QUANTITATIVE TRAIT LOCI ASSOCIATED WITH LODGING, STEM

STRENGTH, YIELD, AND OTHER IMPORTANT AGRONOMIC TRAITS IN DRY

FIELD PEAS

by

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ABSTRACT

In pea, lodging changes canopy structure, increases disease pressure, reduces yield, and reduces harvest efficiency. In order to discover the quantitative trait loci (QTLs) influencing lodging resistance and other important agronomic traits in pea, a recombinant inbred line (RIL) population was created from a relatively wide cross between the commercial variety Delta and an unnamed pea variety. The RIL population was grown for 6 site-years in Bozeman and Moccasin, MT, USA, and phenotypic data was collected for 22 quantitative morphological traits and seven categorical traits which were thought to be associated with lodging resistance. Genotypic data was derived from genotype by sequencing, microsattelite markers, and cleaved amplified sequence tagged sites.

QTL analysis identified a total of 135 putative QTLs for the 22 traits examined in the study. There were 12 specific regions where 115 QTLs co-located, indicating that as few as 12 genes may be responsible for multiple pleiotropic effects. Ten QTLs are found for lodging resistance. Due to the large amount of phenotypic data collected, the putative mechanism of lodging resistance was determined for each QTL. In nearly every case, lodging resistance was associated with reduced plant height, a change in tendril number, or increased stem strength. This conclusion was supported by mathematical modeling. Branch number, which determines the number of tendrils per plant, was also positively associated with lodging resistance during all site-years, indicating that increasing tendril number also increases lodging resistance.

Yield was controlled by eight QTLs. All QTLs for yield had pleiotropic effects on lodging resistance and yield per plant. Seed size was not correlated with yield, and a model was created which explained why no association between yield and seed size was found.

The pleiotropic effects and utility of the QTLs discovered in this study are discussed. The results of this study further refine the ideotype for pea, and can be used for marker assisted selection in this crop.

CHAPTER ONE: INTRODUCTION

The Pisum Genus

Pisum is an important diploid genus in the Fabaceae with a growth habit

resembling a climbing vine. The center of origin for the genus is in the mountainous

regions of Southwest Asia (Afghanistan and India) and Trans-caucasia, with a secondary

center of origin in the Mediterranean region and Ethiopia. There were originally believed

to be four major wild species of pea known as *Pisum elatius*, *Pisum fulvum*, *Pisum*

abyssinicum, and *Pisum syriacum*. (Khvostova 1983). Other individuals familiar with

Pisum diversity have proposed four distinct subgroups of *Pisum* with *Pisum fulvum* and

Pisum elatius as wild species and *Pisum sativum* and *Pisum abyssinicum* as cultivated

species (Jha et al. 2013). However, the classification of *Pisum* has changed over time and

some consider *Pisum sativum* and *Pisum elatius* to be merely subspecies of *Pisum*

sativum, denoted as *Pisum sativum* subsp. *sativum* and *Pisum sativum* subsp. *elatius* (Jha

et al. 2013). *Pisum fulvum* differs from *Pisum sativum* morphologically and also by two

reciprocal translocations (Kosterin and Bogdanova 2015). *Pisum abyssinicum* closely

resembles the garden pea by its growth habit, but has chromosome rearrangements that

set it apart from garden pea (Kosterin and Bogdanova 2015). Taxonomists have recently

suggested that *Pisum* should be incorporated into the genus *Lathyrus*, with *Pisum sativum*

L. becoming *Lathyrus oleraceus* Lamarck (Kosterin 2017).

Pisum sativum contains an enormous variety of wild forms, in addition to a

number of cultivated forms, with variations in height and size being due to adaptations to

certain habitats. Wild forms of *Pisum sativum* are considered to be lumped into a loose classification under *Pisum sativum* subsp. *elatius*. Chromosomal rearrangements and post-zygotic crossing barriers do sometimes exist between wild and cultivated forms, which can cause a drop in fertility when wild *Pisum sativum* subsp. *elatius* accessions are crossed with domesticated *Pisum sativum* subsp. *sativum* (Kosterin and Bogdanova 2015). Wild *Pisum* accessions have been shown to be intercrossable in many cases with *Pisum sativum* L. when the cultivated pea is used as a female donor, and they could potentially be used as a source for desirable alleles (Tayeh et al. 2015b). Crosses between *Pisum sativum* and *Pisum abyssinicum* are difficult regardless of which species is used as the maternal or paternal parent, but *Pisum abyssinicum* could be a good source of alleles for extremely early ripening and bacterial blight resistance (Kosterin 2017). Despite being widely scattered, the primary areas of cultivation and introduction of pea are located in the modern day countries of Georgia, Armenia, Azerbaijan, Turkey, Turkmenistan, the Arabian Peninsula, Iraq, Syria, Iran, and the Punjab region of India (Khvostova 1983). *Pisum fulvum* grows wild in only a small region of the world in Israel, Jordan, Lebanon, Syria, and southern Turkey. *Pisum abyssinicum* is cultivated in Ethiopia and Yemen, and it is highly uniform genetically, having diverged since the beginning of plant domestication, possibly as a result of a hybridization between *Pisum sativum* L. subsp. elatius and *Pisum fulvum* Sibth. et Smith (Kosterin 2017).

Economic Importance of Pea and Varietal Development

Cultivated pea is an excellent rotation crop that participates in biological nitrogen fixation, leaving nitrogen in the soil for the following crop (Sindhu et al. 2014). Pea and other legumes are an essential component of sustainable cropping systems (Duarte et al. 2014, Tayeh et al. 2015b), and it is currently the third most economically important grain legume after soybean and bean (Duarte et al. 2014, Tayeh et al. 2015b, Boutet et al. 2016) with nearly 7 million hectares grown worldwide and 11.1 million metric tons produced (FAOSTAT 2014). When used as a rotation crop in temperate climates, it breaks disease cycles, facilitates weed control, reduces use of fossil fuel based fertilizers, and provides needed crop diversity to wheat cropping systems (Tayeh et al. 2015b, MacWilliam et al. 2014). Dry pea production in the United States (US) as a whole has increased due to demand from southern Asia, with exports to India in 2016 well above their 5-year average (Wells and Bond 2016). Peas and other legumes are part of the local culture and diet of many parts of southern Asia. Dry peas contain 15.8-32.1% protein (typically 22-25% protein), and they are a rich source of dietary fiber (Tayeh et al. 2015b). In 2016 the state of Montana had the highest acreage of peas grown in the US. Field pea production in the Montana has increased nearly 28-fold to an estimated 580,000 acres (235,000 ha) since 2000 (NASS 2016). More importantly, peas have replaced summer fallow in areas of Montana and the prairies of Canada (Nagy 2001). Due to the rapid growth of dry pea production in Montana, more research needs to be done to facilitate the development of new varieties for this important crop. Major traits important in pea varietal development include disease resistance, erectness (lodging resistance),

bleaching resistance, cold tolerance, drought resistance, salinity resistance, insect resistance, yield, seed shape, pod number, flower color, pod position, seed type, height, time of flowering, days to maturity, and leaf type (https://www.coolseasonfoodlegume.org/find/qtl Accessed 2/16/2017., Tayeh et al. 2015b).

Economic Impact of Lodging in Pea

All commercially grown dry pea cultivars must be lodging resistant due to the need for mechanical harvesting. Peas are lodging susceptible due to their growth habit, which depends on tendrils for support (Swinhoe et al. 2001). In the past lodging resistance has ranked only behind yield in importance in pea breeding programs (Stelling 1989). When plants lodge, a humid microclimate is often created that promotes fungal diseases, such as mycosphaerella blight (Kaatz and Gritton 1975, Swinhoe et al. 2001, Banniza et al. 2005, Tar'an et al. 2003, Jha et al. 2013) and premature germination (Swinhoe et al. 2001). Lodging also increases yield losses during harvest (Kaatz and Gritton 1975, Schouls and Langelan 1994, Banniza et al. 2005, Wang et al. 2006, Jha et al. 2016). In soybean it has been shown to cause yield losses of 11-32% (Chen et al. 2011). Yield loss occurs by two mechanisms: when pods fall below the cutter level at harvest, and when normal canopy structure is destroyed, resulting in reduced photosynthetic ability, lower dry matter production, and increased damage from pathogens and pests (Kaatz and Gritton 1975, Chen et al. 2011). Lodging slows harvest because machine operators need to be more careful with header placement, resulting in

more operator hours in each combine. Existing commercial varieties have lodging resistance to varying degrees, but lodging susceptibility is often heavily dependent on environmental conditions. No heritability estimate of lodging has previously been reported.

<u>Climatic and Agronomic Factors Influencing Lodging Susceptibility</u>

The climatic and agronomic factors linked to lodging resistance and other important traits are only partially understood in pea. Climatic and biotic factors that might influence lodging include wind, precipitation, hail, humidity, and disease pressure. Wind might increase the load borne by pea stems. Precipitation could soften stem material and facilitate biotic degradation of stem walls, leading to settling of the crop after a hard rainfall. Hail can cause lodging by mechanical damage. Humidity may affect the stiffness of the stem, and disease pressure might weaken the stem material due to damage.

Agronomic factors that may influence lodging include row spacing, which determines how likely tendrils are to find support from neighboring rows, and seed treatments, which influence germination rates. A recent review article on lodging in small grains indicated that agronomic factors such as delayed sowing, lower seeding rates, crop rotation, soil rolling, and reduced tillage systems all reduced lodging. Sprinkler irrigation, nitrogen fertilization, timing of nitrogen fertilization, phosphorus and potassium fertilization, and silicon content in stems were also shown to influence lodging (Shah et al. 2017).

Chemical or Mechanical Methods Used to Prevent Lodging

Some studies have reported using chemical or mechanical methods to improve lodging resistance in peas (Elkoca et al. 2006, Pullan and Hebblethwaite 1990, Kaatz and Gritton 1975, Kontturi et al. 2011, Klimek-Kopra et al. 2015, Schouls and Langelan 1994). Mepiquat chloride, a plant growth regulator, was shown to decrease lodging susceptibility, increase stem diameter, and reduce height in peas (Elkoca et al. 2006). Seed yield was also increased by ~10-11% when mepiquat chloride was applied at the bloom stage (Elkoca et al. 2006). Given that mepiquat chloride is fairly inexpensive, this may be a fairly good way to decrease lodging and increase yield, but no follow up research was done in peas. In small grains, plant growth regulators have also been shown to reduce lodging (Shah et al. 2017).

Prior research details some of the challenges associated with achieving lodging resistance. For example, it has been shown that internodes in the middle of the stem are stronger than the internodes at the base of the plant (McPhee and Muehlbauer 1999, Skubisz et al. 2007). However, the greatest stress on a pea stem is at the base, where internodes are weaker. Pullan and Hebblethwaite (1990) discovered that peas lodge more at high densities than at low densities and that yield and 100 seed weight declined at higher planting densities due to lodging. It was recommended to plant at a very low density (~30 plants per m^2) in order to reduce lodging (Pullan and Hebblethwaite 1990). It is well known that increasing plant density decreases stem diameter per plant, increases internode length, and influences lodging resistance (Xue et al. 2016). However, other

research indicates that much higher seeding rates are needed to optimize crop yield (Spies et al. 2010).

Dry peas often lodge when they are green. A study which compared shelling peas supported with bamboo with shelling peas interplanted with oats for structural support found that bamboo was very effective in reducing lodging, and it increased yield ~8%, probably due to increased light penetration (Kaatz and Gritton 1975). However, interseeding with oats was not very effective at reducing lodging because pea yield decreased proportionally with increasing oat populations (Kaatz and Gritton 1975, Kontturi et al. 2011). A similar study interseeded peas with linseed for support. Linseed helped prevent lodging, but it also reduced yield (Klimek-Kopra et al. 2015). An additional study looked at mixing various ratios of leafed and semi-leafless cultivars. It was shown that mixing semi-leafless (*afila*) cultivars with normal leafed cultivars was effective at reducing lodging, and it increased yield ~5-6% (Schouls and Langelan 1994). One lodging resistant cultivar was produced by mutagenizing a lodging susceptible cultivar and selecting a lodging resistant mutant, which was then bred into a released variety. The mutant variety had reduced plant height when compared to the original cultivar (Naidenova and Vassilevska-Ivanova 2006).

The Stress Equation, Stem Strength, and Lodging Susceptibility

Tendrils represent a reasonable mechanism for support when other plants with stronger stems grow nearby, but in a commercial field of dry peas stem strength becomes of critical importance for erect growth. Stem strength is dependent on the parameters of

the stem. In engineering, equations have been developed and proven to determine the stress on a tubular beam. In order to analyze the forces acting on the stem of the pea plant (or any plant with an upright hollow stem) the stress equation for a tubular cantilever beam (Gere 2004; http://www.atcpublications.com/Sample_pages_from_FDG.pdf. Accessed 11/10/2016.) can be used to determine the stem stress associated with a given load. This equation identifies the load on the stem, the length of the stem, its diameter, and the thickness of the stem wall as four critical parameters that determine the probability of structural failure. The equation is not completely satisfactory as a model for pea because it does not include such factors as the presence of tendrils, branches, or modifications in the intrinsic strength of the material (more fiber cells or lignin). However, it is a useful starting point for the analysis of the main structural features impacting lodging resistance in pea.

Several studies have focused on improving stem strength and stem characters in order to increase resistance to lodging (Chen et al. 2011, Beeck et al. 2006, and Beeck et al. 2008 a, b), and the response of compressed stem diameter to selection has previously been estimated (Beeck et al. 2008b). Stem diameter traits were shown to be negatively correlated to lodging susceptibility (Chen et al. 2011). In a recent review on lodging in grains, lodging was correlated with plant height, panicle and peduncle length, cell wall thickness, stem diameter, stem wall thickness, area of xylem, and the number of vascular bundles; however, lignin, starch, silicon, hemicellulose, and cellulose content also were shown to play a role (Shah et al. 2017). Stem strength has previously been reported to be positively correlated with internode diameter and internode length and to increase with

the yield potential of various varieties (McPhee and Muehlbauer 1999), indicating that this trait is being selected with yield. Banniza et al. (2005) focused on stem cross-sections and found that there was no difference in the number of schlerenchyma cap fibers among many different cultivars and that the number of sclerenchyma cap fibers was not correlated to lodging susceptibility. Significant negative correlations were found between the proportion of xylem and proportion of supportive tissue at internode 2-3 in pea stems and lodging susceptibility, even though no significant differences among cultivars was observed. Acid detergent fiber, acid detergent lignin, and cellulose were also analyzed and the amount of each was found to be negatively correlated with lodging susceptibility. Higher fiber and lignin content also reduced disease severity. However, only limited variation in lignin content was found in cultivars, a result which is confirmed in other studies (Beeck et al. 2006). It was noted that the diameter of internodes in the upper part of the plant did not influence lodging susceptibility, but internode diameter at node 2-3 explained 16% of the variation in lodging (Banniza et al. 2005). Lodging begins when the basal part of the stem bends over due to the weight of the upper part of the plant. The basal part of the plant has a different architecture than many other parts of the stem (Swinhoe et al. 2001). Since the basal part of the stem is the area where bending occurs, increasing stem strength in the basal region of the stem is likely to increase resistance to lodging. Banniza et al. (2005) noted that there is an increase in stem diameter from lower internodes to higher internodes, which is apparently a characteristic that is found in commercial *Pisum sativum* but not consistently in all *Pisum* germplasm.

Beeck et al. (2006) focused on stem diameter and wall thickness and used a metric of load to determine the traits associated with strength of the pea stem. The best predictor of load as measured in the study was compressed stem thickness (r^2=0.92) followed by stem diameter (r^2=0.80). There was a positive response to selection for compressed stem thickness, which had an estimated broad sense heritability of 0.62 and 0.66 (Beeck et al. 2008a, b). However, the study did not assess whether there was a correlation between lodging resistance and the metric of load used in the study. Beeck et al. (2008a) did indicate that the variance associated with stem strength traits was additive. Compressed stem thickness and black spot resistance were successfully increased by recurrent selection (Beeck et al. 2008b). Skubisz et al. (2007) also concluded that stem wall thickness was correlated with the strength and lodging potential of pea stems.

The Genetics of Lodging Resistance

In other crops it has been shown that plant height, internode length, stem diameter, node number, branch number, stem (breaking) strength, root systems, lignin and cellulose content, silicon content, environmental conditions, fertilization, and disease can influence lodging in crops (Chen et al. 2017). Due to the importance of tendrils for structural support in pea, it is possible that genes influencing plant emergence, which determines the overall number of tendrils in a given field, and tendril width and length, which determine the likelihood of finding support, may influence lodging resistance in pea. In pea or soybean several major genes and quantitative trait loci have been shown to influence lodging susceptibility, particularly genes related to plant height (Jha et al. 2013,

Tar'an et al. 2003), leaf type (Tar'an et al. 2003), and stem strength (Chen et al. 2017).

Lodging susceptibility and plant height have been known to be correlated for much of the

latter part of the last century (Chen et al. 2011). It was dwarfing genes in wheat and rice

that allowed the development of varieties that powered the green revolution (Hedden

2003). A similar trend has been seen in peas, where plant breeders have selected for

varieties that are semi-dwarf (homozygous *le*). Several studies have shown that

quantitative trait loci (QTLs) which influence lodging susceptibility also control plant

height (Tar'an et al. 2003, Inoue et al. 2004).

It is currently known that lodging resistance is a multigenic trait (Tar'an et al.

2003). Singh and Srivastava (2015) indicated that days to maturity is positively correlated

with lodging susceptibility, and that lodging susceptibility was negatively correlated with

seed yield per plant, but the affect may have been due to the study design. Other research

indicates that lodging resistance is positively correlated with branch number, number of

pods per plant, number of seeds per plant, number of fertile nodes per plant, number of

seeds per pod, seed weight per plant, and branch length (Kosev and Mikic 2012).

Lodging resistance was negatively correlated to 1000 seed weight in one study (Kosev

and Mikic 2012).

Mendel's *Le* and Plant Height

The *Le* gene is the most important plant height gene in pea. Mendel first defined

this 'factor' (although he used a different symbol) in his groundbreaking work on the

genetic basis of morphological variation in pea (Mendel 1866). The dominant *Le* allele is

known to be correlated to lodging susceptibility. It codes for a gibberellin 3β-hydroxylase and is located on the lower part of linkage group (LG) III in pea. The specific gene sequence was identified in 1997 (Lester et al. 1997, Martin et al. 1997). The difference in height is caused by a single alanine-to-threonine substitution in the putative active site of the enzyme. This substitution prevents the conversion of GA_{20} to GA_1, a bioactive form of gibberellin, and gibberellin levels are lower in dwarf plants (Lester et al. 1997, Martin et al. 1997). Gibberellin induces *DELLA* protein degradation via ubiquitination, which is followed by destruction by the 26S-proteasome. *DELLA* proteins are known to act in partnership with transcription factors to repress gene expression (Taiz and Zieger 2010). It is well known that ethylene thickens stems, and it has been shown that ethylene controls floral transition via *DELLA*-dependent regulation of floral meristem identity genes, a pathway that is affected by gibberellins (Achard et al. 2007, De Grauwe et al. 2008).

Other research indicates that upregulation of the ethylene response factor 11 (*ERF11*) in *Arabidopsis thaliana* increases bioactive GA levels and decreases ethylene levels (Zhou et al. 2016), indicating that higher levels of ethylene and hence greater stem diameters would be found in dwarf plants, which have reduced concentrations of gibberellins. It is known that there is interaction between gibberellins and ethylene and ethylene appears to decrease GA levels (De Grauwe et al. 2008). In one cross dwarf plants have been reported to have a higher root mass than tall plants (Weeden and Moffet 2002), which also may influence lodging susceptibility due to the differing strength of the respective root systems. However, a contradictory study indicated that tall and dwarf

plants have nearly identical root growth rates, indicating that root vigor would be unaffected (Silva and Davies 2007). It is possible that taller plants have more leaf biomass and less light penetration into the canopy, which prevents basal branches from developing. Fewer basal branches were found in plants with the tall background (Murfet and Reid 1993, Symons and Murfet 1997).

Several QTLs linked to height have been described (Tar'an et al 2003, Prioul et al. 2004), including a number of gibberellin mutants (Silva and Davies 2007). Approximately 15 additional mutants influencing internode length have been described such as *na, ls, lh, sln, lk, lkb, lka, lkc, lkd, la cry^5, lm, lgr, lw, lv, and lip1* (Murfet and Reid 1993). Almost all of these mutants are due to mutations in gibberellin synthesis and response genes, and most have an unknown level of utility in a breeding program. Only some have been mapped (Murfet and Reid 1993). In addition to the *Le* locus, Prioul et al. described a quantitative trait locus (QTL) that controls height located below the *A* locus on LG 2 and near SSR-AD12. An additional QTL was found on the upper part of LG VII near SSR-AA135 (Prioul et al. 2004). Tar'an et al (2003) found a significant QTL for plant height and lodging on the middle of LG III.

<u>The *Afila* Mutation</u>

A breakthrough in lodging resistance in pea breeding came in the early 1980's with the development of varieties with the *afila* (semi-leafless) trait, which converts leaflets to tendrils in the compound leaf, increasing the number of tendrils per plant and allowing the plants to intertwine for mutual support (Stelling 1989; Tar'an et al. 2003;

Kof et al. 2004, Mikel 2013, Klimek-Kopra et al. 2015). Leaves on wild type (*Afila*) pea

lines have a pair of stipules at their base, a distinct petiole, and a pinnately compound

blade. Lines with the *afila* (*af*) mutation have branched tendrils in the proximal half of the

blade and simple tendrils in the distal half (DeMason and Chawla, 2004). This mutation

was first discovered in the early 1960's (Goldenberg 1965). In general, *af* lines are less

competitive with weeds and other plants, making weed control more difficult (Rauber et

al. 2001, Spies et al. 2011), but they can be less susceptible to disease (Banniza et al.

2005). However, splash dispersal of conidia may facilitate the development of some

diseases such as *Mycosphaerella pinoides* more effectively in an open canopy, although

yield losses are generally lower when lodging is not present (McDonald et al. 2013). It is

worth noting that only the proximal half of the leaf blade is different in *af* vs. wild type

lines. The *Afila* locus is located on LG1.

It has been shown that the *af* gene interacts with *Tl* (*acacia/tendrilless*) to

influence leaf development, and that this interaction occurs in a gradient where *Af* has a

strong effect on the proximal leaflets and *Tl* has strong effects on distal and terminal

pinnae. This effect is due to auxin gradients, and branched pinnae are caused where auxin

levels are lower (DeMason and Chawla 2004), although gibberellins are also involved in

pea leaf morphogenesis (DeMason and Chawla 2006). This previous research indicates

that a central auxin gradient controls leaf morphology in pea leaf development.

Mutations that affect auxin levels often have a major impact on the plant because

auxin levels coordinate the function of many biological pathways. Auxin is directly or

indirectly involved in nearly every aspect of plant development (Delker et al. 2008)

including phototropism, geotropism, hydrotropism, and other developmental changes. It has been well documented that auxin stimulates cell wall loosening and cell elongation. Auxin has been documented to interact with numerous other plant hormones and can stimulate ethylene production (Suttle 2003), which influences stem thickening. Auxin induces shoot apical dominance and delays fruit senescence. Auxin also influences the initiation of flowering.

The stipules of most commercial dry pea varieties photosynthesize as much as the *af* leaves. Significantly more photosynthesis occurs in wild type vs *af* leaves (Sharma and Kumar 2012). The conversion of leaflets to tendrils diminished leaf area 1.5 fold when compared to the wild type. A lower leaf area index in *afila* genotypes is likely compensated by stipules of lower leaves, which are usually shaded in wild type varieties (Klimek-Kopra et al. 2015), synthesizing more, increasing the actively functioning assimilation area due to less shading.

All commonly grown dry pea varieties in the western United States are *afila* and *le* but some varieties with the *afila* and *le* alleles heavily lodge. Therefore, leaf morphology is not sufficient to achieve an upright growth habit (McPhee and Muehlbauer 1999). The rate of stem elongation, yield, time to flower, and the total growth period were not apparently affected by the mutation (Kof et al. 2004). However, a different study indicated that *afila* lines matured earlier than wild type lines, and that they were higher yielding, although the effect may not have been due to the *afila* locus (Singh and Srivastava 2015). Varieties with the *afila* mutation had significantly lower stem diameter than wild type varieties (Singh and Srivastava 2015), but they also had larger tendrils

(Klimek-Kopra et al. 2015). It has been determined previously that the *afila* mutation determines plant nitrogen status and decreases seed protein content and yield (Burstin et al 2007).

Basal and Aerial Branching

Basal and aerial branching increases the number of leaves/tendrils per plant, which may affect how tendrils intertwine with each other, thereby influencing lodging resistance. The ideotype is not strictly defined for basal branching in pea (Rameau et al. 1998). Branching genes have been fairly well characterized in pea and they are generally thought to be influenced by strigolatones or carotenoid cleavage (Beveridge et al. 2009). Branching genes described as *Ramosus (Rms)* 1-5 have been characterized (Arumingtyas et al. 1992). *Rms1* is on LG III near the *Hr* locus, a gene involved in the circadian clock and regulation of flowering. *Rms2* is found in the middle of LG I. *Rms3* is on LG II. *Rms4* is on LG VII (Ellis and Poyser 2002), and *Rms5* is on LG V (Apisitwanich et al. 1992). Other mutants such *Asc*, *fr*, *fru*, *ho*, *pro*, and *ram* have been described (Murfet and Reid 1993). The mutations *ascendens (asc), horizontalis (ho)*, and *procumbens (pro)* control the orientation of the basal branches.

The seeding rate for a given variety varies depending on the potential of the variety to branch, and high branching cultivars require lower plant densities to achieve optimum yield potential (Spies et al. 2010), potentially increasing efficiency by reducing seed cost. It appears that basal branching is influenced by changes in auxin, cytokinin, and strigolactone concentrations in the *Rms* mutants (Beveridge 2000, Beveridge 2009,

Dun et al. 2013). Rameau et al. (1998) indicated that mutants with increased aerial branching, such as the 5 ramosus mutants previously reported in the literature, were not appropriate for crop use. *Sn, Dne*, *Ppd*, and *det*, which control plant response to photoperiod, have significantly increased branching. High response to photoperiod (*Hr*) is also known to increase basal branching (Murfet and Reid 1993). The d allele of *Lf* (*Lf^d*), and the flowering genes *g*, and *e* increase aerial branching (Murfet and Reid 1993). Photoperiodic sensitive genotypes have a marked tendency to produce basal lateral branches, whereas day neutral types usually produce only a single stem (Rameau et al. 1998). Several height and internode length mutants such as *le, lh, ls, na, lgr* also pleiotropically increase basal branching, whereas *la*, *cry^5*, and *lv* have reduced branching. The *lk* mutants (*lka, lkb, lkc*, and *lkd*) have short internodes and reduced branching.

