increases the chance that some flowers and pods will develop under more favorable conditions. Together, these make it so pod number, seed number, and flower abortion rates are not very strong indicators of yield under stress. Instead, PHI correlations suggest that most plants end up with sufficient pods and seeds to fill and that the major limit on bean yield is filling of the seeds.

4.6 Drought Tolerance

While drought can negatively impact yield via carbon starvation [73]. the ability to maintain higher photosynthetic rates under drought alone does not impart drought tolerance. Instead, there exists a finer scale ability for plant to utilize available resources which contributes to increased drought tolerance. This is either through use of storage reserves or breaking down of structures in existing tissues, which can be remobilized to fruits and seeds. Plants which are better able to remobilize what is available to them under water deficits do so by maintaining higher sink strength, creating a stronger pull on those resources. Additionally, lines which maintain a higher sink strength are the lines which achieve higher yield under drought and are therefore deemed drought tolerant. In many agricultural plants, markedly improved yields have been associated, not with an increase in total biomass production, but a greater partitioning of the available carbon to the organs being harvested [74,75]. Additionally, in common bean specifically, the metric which shows the strongest correlation to yield under drought is pod harvest index (PHI), which quantifies remobilization efficiency of total pod resources into seeds [11,76].

Yet how inherent sink strength capacities are differently established and regulated between species, genotypes, or even different tissues within the same plant, is not well understood. While studies show differential growth and seed filling rates are determined by cell expansion and resource uptake rates, little is known about how the regulation of these rates differ between genotypes. However, ABA can be linked to these processes through its inhibition of mechanisms that promote expansion and resource uptake, and ABA typically increases under drought. This presents a possible source of regulation, where differences between genotypes could be due to differential sensitivities of ABA response or ABA production itself.

Plants evolved to sense and respond to their environment as a way to optimize their fitness. In the case of drought, this is usually achieved through ABA action. Although ABA has historically been considered a drought tolerance factor, Blum [24]. points out that whether ABA helps or hinders a plant depends on many things. For common bean, wild plants are indeterminate vines living for 8–10 months. They germinate and grow rapidly during the rainy season, developing leaves, flowers, pods and seeds until they sense the dry season beginning. When this occurs, the plants abort flowers, reduce vegetative growth and seed filling, and wait until the rainy season returns (Figure 2). Once rains return, the plants re-initiate both vegetative and reproductive growth, finishing their life cycle by producing beans. However, this response of pausing development during drought has unfortunately been carried into domesticated breeding lines, with the result that mild drought causes plants to slow leaf growth, abort flowers, and slow filling of existing pods and seeds. Therefore, ABA acts as a hindrance to yield in domesticated bush beans which cannot re-initiate reproductive growth like wild lines do. This can cause large impacts on yield, translating to decreased food security for those who depend on beans for protein and sustenance.

However, breeders have produced many lines that are able to perform well under drought. Unlike wild lines, these lines maintain higher rates of leaf growth, pod growth, and seed filling under drought. This results in higher seed yields in these lines. Perhaps in bean lines that are able to maintain higher leaf growth and seed filling under water deficits, their ability to sense or respond to ABA has been lost. It is possible some genotypes produce less or no ABA under drought, rendering them largely drought-insensitive. This would result in many downstream signaling responses not triggering under drought, so less changes in metabolism and uptake would occur. Alternatively, any of those individual responses downstream the ABA signaling pathway could become insensitive to ABA, even when ABA is present. This could lead to partial ABA

insensitivity, reducing some of the ABA-induced drought response mechanisms. If common bean lines which maintain higher PHI under drought have become in some way insensitive to ABA signaling, this could explain why seed filling and leaf growth rates are higher under drought in tolerant lines than sensitive lines. If ABA is not present or sensed such that it inhibits SUT transport or proton pumping, filling rates could remain higher. However, if true that drought tolerant bean has become less sensitive to ABA or its downstream affects, this might also mean that stomata do not close and tolerant lines have increased water loss. Loss of ABA sensitivity in pepper resulted in increased water loss which lead to increased sensitive to drought [77]. Additionally, as mentioned above ABA is often seen as a drought tolerance factor, and increasing ABA sensitivity in some species, such as *Arabidopsis thaliana*, actually increases drought tolerance [78]. This opens many questions as to how ABA could result in opposite responses between different species, increasing drought tolerance in some and sensitivity in others.