Molecular Biology and Marker Assisted Selection in Pea

Pea is an autogamous diploid species with x=7 (2n=14) chromosomes and a genome of 4.3 Gbp that is dominated by a number of transposable elements. The genome size is approximately ten times the size of the model legume species *Medicago truncatula*. The relatively large genome size and the large number of repeats has inhibited the development of genomic breeding tools in this species (Duarte et al. 2014). However, the pea genome is currently being sequenced (Madoui et al. 2015), and medium (Loridon et al. 2005) and high density genetic maps have been developed (Duarte et al. 2014, Sindhu et al. 2014).

Molecular markers are widely used in plant research for marker assisted selection (MAS), candidate gene identification, and QTL mapping (Duarte et al. 2014). Marker assisted selection is an effective tool that can be used to introgress desirable genetic traits into pea (Zhang et al. 2006). Phenotypic selection can be less efficient compared to marker assisted selection (MAS), which has few of the limitations of phenotypic selection, even though it is more time consuming during the process of marker discovery. In pea, a number of marker technologies are available, such as simple sequence repeats (SSR) (Loridon et al. 2005), cleaved amplified polymorphic sequences (CAPS), restriction fragment length polymorphism (RFLP), and random amplified polymorphic DNA (RAPD) markers (Laucou et al. 1998 and Weeden et al. 1998). Numerous QTL studies have defined QTL for various traits in pea (https://www.coolseasonfoodlegume.org/find/qtl Accessed 2/16/2017) using SSR and CAPS markers. However, these technologies require large amounts of time and resources in order to collect data, and genome coverage is often low (Poland et al. 2012, Poland and Rife 2012). Single nucleotide polymorphisms (SNPs) are now the genetic marker of choice since they are highly abundant and uniformly distributed throughout genomes, and they are an excellent genotyping resource (Duarte et al. 2014, Tayeh et al. 2015a). Recently a 13,200 SNP genotyping array has been developed, which has indicated that the pea genome spans ~800 centimorgan (cM) (Tayeh et al. 2015a), similar to the first consensus map (Weeden et al. 1998). Next generation sequencing has advanced at a fast pace, allowing the development of newer technologies such as genotype by sequencing (GBS), which can be used to generate large numbers of SNPs in a fraction of the time of

older technologies, allowing the efficient linking of genotype to phenotype (Poland et al. 2012, Poland and Rife 2012). In other crops, GBS has been a very effective tool to discover QTL and generate high density genetic maps (Celik et al. 2017, Heim and Gillman 2017, Balsalobre et al. 2017, and numerous other studies).

There are several reasons why lodging resistance is better selected for using a genetic marker. Firstly, lodging susceptibility often is dependent on environmental conditions, and it would be ideal to select for lodging resistance using a mechanism that is not dependent on weather conditions (Inoue et al. 2004). Secondly, marker assisted selection can be done year round, speeding up the breeding process. Thirdly, field operations can be reduced by removing lines that are shown to be undesirable, reducing cost. Specific markers linked to genes have been developed for use in breeding in peas (Tayeh et al. 2015b). A highly predictable and easy to use set of genetic markers needs to be developed to predict lodging resistance in pea as well as to predict other commercial traits.

With the development of genetic maps and the identification of the most likely positions of QTLs on these maps, molecular markers for lodging resistance and other traits can be developed. Significant progress has been made toward discovering the genes responsible for various agronomic traits in pea (Tayeh et al. 2015b). Previous research mapped a lodging QTL on both LG III and LG VI, which accounted for 47% and 26% of the variation in lodging, respectively, in a population derived from a cross between two commercial pea lines (Tar'an et al. 2003). A sequence characterized amplified region (SCAR) marker was developed for each of these QTLs. The markers developed in the

previous study were used for selection of lodging resistant new cultivars (Zhang et al. 2006). Unfortunately, the genes underlying these QTLs are not known. A recent study with dry pea in Italy mapped a lodging and height QTL in the middle of LG III, in a similar location as the previous study (Ferrari et al. 2016). In the same study, another QTL for lodging was found on LG IV and the lower part of LG V (Ferrari et al. 2016). However, the map of these QTLs had low resolution, making it difficult to pinpoint individual QTLs.

<p style="text-align:center;">Proposed Work</p>

The purpose of this study was to discover additional QTLs for lodging resistance in pea as well as to quantify the impact of *le* and *af*. This study also attempted to identify pleiotropic effects of these QTLs in order to determine the mechanism by which these genes and traits function. In order to determine the mechanism of lodging resistance for each QTL, traits associated with yield, seed size, branching, stem diameter, leaf size, stem length, seed dormancy, and maturity time were also assessed. QTLs for all of these traits were identified.

CHAPTER TWO: MATERIALS AND METHODS

The RIL Populations

The recombinant inbred line (RIL) population used in this study was developed from a cross between two pea varieties, the lodging resistant cultivar (Delta) and RER, a pea line with more primitive traits. Delta is semi-leafless (*afila*), has short internodes (*le*), and is a well-known commercial line. RER was developed from several crosses by Dr. Norman Weeden at Montana State University. Firstly, a F8 line was derived from a cross between MN313 and OSU1026. The F8 line was crossed with Majoret. A F2 line derived from that cross was crossed further with PI220174, a wild type line from Afghanistan. One of the resulting progeny (RER) was then selfed for 8 generations before it was crossed with Delta. RER was chosen for its erect growth despite having wild type leaves (*Afila*) and long internodes (*Le*). There were 254 F2 lines derived from the Delta x RER cross. Only ninety-four dwarf lines within the 254 lines were subjected to GBS, but phenotypic data was recorded on the entire population. Initial data analysis was on only 94 dwarf lines, but additional RILs in the entire population were genotyped for markers associated with major QTLs in order to confirm their effects in the entire population.

An additional RIL mapping population called the PR population was sourced from the University of Saskatchewan and planted in 2015 in both Bozeman and Moccasin, MT. This population was entirely semi-dwarf (*le/le*) and semi-leafless (*af/af*) being derived from a cross between the commercial lines Carerra and Striker. It was composed of 144 lines including the two parents. Lodging data, stem length, canopy

height, main stem diameter, side branch diameter, and epicotyl diameter was collected in the same manner as the Delta x RER population, but this population was only used to confirm the validity of the stress equation and no genotypic data was collected.

Field Trials

Each F_2 plant from the Delta x RER cross was eventually advanced via single seed descent to the F_7 generation. A single row of 5 F_3 plants per line was planted at Bozeman in 2013, and only rudimentary lodging data was taken. The F_3 was planted in 2013 at the Montana State University Horticulture Farm, and the F_4 generation was advanced in a greenhouse at Montana State University. The F_5 RIL population was planted at the Montana State University Post Agronomy Farm with one replication in 2014, and the generation was again advanced in a greenhouse at Montana State University. In 2015, the F_7 population was planted with three replications using a randomized complete block design at both the Post Agronomy Farm in Bozeman and the Central Agricultural Experiment Station in Moccasin, MT. The seeds were planted at ~40 seeds/m^2 due to lack of seed and to facilitate lodging at the Bozeman location in 2014 and 2015. The seeds were planted at 25 seeds/m^2 in Moccasin in 2015. Data was averaged across replications. The PR population from the University of Saskatchewan was planted in the same manner as the Delta x RER population, except only one replication was planted in both locations, and the population was only planted one year. In 2016, the F_7 families were planted in the same locations with just a single replication in both Bozeman and Moccasin. There were seven check plots for each of the parents within each

replication during both 2015 and 2016. Because it was believed that data would be more similar to field conditions when planted at higher densities, in 2016 the planting density was increased to ~60 seeds/m^2 in Bozeman and ~43 seed/m^2 in Moccasin, MT. Seeding rates are often lower in areas that have lower rainfall and lower yield potential, such as Moccasin. Cultivation regimes were conventional tillage in Bozeman and no-till in Moccasin. Row spacing was 19 cm in Bozeman, MT and 30.5 cm in Moccasin, MT during all site-years except 2013. During all site-years except 2013, seeds were planted in microplots with three rows per plot. During 2014 and 2015, due to lack of seed, three seeds were planted in the border rows, and four seeds were planted in the middle row for a total of ten seeds per plot. During 2016, eight seeds were planted in the two border rows and 9 seeds were planted in the middle row. Due to the difficulty associated with treating each RIL, no seed treatment was used, but seeds were inoculated with N-dure for Peas, Vetch, and Lentil (Verdesian Life Sciences, Cary, North Carolina) during 2014 and 2016.

<div align="center">Phenotyping</div>

Data were collected for canopy height, total stem length, internode length, % lodging, basal branch number, aerial branching, leaf length, leaf width, seed yield, seed size, % emergence, nodes to first flowering, the maximum nodes prior to maturity, number of flowering nodes, maturity time, main stem diameter, compressed main stem thickness, side branch diameter, compressed side branch thickness, epicotyl diameter, stem flexion after crushing, and seed dormancy. If no correlation was found between each trait and lodging susceptibility, the quantitative data was not collected for all site-

years. Mendel's flower color gene (*A*), brown mottle (*M*), high response to photoperiod (*Hr*), black hilum (*Pl*), leaf type (*Af*), and Mendel's height gene (*Le*), which were segregating in the population, were also assessed.

Canopy height was measured on 1-4 individual plants per plot as the distance from soil level to the last node of the main stem. The stem length of these plants was measured from ground level to the last node of each plant. Internode length was estimated by dividing total stem length by total node number. Percent lodging was determined by using the following formula: % lodging for each RIL = (1-(canopy height/stem length))*100. This method was reported by Stelling et al. (1989) to be an accurate method of lodging prediction. Percent lodging was rated at senescence for each individual RIL planted in Bozeman. Due to limited resources, all lines were scored on a single day in Moccasin rather than at the time of senescence for each RIL. Basal branch number was counted on 1 plant in 2013, and 1-2 plants on the edge of the plot in 2014. Four plants in the center of the plot were rated in 2015, and five plants in the center of the plots were rated in 2016. Branch counts included the main stem (e.g. an unbranched plant had a branch count of 1). Sucker branches, which did not bear at least one pod, were not counted. Aerial branching occurs when branches emerge from various nodes along the upper part of the stem, generally above the tenth node. Some aerial branches are as long as the main stem but others are just a few cm long. Aerial branching was assessed on a 1 to 4 scale in 2016 with 1 not having aerial branching and 4 having pronounced aerial branching. It was not assessed in other site-years.

Leaf length of the longest compound leaf on 1-3 plants per plot was measured from the beginning of the petiole to the tip of the last tendril. The plants with the longest tendrils in each plot were consistently chosen to be measured. The individual subsamples were averaged. Leaf width was measured differently in *afila* vs *Afila* genotypes. In *afila* genotypes, the maximum tendril width was obtained by stretching out the main side branches on the leaves measured for leaf length and measuring the distance across the leaf. In *Afila* lines the width was obtained by stretching out the two tendrils closest to the petiole and measuring the diameter across the leaf. Therefore, leaf length cannot be compared between *Afila* and *afila* genotypes.

Seed yield was assessed in Bozeman by harvesting the whole plot in 2014. Because data from 2014 indicated that the border effect was increasing yield, the center row (2015) or the five middle plants in the center row (2016) were assessed for yield in the next two site-years. Emergence was assessed by counting the number of emerged plants per plot after emergence. In the plant growth center in Bozeman, a single study on 96 white and colored flower lines in the RIL population was rated on a 1-3 scale for seed dormancy. Nodes to first flower was assessed prior to harvest by counting the number of nodes from ground level. Each pea plant has ~2 scale nodes below the ground and these were not counted due to the difficulty of assessment. Total node number was assessed as the maximum number of aboveground nodes the plant attained prior to maturity. The number of flowering nodes was assessed by subtracting the nodes to 1st flower from the maximum number of nodes attained by the plant. Maturity time was assessed by rating the individual RIL lines on a 1 to 4 scale with 1 being very early and 4 being very late.

The 1-4 scale was used because many lines flowered at different times over the course of 1 ½ months.

Each plant stem was collected by clipping off the upper part of the plant above the fifth node. The root and stem epicotyl were then removed from the ground using a shovel. The main stem and side branches of pea appear to be very similar at first glance, but there are a number of characteristics that distinguish main stems from side branches. In general, the main stem is continuous from the root to its tip, and it generally has shorter internodes than side branches. Side branches have a visible scar where they attach to the stem (Figure 1). Side branches are easily stripped from the main stem with a gentle tug, leaving a scar on just one side of the main stem. Both the main stem and side branch were measured between the third and fourth node on both the main stem and the side branch. The largest diameter side branch was always rated. Side branches that connected to the main stem close to the ground were always preferred over side branches farther from the ground.
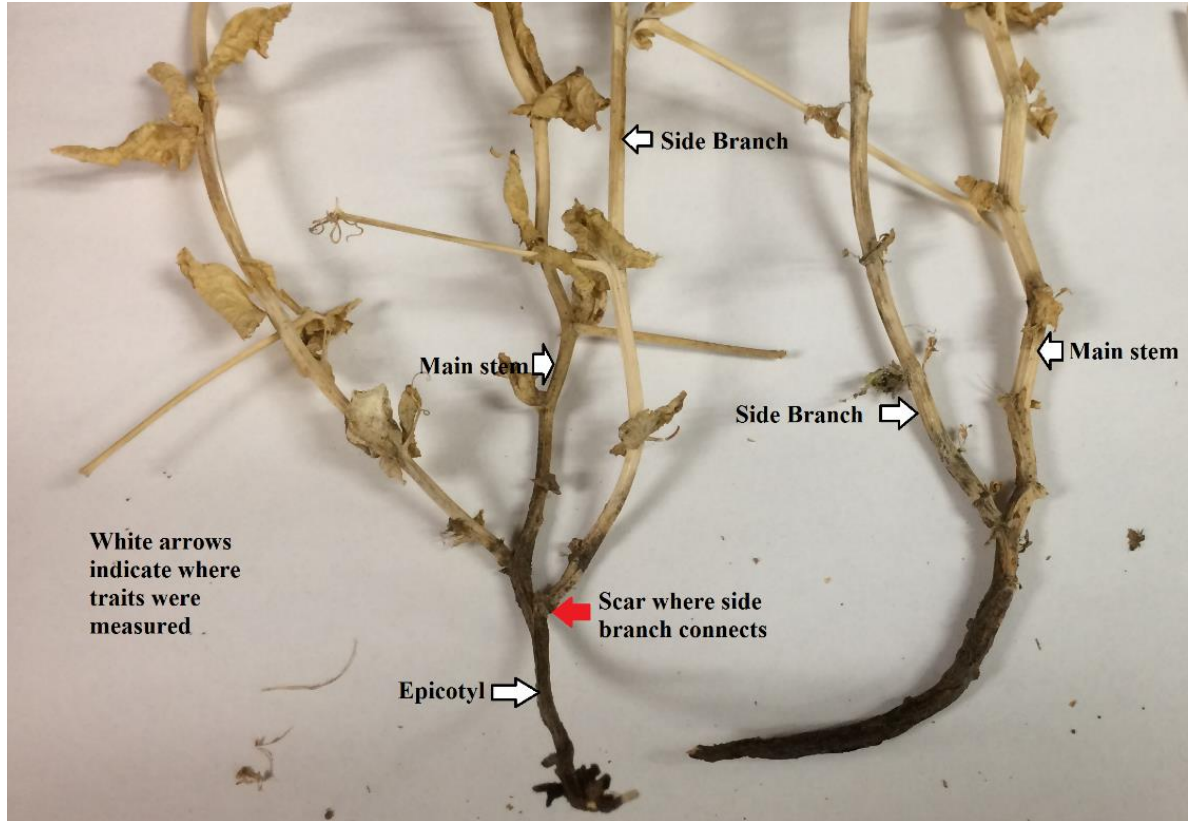
Figure 1: Where stem diameter traits were measured. Stems were measured between the third and fourth nodes on the main stem and side branches (white arrows).

If the stem was buried deeply in the ground (indicated by a brownish discoloration at the third or fourth nodes), the stem was measured between the fourth and fifth nodes or very rarely at the fifth and sixth node. This procedure was used because the belowground stem in pea is not hollow and compressed stem thickness could not be assessed if the stem is solid. It should also be noted that the 2nd to $5th^{th}$ node is the location where bending of the stem during lodging generally occurs, although peas do rarely lodge at the epicotyl. Each stem was measured at the place where bending was thought to occur, if it was not measured at the 3rd and 4th node. Main stem diameter and compressed main stem thickness were assessed at the same location on the stem. Side

branch diameter and compressed side branch thickness were also assessed at the same location. Main stem and side branch diameter were measured using a generic 0-150mm electronic digital caliper with an accuracy of 0.01 millimeters. The compressed stem thickness was assessed by firmly compressing the stem between the calipers by hand until it could not be compressed further. The thickness was then recorded. This method was also used to assess side branch diameter and compressed side branch thickness. Stem flexion was assessed by subtracting the relaxed stem diameter after being compressed from the compressed thickness value. The epicotyl is the stem that emerges from the seed, and this underground portion of the stem is not hollow. This trait was measured during several site-years after some plants were seen to be bent over at the epicotyl. This was especially noted in locations where the ground was rather soft. Epicotyl diameter was assessed in the middle of the epicotyl. In 2014, all plants that emerged (~2000 plants) were assessed for main stem diameter, compressed main stem thickness, side branch diameter, and compressed side branch thickness (~8000 measurements). In 2015, ~2500 plants were assessed for each of the two locations, and epicotyl diameter was included in the analysis (~20,000 measurements). The PR population, the additional RIL population which was sourced from the University of Saskatchewan, was rated for stem diameter, side branch diameter, and epicotyl diameter on ~900 plants in 2015. In 2016, stem diameter, side branch diameter, and epicotyl diameter were assessed on ~2000 plants in the Delta x RER population.

Genotyping

Seven genes segregating in the population were rated qualitatively to develop morphological markers. Flower color (*A*) was assessed by observing the flower color in the field. If the plant was not flowering at the time the rating was taken, the axil color was rated. If the plant had already senesced, the white flower character was assessed by rating the color of the seed. *A* has pleiotropic effects on flower color, leaf axil, and testa pigmentation (Mendel 1866). Brown mottle (*M*) causes brown speckling in both white and purple flowered peas, but the speckling is very faint in white flowered varieties. The seeds of each RIL were carefully rated for speckling, and a magnifying glass was used to rate white flowered lines. The seeds of each RIL were also rated for black hilum (*Pl*). The semi-leafless trait (*af*) was rated in the field during each year and segregating lines were not included in the dataset. *Np* (neoplasm), which causes abnormal pod growths when grown in the absence of ultraviolet light, was rated in the glasshouse during the F_5 generation. Stem length (primarily determined by variation at *Le*) was rated by creating a histogram of the heights of all individuals in the population. The histogram was bimodal with a mode for the dwarf and a mode for the tall genotypes. The genotypes in each mode were considered tall or dwarf, respectively, and the genotypes between the two humps were not included in the analysis. A similar method was used to score the *Hr* locus for maturity time. Initially maturity time appeared to be a quantitative trait. However, a closer examination of multiple years of maturity time data indicated that maturity time could be scored qualitatively due to the relatively obvious effect of the *Hr* gene, and 238 lines were scored and assigned A or B alleles. This marker was mapped on upper LG III

near the *M* locus as expected. In order to create a better marker, forty individuals

intermediate between early or late maturity were removed, creating a marker that closely

mapped in the region known to contain the *Hr* gene.

DNA for genotype by sequencing (GBS) was extracted using the DNAeasy kit

from Qiagen Corporation (QIAGEN, Hilden, Germany). Genotypes of each RIL was

determined with SNPs derived from GBS, simple sequence repeat (SSR) markers, and

cleaved amplified polymorphic sequence (CAPS) markers. DNA for SSR and CAPS

marker amplification was also extracted using a specific DNA extraction protocol for pea

(Appendix A). GBS was run on 94 dwarf RILs and the two parental lines at the USDA

small grains genotyping lab at Washington State University in Pullman, WA, USA. The

ion torrent sequencing platform was used in accordance with a two enzyme GBS protocol

developed at Kansas State University (Poland et al. 2012, Poland and Rife 2012), and

reads were assembled and SNPs identified using an in-house pipeline called Genes in

Order (Skinner et al. 2017).

Previously mapped SSR (Loridon et al. 2005) and CAPS markers were used to

anchor the SNP markers derived from GBS to previously assigned linkage groups.

Polymerase chain reactions (PCR) for SSR markers were conducted with an annealing

temperature of 50-54 degrees C in accordance with recommendations in Loridon et al.

(2005). Taq polymerase was purchased from Promega and used with Promega 5x buffers

(Promega Corporation, Madison, WI, USA). Each individual PCR contained 10.63 μl. of

autoclaved distilled H_2O, 3.75 μl. of buffer, 2.19 μl. of 25 mM $MgCl_2$, 0.69 μl. of dNTP

(containing 10 mM for each dNTP), 0.69 μl. for each primer, and 0.07 μl. of Promega

TAQ polymerase. The PCR was conducted using a PTC-100 Thermocycler and a MJ

Mini Thermocycler (Bio-Rad Inc., Hercules, California, USA) with the following

program (Table 1).

Table 1: SSR and CAPS protocols

SSR Protocol			CAPS Protocol		
Step	°C	Time (minutes)	Step	°C	Time (minutes)
1	92	2:00	1	94	2:00
2	92	0:30	2	92	0:30
3	50-54*	1:00	3	53-65*	2:00
4	72	1:30	4	72	2:00
5	RPT. Steps 2-4, 30 times		5	RPT. Steps 2-4, 30 times	
6	72	4:00	6	72	5:00
7	6	forever	7	6	forever
*=reaction conditions varied with each primer set.					

Table 2: Reaction conditions for SSR and CAPS markers used to anchor linkage groups

Marker Name	Annealing Temp (°C)	Enzyme	Forward Primer (5' to 3')	Reverse Primer (5' to 3')
Scar A004	53*	uncut	GCGCATGAAATCTAGGTTTG	CACAAGAACGAAGAACATCG
SSR-AD51	50#	uncut	TGAAGTAGGCATAGCGAAGAT	GATTAAATAAAGTTCGATGGCG
SSR-AA5	50#	uncut	CCAATCCTGAGGTATTAACACC	CATTTTTGCAGTTGCAATTTCGT
STS.A26	55	Dpn II	TCCAGCAGCACTTCTGCG	GGAATAGCTTACCTGACAG
AGAT FR	53	Hinf 1	ATCATCTCCACACCAGCAAG	TCCTTACATTGACGGTGCTG
CDC-27	53	Ddel/Dpn II	GTTGGAAGTGGTGTATGCTTTG	GCACACTATATACCTGGACCG
LKA	56	Hinf 1	CTCCAACAATTGTCTCTCTGG	CTGCCAATGTGCTTACGCTC
NCPA	56	Rsal	CTGCTATTGGATCTGAACCTC	ACCTTATATAGATCTGGACGC
Mo	56	Ddel	AAATGCCCTCTCAGAGCCA	TATCGAGCCTTTGCACCTC
P628	54#	uncut	ATGTGGTCTCAACTGAC	AGCTATTGCAAACATGAC
PYDC	53#	uncut	CGAGACAGGGGACTCATGG	CAGTTCTTGATCACATTGTATGG
RPL15S	53	Ddel	GATTTGGATGCACTTCTTGAC	GGTTCTGGCTTCTCACCA

*No amplification at 65 °C.

#special reaction conditions. These bands are not thought to be previously described.

Since a methylation sensitive set of restriction enzymes was used in the GBS

procedure, the SNP associated with each specific GBS tag is likely located within a

specific gene. The specific tag for two SNP markers associated with two QTLs was

blasted against the pea transcriptome (Burstin et al. Pea Gene Atlas) and primer sets for

CAPS.A26 and CAPS.CDC-27 were developed from the resulting gene sequence. GBS

tags were blasted for all markers associated with lodging susceptibility, and the resulting

gene associated with each GBS tag was found (data not shown).

SSR markers were all run at 53 C, except as mentioned in Table 2. CAPS markers

were run using the protocol in Table 1, using the specific annealing temperature in Table

2. CAPS markers were cut with a number of restriction enzymes until the appropriate

enzyme was found. All CAPS markers, with the exception of the markers developed from

GBS tags, were sourced from previous projects (unpublished). Amplified DNA fragments

for both SSR and CAPS markers were separated on a 2% agarose gel created by mixing 2

g of agarose (Bioexpress, 420 N. Kays Drive, Kaysville, UT 84037) per 100 ml of SB

buffer (Brody and Kern 2004).

Genetic Map Assembly

Due to the choice of previously mapped morphological, SSR, and CAPS markers

used in this study, the location of scaffold markers in this map can be directly related to

the consensus map of Loridon et al. (2005). With the exception of the lowest part of LG

II, a portion of the upper part of LG IV, and small sections of LG V and LG VII, the

scaffold markers chosen for this study were the same as the scaffold markers used by

Duarte et al. (2014) to anchor ~1400 high quality SNP markers to previous maps. These

scaffold genetic markers were chosen to facilitate future mapping and confirmation of the

QTLs discovered in this study.

The Kosambi mapping function was used for map assembly. Map assembly of the resulting SNP, SSR, and CAPS markers was done in MapDisto (Lorieux 2012) with an LOD of 4 and an r of 0.15. The optimum LOD was determined empirically. There were 4753 markers assembled to be mapped. These markers were filtered by removing markers that had a greater than twofold ratio of either of the two parental alleles. This filtering was performed because many GBS derived SNP markers had a distorted segregation pattern, and distorted markers may map together even when no linkage is present. The AutoOrder, AutoRipple, and AutoCheckInversions commands in MapDisto were used to assemble the map. Approximately 30 markers with a higher than expected number of double recombinants were assessed and removed from the map using the drop locus command in MapDisto. Markers derived from genotype by sequencing commonly had 40% of the data missing. Missing data between markers was imputed by hand by adding missing data between markers in each block of A or B alleles for each individual (Figure 2). Imputation is a preliminary step for data derived from genotype by sequencing (Nazzicarri et al. 2016), and it was used to increase sample size for each marker, thereby increasing statistical significance. The likelihood of discovering an effect by each marker is dependent on sample size, because the probability that a given difference between two means does not exist decreases when sample sizes are larger. Compared to data derived from SNP arrays, Genotype by Sequencing produces many missing genotypes and intrinsically noisy data. Imputation of missing genotypes can be an effective tool for GBS data, and can have error rates near zero (Nazzicarri et al. 2016).

Individual

Marker	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25
A6724		B	A	A	B							A	A	A	A	A	B	A	A		A	B	A	B	B	A
A6737	A	B		A	B			A		A		A			A	B	A			A				B	
A6725	A		A	A			A		A		A			A	A		A	A			B			B	
A6738	A	B	A		B			A						A	A		A		A						A
A6729			A	A	B		A		A		A		A	A			A	A	A		B			B	
A6731	A	B		A			A	A							A				A		B		B		
A6733	A		A	A	B		A		A		A		A		A		A							B	A
SSR.C20b	A	B	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	B	B	A	B	B	A
A7578	A				A	A	A	A	A	A	A				B				A					B	A
A1616			A	A					A		A	A	A	B		A			B	B					
A1623	A						A	A				A			A	A					A	B		A	
A1624					A		B	A	A	A		A		A		B		A	A	B	B	A			
A1615		B		A		A	B		A			A	A	A		B	A	A				B		A	
A1619	A	B	A	A	A	A	B	A	A			A	A	A	B	B	A	A	A		B	A	B	B	

Individual

Marker	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25
A6724		B	A	A	B						A	A	A	A	A	B	A	A		A	B	A	B	B	A
A6737	A	B	A	A	B			A		A	A	A	A	A	A	B	A	A		A	B	A	B	B	A
A6725	A	B	A	A	B		A	A	A	A	A	A	A	A	A		A	A			B	A	B	B	A
A6738	A	B	A	A	B		A	A	A	A	A	A	A	A	A		A	A	A		B	A	B	B	A
A6729	A	B	A	A	B		A	A	A	A	A	A	A	A	A		A	A	A		B	A	B	B	A
A6731	A	B	A	A	B		A	A	A	A	A	A	A	A	A		A	A	A		B	A	B	B	A
A6733	A	B	A	A	B		A	A	A	A	A	A	A	A	A		A	A	A		B	A	B	B	A
SSR.C20b	A	B	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	B	B	A	B	B	A
A7578	A	B	A	A	A	A	A	A	A	A		A	A	A	B	A	A	A	A	B	B	A	B	B	A
A1616	A	B	A	A	A	A		A	A	A		A	A	A	B	A	A	A	A	B	B	A	B	B	A
A1623	A	B	A	A	A	A		A	A	A		A	A	A	B	A	A	A	A	B	B	A	B	B	A
A1624	A	B	A	A	A	A	B	A	A	A		A	A	A	B	B	A	A	A	B	B	A	B	B	A
A1615	A	B	A	A	A	A	B	A	A			A	A	A	B	B	A	A	A		B	A	B	B	A
A1619	A	B	A	A	A	A	B	A	A			A	A	A	B	B	A	A	A		B	A	B	B	

Figure 2: An example of a set of genotypic data derived from GBS (top table) and the imputed data (bottom table). The scaffold marker is SSR.C20b. Red cells indicate individuals for which the recombination fraction cannot be computed because genotypic data is missing. Green cells indicate markers where insufficient data is available to impute the genotype.

Many markers in the original map (Appendix A) mapped within a cM of each other. For clarity in the final map, only one or two markers are presented at a specific

map position. Each marker in the final map was selected based on the high quality of the dataset.

Statistical Analysis

Means for the two parents and the RIL population were averaged over site years. For the two parents, each of the repeated checks was averaged for each year and afterwards the mean value for each year was averaged over site years. To calculate heritabilities for 2015, a linear mixed model was fitted with each phenotypic trait as a dependent variable and RIL and replication as random effects. The variances associated with RIL and replication were calculated using the lmer function in R. The following formula was used to calculate heritabilities: RIL variance/((residual variance/average number of replications)+RIL variance). The same method was used to calculate heritabilities in 2016, except each location was considered to be a replication, since there was only one replication at each location. The Levene's test for unequal variances was used to assess variances among site years in order to determine whether data across site years could be combined. A preliminary map of significant QTLs for lodging susceptibility and other traits was identified by using the QTL/ ANOVA command in MapDisto. The interval mapping and composite interval mapping function in Windows QTL Cartographer was also used to assess the effect of a QTL or putative QTL by computing the LOD associated with important regions for each specific trait.

The QTL analysis was conducted on only 94 dwarf RILs because only 94 dwarf RILs and the two parental lines hwere genotyped during the GBS procedure. Single

marker analysis using all 254 RIL lines was conducted on the *Le, Af, A, Hr*, *Pl*, AD73, AA258, AD147, and A9 markers in addition to the main analysis on the 94 dwarf RIL's. Both analyses are presented for these specific markers. The unadjusted p-values (p) for significant high quality markers were observed using a p of 0.05 as a cutoff value for putative QTLs. Each QTL was then assessed to determine if the same effect was seen during multiple site-years. There were one to six site-years of data for all traits. If the p across at least two site-years was ~0.001, the QTL was considered to be a major QTL. QTLs were not reported unless each allele had a consistent effect across site-years, and additionally, each QTL was required to be significant at the <.05 level for at least two site-years (the analysis for aerial branching was the major exception since just a single site-year was collected). Each QTL was also assessed for genotype by environment interactions. Once a QTL was shown to have the same effect across site-years or locations, an analysis was done to determine the traits that that the QTL affected. A matrix was created in MapDisto with the F-statistic for all 330 markers displayed for each of the 93 columns of phenotypic data resulting in a total of 30,690 F-tests. It is likely, given the number of tests that some false positives would be reported. Each putative effect was assessed based on whether it was seen during multiple site-years, which is unlikely to occur if there is actually no effect. The data was averaged across site-years for the reported statistical analysis, but every site-year was also analyzed separately to determine potential interactions. Each QTL was also assessed based on whether the putative pleiotropic effects were likely to affect lodging susceptibility, and whether those effects were consistent across site-years. If the p for an effect was = or <0.001, it was

considered a major pleiotropic effect. P <0.05 and >0.001 were considered to be minor effects. A Benjamini-Hochberg correction was used to estimate the false discovery rate (FDR) for lodging QTLs (Noble 2009). The false discovery rate is the expected proportion of false positives among the factors for which the existence of a difference is claimed (Noble 2009). P-values were reported that indicate the FDR for all QTLs.

The Stress Equation

An engineering formula called the stress equation was used to model the stress on pea stems of a measured thickness. The stress equation for a tubular cantilever (anchored at one end) beam is stress (force/unit of area)=$WL^2/2Z$ where w= uniform load, L= length or height, and $Z=\pi*(r^4-(r-t)^4)/4r$ with r being the radius and t being the wall thickness. If the beam is a solid tube, z can be simplified to the formula $Z=\pi r^3/4$ (Gere 2004; http://www.atcpublications.com/Sample_pages_from_FDG.pdf Accessed 11/10/2016). Load was held constant at 0.001 Newtons (N) per mm. Stress was measured in N/mm^2. Setting load and length to an arbitrary constant value gives an equation that gives an estimate of stress based entirely on the diameter and wall thickness of a cylindrical tube. The stress equation was used to calculate stress of a model pea stem with a known height, stem diameter, and wall thickness.

CHAPTER THREE: RESULTS OF THE QTL ANALYSES CONCERNING LODGING

Table 3: Parental means, average of 254 progeny, coefficient of variation, range, and estimated heritabilities for the Delta x RER population.

Traits	Mean for parent A (Delta)#	Mean for parent B (RER)#	Average of 254 progeny #	Coefficient of variation (%) #	range (lower limit) #	range (upper limit) #	h^2 2015 Bozeman	h^2 2015 Moccasin	h^2 2016
% Lodging ((1-(canopy height/stem length))*100)	18.3	42.8	36.9	39.6	8.92	70.9	0.74	0.80	0.64
Height (cm)	41.4	57.9	51.5	27.8	25.76	82.01	0.86	0.97	0.92
Basal stem (branch) number (includes main stem)	1.79	2.39	2.62	25.9	1.24	4.63	0.68	NA	0.57
Aerial branching (open ended 1-4 scale)	1.07	2.68	1.95	48.5	1.0	4.5	NA	NA	NA
% Emergence	97.3	89.9	83.5	14.5	23	100	0.66	NA	0.75
Main stem diameter (mm)	2.40	1.86	2.10	10.9	1.68	2.77	0.63	0.84	0.77
Compressed main stem thickness (mm)	0.93	0.86	0.88	14.3	0.59	1.34	0.61	NA	NA
Branch diameter (mm)	2.67	2.05	2.35	9.7	1.7	3.09	0.58	0.75	0.59
Compressed branch diameter (mm)	0.77	0.84	0.81	13.8	0.49	1.28	0.56	0.55	NA
Epicotyl diameter (mm)	1.62	1.74	1.71	11.3	1.3	2.35	0.66	0.71	0.62
Leaf length (cm)	16.5	14.7	15.8	13.6	10	22.3	0.67	NA	0.77
Leaf width (cm)*	15.4	4.83	9.58	47.1	3	18.3	0.91	NA	NA
Maturity time (open ended 1-4 scale)	1.36	2.26	1.88	29.6	0.78	3.13	0.85	NA	NA
Number of Nodes to 1st flower	15.9	19.9	17.8	15.6	11	25	NA	NA	NA
Total node number	20.0	24.5	22.4	12.4	15	30	NA	NA	NA
Number of flowering nodes	4.13	4.61	4.63	30.7	1	9.5	NA	NA	NA
Yield (kg/ha)	4814	3733	3457	30.1	217	6649	0.30	NA	NA
Yield per plant (g)	9.17	7.85	8.25	30.1	1.56	20.24	0.30	NA	NA
Seed weight (g/100 seeds)	24.7	9.70	15.75	19.3	9.51	25.2	NA	NA	NA

Data was averaged across all site years that data was collected.

*Leaf width was measured differently for Afila vs afila genotypes, therefore a direct comparison cannot be made between the two pa

Seed dormancy, internode length, and canopy height are not reported. These traits are estimated by % Emergence, Height, and % Lodging.

Linkage Map Development for the Delta x RER Population

The GBS procedure generated a total of 4753 markers for the 94 RILs that were genotyped. Of these 2895 gave a segregation pattern reasonably close to the expected 1:1 ratio and were included in the initial mapping calculations. On average these remaining SNP markers had 37.6% missing data, and 29 markers were eliminated because they had fewer than 25 data points. The resulting 2866 SNP markers were mapped with 7 morphological markers, 26 SSR, and 11 CAPS markers as a scaffold. There were 36 of

these markers that mapped in previously mapped locations. Only 282 SNP markers

mapped at an LOD of 4, and 2613 SNP markers were discarded. A total of 330 markers

were mapped in the original map (Appendix A).

The A001 and A004 SCAR markers previously mapped to lodging QTLs in a

prior population (Zhang et al 2005) were hard to interpret in this population. Only A004

was mapped, and bands were very faint at all annealing temperatures tried. A004 did not

map in the location previously reported. SSR.AD51 and SSR.AA5 had three different

polymorphisms each. Only one polymorphism for each marker was previously reported.

After markers within the same map unit were binned, the final genetic map of the

Delta x RER population consisted of 196 markers grouped into 8 linkage groups (Figure

3) with the largest gap between adjacent markers being 15.5 cM (on LG I). The total

length of the map was 452 cM. The average marker density was therefore one marker for

every 2.3 map units.

The positions of previously mapped markers anchored the linkage groups to

previous pea consensus maps and indicated the degree of coverage. Based on prior maps,

part of LG IV, the lower end of LG II (as presented in Figure 3), and the lower arms of

LGV and LG VII appeared to be missing. Previously reported SSR and CAPS markers

examined in these missing regions were monomorphic. All polymorphic CAPS markers

are reported in Table 2, in addition to SSR markers with special reaction conditions.

Identification of QTLs Affecting Lodging Susceptibility in the Delta x RER Population

Four major and six minor QTLs were identified for lodging susceptibility in the Delta x RER population on LG I, II, III, and VI (Figure 3, and Table 4). The QTLs were identified by single marker analysis in MapDisto, and confirmed by interval mapping, and composite interval mapping in Windows QTL Cartographer. The minor QTLs had generally weak effects on lodging but had the same effect across multiple site-years.

LG IVB (cM / Loci):
- A30 (0.0 cM)
- 2.7 — A5406 (2.7 cM)
- 0.5 — A5399 (3.3 cM)
- 1.6 — A5402 (4.9 cM)
- 1.1 — A5404 (6.0 cM)
- 0.0 — A5398 (6.0 cM)
- 7.3 — SSR.AA315 (13.2 cM)

LG V (cM / Loci):
- A4327 (0.0 cM)
- 3.4 — A5227 (3.4 cM)
- 0.0 — A615 (3.4 cM)
- 3.1 — A5157 (6.5 cM)
- 3.9 — A7604 (10.4 cM)
- 0.0 — A7621 (10.4 cM)
- 5.3 — CAPS.PYDC (15.7 cM)
- 3.9 — A2144 (19.5 cM)
- 1.1 — A4824 (20.7 cM)
- 2.5 — A444 (23.1 cM)
- 0.6 — A448 (23.7 cM)
- 0.5 — A447 (24.3 cM)
- 8.1 — SSR.AA81 (32.4 cM)
- 2.7 — SSR.AB23 (35.1 cM)
- 1.1 — A4827 (36.2 cM)
- 1.8 — A1822 (38.0 cM)
- 0.6 — A4806 (38.6 cM)

LG VI (cM / Loci):
- SSR.AA374 (0.0 cM)
- 2.8 — SSR.AA224 (2.8 cM)
- 2.7 — A7224 (5.5 cM)
- 1.8 — A1580 (7.3 cM)
- 0.9 — A5439 (8.2 cM)
- 5.8 — A5420 (14.0 cM)
- 1.8 — A7133 (15.8 cM)
- 0.0 — A7134 (15.8 cM)
- 6.3 — SSR.AA335 (22.1 cM)
- 6.9 — A1000 (29.0 cM)
- 0.0 — A999 (29.0 cM)
- 0.0 — A1008 (29.0 cM)
- 0.0 — A1003 (29.0 cM)
- 0.0 — A997 (29.0 cM)
- 3.5 — PI (32.5 cM)
- 1.3 — A6634 (33.7 cM)
- 2.6 — SSR.AC76b (36.3 cM)

(Lodge VI-1 shown on LG VI)

LG VII (cM / Loci):
- A638 (0.0 cM)
- 1.4 — A6561 (1.4 cM)
- 0.6 — A6563 (2.0 cM)
- 0.6 — A1915 (2.5 cM)
- 0.0 — A1916 (2.5 cM)
- 0.5 — CAPS.RPL15S (3.1 cM)
- 1.6 — A640 (4.7 cM)
- 4.1 — A4825 (8.9 cM)
- 0.0 — A4828 (8.9 cM)
- 1.1 — A7763 (10.0 cM)
- 1.7 — SSR.AA456 (11.7 cM)
- 4.6 — A1978 (16.2 cM)
- 0.0 — A1980 (16.2 cM)
- 3.4 — SSR.AA416 (19.6 cM)

Figure 3: The linkage groups and lodging QTLs. Major QTLs are shown in red. Minor QTLs are shown in black.

Table 4: Lodging QTLs in the Delta x RER population. With the exception of *Le*, all analyses were conducted on 94 dwarf RILs.

QTL #	QTL Name and/or Closest Genetic Marker	LG	% Variation (Averaged across all site years) (1)	p-value (unadj., data averaged across site years) (2)	False discovery rate (Benjamini-Hochberg) (3)	LOD (Int. Map.) (4)	Source of Desirable allele (5)	% Lodging (Averaged across all site years) (6)
Major QTL (strong or moderate evidence). Listed in order of importance based on p-value.								
1	Lodge III-1 (*Le*) (analyzed on all 254 lines)	III	49.90%	7.53×10^{-39}	<.001	59.0	Delta Parent	20.43%
2	Lodge I-1 (*Afila*)	I	22.90%	8.99×10^{-7}	0.0002	5.5	Delta Parent	10.03%
3	Lodge III-2 (*Hr/Rms1/M*)	III	9.90%	0.00200	0.041	2.8	RER Parent	6.64%
4	Lodge III-3 (SSR-AD73)	III	7.80%	0.00600	0.079	1.6	Delta Parent	5.84%
Putative QTL. Due to interactions with other QTL and the environment, QTL are listed based on the strength of the evidence for each QTL.								
5	Lodge II-1 (*A*)	II	5.80%	0.01800	0.107	2.2	Delta Parent	5.03%
6	Lodge I-2 (Marker A7384) (#)	I	7.9%/12.0%	0.0065, 0.00081#	0.051, 0.023#	0.5, 1.1	Delta Parent	2.90%
7	Lodge II-2 (A7258)	II	5.60%	0.02200	0.138	1.0	Delta Parent	4.94%
8	Lodge II-3 (*Mo*)	II	6.00%	0.02647	0.150	1.0	Delta Parent	6.28%
9	Lodge I-3 (A5967, A2259) (?)	I	6.30%	0.008?	0.0918?	5.0	Delta Parent	6.86%
10	Lodge VI-1 (A1580)	VI	2.60%	0.13400	0.388	0.2	Delta Parent	3.50%

#Data is reported for 2015 and 2016 in Moccasin, respectively. There was no apparent effect in Bozeman.

?= Actual effect unknown, strongly influenced by the nearby Lodge I-1 (*Afila*) QTL

(1) % Variation is the percentage of variation explained by the QTL.

(2) P-value (Unadj.)=this p-value is not adjusted for multiple comparisons.

(3) False Discovery Rate=The expected proportion of false positives among the factors for which the existence of a difference is claimed.

(4) LOD score=logarithm (base 10) of odds using interval mapping or composite interval mapping.

(5) The parent from which the reduced lodging allele is derived.

(6) % Lodging is the mean difference in % lodging when the means of the two genotypes are compared.

A subset of markers near significant QTL was analyzed for significance on the entire population. These results are shown in Table 5 and were used to confirm QTLs.

Table 5: Results of single marker analyses for lodging on all 254 lines.

Genetic Markers	LG	% Variation (Averaged across all site years) (1)	p-value (unadj., data averaged across site years (2)	False Discovery Rate (Benjamini-Hochberg) (3)	Source of Desirable allele (4)	% Lodging (Averaged across all site years) (5)
Le (Lodge III-1)	III	49.90%	7.53×10^{-39}	$\sim 1 \times 10^{-37}$	Delta Parent	20.43%
Hr (Lodge III-2) (Int) (only tall lines)	III	25.70%	7.02×10^{-8}	1.4×10^{-5}	RER Parent	11.69%
Hr (Lodge III-2) (Int) (only dwarf lines)	III	7.60%	0.003	0.050	RER Parent	6.24%
Hr (Lodge III-2) (Int) (all lines)	III	3.60%	0.006	0.074	RER Parent	5.72%
Afila (Lodge I-1)	I	7.40%	0.00001	0.007	Delta Parent	7.85%
A (Lodge II-1	II	5.40%	0.001	0.037	Delta Parent	6.96%
SSR-AD73 (Lodge III-3)	III	1.70%	0.044	0.170	Delta Parent	3.86%

Int= interaction between tall and dwarf genotypes, Same effect but the strength of effect differed

(1) % Variation is the percentage of variation explained by the QTL.

(2) P-value (Unadj)=this p-value is not adjusted for multiple comparisons.

(3) False Discovery Rate=The expected proportion of false positives given the unadjusted p-value.

(4) The parent from which the reduced lodging allele is derived.

(5) % Lodging is the mean difference in % lodging when the means of the two genotypes are compared.

Lodge III-1 at the Distal End of LG III (*Le*)

When lodging data was averaged over all site-years from 2014 to 2016, a very strong QTL was found at the bottom of LG III, centered around *Le*. On average, the segregation at *Le* explained 49.9% of the variation in lodging, and it was the most significant marker for lodging in this area. The peak for the Lodge III-1 was at *Le*, although a flanking marker was not present on one side of the QTL. There was 20.4% less lodging in lines with the dwarf allele than lines with the tall allele.
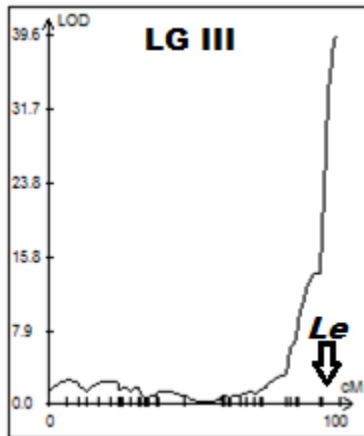
Figure 4: QTL Lodge III-1 identified on pea LG III using interval mapping in Windows QTL Cartographer. The analysis for *Le* was run on all 254 lines in the entire population. See Figure 6 for the LOD curve for LG III on just dwarf lines.

Lodge I-1 on the Distal End of LG I (Centered at *Afila*)

When data was averaged over all site-years from 2014 to 2016, a very strong QTL was found on the bottom of LG I, centered around the *Afila* gene (*Af*) (p =0.000013), which was clearly segregating in the population. Flanking markers were less significant. *Af* is known to control leaf morphology, which has an influence on lodging susceptibility, therefore, it is likely that *Af* is the gene underlying this QTL, based on the location and effects of this lodging QTL. *Af* explained 7.4% of the variation in lodging susceptibility in this population across all RILs. When tall lines were excluded from the analysis, this QTL explained 18.1% of the variation in lodging susceptibility in dwarf lines versus 11.7% in tall lines. The two means for *Af* and *af* lines were computed. Averaged over all site-years and genotypes, lines with the domesticated *af* allele had 7.9% less lodging than lines with the *Af* allele. When tall lines were excluded from the statistical analysis, the p for an F-test was 5.29×10^{-7} for the *Afila* locus, and when dwarf lines were excluded from

the statistical analysis the p was 0.00008. Lodging was 9.5% less (average lodging was 31.4% in *Afila* vs 21.9% in *afila*) in a dwarf background, and 7.3% less in a tall background (49.4% vs. 42.1%, respectively).
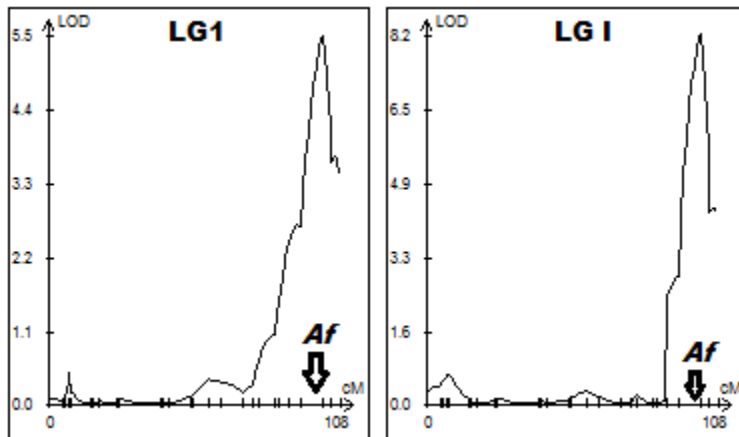
Figure 5: QTL Lodge I-1 identified on pea LG I using interval mapping (left) and composite interval mapping (right) in Windows QTL Cartographer.

Lodge III-2 the Upper End of LG III (Putatively the *M/Hr/Rms1* Region)

The effect of this QTL was variable, and data was analyzed for each site-year in addition to across site-years. In Bozeman in 2015 this QTL was significant for lodging (p=0.0009). Over all genotypes, this locus explained ~6% of variation in lodging in Bozeman in 2015. In Moccasin in 2015 this QTL explained 15.2% of the variation in lodging across all genotypes, 31.1% of the variation in lodging in dwarf genotypes, and 33.6% of the variation in lodging in tall genotypes. A greater percentage of the variation in lodging was explained in either tall or dwarf genotypes due to the effect of *Le*, which explains 49.9% of the variation in lodging. Over all genotypes the locus had a p= 1.65×10^{-8} with the allele from Delta having 13.2% more lodging than the allele derived

from the RER line (41.0% vs 27.8% respectively). This locus was not significant in Bozeman in 2016, but it was significant in Moccasin in 2016 (p-value= 0.00019). It explained 7.0% of the variation in lodging across all genotypes, 11.0% of the variation in dwarf genotypes, and 15.8% of the variation in tall genotypes. Over all genotypes, individuals possessing the allele from Delta had 8.6% more lodging than individuals with the RER derived allele (35.5% vs 26.9%).

When data was averaged over all site-years, this QTL explained 3% of the variation in lodging in all genotypes (p=0.006), 7.6% in dwarf genotypes (p=0.003), and 25.7% of the variation in tall genotypes (p 7.0x 10^{-8}). The low percentage of lodging explained when the data is analyzed across all genotypes is due to variance from *Le*. The allele derived from Delta significantly increased lodging 5.7% across all genotypes (39.4% vs 33.7%), 6.2% in dwarf varieties (29.8% vs 23.6%), and 11.7% in tall varieties (54.2% vs 42.5%).

This QTL spanned 22 cM. Interval mapping of this QTL indicated three peaks within the QTL, indicating that more than one gene may be responsible. Composite interval mapping indicated that the QTL was centered around the *Hr* gene.
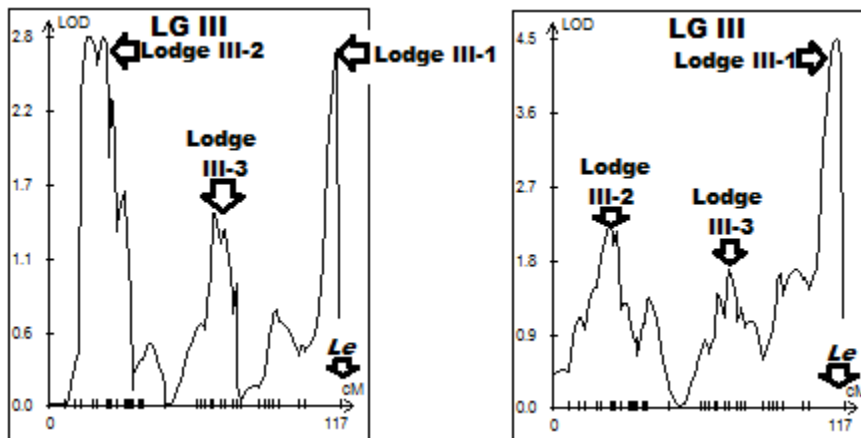
Figure 6: QTL Lodge III-1, Lodge III-2, and Lodge III-3 identified on pea LG III using composite interval mapping (left) and interval mapping (right) in Windows QTL Cartographer.