With studies showing that leaf size, seed size, sucrose uptake rates, and other sink strength indicators all correlate with yield, it is clear that establishing and maintaining strong sink strength is a large component of achieving higher yields in common bean. Additionally, indeed, bean breeders are strongly focused on finding lines that have higher harvest indexes, namely PHI. However, much work is still needed to understand control of sink strength, the mechanisms drought impacts and how, and whether, as proposed, loss of ABA signaling and/or sensitivity acts as a factor in maintaining sink strength and therefore increasing drought tolerance in domesticated common bean. Better understanding of this control will hopefully help improve breeding efficiency in common bean as well as contribute to a better scientific basis for the understanding of sink strength in crops.

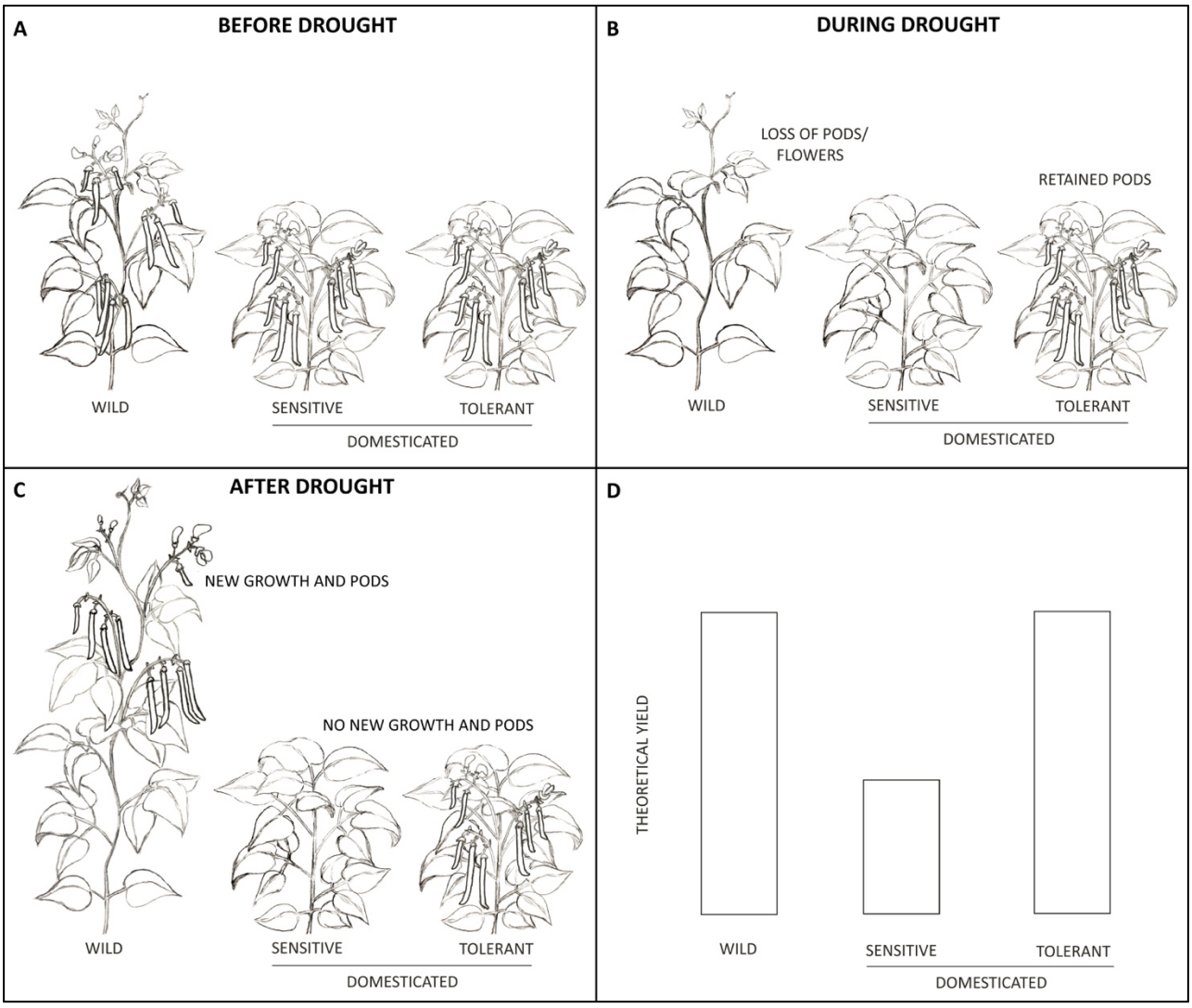

Figure 2. Effects of drought on flowers, pods, and yield in wild and domesticated common bean. (**A**) shows reproductive development in wild, vine beans compared to domesticated bush beans. Both have flowered and are producing pods. In (**B**), drought has caused both the wild and drought-sensitive domesticated lines to abort flowers and pods, however, the drought-tolerant line has retained its pods. All flowers and pods have been removed from these plants to accentuate drought's affect, however, many times not all flowers and pods aborted under drought. (**C**) shows the wild line re-initiating both vegetative and reproductive growth once the drought has passed, allowing it to set new flowers and pods while the domesticated drought-sensitive line is unable to do the same. Theoretical differences in yields under drought (**D**) show that both the wild and drought-tolerant line are able to achieve a higher yield than the drought-sensitive line.

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Chapter 5: General conclusions and future directions

The work contained in this dissertation set out to explore the overarching question of what underlies differences in allocation between drought tolerant and drought sensitive common bean (*Phaseolus vulgaris* L.) lines. As breeding of common bean has produced increasingly drought tolerant lines, breeders have found that a trait called Pod Harvest Index (PHI), which quantifies the allocation of biomass from pod walls to seeds, acts as the strongest predictor of drought tolerance in bean. However, what ultimately determines differences in allocation, and therefore PHI, between drought tolerant and drought sensitive lines is not known. Nor has it been studied how the trait of PHI relates to other allocation processes occurring with a plant. To address these questions, we started out working in the field at the whole plant scale, measuring traits which relate to