Lodge III-3 in the Middle of LG-III (Near AD73)

The region near the SSR-AD73 locus had a moderately strong effect in Bozeman in 2014 and 2016, with the allele from Delta providing tolerance to lodging. Averaged across all site-years and genotypes, this allele explained 1.7% of the variation in lodging (p= 0.044). This allele decreased lodging 3.9% when averaged over site-years and 233 genotypes. Lines with the Delta allele consistently had better lodging resistance than lines with the RER allele during all six site-years. This QTL had a strong effect when analyzed across dwarf lines in the 2015 Bozeman planting (p=.0005 for the AD73 locus).

Lodge II-1 in the Middle of LG II (Near *A*)

A strong QTL was detected on LG II near the *a* locus based on data averaged across all site-years. Lodging was 7.0% lower in lines with the *a* allele when compared to lines with the *A* allele (32.7% vs 39.7% respectively). Averaged across all site-years this

QTL explained 5.4% of the variation in lodging. The effect was consistent every site year.
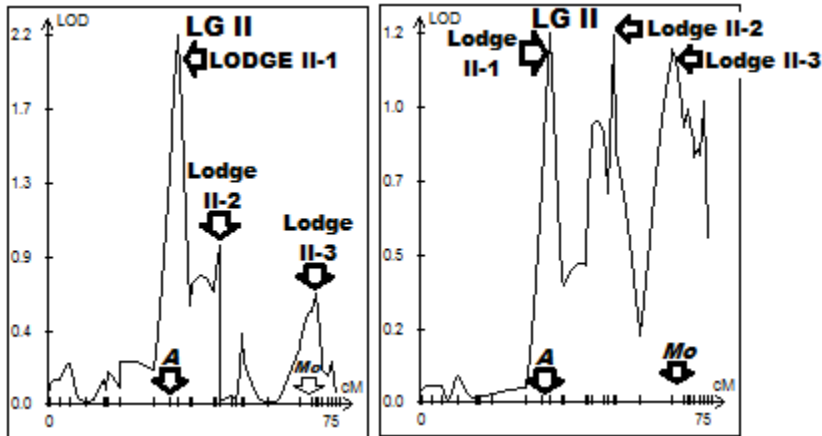
Figure 7: QTLs for lodging identified on pea LG II using composite interval mapping (left) and interval mapping (right) in Windows QTL Cartographer.

Lodge I-2 (Near A7384)

A significant QTL for lodging was found in the middle of LG I during both site-years in Moccasin, MT, but there was no apparent effect in Bozeman, MT. This QTL was centered around the A7384 and A7380 markers. This QTL explained 7.9% of the variation in lodging in Moccasin in 2015 (p= 0.0065). Individuals with the RER allele had 7.8% more lodging than individuals with the Delta allele (27.4% vs 19.6% respectively).

In 2016, this QTL explained 12% of the variation in lodging resistance (p=0.0008) in Moccasin. Individuals with the RER allele had 9.8% higher lodging than individuals with the allele from Delta (27.8% vs 18.0% respectively). It is important to note that the effect was the same in only 5 out of the 6 site-years. In 2014 in Bozeman

more lodging was found in lines with the Delta derived allele than lines with the RER

allele, although this effect was non-significant.
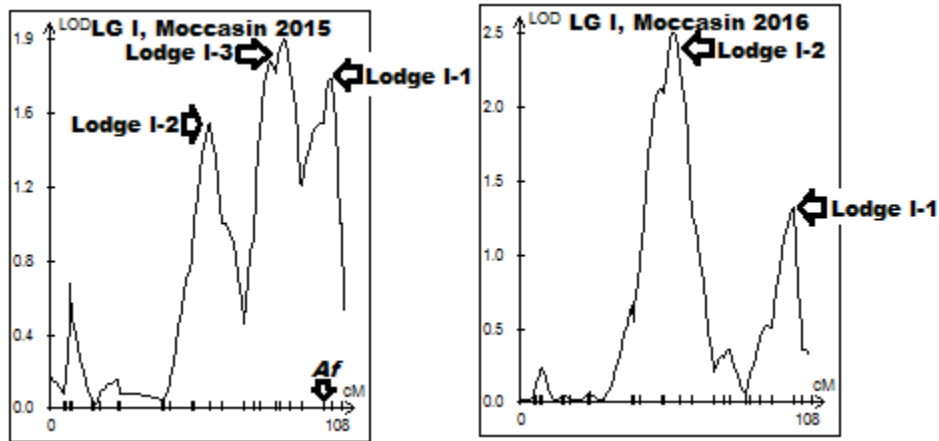
Figure 8: Lodging QTL were identified on LG 1 for the 2015 and 2016 Moccasin site-years using interval mapping in Windows QTL Cartographer.

Lodge II-2 (Near A7258)

A 4 cM long region located approximately 15 cM below the *A* locus was

significant when averaged over all site-years, although the effect was weak in any given

site-year (Figure 7). This region is highly significant for nodes to first flower and total

node number. Composite interval mapping in Windows QTL Cartographer indicated 2

lodging QTL in this region including the QTL associated with *A*. Across all site-years the

A7258 marker explained 5.6% of the variation in lodging (p= 0.022). Lines with the

Delta derived allele had 4.9% lower lodging than lines with the RER derived allele.

Lodge II-3 (Near *Mo*)

A weak QTL for lodging was identified near *Mo* (Figure 7). This QTL had the same effect during all six site-years. Averaged across all site-years, this QTL explained 8.6% of the variation in lodging in dwarf lines (p=0.0114). The allele derived from Delta decreased lodging by 6.3% when compared to the RER derived allele (23.9% vs 29.2%, respectively).

Lodge I-3 (Near A2259)

The QTL around the morphological marker for the *Af* locus, which spans 22 cM, may be controlled by two linked QTL, designated Lodge I-1 and Lodge I-3 (Figure 8). It is possible that one QTL is located at 86 cM and then another QTL is located at 102.5 cM. This locus decreased lodging 6.9% in lines with the Delta derived allele.

Lodge VI-1 (Upper LG VI)

This study identified a very weak QTL on the upper part of LG VI that was associated with lodging in Bozeman in 2015 and 2016. It was associated with the marker A1580. It was only statistically significant at the <.05 level during two site-years, and only at the Bozeman site, although lines with the Delta derived allele had less lodging 5 out of 6 site-years. In 2014, the opposite effect was seen and the RER allele had less lodging than the Delta allele, although the effect was not significant.

This QTL explained 5.8% of the variation in lodging in 2015 in Bozeman (p =0.028). Lodging was 7.12% lower in lines with the Delta derived allele when compared to the RER allele (28.7% vs. 35.8% respectively).

In 2016 in Bozeman, this QTL explained 7.4% of the variation in lodging in this population (p=0.012) On average, the allele from Delta decreased lodging by 7.9% when compared to the RER line (12.3% vs. 20.2% respectively).
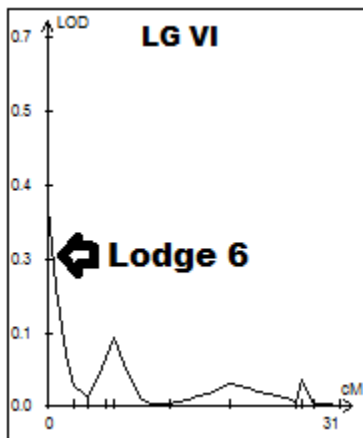
Figure 9: Interval mapping of Lodge VI-1 during 2016.

Additional QTL Analyses

Because lodging is believed to be associated with plant height, stem strength, maturity time, leaf characteristics, emergence, and yield, the results from QTL analyses of these traits are also presented. As mentioned previously, the major analysis utilized a subset of 94 dwarf RILs. However, single marker analysis for specific markers linked to QTLs was also conducted using the entire population of 254 lines in the RIL population.

QTLs for Plant Height

Le appeared to be the primary gene influencing plant height and lodging in the population, but one other major QTL and one minor QTL were also identified (Table 6 and 7). For the QTL at *Le,* reduced lodging was correlated with an increase in plant height, but all other lodging QTLs did not have an effect on plant height. Two of the QTLs for height co-located in the same positions as lodging QTLs.

Table 6: QTLs for plant height (analyzed on 94 dwarf RILs, unless noted).

QTL Name and/or Closest Genetic Marker	LG	% Variation (Averaged across all site years) (1)	p-value (unadj., data averaged across site years) (2)	False discovery rate (Benjamini-Hochberg) (3)	Shortest Plant (Allele) (4)	Effect (cm) (5)
Major QTL (strong evidence). Listed in order of importance (based on p-value)						
Lodge III-1 (Le) (analyzed on all 254 lines)	III	78.90%	1.08×10^{-85}	$\sim 1 \times 10^{-83}$	Delta	25.49 cm
Putative QTL (moderate or weak evidence). Listed in order of importance (based on p-value)						
STS.AGAT#	IV	7.90%	0.005	0.1890	RER	3.85 cm
Lodge III-2 *(Hr/Rms1/M)*	III	5.10%	0.031	0.399	Delta	3.14 cm

(1) % Variation is the percentage of variation explained by the QTL.

(2) P-value (Unadjusted)=this p-value is not adjusted for multiple comparisons.

(3) False Discovery Rate=The expected proportion of false positives after correcting for multiple comparisons.

(4) Shortest plant= The parental allele associated with the shortest height.

(5) Effect= the mean difference in height between the two genotypes.

Table 7: Results of single marker analyses for plant height on all 254 lines.

Genetic Markers	LG	% Variation (Averaged across all site years) (1)	p-value (Unadjusted, data averaged across site years) (2)	False Discovery Rate (Benjamini-Hochberg) (3)	Shortest Plant (Allele) (4)	Effect (cm) (5)
Le (Lodge III-1)	III	78.90%	1.08×10^{-85}	$\sim 1 \times 10^{-83}$	Delta	25.49 cm
Hr (Lodge III-2)	III	8.20%	0.00003	0.0027	Delta	8.26 cm

(1) % Variation is the percentage of variation explained by the QTL.

(2) P-value (Unadjusted)=this p-value is not adjusted for multiple comparisons.

(3) False Discovery Rate=The expected proportion of false positives.

(4) Shortest plant= The parental allele associated with the shortest height.

(5) Effect= the mean difference in height between the two genotypes.

<u>QTLs for Stem Diameter, Compressed Stem Thickness, and Epicotyl Diameter</u>

Four major and nine minor QTLs were identified for traits related to stem strength

in the Delta x RER population (Tables 8 through 17). These were distributed across all

seven linkage groups, with multiple QTLs on LG I, II, and III. Eight of the QTL for stem

diameter co-located in the same positions as the ten reported lodging QTLs.

Table 8: QTLs for main stem diameter (analyzed on 94 dwarf RILs)

QTL Name and/or Closest Genetic Marker	LG	% Variation (Averaged across all site years) (1)	p-value (unadj., data averaged across site years) (2)	False discovery rate (Benjamini-Hochberg) (3)	Source of desirable allele (4)	Effect (mm) (5)
Major QTL (strong evidence). Listed in order of importance (based on p-value)						
Lodge III-1 (*Le*) (analyzed on all 254 lines)	III	34.20%	3.635×10^{-24}	$\sim 1 \times 10^{-22}$	Delta Parent	.27 mm
Lodge I-1 *(Afila)*	I	24.90%	2.57×10^{-7}	5.04×10^{-5}	RER Parent	.22 mm
Putative Tsw1.1 (A6724)	I	12.10%	0.0005	0.011	Delta Parent	.15 mm
Lodge III-3 (CAPS.CDC27)	III	11.10%	0.001	0.021	Delta Parent	.14 mm
Putative QTL (moderate or weak evidence). Listed in order of importance (based on p-value)						
Lodge II-1 (*A*)	II	9.80%	0.002	0.023	Delta Parent	.13 mm
Lodge II-2 (A7258)	II	7.60%	0.008	0.052	Delta Parent	.12 mm
Marker A1915	VII	5.80%	0.019	0.117	Delta Parent	.10 mm
Lodge VI-1 (SSR-AA374)	VI	4.10%	0.050	0.203	Delta Parent	.11 mm
Lodge III-2 *(Hr/Rms1/M)*	III	3.20%	0.086	0.263	Delta Parent	.07mm

!=RER parent has increased lodging but larger main stem diameter.

(1) % Variation is the percentage of variation explained by the QTL.

(2) P-value (Unadj)=this p-value is not adjusted for multiple comparisons.

(3) False Discovery Rate=The proportion of false positives among the factors for which the existence of a difference is claimed.

(4) Source of desirable allele= The parental allele that increases main stem diameter.

(5) The effect size is the mean difference in stem diameter between the two genotypes.

A subset of markers near significant QTL was analyzed for significance on the entire

population. These results are shown below and were used to confirm QTLs.

Table 9: Results of single marker analyses for main stem diameter on all 254 RILs.

Genetic Markers	LG	% Variation (Averaged across all site years) (1)	p-value (Unadjusted, data averaged across site years) (2)	False Discovery Rate (Benjamini-Hochberg) (3)	Parental allele associated with the largest stem diameter (4)	Effect (mm) (5)
Le (Lodge III-1)	III	34.2%	3.635×10^{-24}	$\sim 1 \times 10^{-22}$	Delta Parent	.27 mm
Afila (Lodge I-1)	I	20.9%	2.085×10^{-14}	2.31×10^{-12}	RER Parent	.21 mm
Hr (Lodge III-2)	III	6.1%	0.0003	0.011	Delta Parent	.12 mm
SSR-AD73 (Lodge III-3)	III	4.1%	0.002	0.023	Delta Parent	.09 mm
A (Lodge II-1)	II	3.0%	0.002	0.052	Delta Parent	.07 mm
SSR-AD147 (Lodge I-2)	I	3.0%	0.011	0.081	Delta Parent	.08 mm

(1) % Variation is the percentage of variation explained by the QTL.

(2) P-value (Unadj)=this p-value is not adjusted for multiple comparisons.

(3) False Discovery Rate=The expected proportion of false positives given the unadjusted p-value.

(4) Source of desirable allele= The parental allele that increases main stem diameter.

(5) The effect size is the mean difference in stem diameter between the two genotypes.

Table 10: QTLs for compressed main stem thickness (analyzed on 94 dwarf RILs).

QTL Name and/or Closest Genetic Marker	LG	% Variation (Averaged across all site years) (1)	p-value (unadj., data averaged across site years) (2)	False discovery rate (Benjamini-Hochberg) (3)	Source of desirable allele (4)	Effect (mm) (5)
Major QTL (strong evidence). Listed in order of importance (based on p-value)						
Lodge I-1 *(Afila)*	I	16.40%	4.7×10^{-5}	0.009	RER!	.10 mm
Lodge III-3 (CAPS.CDC27)	III	9.90%	0.001	0.050	Delta	.08 mm
Putative QTL (moderate or weak evidence). Listed in order of importance (based on p-value)						
Neoplasm (A671 or *Np*)	III	7.70%	0.003	0.059	Delta	.07 mm
Lodge II-2 (A7258)	II	5.40%	0.025	0.247	Delta	.06 mm
A1404	I	5.00%	0.027	0.250	RER	.05 mm
A1915	VII	7.30%	0.041	0.262	Delta	.06 mm
Lodge VI-1 (SSR-AA374)	VI	4.30%	0.044	0.267	Delta	.06 mm

!=RER parent has increased lodging but larger compressed stem thickness

(1) % Variation is the percentage of variation explained by the QTL.

(2) P-value (Unadjusted)=this p-value is not adjusted for multiple comparisons.

(3) False Discovery Rate=The expected proportion of false positives after correcting for multiple comparisons.

(4) Source of desirable allele= The parental allele that increases compressed main stem diameter

(5) The effect size is the mean difference in compressed main stem diameter between the two genotypes.

Table 11: Results of single marker analyses for compressed main stem thickness on all 254 RILs.

Genetic Markers	LG	% Variation (Averaged across all site years) (1)	p-value (Unadjusted, data averaged across site years) (2)	False Discovery Rate (Benjamini-Hochberg) (3)	Parental allele associated with the largest stem thickness (4)	Effect (mm) (5)
Afila (Lodge I-1)	I	25.60%	1.79×10^{-16}	2.52×10^{-11}	RER#	.13 mm
SSR-AD73 (Lodge III-3)	III	8.70%	4.79×10^{-6}	0.0004	Delta	.07 mm
Neoplasm (*Np*)	III	6.40%	0.0001	0.007	Delta	.06 mm

\# The RER parent has increased lodging but larger compressed stem diameter

(1) % Variation is the percentage of variation explained by the QTL.

(2) P-value (Unadjusted)=this p-value is not adjusted for multiple comparisons.

(3) False Discovery Rate=The expected proportion of false positives.

(4) Source of desirable allele= The parental allele that increases compressed main stem diameter

(5) The effect size is the mean difference in compressed main stem diameter between the two genotypes.

Table 12: QTLs for side branch diameter (analyzed on 94 dwarf RILs).

QTL Name and/or Closest Genetic Marker	LG	% Variation (Averaged across all site years) (1)	p-value (unadj., data averaged across site years) (2)	False discovery rate (Benjamini-Hochberg) (3)	Source of desirable allele (4)	Effect (mm) (5)
Major QTL (strong evidence). Listed in order of importance (based on p-value)						
Putative Tsw1.1 (A6724)	I	15.50%	0.00007	0.004	Delta	.19 mm
Lodge I-1 (*Afila*)	I	14.40%	0.0001	0.004	RER *	.18 mm
Lodge III-1 (*Le*) (analyzed on all 254 lines)	III	5.40%	0.0002	0.004	Delta	.10 mm
Lodge II-1 (A)	II	12.40%	0.00044 (int1)	0.007	Delta	.17 mm
Putative QTL (moderate or weak evidence). Listed in order of importance (based on p-value)						
SSR-A9	IV	9.40%	0.0023 (int2)	0.017	Delta	.14 mm
Lodge I-2 (SSR-AD147)	I	8.70%	0.006 (int3)	0.037	Delta	.14 mm
Lodge II-2 (A7258)	II	6.90%	0.011	0.052	Delta	.12 mm
Lodge VI-1 (A1580)	VI	6.30%	0.014	0.069	Delta	.12 mm
A1025 (near Lodge III-3)	III	6.10%	0.016	0.070	Delta	.07 mm
Lodge III-2 (*Hr/Rms1/M*)	III	4.60%	0.038	0.196	RER	.10 mm

*=RER parent has poor lodging resistance but also had a larger side branch diameter.

int1= Interaction, strong effect some years (p-value: $2014=2.57 \times 10^{-7}$; 2015=.00025,) but not others.

int2= Interaction, stronger effect in dwarf lines than tall lines. Consistent effect across all site years.

int3=Interaction, no effect in Bozeman, but a fairly strong effect in Moccasin, MT. (P-VALUE=.0001)

(1) % Variation is the percentage of variation explained by the QTL.

(2) P-value (Unadjusted)=this p-value is not adjusted for multiple comparisons.

(3) False Discovery Rate=The expected proportion of false positives after correcting for multiple comparisons.

(4) Source of desirable allele= The parental allele that increases side branch diameter

(5) The effect size is the mean difference in side branch stem diameter between the two genotypes.

Table 13: Results of single marker analyses for side branch diameter on all 254 lines

Genetic Markers	LG	% Variation (Averaged across all site years) (1)	p-value (Unadjusted, data averaged across site years) (2)	False Discovery Rate (Benjamini-Hochberg) (3)	Source of Desirable allele (4)	Effect (mm) (5)
Afila (Lodge I-1)	I	12.30%	2.36×10^{-8}	8.620×10^{-6}	RER *	.16 mm
SSR-AD147 (Lodge I-2)	I	8.00%	0.00003	0.004	Delta	.13 mm
Hr (Lodge III-2)	III	6.30%	0.00009	0.004	RER	.11 mm
Le (Lodge III-1)	III	5.40%	0.0002	0.004	Delta	.10 mm
A (Lodge II-1)	II	2.00%	0.034 (int)	0.119	Delta	.07 mm
SSR-A9	IV	1.70%	0.049	0.172	Delta	.06 mm

*=RER parent has poor lodging resistance but also had a larger side branch diameter.

int= Interaction, strong effect some years (p-value: $2014=2.57 \times 10^{-7}$; 2015=.00025) but not others.

(1) % Variation is the percentage of variation explained by the QTL.

(2) P-value (Unadjusted)=this p-value is not adjusted for multiple comparisons.

(3) False Discovery Rate=The expected proportion of false positives.

(4) Source of desirable allele= The parental allele that increases side branch diameter

(5) The effect size is the mean difference in side branch stem diameter between the two genotypes.

Table 14: QTLs for compressed side branch thickness (analyzed on 94 dwarf RILs).

QTL Name and/or Closest Genetic Marker	LG	% Variation (Averaged across all site years) (1)	p-value (unadj., data averaged across site years) (2)	False discovery rate (Benjamini-Hochberg) (3)	Source of desirable allele (4)	Effect (mm) (5)
Major QTL (strong evidence). Listed in order of importance (based on p-value)						
Lodge III-2 (*Hr/Rms1/M*)	III	34.10%	9.44×10^{-10}	1.85×10^{-7}	RER	.11 mm
Putative Tsw1.1 (A6724)	I	11.60%	0.00023	0.01	Delta	.06 mm
Putative QTL (moderate or weak evidence). Listed in order of importance (based on p-value)						
Lodge VI-1 (A1580)	VI	7.70%	0.006	0.04	Delta	.05 mm
Lodge I-1 (*Afila*)	I	6.30%	0.014	0.08	RER	.05 mm
Lodge III-3 (SSR-AD174)	III	5.30%	0.025	0.13	Delta	.04 mm

(1) % Variation is the percentage of variation explained by the QTL.

(2) P-value (Unadjusted)=this p-value is not adjusted for multiple comparisons.

(3) False Discovery Rate=The expected proportion of false positives after correcting for multiple comparisons.

(4) Source of desirable allele= The parental allele that increases compressed side branch diameter.

(5) The effect size is the mean difference in compressed side branch stem diameter between the two genotypes.

Table 15: Results of single marker analyses for compressed side branch thickness on all 254 lines.

Genetic Markers	LG	% Variation (Averaged across all site years) (1)	p-value (Unadjusted, data averaged across site years) (2)	False Discovery Rate (Benjamini-Hochberg) (3)	Source of Desirable allele (4)	Effect (mm) (5)
Hr (Lodge III-2)	III	42.40%	4.56×10^{-26}	$\sim 1 \times 10^{-24}$	RER	.14 mm
Afila (Lodge I-1)	I	9.30%	0.0005	0.0399	RER	.06 mm
SSR-AD73 (Lodge III-3)	III	4.10%	0.002	0.014	Delta	.04 mm

(1) % Variation is the percentage of variation explained by the QTL.

(2) P-value (Unadjusted)=this p-value is not adjusted for multiple comparisons.

(3) False Discovery Rate=The expected proportion of false positives after correcting for multiple comparisons.

(4) Source of desirable allele= The parental allele that increases compressed side branch diameter.

(5) The effect size is the mean difference in compressed side branch stem diameter between the two genotypes.

Table 16: QTLs for epicotyl diameter (analyzed on 94 dwarf RILs).

QTL Name and/or Closest Genetic Marker	LG	% Variation (Averaged across all site years) (1)	p-value (unadj., data averaged across site years) (2)	False discovery rate (Benjamini-Hochberg) (3)	Source of desirable allele (4)	Effect (mm) (5)
Major QTL (strong evidence). Listed in order of importance (based on p-value)						
Lodge III-2 *(Hr/Rms1/M)*	III	28.40%	4.20×10^{-8}	8.22×10^{-6}	RER	.20 mm
Lodge I-1 *(Afila)*	I	10.70%	0.00120	0.024	RER	.12 mm
Putative QTL (moderate or weak evidence). Listed in order of importance (based on p-value)						
Lodge III-3 (A1025)	III	7.20%	0.0090	0.1070	Delta	.10 mm
Lodge III-1 *(Le)* (analyzed on all 254 lines)	III	2.20%	0.0200	0.3227	Delta	.06 mm
SSR-AA81	V	4.80%	0.0320	0.2750	RER	.08 mm

(1) % Variation is the percentage of variation explained by the QTL.

(2) P-value (Unadjusted)=this p-value is not adjusted for multiple comparisons.

(3) False Discovery Rate=The expected proportion of false positives after correcting for multiple comparisons.

(4) Source of desirable allele= The parental allele that increases epicotyl diameter.

(5) The effect size is the mean difference in epicotyl diameter between the two genotypes.

Table 17: Results of single marker analyses for epicotyl diameter on all 254 lines.

Genetic Markers	LG	% Variation (Averaged across all site years) (1)	p-value (Unadjusted, data averaged across site years) (2)	False Discovery Rate (Benjamini-Hochberg) (3)	Source of Desirable allele (4)	Effect (mm) (5)
Hr (Lodge III-2)	III	26.90%	1.79×10^{-15}	$\sim 1 \times 10^{-11}$	RER	.21 mm
Afila (Lodge I-1)	I	13.60%	6.77×10^{-9}	0.00001	RER	.14 mm
Le (Lodge III-1)	III	2.20%	0.020	0.32	Delta	.06 mm

(1) % Variation is the percentage of variation explained by the QTL.

(2) P-value (Unadjusted)=this p-value is not adjusted for multiple comparisons.

(3) False Discovery Rate=The expected proportion of false positives.

(4) Source of desirable allele= The parental allele that increases epicotyl diameter.

(5) The effect size is the mean difference in epicotyl diameter between the two genotypes.

QTLs for Basal Branch Number and Aerial Branching

A positive correlation (r=.46) between lodging tolerance and basal branch number was observed in each site-year based on correlation matrices (Chapter 7). Aerial branching was also seen in a number of lines. Two major and seven minor QTLs were identified for branching and aerial branching in the Delta x RER population (Tables 18-21). Six of the QTLs for branching or aerial branching co-located in the same position as lodging QTLs.

Table 18: QTLs for basal branch number (analyzed on 94 dwarf RILs, unless noted).

QTL Name and/or Closest Genetic Marker	LG	% Variation (Averaged across all site years) (1)	p-value (unadj., data averaged across site years) (2)	False discovery rate (Benjamini-Hochberg) (3)	Fewest Branches (Allele) (4)	Effect (branch number) (5)
Major QTL (strong evidence). Listed in order of importance (based on p-value)						
Lodge III-2 *(Hr/Rms1/M)*	III	30.20%	1.33×10^{-8}	2.60×10^{-6}	Delta	0.80
Lodge III-1 (*Le*) (analyzed on all 254 lines)	III	6.50%	4.62×10^{-5}	0.001	RER	0.35
Putative QTL (moderate or weak evidence). Listed in order of importance (based on p-value)						
Putative Tsw 1.1 (A6724)	I	9.70%	0.002	0.047	Delta	0.45
Lodge VI-1 (A1580)	VI	8.30%	0.006	0.048	Delta	0.42
Gap locus (A1330)	I	6.70%	0.011	0.072	Delta	0.38

(1) % Variation is the percentage of variation explained by the QTL.

(2) P-value (Unadjusted)=this p-value is not adjusted for multiple comparisons.

(3) False Discovery Rate=The expected proportion of false positives after correcting for multiple comparisons.

(4) Fewest branches= The parental allele associated with the fewest branches.

(5) Effect= the mean difference in branch number between the two genotypes.

Table 19: Results of single marker analyses for basal branch number on all 254 lines.

Genetic Markers	LG	% Variation (Averaged across all site years) (1)	p-value (Unadjusted, data averaged across site years) (2)	False Discovery Rate (Benjamini-Hochberg) (3)	Fewest Branches (Allele) (4)	Effect (branch number) (5)
Hr (Lodge III-2)	III	21.50%	2.43×10^{-12}	8.07×10^{-10}	Delta	0.64
Le (Lodge III-1)	III	6.50%	4.62×10^{-5}	0.001	RER	0.35

(1) % Variation is the percentage of variation explained by the QTL.

(2) P-value (Unadjusted)=this p-value is not adjusted for multiple comparisons.

(3) False Discovery Rate=The expected proportion of false positives.

(4) Fewest branches= The parental allele associated with the fewest branches.

(5) Effect= the mean difference in branch number between the two genotypes.

Table 20: QTLs for aerial branching (analyzed on 94 dwarf RILs).

QTL Name and/or Closest Genetic Marker	LG	% Variation (one site year) (1)	p-value (Unadusted, one site year) (2)	False discovery rate (Benjamini-Hochberg) (3)	Fewest Aerial Branches (Allele) (4)	Effect (points on a 1-4 rating scale) (5)
Major QTL (strong evidence). Listed in order of importance (based on p-value)						
Lodge III-2 *(Hr/Rms1/M)*	III	24.40%	7.11×10^{-7}	$\sim 1 \times 10^{-5}$	Delta	0.96
Putative QTL (moderate or weak evidence). Listed in order of importance (based on p-value)						
Lodge I-3 (A5967)	I	11.30%	0.0014	0.044	RER	0.64
Putative Tsw 1.1	I	9.00%	0.004	0.059	Delta	0.58
Lodge I-2 (A7384)	I	6.50%	0.014	0.136	RER	0.49
STS.RPL15S	VII	4.60%	0.039	0.241	Delta	0.42

(1) % Variation is the percentage of variation explained by the QTL.
(2) P-value (Unadjusted)=this p-value is not adjusted for multiple comparisons.
(3) False Discovery Rate=The expected proportion of false positives after correcting for multiple comparisons.
(4) Fewest branches= The parental allele associated with the fewest branches.
(5) Effect= the mean difference in points between the two genotypes.

Table 21: Results of single marker analyses for aerial branching on all 254 lines.

Genetic Markers	LG	% Variation (one site year) (1)	p-value (Unadusted, one site year) (2)	False Discovery Rate (Benjamini-Hochberg) (3)	Fewest Aerial Branches (Allele) (4)	Effect (points on a 1-4 rating scale) (5)
Hr (Lodge III-2)	III	29.60%	1.711×10^{-16}	$\sim 1 \times 10^{-14}$	Delta	1.05
Np (Lodge III-1)	III	1.70%	0.045	0.27	Delta	0.25

(1) % Variation is the percentage of variation explained by the QTL.
(2) P-value (Unadjusted)=this p-value is not adjusted for multiple comparisons.
(3) False Discovery Rate=The expected proportion of false positives.
(4) Fewest branches= The parental allele associated with the fewest branches.
(5) Effect= the mean difference in points between the two genotypes.

QTLs for Maturity Time, Node Number, and Number of Flowering Nodes

The results of QTL analyses on maturity time, nodes to first flower, total node number, and average number of flowering nodes are presented in Tables 22 through 29.

Nine of the ten lodging QTLs (Table 4) also co-located with QTLs for traits related to

maturity time. A very weak negative correlation between % lodging and maturity time

was seen in each site-year based on correlation matrices (See Chapter 7 and Appendix B),

but no significant associations were found between lodging and the other traits.

Table 22: QTLs for maturity time (analyzed on 94 dwarf RILs).

QTL Name and/or Closest Genetic Marker	LG	% Variation (Averaged across all site years) (1)	p-value (Unadjusted, data averaged across all site years) (2)	False discovery rate (Benjamini-Hochberg) (3)	Earliest Maturity Time (Allele) (4)	Effect size (Number of points) (5)
Major QTL (strong evidence). Listed in order of importance (based on p-value)						
Lodge III-2 (*Hr/Rms1/M*)	III	64.80%	2.64×10^{-22}	$\sim 1 \times 10^{-20}$	Delta	.89 pts
Lodge III-3 (A7384)	III	11.90%	0.00063	0.0058	RER	.28 pts.
Putative QTL (moderate or weak evidence). Listed in order of importance (based on p-value)						
Lodge I-3 (A2259)	I	9.60%	0.0029	0.024	RER	.33 pts.
A7193	IV	7.90%	0.0062	0.047	RER	.31 pts.

(1) % Variation is the percentage of variation explained by the QTL.

(2) P-value (Unadjusted)=this p-value is not adjusted for multiple comparisons.

(3) False Discovery Rate=The expected proportion of false positives after correcting for multiple comparisons.

(4) Earliest Maturity Time= The parental allele that decreases maturity time.

(5) The effect size is the mean difference in maturity between the two parental genotypes based on a 1-4 scale.

Table 23: Results of single marker analyses for maturity time on all 254 lines.

Genetic Markers	LG	% Variation (Averaged across all site years) (1)	p-value (Unadjusted, data averaged across all site years) (2)	False Discovery Rate (Benjamini-Hochberg) (3)	Earliest Maturity Time (Allele) (4)	Effect size (Number of points) (5)
Hr (Lodge III-2)	III	75.80%	8.47×10^{-65}	$\sim 1 \times 10^{-63}$	Delta	1.05 pts.
A (Lodge II-1)	II	3.70%	0.0023	0.020	Delta	.22 pts.
SSR-AD147 (Lodge III-3)	III	2.50%	0.021	0.11	RER	.17 pts

(1) % Variation is the percentage of variation explained by the QTL.

(2) P-value (Unadjusted)=this p-value is not adjusted for multiple comparisons.

(3) False Discovery Rate=The expected proportion of false positives.

(4) Earliest Maturity Time= The parental allele that decreases maturity time.

(5) The effect size is the mean difference in maturity between the two genotypes based on a 1-4 scale.

Table 24: QTLs for nodes to first flower (analyzed on 94 dwarf RILs).

QTL Name and/or Closest Genetic Marker	LG	% Variation (averaged across two site years) (1)	p-value (Unadjusted, data averaged across two site years) (2)	False discovery rate (Benjamini-Hochberg) (3)	Earliest Nodes to 1st Flower (Allele) (4)	Effect size (Number of nodes) (5)
Major QTL (strong evidence). Listed in order of importance (based on p-value)						
Lodge III-2 (*Hr/Rms1/M*)	III	24.50%	4.96×10^{-7}	2.93×10^{-5}	Delta	2.56
Lodge II-2 (A7258)#	II	22.60%	1.58×10^{-6}	4.01×10^{-5}	Delta	2.40
Lodge II-1 (A854)#	II	22.55%	2.25×10^{-6}	4.90×10^{-5}	Delta	2.41
Putative QTL (moderate or weak evidence). Listed in order of importance (based on p-value)						
Lodge I-2 (A7384)	I	6.70%	0.012	0.039	RER	1.33
Lodge I-1 (*Afila*)	I	6.60%	0.012	0.056	RER	1.32

#Lodge II-2 and Lodge II-1 are closely linked.

(1) % Variation is the percentage of variation explained by the QTL.

(2) P-value (Unadjusted)=this p-value is not adjusted for multiple comparisons.

(3) False Discovery Rate=The expected proportion of false positives after correcting for multiple comparisons.

(4) Earliest Nodes to 1st Flower= The parental allele that decreases nodes to first flowering.

(5) The effect size is the mean difference in nodes to first flowering between the two genotypes.

Table 25: Results of single marker analyses for nodes to first flower on all 254 lines.

Genetic Markers	LG	% Variation (averaged across two site years) (1)	p-value (Unadjusted, data averaged across two site years) (2)	False Discovery Rate (Benjamini-Hochberg) (3)	Earliest Nodes to 1st Flower (Allele) (4)	Effect size (Number of nodes) (5)
Hr (Lodge III-2)	III	35.70%	3.48×10^{-21}	$\sim 1 \times 10^{-18}$	Delta	3.44
A (Lodge II-1)	II	14.30%	8.84×10^{-10}	6.36×10^{-8}	Delta	2.08
Afila (Lodge I-1)	I	2.50%	0.012	0.047	RER	0.88
Np (Lodge III-1)	III	1.90%	0.03	0.11	Delta	0.77

(1) % Variation is the percentage of variation explained by the QTL.

(2) P-value (Unadjusted)=this p-value is not adjusted for multiple comparisons.

(3) False Discovery Rate=The expected proportion of false positives.

(4) Earliest Nodes to 1st Flower= The parental allele that decreases nodes to first flowering.

(5) The effect size is the mean difference in nodes to first flowering between the two genotypes.

Table 26: QTLs for total node number (analyzed on 94 dwarf RILs).

QTL Name and/or Closest Genetic Marker	LG	% Variation (averaged across two site years) (1)	p-value (unadj., data averaged across site years) (2)	False discovery rate (Benjamini-Hochberg) (3)	Fewest Nodes Prior to Senescence (Allele) (4)	Effect size (Number of nodes) (5)
Major QTL (strong evidence). Listed in order of importance (based on p-value)						
Lodge III-2 (*Hr/Rms1/M*)	III	36.60%	1.49×10^{-10}	2.59×10^{-8}	Delta	3.04
Lodge III-1 (*Le*) (analyzed on all 254 lines)	III	5.80%	0.0001	0.004	Delta	1.34
Lodge II-2 (A7258)	II	11.20%	0.0008	0.023	Delta	1.59
Putative QTL (moderate or weak evidence). Listed in order of importance (based on p-value)						
Lodge I-2 (A7384)	I	9.60%	0.003	0.05	RER	1.53

(1) % Variation is the percentage of variation explained by the QTL.

(2) P-value (Unadjusted)=this p-value is not adjusted for multiple comparisons.

(3) False Discovery Rate=The expected proportion of false positives after correcting for multiple comparisons.

(4) Fewest nodes to senescence= The parental allele associated with the fewest total number of nodes per plant.

(5) The effect size is the mean difference in total node number between the two genotypes.

Table 27: Results of single marker analyses for total node number on all 254 lines.

Genetic Markers	LG	% Variation (averaged across two site years) (1)	p-value (Unadjusted, data averaged across site years) (2)	False Discovery Rate (Benjamini-Hochberg) (3)	Fewest Nodes Prior to Senescence (Allele) (4)	Effect size (Number of nodes) (5)
Hr (Lodge III-2)	III	43.80%	7.565×10^{-27}	$\sim 1 \times 10^{-24}$	Delta	3.79
A (Lodge II-1)	II	6.00%	0.0001	0.004	Delta	1.36
Le (Lodge III-1)	III	5.80%	0.0001	0.004	Delta	1.34

(1) % Variation is the percentage of variation explained by the QTL.

(2) P-value (Unadjusted)=this p-value is not adjusted for multiple comparisons.

(3) False Discovery Rate=The expected proportion of false positives after correcting for multiple comparisons.

(4) Fewest nodes to senescence= The parental allele associated with the fewest total number of nodes per plant.

(5) The effect size is the mean difference in total node number between the two genotypes.

Table 28: QTLs for average number of flowering nodes (analyzed on 94 dwarf RILs, unless otherwise noted).

QTL Name and/or Closest Genetic Marker	LG	% Variation (averaged across two site years) (1)	p-value (unadj., data averaged across site years) (2)	False discovery rate (Benjamini-Hochberg) (3)	Most flowering nodes (Allele) (4)	Effect size (Number of nodes) (5)
Major QTL (strong evidence). Listed in order of importance (based on p-value)						
Lodge III-1 (*Le*) (analyzed on all 254 lines)	III	8.20%	5.53×10^{-6}	0.0031	RER	0.82
Lodge II-1 (*A*)	II	14.90%	0.0001	0.0217	Delta	0.89
Putative QTL (moderate or weak evidence). Listed in order of importance (based on p-value)						
Lodge II-2 (A7258)	II	10.90%	0.0013	0.0507	Delta	0.77
Lodge III-2 (*Hr/Rms1/M*)	III	6.50%	0.0150	0.1516	RER	0.59
Lodge II-3 (*Mo*)	II	6.90%	0.0180	0.1586	Delta	0.53
Lodge VI-1 (A1580)	VI	5.90%	0.0250	0.1952	RER	0.55

(1) % Variation is the percentage of variation explained by the QTL.

(2) P-value (Unadjusted)=this p-value is not adjusted for multiple comparisons.

(3) False Discovery Rate=The expected proportion of false positives after correcting for multiple comparisons.

(4) Most flowering nodes= The parental allele associated with the most flowering nodes per plant.

(5) The effect size is the mean difference in flowering nodes between the two genotypes.

Table 29: Results of single marker analyses for number of flowering nodes on all of the 254 lines.

Genetic Markers	LG	% Variation (averaged across two site years) (1)	p-value (Unadjusted, data averaged across site years) (2)	False Discovery Rate (Benjamini-Hochberg) (3)	Most flowering nodes (Allele) (4)	Effect size (Number of nodes) (5)
Le (Lodge III-1)	III	8.20%	5.53×10^{-6}	0.0031	RER	0.82
A (Lodge II-1)	II	5.30%	0.0003	0.040	Delta	0.67
Hr (Lodge III-2)	III	2.20%	0.034	0.31	RER	0.44

(1) % Variation is the percentage of variation explained by the QTL.

(2) P-value (Unadjusted)=this p-value is not adjusted for multiple comparisons.

(3) False Discovery Rate=The expected proportion of false positives.

(4) Most flowering nodes= The parental allele associated with the most flowering nodes per plant.

(5) The effect size is the mean difference in flowering nodes between the two genotypes.

QTLs Affecting Leaf Length and Width in Dry Peas

Two major and four minor QTLs were identified for traits related to leaf length

and width in the Delta x RER population (Tables 30-32). Two of the QTLs, which

affected both leaf length and width, co-located in the same positions as Lodge III-1 and

Lodge VI-1. The most important of the two, that overlapping Lodge III-1, was found

significant for all site-years and displayed a negative relationship with lodging tolerance

(increasing leaf size decreased lodging tolerance). Lodge VI-1, in contrast, was positively

correlated with lodging tolerance, but the effect was minor. The second major QTL for

leaf length, Tsw 1.1, a QTL putatively shown in previous studies to be associated with

seed size, co-locates with QTLs for height, branching, and stem diameter. There is weak

evidence to suggest that a section of LG I located 20 map units above AD147 is

significant for leaf width. This section of LG I has a wide gap of 14.6 cM, and the

markers on either side of the gap appear to be equally significant. A QTL may exist in the

middle of the gap.

Table 30: QTLs for leaf length (analyzed on 94 dwarf RILs, unless noted).

QTL Name and/or Closest Genetic Marker	LG	% Variation (Averaged across all site years) (1)	p-value (Unadusted, data averaged across site years) (2)	False discovery rate (Benjamini-Hochberg) (3)	Longest length (Allele) (4)	Increase in Length (cm) (5)
Major QTL (strong evidence). Listed in order of importance (based on p-value)						
Lodge III-1 *(Le)* (analyzed on all 254 lines)	III	47.90%	5.834×10^{-37}	$\sim 1 \times 10^{-35}$	RER	2.93 cm
Putative Tsw 1.1 (A6724)	III	23.30%	7.10×10^{-7}	0.0003	Delta	1.53 cm
Putative QTL (moderate or weak evidence). Listed in order of importance (based on p-value)						
Lodge VI-1 (A1580)	VI	9.00%	0.0049	0.0650	Delta	1.00 cm
STS.AGAT	IV	6.70%	0.0111	0.1074	Delta	.82 cm

(1) % Variation is the percentage of variation explained by the QTL.

(2) P-value (Unadjusted)=this p-value is not adjusted for multiple comparisons.

(3) False Discovery Rate=The expected proportion of false positives after correcting for multiple comparisons.

(4) Longest length= The parental allele associated with the longest leaves.

(5) Increase in length= the mean difference in centimeters between the two genotypes.

Table 31: Results of single marker analyses for leaf length on all of the 254 RIL lines.

Genetic Marker	LG	% Variation (Averaged across all site years) (1)	p-value (Unadusted, data averaged across site years) (2)	False Discovery Rate (Benjamini-Hochberg) (3)	Longest length (Allele) (4)	Increase in Length (cm) (5)
Le (Lodge III-1)	III	47.90%	5.83×10^{-37}	$\sim 1 \times 10^{-35}$	RER	2.93 cm
SSR-AD147 (Lodge I-2)	I	1.70%	0.056	0.33	Delta	.56 cm

(1) % Variation is the percentage of variation explained by the QTL.

(2) P-value (Unadjusted)=this p-value is not adjusted for multiple comparisons.

(3) False Discovery Rate=The expected proportion of false positives after correcting for multiple comparisons.

(4) Longest length= The parental allele associated with the longest leaves.

(5) Increase in length= the mean difference in centimeters between the two genotypes.

Table 32: QTLs for leaf width (analyzed on 94 dwarf RILs unless noted)

QTL Name and/or Closest Genetic Marker	LG	Lines used (1)	% Variation (one site year) (2)	p-value (Unadusted, one site year) (3)	False discovery rate (Benjamini-Hochberg) (3)	Largest Width (Allele) (5)	Increase in width (cm) (6)
Major QTL (strong evidence). Listed in order of importance (based on p-value)							
Lodge III-1 *(Le)* (analyzed on all 254 lines)	III	*af*	12.80%	0.0001	0.0431	RER	1.61 cm
Putative QTL (moderate or weak evidence). Listed in order of importance (based on p-value)							
Lodge VI-1 (A1580)	VI	sub-*af*/sub-*Af*	27.9%/9.1%	0.00125/.037	.209/.483	Delta	2.06/.84 cm
Gap locus (A1325-A1332)#	I	sub-*af*/sub-*Af*	11.1/12.6%	0.044/.0078	.458/.383	Delta	1.43/.98 cm
SSR-AA285	IV	sub-*Af*	16.70%	0.0019	0.3830	Delta	1.13 cm
Putative Tsw 1.1 (A6724)	I	sub-*af*	17.10%	0.0110	0.3550	Delta	1.74 cm

#Near a very large gap on LG 1. A major QTL could be in the gap.

(1) Lines used [*af* =only *afila* lines analyzed]; [sub-*af* = only ~35 *afila* Lines used (1)]; [sub-*Af* = only ~55 Af Lines used]

(2) % Variation is the percentage of variation explained by the QTL.

(3) P-value (Unadjusted)=this p-value is not adjusted for multiple comparisons.

(4) False Discovery Rate=The expected proportion of false positives after correcting for multiple comparisons.

(5) Largest width= The parental allele associated with the widest leaves.

(6) Increase in width= the mean difference in centimeters between the two genotypes.

<u>QTLs for Emergence and Seed Dormancy</u>

Results from this study indicate that reduced emergence may increase lodging.

One major and four minor QTLs were identified for traits related to emergence and seed

dormancy in the Delta x RER population (Tables 33-35). Three of the QTLs for

emergence and seed dormancy co-located in the same positions as reported lodging

QTLs.

Table 33: QTLs for emergence (analyzed on 94 dwarf RILs)

QTL Name and/or Closest Genetic Marker	LG	% Variation (Averaged across all site years) (1)	p-value (Unadusted, averaged across site years) (2)	False discovery rate (Benjamini-Hochberg) (3)	Highest Germination (Allele) (4)	Effect (% Germination) (5)
Major QTL (strong evidence). Listed in order of importance (based on p-value)						
AA107	III	11.80%	0.0006	0.021	Delta	5.55%
Putative QTL (moderate or weak evidence). Listed in order of importance (based on p-value)						
Lower LG 6 (A1008)	VI	6.70%	0.012	0.096	RER	4.16%
Lodge II-1 *(A)*	II	5.40%	0.022	0.161	Delta	3.73%
A421	III	4.80%	0.038	0.213	RER	3.58%

(1) % Variation is the percentage of variation explained by the QTL.

(2) P-value (Unadjusted)=this p-value is not adjusted for multiple comparisons.

(3) False Discovery Rate=The expected proportion of false positives after correcting for multiple comparisons.

(4) Highest germination= The parental allele associated with the highest germination rate.

(5) Effect= the mean difference in % germination between the two genotypes.

Table 34: Results of single marker analyses for % emergence on all of the 254 RIL lines.

Genetic Markers	LG	% Variation (Averaged across all site years) (1)	p-value (Unadusted, averaged across site years) (2)	False Discovery Rate (Benjamini-Hochberg) (3)	Highest Germination (Allele) (4)	Effect (% Germination) (5)
A (Lodge II-1)	II	17.50%	8.92×10^{-12}	$\sim 1 \times 10^{-10}$	Delta	9.82%
Hr (Lodge III-2)	III	7.10%	0.0001	0.01	Delta	5.01%
SSR-A9	IV	2.50%	0.017	0.13	RER	3.49%

(1) % Variation is the percentage of variation explained by the QTL.

(2) P-value (Unadjusted)=this p-value is not adjusted for multiple comparisons.

(3) False Discovery Rate=The expected proportion of false positives after correcting for multiple comparisons.

(4) Highest germination= The parental allele associated with the highest germination rate.

(5) Effect= the mean difference in % germination between the two genotypes.

Table 35: QTLs for seed dormancy (analyzed on 94 dwarf RILs).

QTL Name and/or Closest Genetic Marker	LG	% Variation (single site year) (1)	p-value (Unadusted, single site year) (2)	False discovery rate (Benjamini-Hochberg) (3)	Fastest imbibition (Allele) (4)	Effect (Points on a 1-3 rating scale) (5)
Putative QTL (moderate or weak evidence). Listed in order of importance (based on p-value)						
Lodge II-1 *(A)*	II	8.60%	0.004	0.332	Delta	0.56
Lodge II-2 (A7258)	II	6.10%	0.017	0.349	Delta	0.47
Lower LG 6 (A1008)	VI	5.30%	0.027	0.349	RER	0.43
SSR.A9	IV	4.60%	0.036	0.349	RER	0.41

(1) % Variation is the percentage of variation explained by the QTL.

(2) P-value (Unadjusted)=this p-value is not adjusted for multiple comparisons.

(3) False Discovery Rate=The expected proportion of false positives after correcting for multiple comparisons.

(4) Fastest imbibition= The parental allele associated with the lowest amount of seed dormancy.

(5) Effect= the mean difference in seed dormancy when rated on a 1-3 point rating scale.

The region between SSR.A9 and AA92 may be important for seed dormancy. There is a gap between markers in this region that likely is where the QTL is located. Markers on both sides of the gap are significant.

QTLs for Yield and Seed Size

Yield theoretically increases lodging by increasing the load on plant stems. Two major and six minor QTLs were identified for traits related to yield in the Delta x RER population (Table 36-39). All QTLs for yield co-located in the same positions as reported lodging QTLs, and 4 QTLs for hundred seed weight co-located in the same position as lodging QTLs

Table 36: QTLs for yield (analyzed on 94 dwarf RILs).

QTL Name and/or Closest Genetic Marker	LG	% Variation (Averaged across two site years) (1)	p-value (unadj., data averaged across site years) (2)	False discovery rate (Benjamini-Hochberg) (3)	Source of desirable allele (4)	Effect (kg/ha) (5)
Major QTL (strong evidence). Listed in order of importance (based on p-value)						
Lodge III-2 (A55)	III	13.50%	0.0002	0.013	RER	755 kg/ha
Lodge II-1 (*A*)	II	11.00%	0.0009	0.017	Delta	679 kg/ha
Putative QTL (moderate or weak evidence). Listed in order of importance (based on p-value)						
Lodge III-3 (AA5c)	III	10.50%	0.001	0.022	Delta	659 kg/ha
Lodge II-2 (A5224-A5967)	I	9.20%	0.003	0.033	Delta	616 kg/ha
Lodge II-2 (A7258)	II	8.50%	0.004	0.048	Delta	610 kg/ha
Lodge II-3 (*Mo*)	II	11.80%	0.007	0.058	Delta	704 kg/ha
Lodge III-1 (*Le*)	III	2.10%	0.022	0.098	Delta	302 kg/ha
Lodge I-1 (*Afila*)	I	3.70%	0.063	0.218	RER	396 kg/ha

(1) % Variation is the percentage of variation explained by the QTL.

(2) P-value (Unadjusted)=this p-value is not adjusted for multiple comparisons.

(3) False Discovery Rate=The expected proportion of false positives after correcting for multiple comparisons.

(4) Source of desirable allele= The parental allele that increases small plot yield.

(5) The effect size is the mean difference in small plot yield between the two genotypes.

Table 37: Results of single marker analyses for yield on all of the 254 RIL lines.

Genetic Markers	LG	% Variation (Averaged across two site years) (1)	p-value (Unadjusted, data averaged across site years) (2)	False Discovery Rate (Benjamini-Hochberg) (3)	Source of Desirable allele (4)	Effect (lbs/A) (5)
A (Lodge II-1)	II	11.10%	1.04×10^{-7}	0.00003	Delta	688 kg/ha
Afila (Lodge I-1)	I	4.10%	0.0013	0.022	RER	416 kg/ha
Le (Lodge III-1)	III	2.10%	0.022	0.098	Delta	302 kg/ha

(1) % Variation is the percentage of variation explained by the QTL.

(2) P-value (Unadjusted)=this p-value is not adjusted for multiple comparisons.

(3) False Discovery Rate=The expected proportion of false positives after correcting for multiple comparisons.

(4) Source of desirable allele= The parental allele that increases small plot yield.

(5) The effect size is the mean difference in small plot yield between the two genotypes.

Table 38: QTLs for seed size (analyzed on 94 dwarf RILs).