allocation – leaf growth rate (LGR), pod growth rate (PGR), yield, biomass accumulation, and PHI – to see how these processes were related within and across genotypes and how they are affected by water deficit. From there, we moved to growth chamber experiments, working at the tissue level, in order to pinpoint the developmental timepoint and location where differences in allocation arise. This allowed us to focus in at a specific time and place on some of the finer physiological processes which control allocation; sucrose uptake, cell wall acidification via H^+-ATPases, and abscisic acid (ABA) sensitivity, to compare how these mechanisms are differentially regulated between sensitive and tolerant genotypes.

For the field study, our initial question was: Are leaf growth and seed growth regulated by similar processes? Early work from a pilot greenhouse study showed that LGR predicted yield, and we set out to test this finding across a larger set of genotypes in field conditions. This work was carried out at CIAT, the International Center for Tropical Agriculture, where many drought tolerant bean lines are bred and studied. In the field, 20 genotypes which ranged from drought tolerant to drought sensitive were grown under both well-watered and water-stressed conditions. For all 20 genotypes, under both conditions, we measured LGR, PGR, biomass accumulation, PHI and yield and calculated how impacted each of these values were under drought. We first looked at LGR and found that how impacted leaf growth rates were under drought varied strongly by genotype. Interestingly, opportunistic fast growers under well-watered conditions were the genotypes most knocked back by water stress. When looking across all traits to

determine how they were impacted by drought, we found that how sensitive each of these processes was to drought, that is how strongly each trait was decreased under water stress when compared to well-watered values, was related across all traits measured except PGR. Genotypes which had leaf growth rates strongly reduced by drought also had PHI, biomass accumulation and yields strongly reduced by drought. This suggested that these processes may be controlled by a common mechanism which is similarity regulated under drought. In addition, although biomass did correlate with yield, allocation of biomass was a stronger predictor of yield, since genotypes which achieved the same biomass sometimes differed in yield 4-fold. Therefore, while allocation can be either source or sink limited, it appears that sink limitations play a stronger role in determining yield. Together, these data suggest that sink strength, the ability of a growing sink tissue to take up resources, is the shared mechanism underlying differences in allocation and that each genotype has a set sink strength which is differentially affected by drought.