QTL Name and/or Closest Genetic Marker	LG	% Variation (Averaged across two site years) (1)	p-value (Unadusted, data averaged across both site years) (2)	False discovery rate (Benjamini-Hochberg) (3)	Source of desirable allele (4)	Effect (g/100 seed) (5)
Major QTL (strong evidence). Listed in order of importance (based on p-value)						
Putative Tsw 1.1 (A6724)	I	39.20%	7.88×10^{-12}	9.64×10^{-10}	Delta	3.87 g
Lodge III-2 *(Hr/Rms1/M)*	III	20.20%	6.66×10^{-6}	0.00012	Delta	2.75 g
Lodge VI-1 (AA374)	VI	11.40%	0.0009	0.008	Delta	2.07 g
Putative QTL (moderate or weak evidence). Listed in order of importance (based on p-value)						
Lodge III-3 (AD73)	III	9.50%	0.002	0.014	Delta	1.91 g
A1915	VII	6.30%	0.015	0.055	Delta	1.57 g
Lodge III-1 *(Np)*	III	5.60%	0.021	0.070	Delta	1.63 g

(1) % Variation is the percentage of variation explained by the QTL.

(2) P-value (Unadjusted)=this p-value is not adjusted for multiple comparisons.

(3) False Discovery Rate=The expected proportion of false positives after correcting for multiple comparisons.

(4) Source of desirable allele= The parental allele that increases seed size.

(5) The effect size is the mean difference in 100 seed weight between the two genotypes.

Table 39: Results of single marker analyses for seed size on all of the 254 RIL lines.

Genetic Markers	LG	% Variation (Averaged across two site years) (1)	p-value (Unadusted, single site year) (2)	False Discovery Rate (Benjamini-Hochberg) (3)	Source of Desirable allele (4)	Effect (g/100 seed) (5)
Hr (Lodge III-2)	III	18.90%	9.28×10^{-11}	3.85×10^{-9}	Delta	2.71 g
SSR-AD73 (Lodge III-3)	III	7.70%	0.00002	0.0003	Delta	1.73 g
Np (Lodge III-1)	III	5.40%	0.0003	0.002	Delta	1.45 g
SSR-AD147 (Lodge I-2)	I	2.80%	0.014	0.052	Delta	1.06 g

(1) % Variation is the percentage of variation explained by the QTL.

(2) P-value (Unadjusted)=this p-value is not adjusted for multiple comparisons.

(3) False Discovery Rate=The expected proportion of false positives after correcting for multiple comparisons.

(4) Source of desirable allele= The parental allele that increases seed size.

(5) The effect size is the mean difference in 100 seed weight between the two genotypes.

CHAPTER FOUR: DISCUSSION

Means for Each Trait and Heritability Estimates

Levene's test for homogeneity of variances indicated that no traits had significantly different variances at a p=.01, but branch number, germination, maturity time, yield, and yield per plant were all significant at the .05 level due to significant variation in 2014. Each of these traits was analyzed during each site-year prior to combining data. Because the effects of these traits were fairly consistent each site year, separating the data by site-year did not change the outcome of the analysis so data was averaged across site years for every trait. Lodging resistance was fairly heritable across site-years with broad sense heritabilities ranging from .74 to .80 in 2015, indicating that this trait would improve rapidly by selection. The means of the two parents were different for every trait measured in this study, and a wide range of means were measured for each trait, indicating that genes controlling each trait were segregating in this population. Yield was the trait with the lowest heritability during both site years heritabilities were measured, indicating the difficulty in selecting for high yielding genotypes. Heritability does not provide an estimate of subsampling error. Subsampling error was low for traits such as plant height and 100 seed weight which had high levels of heritability, but subsampling error was high for traits such as branch number and stem diameter. Because the average of several subsamples was used to calculate plot means, subsampling variance was minimized in the estimates for plot means, likely influencing heritability estimates due to reduced environmental variance within each microplot. Should future

studies on lodging resistance be conducted in pea, the importance of subsampling

variance should be considered in the study design when assessing traits with a high

degree of subsampling variance.

Genetic Mapping

While there were originally 4753 SNP markers, only 282 high quality SNP

markers were used to create the genetic map. Many of the markers did not have sufficient

data to be mapped at an LOD of 4. At an LOD of 3 and 3.5, several thousand markers

were arranged into one long linkage group, indicating that a higher LOD was required. It

is also possible that these unmapped genetic markers were the result of sequencing errors

or that data was poor quality. Ultimately, the number of markers found using the GBS

procedure is dependent on the number of polymorphic SNPs in unmethylated regions

between the two parents (the two enzyme system selects unmethylated DNA). There

were 330 markers that were mapped across 422 cM, indicating an average density of one

marker per 1.28 cM. However, many markers were often within the same map unit. In

order to increase clarity, the markers that were within the same map unit were removed,

and a map of 196 high quality markers was created that spanned 452 map units. The 30

cM increase in map size with the final map was due to fewer unreported recombinants

(see Figure 2). This effect occurs because recombination cannot be computed for

individuals that have a missing data point between genetic markers (see Figure 2)

The upper part of LG III above the *M* locus did not always map in any location

when different permutations (varying LOD or minimum recombination) of the mapping

function were tried, indicating that the upper part of LG3 may not be correctly mapped. This total map distance in the genetic map of the Delta x RER population was shorter than other maps by Weeden et al. (1998), Loridon et al. (2005), or Duarte et al. 2014. Based on these prior maps, pea has 7 complete linkage groups that range from 142-285 cM in length. The map for this population was not complete when compared to prior pea consensus maps. LG II and IV both had missing sections >20 cM each, and LG V and LG VII had large sections >50 cM long with no polymorphism. LG I is believed to be complete because SSR-C20b is near the top end of the map by Loridon et al. (2005), and *Af* is known to be near the opposite end of that linkage group (Ellis et al 1992, Loridon et al. 2005). Similarly, SSR-AA473 maps near the top of LG II (Loridon et al) and *Mo* maps about 75% of the way down this linkage group (Weeden et al. 1998). *M* and *Le* map near the proximal and distal ends, respectively, of their respective arms of LG III, and SSRAA374 and *Pl* are on opposite ends of LG VI. Fifty-four SSR and several dozen CAPS markers which were previously mapped in the missing regions were not polymorphic, suggesting that these regions are highly similar in genetic background. However, with the exception of the lowest part of LG II, a portion of the middle of LG IV, and the lower sections of LG V and LG VII, the scaffold markers indicate that coverage of the pea linkage map for the current RIL population is relatively comprehensive.

Phenotypic Basis of Lodging QTLs

Each of the lodging QTLs were associated with at least two QTLs found for the other traits investigated (Table 40). The associations can provide a strong indication of the mechanisms associated with lodging resistance for each lodging QTL described in Table 4.

Table 40: Correlation of lodging QTLs with QTLs identified for other traits.

Traits (Most lodging resistant phenotype shown)	Lodge III-1 (Putatively Le)	Lodge I-1 (Putatively Af)	Lodge III-2 (Putatively Hr/Rms1/M)	Lodge III-3 (near AD73)	Lodge II-1 (Putatively A/Lf)	Lodge I-2 (near A7384)	Lodge II-2 (near A7258)	Lodge II-3 (near Mo)	Lodge I-3 (A2259, A5967)	Lodge VI-1 (A1580)
Lodging resistant parent	1* Delta (D)	2* Delta (D)	3* RER (R)	4* Delta (D)	5* Delta (D)	6* Delta (D)	7* Delta (D)	8* Delta (D)	9* Delta (D)	10* Delta (D)
Plant height (shortest allele shown)	1* (D)	--	3 (D)	--	--	--	--	--	--	--
Main stem diameter (largest diameter shown)	1 (D)	2 (R)&	9 (D)	4 (D)	5 (D)	--	6 (D)	--	--	8 (D)
Comp. mn. stm thickness (Thickest diam. shown)	3? (D)	1 (R)	--	2 (D)	--	--	4 (D)	--	--	7 (D)
Side branch diam. (largest stem diam. shown)	3 (D)	2 (R)	10 (R)	9 (D)	4 (D)	6 (D)	7 (D)	--	--	8 (D)
Compressed side branch thickness	--	4 (R)	1 (R)	5 (D)	--	--	--	--	--	3 (D)
Epicotyl diameter (largest diameter)	4 (D)	2 (R)	1 (R)	3 (D)	--	--	--	--	--	--
Basal branching (most basal branches)	2 (D)	--	1 (R)	--	--	--	--	--	--	4 (R)
Aerial branching (most aerial branches)	--	--	1 (R)	--	--	4 (D)	--	--	2 (D)	--
Maturity time (Latest maturing)	--	--	1 (R)	--	--	2 (D)	--	--	3 (D)	--
Nodes to 1st flower (fewest nodes)	--	5 (R)	1 (D)	--	3 (D)	4 (R)	2 (D)	--	--	--
Total node # (fewest nodes designated)	2 (D)	--	1 (D)	--	--	4 (R)	3 (D)	--	--	--
Avg # flowering nodes (most nodes)	1 (R)	--	4 (R)	--	2 (D)	--	3 (D)	5 (D)	--	6 (R)
Leaf length (longest leaves)	1 (R)	--	--	--	--	--	--	--	--	3 (D)
Leaf width (widest leaves)	1 (R)	--	--	--	--	--	--	--	--	2 (D)
% Emergence	--	--	1? (D)	--	3 (D)	--	--	--	--	--
Seed dormancy (lowest seed dormancy)	--	--	--	--	1 (D)	--	2 (D)	--	--	--
QTL for small plot yield (Highest yield)	7 (D)	8 (R)	1 (R)	3 (D)	2 (D)	5? (D)	4 (D)	6 (D)	--	--
Seed size (largest seed size)	6 (D)	--	2 (D)	4 (D)	--	--	--	--	--	3 (D)

*The number indicates the rank of the QTL for each trait
-- Indicates that the QTL has no effect on that specific trait
Green cells indicate a trait for which a lodging effect in favor of either allele is not apparent
& Red cells indicate a trait where the favorable allele for lodging and the favorable allele for the trait are not the same
? Indicates that the genes affecting lodging and affecting the trait may not be the same

Lodge III-1 (Putatively *Le*)

The results of this study indicate that the *Le* mutation may be the most important gene influencing lodging in pea. However, it should be noted that there may be an additional gene influencing lodging within the Lodge III-1 QTL. When the region 20-30 cM above *Le* was analyzed on just dwarf lines, some markers were weakly significant, indicating that multiple genes may be within this QTL. However, by far, the most significant region for lodging was centered at *Le*. The obvious mechanism for the

influence of *Le* on lodging is the decrease in height produced by the recessive allele. Few height genes were segregating in this population, and *Le* explained nearly 80% of the variation in height. However, as is apparent from Table 40, the effect of variation at *Le* may not be just variation in height. *Le* (or a tightly linked locus) also was the primary QTL for stem diameter ($p=3.6\text{x}10^{-24}$), so that possession of the Delta allele not only decreased stem height but also increased stem diameter. In addition, this region contained the second most important QTL for branching, with the allele from Delta producing more basal branches. Increased basal branching increases tendril number per plant because each additional branch has 5 or more tendrils at each node. Therefore, increased branching will allow plants to intertwine tendrils for greater support. The *Le* region also contained the most important QTL for leaf length and width, which influences contact between adjacent plants and the probability that tendrils will find support. The primary QTL for leaf length mapped at *Le*, and increased leaf length was associated with the dominant allele. Any impact longer leaves might have had on lodging tolerance was overshadowed by the benefits of the dwarf phenotype such as increased stem diameter and branching. Thus, three major changes in phenotype (dwarfing, increased stem diameter, and increased branching) contribute to the increased lodging tolerance of the Delta allele. Interestingly, the second most important QTL for height (that in the *M/Hr/Rms1* region) did not affect lodging in the same manner as *Le,* with shorter plants having lower lodging tolerance.

The finding that the dwarfing allele, *le*, is putatively the most important factor controlling lodging resistance in the RIL population stands in contrast to a finding by

McPhee and Muehlbauer (1999) who indicated that stem strength was positively correlated with internode length. However, McPhee and Muehlbauer assessed a diverse array of genotypes where solid stems were common, unlike the Delta x RER population. Based on the stress equation, the 0.27 mm reduction in stem diameter caused by the *Le* locus increases stem stress 1.43 fold, and the increase in height also increases the stress on the stem 2.82 fold. When all factors are considered together, tall lines would have 3.96-fold higher stem stress than dwarf lines. It is also important to note that *Le* lines also had a very strong reduction in side branch diameter across all site-years when compared to *le* lines. A weak effect was seen on epicotyl diameter. These effects are not accounted for in the stress equation.

Lodge I-1 (Putatively the *Afila* Locus)

The *af* allele displayed a strong positive correlation with lodging resistance. It is widely accepted that the increase in lodging tolerance is caused by the ability of the multiple tendrils and novel leaf shape of *af* genotypes to allow adjacent plants to intertwine their tendrils and support each other (Stelling 1989; Tar'an et al. 2003; Kof et al. 2004, Mikel 2013, Klimek-Kopra et al. 2015). Several major QTLs for traits involved in lodging resistance (including main stem diameter and compressed stem thickness) co-located at the *Af* locus but in these cases the Delta derived allele pleiotropically decreased main stem, side branch, and epicotyl diameter, with the *Af* gene being the second most important gene influencing main stem diameter after *Le* (p= 2.1×10^{-14}). In the RIL population *af* accounted for 7-9% in the variance in lodging, but the actual impact of the

semi-leafless habit probably was greater because the effect of increased tendril number had to overcome the effect of reduced stem diameter. The stress equation predicts the decrease in stem diameter and stem wall thickness in *af* lines increases stress on the stem by 1.37 fold, indicating that this locus has contradictory effects on lodging resistance.

The predicted increase in lodging susceptibility due to reduced stem diameter in *af* lines appears to be more than offset by the increased tendril number present in *af* lines. The 0.20 mm increase in stem diameter in *Af* lines also increased compressed stem thickness in *Af* lines by 0.12 mm when averaged over site-years. The *af* locus also affected side branch diameter when averaged over all site-years and the same effect on wall thickness was seen, but the effect was weaker. There was very strong evidence that epicotyl diameter was affected in the same manner as stem and side branch diameter ($p=6.8 \times 10^{-9}$), with *af* types having narrower epicotyls.

Lodge III-2 (The *Hr/Rms1/M* Region)

The RER parent was originally chosen to cross with Delta because it had good lodging resistance. It was hoped that introgression of alleles from RER into Delta would improve lodging resistance. This QTL represents the major contribution of RER to lodging resistance. It appears to act by increasing the amount of branching (both basal and aerial) (Table 40). The *Rms1* locus would be the most likely candidate for controlling branching in this region, although *Hr* has also been suggested to increase branching (Murfet and Reid 1993). Unfortunately, it was not determined whether RER has a different allele at the *Rms1* locus than Delta, and the identity of the gene responsible for

the increased branching remains unresolved. Increasing the number of side branches increases the number of tendrils per plant, presumably increasing lodging resistance. This is a similar effect as the *af* allele, which concurrently increases the number of tendrils per plant and lodging resistance.

Aerial branching occurs when the upper part of the plant (generally above the tenth node) develops side branches coming from each node. Lodging may be affected by aerial branching because plant weight and the number of tendrils per plant are increased, but the size of the effect of aerial branching is unknown. Aerial branching is diagnostic of *Rms1* (Murfet and Reid 1993) and mutations in genes affecting strigolactones (Beveridge et al. 2009).

In addition to increasing branching and reducing lodging, the RER allele also decreased main stem diameter, a change that would predict higher stem stress. However, side branch diameter and compressed side branch thickness was increased in lines with the RER-derived allele, indicating that this allele shifted resources to the side branches at the expense of the central stem, likely compensating for the decreased main stem diameter. This QTL was the most important QTL for compressed side branch thickness in the population. This QTL was the most important QTL for epicotyl diameter, with the RER allele increasing epicotyl diameter when compared to the Delta allele. From the data available, it is likely that increases in branch number and stem diameter are the likely sources of lodging resistance for this QTL.

Lodge III-3 (SSR-AD73)

This study may confirm a lodging QTL found in the middle of LG III in two previous studies (Tar'an et al. 2003, Ferrari et al. 2016). The QTL found in Ferrari et al was approximately 30 cm below the ADH1 marker, which was mapped very close to the LKA marker on upper LG 3 in this population (ADH1 not shown). The QTL found in Tar'an et al. (2003) was in the same region as the QTL found in this study based on the position of anchor markers J12_1280, NI_720, and *Le* when the maps of Laucou et al. (1998) and Tar'an (2003) are compared. However, in contrast to the QTL found in Tar'an et al. (2003) and Ferrari et al. (2016), Lodge III-3 did not appear to affect plant height. In alfalfa, a QTL for lodging was found in approximately the same location in the middle of LG III (McCord et al. 2013) based on a map of synteny with pea (Zhu et al. 2005). This QTL also controls hundred seed weight, which was also reported previously (Ma et al. 2017).

Lines with the allele from the Delta parent always had a larger main stem, side branch, and epicotyl diameters than lines with the RER derived allele. Compressed main stem and side branch thicknesses increased concurrently with stem and side branch diameter, suggesting a model where the allele from the Delta parent increases stem diameter by increasing compressed stem thickness. The increase in stem diameter reduces lodging by increasing mechanical strength. Based on the stress equation, the increase in stem diameter and compressed stem thickness will reduce stress on the stem by 1.15 fold. This allele had the same affect across all site-years.

When averaged across all site-years, there was weak evidence to indicate that lines with the allele from Delta had higher yield and yield per plant. Normally it would be expected that increased yield would increase lodging, but this effect was not seen at this locus.

<u>Lodge II-1 (Putatively *A*)</u>

The mechanism of the lodging effect produced by the QTL overlapping the *A* locus is difficult to assign. In theory, the effect may be directly attributable to variation at *A* through lignin content in the stems because lignin, as well as anthocyanins and tannins are products of the phenylpropanoid pathway. In lentil it has been shown that the gene orthologous to the *A* gene in pea controls the production of dihydromyricetin, myricetin-3-O-rhamnoside, flavan-3-ols, and proanthocyanidin oligomers in the phenylpropanoid pathway (Mirali et al. 2016). However, lignin biosynthesis is in a different branch of the phenypropanoid pathway than synthesis of flavonoids (Ring et al. 2013). This study did not assess stem lignin content, and an interesting area of future research would be to assess whether there was a significant difference between stem lignin content in *a* vs. *A* lines.

Pullan and Hebblethwaite (1990) predicted that high seeding rates increase lodging. However, in this study % emergence was negatively correlated with lodging during every site-year (see Chapter 7), indicating that lodging decreases as % emergence and stand density increases. This QTL for lodging might be directly attributable to variation in *A* because the QTL centered at *A* had a strong effect on % emergence (p=

8.92×10^{-12} when analyzed on all 254 lines). Ideally lodging should be assessed at a uniform plant density, but the effect of this region on germination was previously unknown. The effect on emergence by the *A* locus is possibly due to the increased tannin content in the seed in colored flowered (*A)* lines. There is moderate evidence to indicate that tannin content influences seed dormancy (Mirali et al. 2016). Variation in seed dormancy was implicated because removal of a small portion of the seed coat caused the seeds from colored flowered lines to germinate in this study. Physical dormancy as found in legume crops involves development of a water-impermeable seed coat, caused by the presence of phenolics and suberin-impregnated layers in cell walls in the seed coat. Domesticated varieties of peas, which have rapid germination, have been shown to possess thinner seed coats and substantially lower lignin in their seed coats than wild type peas (Smykal et al. 2014). If the *A* locus is influencing lodging through an effect on % emergence, the most likely cause would be by increasing plant density and thereby the ability of adjacent plants to intertwine tendrils. This region also contained the second most important QTL for yield in the Delta x RER population. The QTL for % emergence co-located with the QTL for yield, suggesting that at the planting density used, more plants generated greater yield. The positive correlation between the major yield QTL and lodging resistance indicates that yield was not a major factor influencing lodging although the stress equation predicts that an increase in yield should increase stem stress. It should be noted that the planting density associated with the most lodging resistance is unknown. The other QTLs that overlapped the *A* region were not centered at the *A* locus,

and are not considered possible alternative explanations for the effect of this region on lodging.

Lodge I-2 (Marker A7384)

This lodging QTL explained 8.7% of the variation in basal branch diameter with the allele from the Delta parent increasing side branch diameter when compared to the RER parent (p=0.006). Stronger effects were found at the Moccasin location than the Bozeman location, but the effect was consistent across all site-years. An increase in basal branch diameter will decrease stem stress and reduce lodging. This QTL was found during both site-years in Moccasin, MT, but not in Bozeman, MT. Across all site-years there was strong evidence that the Delta derived allele of this QTL increased maturity time, nodes to first flower, and nodes to senescence. The amount of lodging that occurs is dependent on the amount of time that passes between crop senescence and harvest. Fields harvested at senescence will generally have less lodging than fields harvested a month later, due to the effects of rainfall, wind, and decomposition. While lines in Bozeman were rated at senescence, lines in Moccasin were rated for lodging only once during each site-year. Therefore, it is possible that earlier maturing RILs would have lower ratings of lodging resistance because the period between senescence and the lodging evaluation was longer for early maturing lines. Crop standability declines after senescence due to the effects of rain, wind, and decomposition. Therefore, this QTL should be confirmed in a study where maturity time is controlled. The method of data collection and the effect on basal branch diameter are the putative mechanisms for the lodging effect of this QTL.

Lodge II-2 (A7258)

There was moderate evidence to indicate that this allele influenced main stem diameter and compressed main stem diameter, lines with the Delta allele had 0.12 mm larger main stem diameters than lines with the RER derived allele. The same effect was seen for side branch diameter. Lodging resistance is positively correlated with increased main stem and side branch diameter. Based on the stress equation, lines with the RER derived allele would have 1.18-fold greater stem stress than lines with the Delta derived allele.

Lodge II-3 (*Mo*)

There was moderate evidence to indicate that the Delta allele increased side branch diameter during 2014 and 2015, but the effect was weak when averaged across all site-years (p=0.092). There is strong evidence to indicate that lines with the allele from the delta parent had higher yield when averaged across all site-years. Due to the location of this QTL and its effect on yield, it is possible that the gene responsible for this lodging QTL is *Mo*, a gene which bestows resistance to several viruses (Choi et al. 2012). Symptoms of pea seed-borne mosaic virus were present during both 2014 and 2015, but the specific virus causing the infection was not identified. A model is suggested whereby *Mo* mediates the plant's response to viral infection. It is therefore quite possible that this QTL has no effect on lodging by itself, and that viral infection merely affects lodging. The strong reduction in yield found in 2014 when virus symptoms were prevalent in the trial indicate that this locus was having an effect on virus resistance; however, a region 10

cM long was important for lodging when averaged over all site-years, so the effect of other genes cannot be ruled out.

Lodge I-3 (A5967, A2259)

The putative Lodge I-3 QTL is located 17 cM above the *Af* locus on LG I. This QTL may be merely significant because of linkage drag with *Af*, but there is some evidence that it is a separate QTL. Firstly, there was one site-year where this QTL had a greater effect on lodging than the *Af* locus, despite being 17 map units away, and secondly, there is moderately strong evidence that this section of LG 1 is a QTL for aerial branching (p=0.0014). The *Af* gene has no effect on aerial branching, and it is likely that the mechanism of this QTL is through affecting aerial branching. Aerial branching can result in more tendrils being produced per plant, but it also increases the weight of the upper part of the plant. It is unknown how much aerial branching affects lodging, a question which should be addressed in future studies. This QTL is very close to the *Af* locus, and additional work needs to be done to confirm this QTL when the effect of *Af* is excluded. Analysis of this QTL when the effect of *Af* was excluded failed to find a significant difference at Lodge I-3, but sample size was very small, with only 12 lines in one group. Until confirmed, this QTL is only putative.

Lodge VI-1 (A1580)

Tar'an et al (2003) identified a lodging QTL on LG VI accounting for 24.6% of the variation in lodging. The present study may confirm this QTL, but it is difficult to

assess the specific map positions of the two QTL. Laucou et al. (1998) places the markers G4_2000 and B7_1750 on the proximal and distal ends of LG VI. However, Tar'an et al. (2003) places the two markers on the top and upper middle regions of a much longer LG. The discrepancy between the two maps may be due to different populations being investigated. If the Tar'an map is inverted, it is likely that the QTL found in this study and the previous study are one and the same. Moreover, it appears that the marker B7_1750 is on the lower section of LG VI in the Loridon et al. (2005) map, which would place the QTL found in this study in the appropriate location. However, the lack of consensus markers makes it difficult to determine the location of the QTLs. The marker developed by Tar'an was not repeatable in this population.

The effect of this putative QTL was very weak. It had a p= 0.13 and an expected false discovery rate of 0.388 when averaged over all site-years. The high p reflects that this QTL had the same effect in only 5 site-years, with a non-significant interaction occurring in 2014. While the evidence for this QTL was weak, the increase in side branch diameter in lines with the Delta derived allele would decrease side branch stress by 1.17 fold according to the stress equation. Main stem diameter appeared to be affected in the same manner. This QTL was the second most important QTL for leaf width and the third most important QTL for leaf length. The secondary effect on basal branching was not directly centered over Lodge VI-1, and probably should be disregarded for the present. The putative effects of this QTL may suggest a mechanism of lodging resistance whereby this QTL increases stem diameter concurrently with leaf size.

Yield and Lodging

The stress equation predicts that increasing the load on the plant stem (e.g. increasing yield) would decrease lodging resistance. However, yield and lodging resistance were positively correlated in this population, with six of the lodging resistance QTLs also appearing to increase yield. The exception to the rule was the *af* allele, which has been previously shown to decrease yield based on the semi-leafless habit. It is well known that lodging destroys canopy structure and reduces yield. Therefore, while it is likely that increases in yield will increase stem stress, the reduction in lodging associated with other effects of lodging QTLs will increase yield.

Summary of the Lodging QTLs

This study determined that there are a number of loci likely to be responsible for lodging in peas. Most of these loci control stem diameter, but there are also a number of QTLs and putative QTLs that control plant height, basal branching, aerial branching, seed dormancy, maturity time traits, or leaf (tendril) length. Basal branching appeared to increase lodging resistance and yield. It is important to note that sucker branches, which produced less than one pod, were not counted in this experiment. In almost every instance, the basal branches counted in this experiment possessed pods that could be harvested mechanically and were a similar height as the main stem. It should be noted that the branching QTL on upper LG 1 likely was not associated with lodging due to a pleiotropic effect on stem diameter. Increases in stem diameter appeared to consistently decrease lodging, with the *Af* locus an exception to the rule. Leaf area determines the

likelihood that a plant will find support, but there was inconclusive evidence that leaf

length or width influenced lodging in this study. There was no evidence that lodging

susceptibility and yield were positively correlated, indicating that breeding for higher

yield will not decrease lodging resistance. There was also no evidence that lodging was

correlated with nodes to flowering or total node number. The results of this trial also

indicated that lower emergence may increase lodging susceptibility, but the evidence was

inconclusive. In almost every instance, the favorable allele for lodging resistance was

derived from Delta, which has good lodging resistance when compared to many other

commercially grown varieties.

The Stress Equation and the Empirical Data (Delta x RER population)

The stress equation for a tubular cantilever beam [**stress** (force/unit of

area)=$WL^2/2Z$] identifies 4 specific factors that should influence lodging in a hollow-

stemmed plant such as pea: load or yield potential (W), stem height (L), stem diameter

(r), and stem wall thickness (t). In the equation, $Z=\pi*(r^4-(r-t)^4)/4r$ (Gere 2004;

http://www.atcpublications.com/Sample_pages_from_FDG.pdf Accessed 11/10/2016.)

The stress equation is frequently used to predict the relative amount of stress on tubular

beams in many everyday applications in engineering. The variation in each of these

factors and the genetic basis for this variation were analyzed in two populations derived

from two relatively erect (lodging resistant) but genetically very divergent parents.

Although two of the above factors proved to strongly influence lodging in the population,

other factors not intrinsic to the equation also were important. These additional factors

included the ability of the plant to form an interlocking network of tendrils with

neighboring plants, the number of branches produced by the plant, stand density, and

other unidentified factors that may involve maturity, resistance to seed-borne mosaic

virus, and yield.

The Delta x RER population was analyzed to determine how well the stress

equation predicted lodging in the empirical dataset. With the component of height

removed, main stem diameter alone predicted less than 6.9% of the variation in lodging

in this population. When height was included in the model, the engineering stress

equation for a tubular cantilever beam explained 28.8% of the variation in lodging in the

Delta x RER population. With stem wall thickness held constant at 0.44 mm (the average

for all lines), the model functioned slightly better, explaining 31.1% of the variation in

lodging. In comparison, height alone predicted 45.8% of the variation in lodging. It is

important to note that the low percentage of lodging predicted by the stress equation is

likely caused by Lodge I-1 (putatively *Afila*), which decreases stem diameter but reduces

lodging. Within *afila* and *Afila* lines alone, respectively, the stress equation predicted

45.6% and 46.5% of the variation in lodging based on main stem radius, main stem wall

thickness, and height. When main stem radius, side branch radius, and epicotyl radius

were averaged and main stem and side branch wall thickness were averaged in the model,

the model explained 57.4% and 58.6% of the variation in lodging in *af* and *Af* lines,

respectively. Overall, the stress equation fit the empirically derived data fairly well and it

explained more of the variation in lodging than height alone.

Correlation Matrix for Lodging and Stem Strength Traits (Delta x RER Population
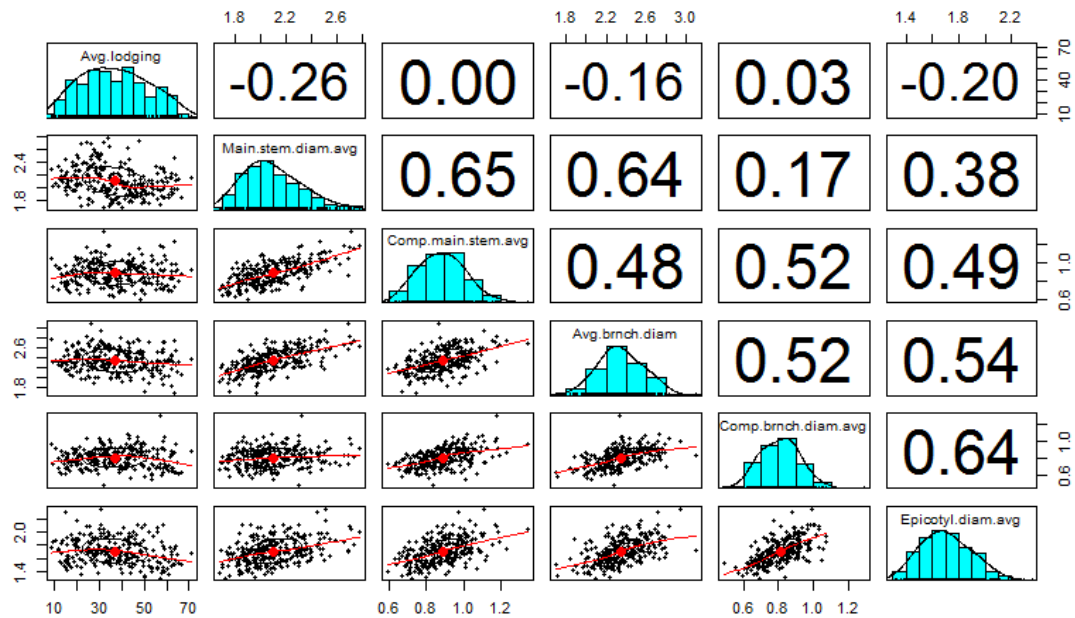
Figure 10: Correlations with lodging and other traits along the diagonal are displayed in the top row for the Delta x RER population. The correlation between lodging and main stem diameter, branch diameter, and epicotyl diameter (histograms 2, 4, and 6) are significant (p= 0.00002, 0.013, 0.0016, respectively) and negative across all site-years, indicating that these traits influence lodging. Compressed main stem diameter (row 3 and column 3) and compressed branch diameter (row 5 and column 5) had no significant correlation with lodging.

The Stress Equation and the Empirical Data (PR population)

An analysis was done of the PR population (Appendix C), which was not segregating for *Le* or the *Afila* locus, to determine how well the stress equation predicted lodging. When main stem, side branch, and epicotyl diameter were averaged, the average value predicted 26.4% of the variation in lodging. In contrast, the stress equation predicted 30.0% of the variation in lodging in this population based on stem diameter alone. Height alone predicted 66.15% of the variation in lodging in the PR population. In contrast, including height in the stress equation predicted nearly 61% of the variation in

lodging (Figure 11). It is important to note that stem diameter and height are strongly and negatively correlated in peas which is why height predicted lodging as well as the stress equation in this instance. However, based on how well the empirically derived data fits the stress equation, it is apparent that the stress equation is a highly credible model for lodging resistance in peas.
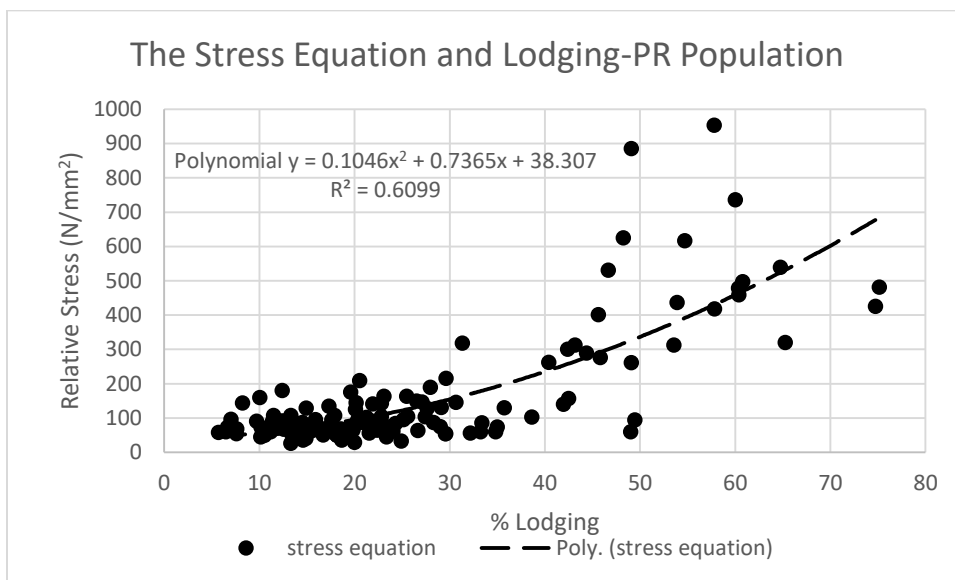
Figure 11: The stress equation and lodging (PR population)

<u>The Effect of Wall Thickness on Stem Stress</u>

It is clear, based on the stress equation and empirical data that stem diameter, wall thickness and plant height do play a role in lodging. Therefore, mathematical calculations using the stress equation can be made to predict which component is most important. The effect of stem wall thickness was analyzed by holding the load, radius, and length of the tube constant. Holding these parameters constant and varying stem wall thickness will allow the effect of wall thickness on stem stress to be determined (Figure 12).

Stress on a Stem with Radius Held Constant at 2mm and Wall Thickness Varying between .1 and 2 (Increasing at .05mm Increments)
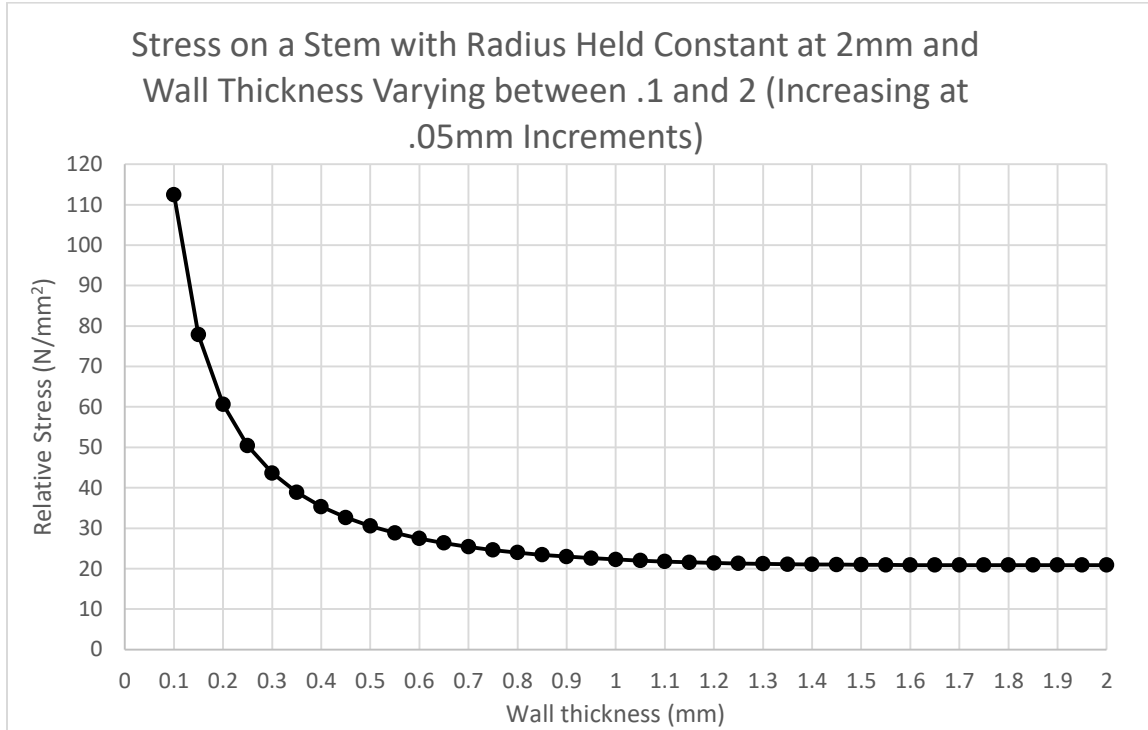
Figure 12: Stem diameter and stem stress. Load, height, and stem diameter were held constant at .001 N/mm2, 514 mm, and 4mm, respectively. Stem wall thickness was varied from .1 mm (thin walled) to 2 mm (solid stemmed) in order to determine the effect of wall thickness on stem stress. Based on this analysis, stem stress is reduced with increases in stem wall thickness but at a declining rate. Based on the average stem wall thickness observed in the Delta x RER population (.44 mm), it is expected that increases in stem wall thickness would not have a significant effect on stem stress.

Based on the stress equation, RILs with a larger stem wall thickness will have less stress than genotypes with a lesser radius and smaller inner stem diameter, but since the maximum bending stress of a beam is focused on the outer portion of the stem where it is either being compressed or stretched, adding material to the middle portion of a beam has only a slight effect (Figure 12). This is why hollow tubes are preferred in many structural engineering applications. Breeding for a more solid stemmed pea line would decrease stem stress, but only slightly. As can be seen in Figure 12, the stress on a hollow stem follows an exponential decay curve with each incremental increase in wall thickness

reducing stress on the stem less than the previous increase in wall thickness. The relationship is not linear.

The stress equation indicates that increasing the wall thickness of a stem beyond 20% of the radius's width would decrease stress on the stem very little. Increases in stem area increase the load on a stem due to added weight, although it is unknown what percentage of the load on a stem is due to stem material itself. Figure 12 shows that stress is reduced 5.39 fold by making the stem solid. However, increasing the wall thickness of a stem increases the area of stem material by a factor of x where $x=(\pi r^2-\pi(r-T2))/(\pi r^2-\pi(r-T1))$, r=radius, and T1 and T2= thickness 1 and 2 respectively. In this case stem area increases 10.3 fold, which would drain resources from the plant in plants with solid stems and also add weight to the stem. It is important to note that the average stem wall thickness in the Delta x RER population was 0.44 mm, which is already ideal. Empirical data from this study indicates that in many pea varieties the wall thickness of a stem is generally around 1/3 of the radial diameter. Therefore, it is unlikely that selecting for increased compressed stem thickness would have an effect on lodging resistance, since the model shows that the gains in stress resistance are nearly asymptotic at that point. Therefore, breeding efforts should not be focused on increasing stem wall thickness in pea if current germplasm has wall thicknesses that are sufficient.

The Effect of Stem Diameter on Stem Stress

In many cases in nature, plant species have hollow stems. In the angiosperms, this trait is very prevalent, probably due to the fact that hollow stems are a much more

efficient use of plant material, which cost the plant in terms of energy. However, it is

worth noting that increasing stem diameter also comes at a cost to the plant since the

amount of plant material increases by a factor of y with every increase in radius

(assuming wall thickness is held constant), where $y= \pi r_2^2/\pi r_1^2$ and where r_1 and r_2 are the

initial radius and the increased radius, respectively. The relationship is not linear. The

model below (Figure 13) estimates stress on stems that vary from 1-5 mm in diameter

(radii from 0.5 to 2.5mm), which is the normal range in pea. The model held wall

thickness constant at 0.44 mm (the average wall thickness in the Delta x RER population)

and plant height constant at 51.2 cm (the average height in the Delta x RER population).

Only stem diameter was varied.
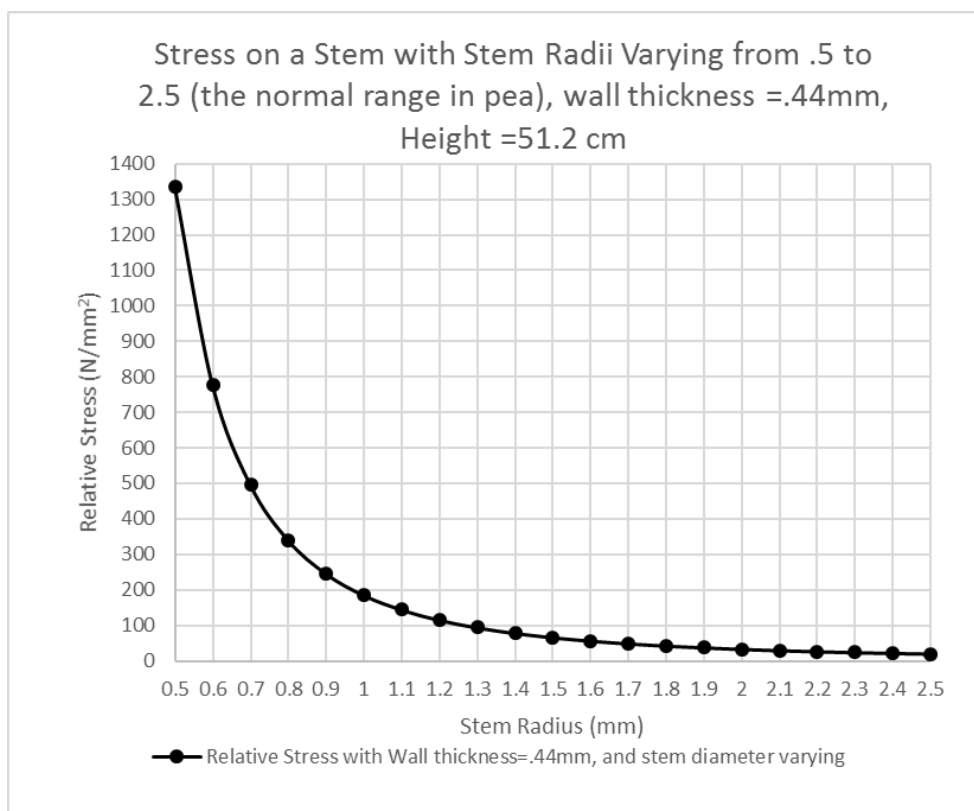
Figure 13: The stress equation and stem diameter

This model shows that increasing stem radius five-fold from 0.5 to 2.5 mm would decrease the amount of stress on the stem by 308.3 fold when wall thickness is held constant at .44 mm. The stem area would also increase by 17.1 fold. The fold reduction in stress decreases with decreases in stem wall thickness and increases with increases in stem wall thickness. For example, cutting stem wall thickness by half to 0.22mm while simultaneously increasing stem radius from 0.5 to 2.5 mm would reduce stem stress by only 182.7 fold rather than 308.3 fold. However, the stem area would only increase by 12.5 fold rather than 17.1 fold. As mentioned previously, increasing stem wall thickness has a pronounced effect on stress reduction when the wall thickness is less than 25% of the radial diameter, but the reduction in stress eventually becomes asymptotic as a stem becomes more solid.

The stress equation indicates that increasing stem diameter is the key to reducing the stress on the pea stems and hence lodging resistance in pea cultivars. A previous study by Beeck et al. (2006) suggested that compressed stem thickness was the most important factor in determining lodging resistance. However, that previous study did not collect empirical data on lodging, and this study indicates the opposite, finding weak evidence to indicate that compressed stem thickness plays a role in lodging, based on empirical data and mathematical modeling. This study indicates that main stem diameter, epicotyl diameter, and side branch diameter are the most important factors when modeling the effect of stem diameter traits on lodging resistance.

The Importance of the Stress Equation: Conclusions

If load doubles due to a doubling in yield, the amount of stress on a stem will also double. Stress increases in a completely linear fashion when load is increased. Therefore, increasing yield by 10% will also increase stem stress by 10% based on the stress equation.

If plant height is changed without a change in other parameters, the amount of stem stress will increase by a factor of H_2^2/H_1^2, where H_2 indicates the final height and the H_1 indicates the initial height. Doubling plant height will increase stress by fourfold because length is multiplied to the second power.

Pea stem diameter, which ranges from ~1-5 millimeters in peas, also has a dramatic effect on the stress equation since the radius is multiplied to the fourth power, this effect is highest when stem wall thickness is at least ¼ of the radial thickness. Because the value of Z is in the denominator of the stress equation, larger values of Z (larger radii or greater stem wall thickness) will always result in smaller stresses on the stem.

Pea stems are nearly always hollow, sometimes with or without a weak pith in the middle of the stem. It is likely that hollow stems were an adaptation in peas and other plants because they have higher mechanical strength per unit of plant material. It is important to understand that the effect of compressed stem thickness on stem stress is highly dependent on the diameter of the stem. The average stem wall thickness in the Delta x RER population was approximately 0.44 mm when averaged over all site-years, with a range of 0.30 to 0.67 mm. When radius is held constant at 1.05 mm (the average

stem diameter in the Delta x RER population), there is a 1.34-fold difference in stem stress between the inner bound (.30 mm) and outer bound (0.67 mm) of the range of stem wall thicknesses found in the Delta x RER population. Stem area also is affected by 1.8 fold. This is a relatively weak effect when compared to the effect of stem diameter. Doubling stem radius from 1.05 mm to 2.10 mm will decrease stem stress by 5.5 fold with a 2.27-fold increase in stem area, indicating that wall thickness has a much lower effect per unit of plant material than stem radius, when values are restricted to the normal range in pea.

The stress equation is a robust engineering model that can predict lodging when estimates of stem diameter, stem wall thickness, and plant height are known, but more importantly, it allows the effects of changes in different parameters of the model to be determined in a theoretical sense. The stress equation creates a solid theoretical framework for lodging resistance in pea. However, the stress equation also provides a theoretical model to predict lodging resistance in rice, corn, wheat, and other crops where lodging susceptibility is an issue. While the stress equation predicted 30% and 60% of the variation in lodging in the two RIL populations, respectively, it should be noted that the stress equation would likely be far more accurate in other crops, because the effect of tendril vigor would be eliminated. However, more research would need to be done to prove the model in other crops.

CHAPTER FIVE: QUANTITATIVE TRAIT LOCI FOR SEED WEIGHT AND YIELD

IN DRY PEA

Introduction: An Analysis of Source Sink Relationships in Pea

Yield potential is the genetically determined ability of a crop to generate optimal

yield in a given growth environment (Patrick and Colyvas 2014). Yield potential is

thought to be partially determined by seed size, and a number of studies have tried to

understand the relationship between seed size and yield in pea and other pulses (Gusmao

et al. 2012, Bing and Lui 2011, Bicer 2009, Krajewski et al 2012, Irzykowska and Wolko

2004, and Timmerman-Vaughn et al 1996 and 2005). Weeden (2007) proposed that

increases in seed size was a major part of the domestication of peas.

The results of research on seed size and yield in various legumes are contradictory

in many respects. A lack of correlation between seed size and yield was found in grass

pea under drought conditions (Gusmao et al. 2012). In chickpea, a positive correlation

was found between seed size and yield, but no effect was seen in lentil (Bicer 2009). In

one study in pea, a strong correlation was found between seed size and yield (Bing and

Lui 2011), and Krajewski et al. (2012) found that seed size was positively correlated with

yield, explaining 20% of the variation in yield in pea, but peduncle number explained

nearly 50% of the variation in yield, indicating that seed size is not the most important

factor in determining seed yield. Other research has also shown a correlation between the

number of fertile nodes and seed yield rather than seed weight and seed yield (Kosev and

Mikic 2012). Timmerman-Vaughn et al. (2005) indicated that seed size is negatively

correlated with seed yield, contradicting the study by Krajewski et al (2012). Irzykowska and Wolko (2004) indicated that QTLs for seed size and yield were not co-located. Gambin and Borras (2010) concluded that crop yield within a species was more related to variations in seed number than in seed weight. Hence, there appears to be no consensus regarding the relationship, and the ideotype for seed size has not been defined in pea.

The production of seed in pea requires fixed (eg. peduncle, flowering nodes, and stem material) and variable (e.g. seed protein, mineral content) costs to the plant. A hypothetical plant that could produce 100 g. of seed, could produce 400 seeds that weighed a 0.25 g., or 100 seeds that weighed 1 g (Sadras 2007). The consideration of the plasticity of yield components is important when determining the trade-offs between seed size and seed number (Sadras 2007). Photosynthates can be partitioned into pods or seeds in pea. Assuming that pod wall thickness is the same in large seeded and small seeded genotypes and that pod length is the same, we can determine the relative amount of photosynthate required to produce pods and seeds of a certain size. Pod material is photosynthetic so it may not be a major burden for the plant to produce, but it is likely that the pods are a sink, especially during their formation.

A previous study in lupin estimated that for every 100 units of carbon imported from the parent plant into pods or seeds, 52 are incorporated into seeds, 37 into the pod and the remaining 11 units are lost as CO_2, indicating that up to 41.5% of photosynthate may end up in pod material rather than seed in lupin (Pate et al. 1977). However, 96% of the fruits nitrogen becomes incorporated into seeds, with 16% of that being remobilized from pod material, indicating that seeds may be more nutrient rich than pods. Data

indicates that approximately 7% of carbon is photosynthesized from the pod itself (Pate et al. 1977). It has been noted that photosynthates can travel relatively long distances in the plant (Liu et al. 2010). When compared to other legumes, peas have a much lower proportion of pod walls to percent pod biomass, and it is estimated that this percentage is around 13% (Huyghe 1998). There appears to be an association between low pod wall proportion and high seed yield (Huyghe 1998). In essence, it is reasonable to predict that it takes the same amount of photosynthate to create 1 kg of small seeded peas vs 1 kg of large seeded peas, but in reality the cost to the plant of producing small seeded peas is higher than the cost of producing large seeded peas because small seeded peas require more pod surface area per unit of mass and more flowering nodes. While it often increases fitness for wild germplasm to produce large numbers of small seeds rather than a few large seeds, wild varieties of pea are not engineered for high yields.

The ideotype for seed size needs to be defined in pea because seed size plays a major role in actual yield (seed yield – weight of seed planted). The recommended seeding rate in Montana is 80 seeds per m^2 (323,866 seeds per acre) (Dr. Perry Miller personal communication). In Canada the ideal seeding density is 88 plants m^2, but ideal seeding density varies depending on variety (Spies et al. 2010). It can be expected that a 1 g. increase in 100 seed weight would require 8 kg (17.6 lb) more seed per ha based on a seeding rate of 80 seeds per m^2. Based on the average annual pea yield in Montana, which is 1740 kg/ha (1548 lb/A) when averaged across 2001-2016 (www.nass.usda.gov), actual crop yield declines 0.46% with every 1 g. increase in hundred seed weight. Understanding the genes responsible for seed weight in pea will facilitate the

development of pea cultivars with the ideal seed weight, which is the seed size that maximizes actual yield at a specific yield potential.

This study attempted to determine whether QTLs for seed weight, seed yield per plant, and yield were co-located in pea. Therefore, a QTL analysis of 100 seed weight and yield was conducted in the Delta x RER RIL population. There are a number of studies that have attempted to discover QTLs for yield in dry peas, but the nature of yield related traits is that QTLs are often not stable across environments. A great need exists to confirm QTLs in multiple environments, which would allow plant breeders to select for QTLs that have a stable effect on yield and seed weight. This analysis on seed weight and yield could potentially be used to confirm QTLs that were found in other crosses.