How do differences in sink strength change where biomass is allocated between sensitive and tolerant genotypes? How do rates of seed growth change over time between sensitive and tolerant genotypes? Using one sensitive and one tolerant line, chamber studies were carried out which allowed us to probe these questions by quantifying biomass pools in the two genotypes over time. In both a well-watered and water-stressed treatment, destructive harvests at 4 timepoints were performed on each genotype where each plant's biomass was measured by tissue type; leaves, stems, pods and seeds. Fresh and dry weights were obtained to allow for visualization of biomass shifts overtime. These data revealed three important differences between the tolerant and the sensitive line. First, the tolerant line, in both well-watered and water-stressed conditions, filled seeds earlier than the sensitive line. Second, the tolerant line under both well-watered and water-stressed conditions allocated a higher percentage of total biomass to seeds than did the sensitive line. And third, the tolerant line allocated a higher percent of total biomass into seeds by allocating more out of the pod wall, while the sensitive line has a lower percent of total biomass in seeds due to not allocating as much percent out of the pod walls into the seeds. Similar percentages of total biomass remained in the leaves and stems when comparing between the sensitive to the tolerant, indicating that the pod wall/seed interface is the location of the bottleneck. Differences in the timing of maximum seed filling rates between the

tolerant and sensitive line revealed key timepoints to study to gain insights into mechanisms controlling seed filling.

Determining that allocation of biomass from the pod wall to the seed was limiting seed filling fit with what is known in regards to the physical structures which facilitate the flow of resources between these structures. For resources to reach the seeds, largely carbon and nitrogen, they must be transported there. This is through the system of vasculature called phloem. Where resources are produced or taken up (eg. leaves for C, roots for N), they are loaded into the phloem. From there, interconnected phloem tubes (sieve tube elements) facilitate for the movement of resources, according to concentration gradients. As these gradients typically favor movement from sources towards sinks (from high to low concentration), this movement is passive from the leaves, through the stems, up to the fruit (pod, in bean). However, at the pod wall/seed interface, the interconnected system of phloem stops. This is because, while the leaves, stems and fruit all are a part of the mother plant and are therefore connected, the seed is a separate entity, a filial tissue, which is not physically connected to the phloem. Therefore, at this interface, active steps are required to unload sugar out of the seed coat (the last step of transport which is maternal tissue), into the apoplast surrounding the embryo (the seed), then actively transported into the seed itself. These steps require sucrose effluxers for export from the seed coat, sucrose uptake cotransporters (SUCs) for import into the seed, H^+-ATPases to set proton gradients which drive transport activity, as well as enzymes which help convert or break down sugars into the correct form for transport and metabolism.

We performed a series of seed assays to determine whether two of the mechanisms which control seed filling, sucrose uptake and proton pumping, differed between the two genotypes in both well-watered and water-stressed treatments. Seeds were dissected out of seed coats and floated in solutions similar to the apoplast surrounding the seeds *in planta*. Biomass gains, sucrose uptake rates (inferred from sucrose depletion from solution), and changes in pH were measured over 24 hours. We found that changes in pH of the solution suggest lower rates (or abundance) of proton pumps in the tolerant line under both the well-watered and water-stressed treatments. However, the sensitive line had lower rates under the well-watered treatment and higher rates under water-stressed treatment. Sucrose uptake rates closely matched biomass fresh weight gains. Like the

changes in pH, the tolerant line under both well-watered and water-stressed had low rates of both sucrose uptake and biomass gain. The sensitive line had higher rates of both sucrose uptake and biomass gain. These results were again similar to the changes in pH, except for the sensitive line under the well-watered treatment.

The seed assays were performed on seeds which corresponded developmentally to between timepoints 2 and 3 from the destructive harvest experiment (mid seed fill to max seed fill), therefore sucrose uptake rates and biomass gains should be compared at that time point. When comparing to the appropriate timepoint for each genotype-treatment pair, sucrose uptake rates and biomass gains matched the dry weight biomass gains seen in the biomass allocation experiment detailed previously. That is to say, both the tolerant treatments (well-watered and water-stressed) had lower sucrose uptake and fresh biomass gains in the seed assays and this corresponded to low seed dry biomass gains in the destructive harvest experiment when compared to the appropriate time point. Likewise, for both the sensitive treatments, higher sucrose uptake and fresh biomass gains in the seed assays match with high seed dry biomass gains in the destructive harvests. Of note, we performed seed assays on pods which were already past mid seed filling. However, it is only before mid seed filling that the tolerant line achieved a higher filling rate than the sensitive line. Therefore, we only captured the difference that occurred when the tolerant line filled slower than the sensitive. Assays done on younger pods/filling seeds will need to be performed in order to capture the timepoint where the tolerant line fills faster than the sensitive.

Lastly, we tested the effect of a drought induced phytohormone, abscisic acid (ABA) on sucrose uptake and fresh biomass gains in seed assays. ABA is known to impact processes relating to allocation, including proton pumping, which sets up the proton motive force that drives sucrose uptake. We found that while ABA had a small effect on both the well-watered and the water-stressed tolerant treatments rates of sucrose uptake and biomass gains, ABA had a large effect on both the well-watered and water-stressed sensitive treatments. This suggested that the tolerant line is not as sensitive to ABA as the sensitive line. If true, this could explain how the tolerant line maintains proton pumping and therefore sucrose uptake, maintaining seed sink strength and increasing allocation and PHI for the tolerant line.

Together, this body of work suggests that sink strength underlies differences in allocation, where maintenance of sink strength results in continuation of growth or seed filling processes, increasing allocation efficiencies (measured via PHI) and ultimately yields. Sink strength itself was seen to be differentially regulated via differences in sucrose uptake rates into seeds, which in turn are regulated by proton gradients set up by the proton pump. Furthermore, mechanisms underlying sink strength in the sensitive line were found to be more sensitive to a drought induced hormone, ABA. Further experimentation is needed to further dissect the mechanisms controlling these differences, such as determining whether increased proton pumping and sucrose uptake establishes early fluxes in seed filling within tolerant lines, and confirm hormone sensitivity or lack thereof in regulation of these fluxes. Additionally, the study of other mechanisms which are known to contribute to allocation and sink strength processes, such as enzymes involved in carbohydrate metabolism and synthesis, carbohydrate transporters beyond SUCs, as well as nitrogen transporter and metabolism may contribute other key findings relating to the control of resource allocation.

Our findings also bring to light questions regarding how the timing, duration and severity of drought alter allocation processes. Likely, earlier water deficits have stronger affects, since drought can result in decreased photosynthetic rates, leading to decreased resource availability and in turn, impacts to nearly every aspect of growth and development. Likewise, drought is known to have especially strong impacts on yield if the water stress coincides with flowering and fertilization since these processes are highly sensitive to water stress. Is this same finding true in drought tolerant bean genotypes which display decreased sensitivity to drought? Perhaps lines which are able to maintain sink strength in leaves and seeds also have decreased sensitivity to water stress at this key developmental timepoint, flowering, leading to less abortion of flowers and young pods. In our studies, we introduced water stress after flowering and fertilization has occurred in order to exclude impacts to these processes, so affects to these processes were not quantified. In addition, the drought treatments applied to (1) the field study and (2) the chamber study differed strongly as did some key results between the two bodies of work. While tolerant lines had higher allocation of resources in both studies, the tolerant line and the sensitive line (designated such based on high and low yields under water stress in the field study, respectively)

did not differ in yields under water stress in the chamber study. This is likely due (at least partially) to differences in the severity of drought. In the field study, water was withheld starting from 6 days after flowering through the remainder of pod and seed development. However, in the chamber study, plants in the water-stressed treatment received water periodically (upon wilting) throughout pod and seed development, resulting in a less severe drought. How this changed allocation patterns between the field and chambers is not known. Future studies are needed to determine whether more severe water deficits would exacerbate trends in allocation identified in the chamber studies, such as differences in timing and rates of seed filling.

Moving from coarse to fine physiology, we found that field level trends can be explained by differences in allocation processes controlled at the level of the cell. Explorations in understanding mechanisms underlying these differences was begun, with results which could guide future studies. This work aimed to contribute findings which could help breeders to better understand and produce more drought tolerant lines. Findings which showed that sensitivity of leaf growth rate could predict sensitivity to yield may be used as a predictive trait which could allow identification of drought tolerant lines earlier in a plant's life cycle. Gaining insights into these processes may facilitate future gains in bean yields under water deficits and may prove relevant to understanding growth and allocations process in plants generally.