Results: QTLs for 100 Seed Weight, Yield, and Yield Per Plant

Eleven QTLs were identified for traits related to seed size and yield in the Delta x RER population. Three of the QTLs for hundred seed weight co-located in the same positions as yield QTLs. All seven of the QTLs for yield co-located with the seven QTLs for yield per plant.
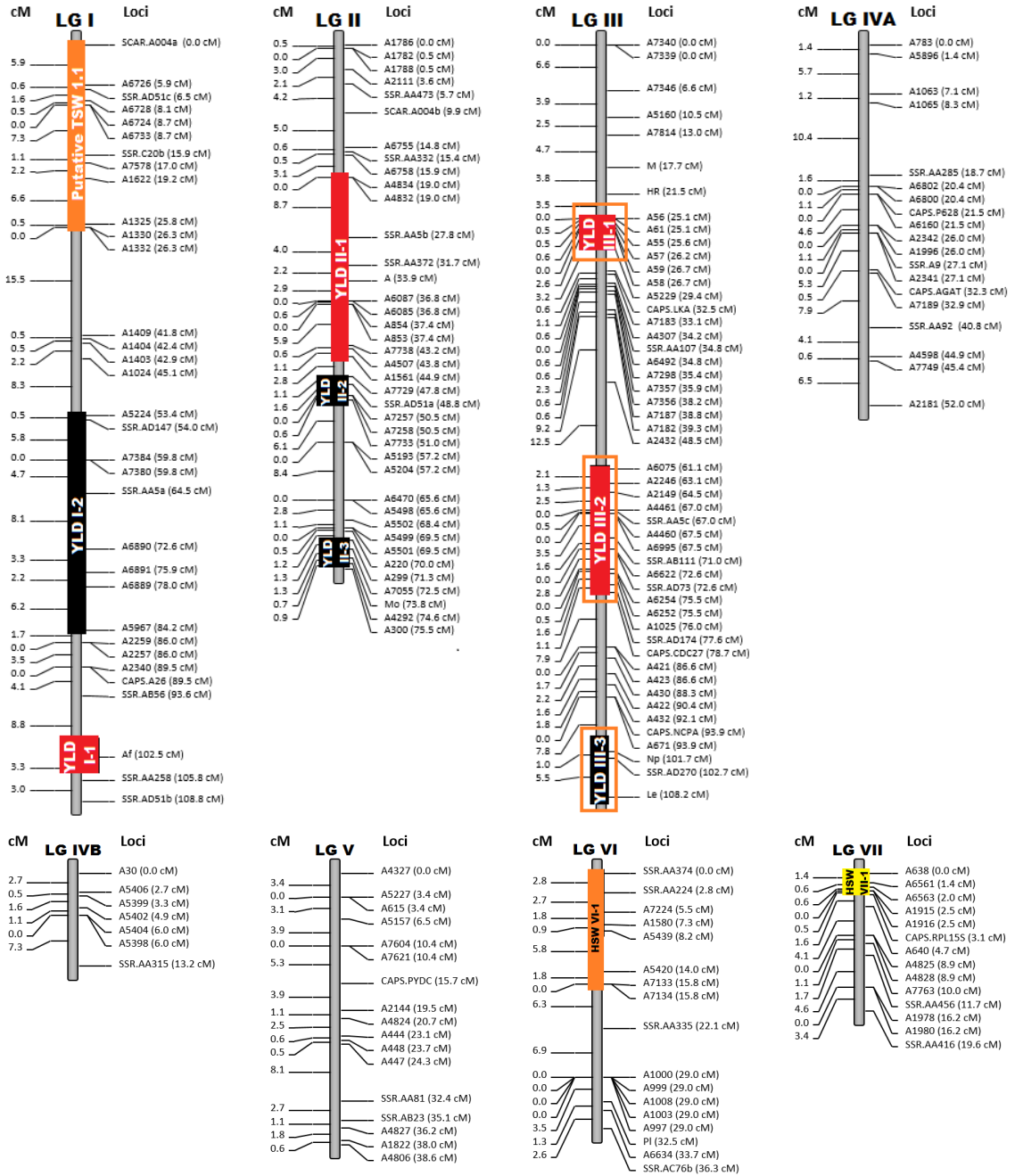
Figure 14: The genetic map showing the yield and 100 seed weight QTLs. Major QTLs for yield are in red. Minor QTLs for yield are shown in black. Major QTLs for hundred seed weight are shown in orange. Minor QTLs for hundred seed weight are shown in yellow. Orange boxes indicate where major QTLs for hundred seed weight and yield co-located on the chromosomes.

Table 41: QTLs for yield (analyzed on 94 dwarf RILs unless noted)

QTL Name and/or Closest Genetic Marker	LG	% Variation (Averaged across two site years) (1)	p-value (unadj., data averaged across site years) (2)	False discovery rate (Benjamini-Hochberg) (3)	Source of desirable allele (4)	Effect (lbs/A) (5)
Major QTL (strong evidence). Listed in order of importance (based on p-value)						
YLD III-1 (A55)	III	13.50%	0.0002	0.013	RER	755 kg/ha
YLD II-1 (*A*)	II	11.00%	0.0009	0.018	Delta	680 kg/ha
YLD III-2 (AA5c)	III	10.50%	0.001	0.022	Delta	659 kg/ha
Putative QTL (moderate or weak evidence). Listed in order of importance (based on p-value)						
YLD I-2 (A2259-A5967)	I	9.20%	0.0027	0.03	Delta	616 kg/ha
YLD II-2 (A7258)	II	8.50%	0.0044	0.05	Delta	610 kg/ha
YLD II-3 (*Mo)*	II	11.80%	0.0073	0.06	Delta	704 kg/ha
YLD III-3 (*Le*) (analyzed on all 254 lines)	III	2.10%	0.0220	0.10	Delta	302 kg/ha
YLD I-1 (*Afila)*	I	3.70%	0.063	0.21	RER	396 kg/ha

(1) % Variation is the percentage of variation explained by the QTL.

(2) P-value (Unadjusted)=this p-value is not adjusted for multiple comparisons.

(3) False Discovery Rate=The expected proportion of false positives.

(4) Source of desirable allele= The parental allele associated with the highest small plot yield.

(5) Effect= the mean difference in small plot yield between the two genotypes.

Table 42: Single marker analysis for yield on specific markers using all 254 lines in the Delta x RER population

Genetic Markers	LG	% Variation (Averaged across two site years) (1)	p-value (Unadjusted, data averaged across site years) (2)	False Discovery Rate (Benjamini-Hochberg) (3)	Source of Desirable allele (4)	Effect (lbs/A) (5)
A (YLD II-1)	II	11.10%	1.04×10^{-7}	0.00003	Delta	688 kg/ha
Afila (YLD I-1)	I	4.10%	0.001	0.022	RER	416 kg/ha
Le (YLD III-3)	III	2.10%	0.022	0.098	Delta	302 kg/ha

(1) % Variation is the percentage of variation explained by the QTL.

(2) P-value (Unadjusted)=this p-value is not adjusted for multiple comparisons.

(3) False Discovery Rate=The expected proportion of false positives after correcting for multiple comparisons.

(4) Source of desirable allele= The parental allele associated with the highest small plot yield.

(5) Effect= the mean difference in small plot yield between the two genotypes.

Table 43: QTLs for yield per plant (analyzed on 94 dwarf RILs unless noted)

QTL Name and/or Closest Genetic Marker	LG	% Variation (Averaged across all site years) (1)	p-value (unadj., data averaged across site years) (2)	False discovery rate (Benjamini-Hochberg) (3)	Source of desirable allele (4)	Effect (g/plant) (5)
Major QTL (strong evidence). Listed in order of importance (based on p-value)						
YLD III-1 *(A55)*	III	25.10%	2.616×10^{-7}	0.00003	RER	2.28 g
Putative QTL (moderate or weak evidence). Listed in order of importance (based on p-value)						
YLD II-1 *(A)*	II	8.50%	0.004	0.053	Delta	1.32 g
YLD II-2 (A7258)	II	7.90%	0.006	0.067	Delta	1.29 g
YLD III-1 (AA5c)	III	6.20%	0.015	0.111	Delta	1.04 g
YLD III-3 *(Le)* (Analyzed on all 254 lines)	III	2.30%	0.019	0.111	Delta	.74 g
YLD II-3 *(Mo)*	II	7.90%	0.039	0.179	Delta	1.29 g
YLD I-2 (A6891)	I	4.10%	0.049	0.263	Delta	.92 g
YLD I-1 *(Afila)*	I	2.60%	0.116	0.291	RER	.74 g

(1) % Variation is the percentage of variation explained by the QTL.

(2) P-value (Unadjusted)=this p-value is not adjusted for multiple comparisons.

(3) False Discovery Rate=The expected proportion of false positives after correcting for multiple comparisons.

(4) Source of desirable allele= The parental allele associated with the highest yield per plant.

(5) Effect= the mean difference in yield per plant between the two genotypes.

Table 44: Single marker analysis for yield per plant on specific markers using all 254 lines in the Delta x RER population.

Genetic Markers	LG	% Variation (averaged across all site years) (1)	p-value (Unadjusted, data averaged across site years) (2)	False Discovery Rate (Benjamini-Hochberg) (3)	Source of Desirable allele (4)	Effect (g/plant) (5)
A (Lodge II-1)	II	3.20%	0.005	0.05	Delta	.88 g
Hr (Lodge III-2)	III	3.70%	0.006	0.05	RER	.95 g
Afila (Lodge I-1)	I	2.70%	0.009	0.10	RER	.82 g
Le (Lodge III-1)	III	2.30%	0.019	0.11	Delta	.74 g

(1) % Variation is the percentage of variation explained by the QTL.

(2) P-value (Unadjusted)=this p-value is not adjusted for multiple comparisons.

(3) False Discovery Rate=The expected proportion of false positives.

(4) Source of desirable allele= The parental allele that increases yield per plant.

(5) The effect size is the mean difference in yield per plant between the two genotypes.

Table 45: QTLs for 100 seed weight (analyzed on 94 dwarf RILs)

QTL Name and/or Closest Genetic Marker	LG	% Variation (Averaged across two site years) (1)	p-value (Unadusted, single site year) (2)	False discovery rate (Benjamini-Hochberg) (3)	Source of Desirable allele (Larger seed size) (4)	Effect (g/100 seed) (5)
Major QTL (strong evidence). Listed in order of importance (based on p-value)						
Putative Tsw 1.1 (A6724)	I	39.20%	7.88×10^{-12}	9.64×10^{-10}	Delta	3.87 g
YLD III-1 *(Hr/Rms1/M)*	III	22.60%	1.41×10^{-6}	0.00002	Delta	2.97 g
HSW VI-1 (1580)	VI	11.40%	0.00085	0.008	Delta	2.07 g
Putative QTL (weak evidence). Listed in order of importance (based on p-value)						
YLD III-2 (AD73)	III	9.50%	0.002	0.01	Delta	1.91 g
HSW VII-1 (A1915)	VII	6.30%	0.015	0.05	Delta	1.57 g
YLD III-3 *(Np)*	III	5.60%	0.021	0.06	Delta	1.63 g

(1) % Variation is the percentage of variation explained by the QTL.

(2) P-value (Unadjusted)=this p-value is not adjusted for multiple comparisons.

(3) False Discovery Rate=The expected proportion of false positives.

(4) Source of desirable allele= The parental allele associated with the largest seed size.

(5) Effect= the mean difference in 100 seed weight between the two genotypes.

Table 46: Single marker analysis for seed weight on specific markers using all 254 lines in the Delta x RER population.

Genetic Markers	LG	% Variation (Averaged across two site years) (1)	p-value (Unadusted, single site year) (2)	False Discovery Rate (Benjamini-Hochberg) (3)	Source of Desirable allele (Larger seed size) (4)	Effect (g/100 seed) (5)
Major QTL (strong evidence). Listed in order of importance (based on p-value)						
Hr (YLD III-1)	III	18.90%	9.28×10^{-11}	3.85×10^{-9}	Delta	2.71 g
SSR-AD73 (YLD III-2)	III	7.70%	0.00002	0.0002	Delta	1.73 g
Np (YLD III-3)	III	5.40%	0.0003	0.002	Delta	1.45 g

(1) % Variation is the percentage of variation explained by the genetic marker.

(2) P-value (Unadjusted)=this p-value is not adjusted for multiple comparisons.

(3) False Discovery Rate=The expected proportion of false positives after correcting for multiple comparisons.

(4) Source of desirable allele= The parental allele associated with the largest seed size.

(5) Effect= the mean difference in 100 seed weight between the two genotypes.

Discussion: The Yield and Seed Size QTLs

All QTLs for plot yield and yield per plant co-located with each other, although the rank of importance for yield and yield per plant QTLs was not the same. Three QTLs for 100 seed weight co-located with QTLs for yield. It is important to note that these trials were planted in microplots at seeding rates ranging from 25 to 60 seeds/m^2, which is lower than the current recommended rate of 80 seeds/m^2. It is unknown whether plot size and seeding rate influenced the results of this study, therefore these QTLs should ideally be confirmed in additional research prior to being used in marker assisted selection.

Yield III-1: The *Hr/Rms1/M* Region (Near Marker A55)

A seed weight QTL in this region was previously reported by Timmerman Vaughan et al. (1996). This QTL was very significant for seed size (p=9.28x10^{-11} at the *Hr* marker) and more than a dozen other traits (Chapter 7) including yield and yield per plant. Lines with the RER derived allele yielded more than the Delta derived allele. It is unknown which gene is actually influencing the trait. The main effect appeared to be centered slightly below *Hr*.
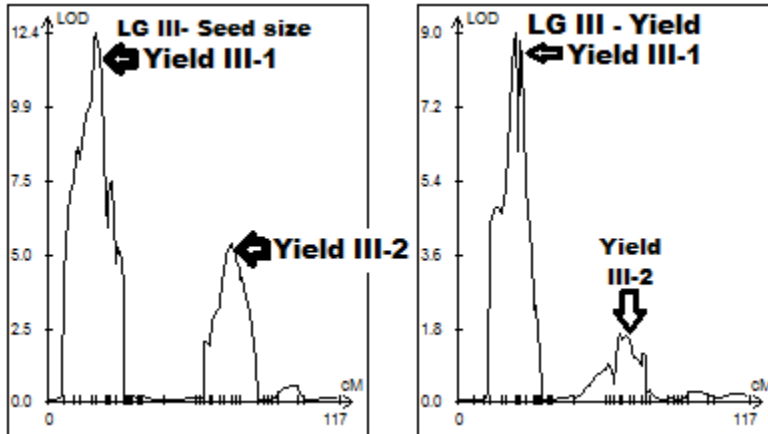
Figure 15: Composite interval mapping of QTLs for seed size (left) and yield (right) on LG III. This QTL had a relatively strong effect on seed size, and a weak effect on yield.

Yield II-1: The *A* Region

A QTL centered at the *A* locus was the second most significant QTL for yield, possibly because this QTL strongly affected % emergence and seed dormancy (Chapter 7). This locus also affected yield per plant. The effect on yield was centered at the *A* locus with flanking markers being less significant, indicating *A* may be the gene responsible.
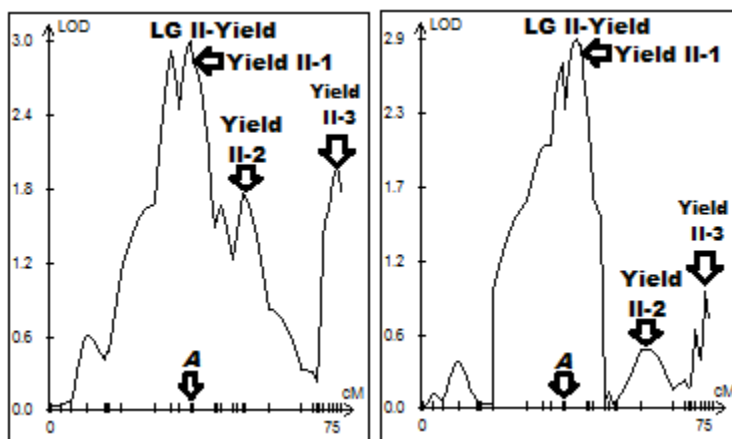
Figure 16: Interval mapping (left) and composite interval mapping (right) of yield QTL on LG II using Windows QTL Cartographer.

Yield III-2: The AD73 Marker

A recent paper indicates that the AD73 locus is a major QTL for 100 seed weight (Ma et al. 2017). This study confirms their findings, but it also indicates that this QTL influences yield and yield per plant. According to the model for 100 seed weight (Chapter 6), yield is slightly increased when seed size increases. This QTL was centered in the 6 cM region between SSR.AA5c and SSR.AD73. The gene underlying this QTL is unknown.

Yield I-1: The *Afila* Region

This field study appears to confirm previous studies that indicate that the *af* allele reduces yield (Burstin et al. 2007, Djordjevic et al. 2002). The effect on yield was centered at the *af* locus and flanking markers were less significant, indicating that *af* was the gene responsible. This result is consistent with the explanation that significantly more photosynthesis occurs in wild type vs *af* leaves (Sharma and Kumar 2012). The conversion of leaflets to tendrils diminished leaf area 1.5 fold when compared to the wild type. A lower leaf area index in *afila* genotypes is likely compensated by stipules of lower leaves, which are usually shaded in wild type varieties (Klimek-Kopra et al. 2015), synthesizing more, increasing the actively functioning assimilation area due to less shading. Given the same plant dimensions for each phenotype, wild type *Af* plants have more leaf area than *af* mutants, which likely has an effect on photosynthate production.
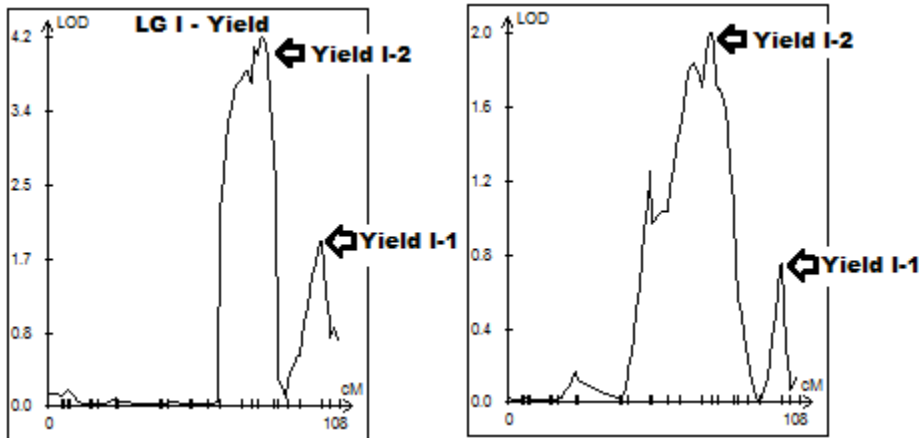
Figure 17: Composite interval mapping (left) and interval mapping (right) of QTLs for yield on LG I. Evidence for an effect at the *afila* marker (Yield I-1) was weak when analyzed on just dwarf lines, but it was strong (p= .001) when analyzed on all lines.

<u>Minor QTLs for Yield</u>

A QTL tentatively named Yield I-2 was found in the 30 cM region between markers A5224 and A5967, overlapping with a lodging QTL named Lodge I-2. The A6891 marker was the most important marker for yield, but it was not the most important marker for yield per plant. Marker coverage was poor in this region, and a greater marker density would be needed to pinpoint the exact location of the QTL.

It is likely that there is a yield QTL below *A* near marker A7258 (Figure 16). As it stands, a 15 cM region is significant for yield below and including the white flower locus. Although *A* is the most significant marker in that region, composite interval mapping indicated that there were two QTLs for yield on LG 2 with the second centered near the A7258 locus. This QTL has been named Yield II-2.

There appears to be a yield QTL centered at the *Mo* locus on LG II (Figure 16). This QTL has been tentatively named Yield II-3. The *Mo* locus is a gene which

influences resistance to several viruses, indicating that the putative mechanism of this

QTL is virus resistance. Symptoms of pea seed-borne mosaic virus were common during

the 2014 and 2015 field trials.

This field study indicates that a QTL encompassing both *Le*, Mendel's height

gene, and *Np*, a gene linked to bruchid resistance and pod structure, influences 100 seed

weight, yield, and yield per plant. Averaged across both site-years there was strong

evidence to indicate that this region influences 100 seed weight, which has been shown

by a previous study (Weeden 2007). The QTL for 100 seed weight was centered near *Np*,

but the QTL for yield was centered near the *Le* locus, indicating that separate QTL might

be involved. It is possible that the increased lodging associated with *Le* would influence

yield. This QTL is tentatively named Yield III-3. Due to the nature of the QTL, no

candidate genes are being proposed.

QTLs for 100 Seed Weight: Putative Tsw1.1

A major QTL was found for 100 seed weight. It was centered near a high quality

SNP marker named A6724. This QTL was located on the upper part of LG I. It appears

that this QTL is in a similar position as a QTL found in six previous studies

(Timmerman-Vaughan et al 1996 and 2005, Irzykowska and Wolko 2004, Gondo et al.

2007, Krajewski et al. 2012, Ferrari et al. 2016). However, due to a lack of consensus

markers between the studies, the correct orientation of LG I has to be assumed. This QTL

was previously called Tsw1.1. This QTL was the most important seed weight QTL when

averaged across both site-years, explaining 39.2% of the variation in 100 seed weight.

Lines with the Delta allele had higher 100 seed weights than lines with the RER derived

allele (18.08 g vs. 14.20 g, respectively). This QTL was not a QTL for yield
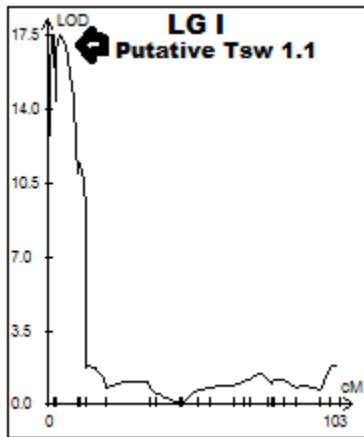
Figure 18: Composite interval mapping of putative Tsw 1.1 in Windows QTL
Cartographer

QTLs for 100 Seed Weight: HSW VI-1

There is moderate evidence of a QTL on the upper part of LG VI near the Lodge

VI-1 locus. This locus was centered on the SSR.AA374 marker. This locus explained

11.4% of the variation in 100 seed weight. This locus also affected lodging (see Chapter

4).
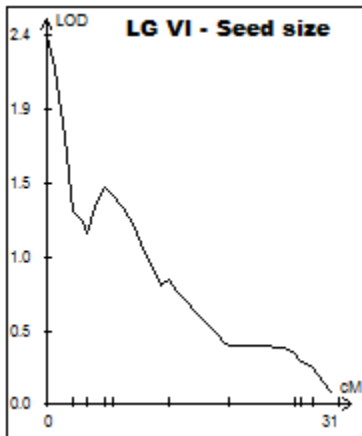
Figure 19: Interval mapping of LG VI in Windows QTL Cartographer.

HSW VII-1

There is weak evidence of a QTL on the upper part of LG VII near marker A1915. This QTL was tentatively named HSW VII-1. This QTL also had putative pleiotropic effects on aerial branching, compressed main stem thickness, and main stem diameter, but the effect was weak.

The Mechanism for Crop Yield Increases

Understanding the mechanism for crop yield increases would allow breeders to determine how crop yields are increased and what genes and traits to target. While some of this analysis is speculative in nature, it could be used as a guide to identify traits putatively linked to yield.

Table 47: QTLs for yield and QTLs for other traits.

Traits (Most lodging resistant phenotype shown)	YLD III-1 (Near A55, Hr, Rms1)	YLD II-1 (Putatively A)	YLD III-2 (near AA5c)	YLD I-2 (A5224-A5967)	YLD II-2 (near A7258)	YLD II-3 (near Mo)	YLD III-3 (Near Le, Np)	YLD I-1 (Putatively Af)
QTL for small plot yield (Highest yield)	1* RER (R)	2* Delta (D)	3* Delta (D)	4* Delta (D)	5* Delta (D)	6* Delta (D)	7* Delta (D)	8* RER (R)
Yield per plant (Highest yield)	1 (R)	2 (D)	4 (D)	7 (D)	3 (D)	6 (D)	5 (D)	8 (R)
Seed size	2 (D)	--	4 (D)	--	--	--	6 (D)	--
Lodging resistant parent	3 (R)	5 (D)	4 (D)	9 (D)	7 (D)	8 (D)	1 (D)	2 (D)
Plant height (shortest allele shown)	3 (D)	--	--	--	--	--	1 (D)	--
Main stem diameter (largest diameter shown)	9 (D)	5 (D)	4 (D)	--	6 (D)	--	1 (D)	2 (R)
Comp. mn. stm thickness (Thickest diam. shown)	--	--	2 (D)	--	4 (D)	--	3? (D)	1 (R)
Side branch diam. (largest stem diam. shown)	10 (R)	4 (D)	9 (D)	6 (D)	7 (D)	--	3 (D)	2 (R)
Compressed side branch thickness	1 (R)	--	5 (D)	--	--	--	--	4 (R)
Epicotyl diameter (largest diameter)	1 (R)	--	3 (D)	--	--	--	4 (D)	2 (R)
Basal branching (most basal branches)	1 (R)	--	--	--	--	--	2 (D)	--
Aerial branching (most aerial branches)	1 (R)	--	--	2? (D)	--	--	--	--
Maturity time (Latest maturing)	1 (R)	--	2 (D)	3 (D)	--	--	--	--
Nodes to 1st flower (fewest nodes)	1 (D)	3 (D)	--	4? (R)	2 (D)	--	--	5 (R)
Total node # (fewest nodes designated)	1 (D)	--	--	4? (R)	3 (D)	--	2 (D)	--
Avg # flowering nodes (most nodes)	4 (R)	2 (D)	--	--	3 (D)	5 (D)	1 (R)	--
Leaf length (longest leaves)	--	--	--	--	--	--	1 (R)	--
Leaf width (widest leaves)	--	--	--	--	--	--	1 (R)	--
% Emergence	1? (D)	3 (D)	--	--	--	--	--	--
Seed dormancy (lowest seed dormancy)	--	1 (D)	--	--	2 (D)	--	--	--

*The number indicates the rank of the QTL for each trait

-- Indicates that the QTL has no effect on that specific trait.

Red cells indicate a trait where the favorable allele for small plot yield and the favorable allele for the trait are not the same

? Indicates that the genes affecting yield and affecting the trait may not be the same

All genes that affected yield also affected lodging, and seven of the eight yield QTL showed positive associations between yield and lodging resistance. These data indicate that lodging resistance is not merely important for mechanical harvesting but also because it increases yield. When averaged across all site-years, the correlation between % lodging and yield was -0.30 (See chapter 7), indicating that lodging explained 9% of the variation in yield. Traits associated with lodging resistance such as stem diameter were also positively associated with yield in nearly every instance. With the exception of *Le*, all QTLs that increased the number of flowering nodes, also increased yield. In contrast, only two of the six QTLs for 100 seed weight showed a positive correlation between yield and lodging resistance. QTLs for basal branching and aerial branching appeared to be associated with yield (r= 0.34; Chapter 7). There appeared to be no clear pattern for QTLs associated with maturity time, total node number, leaf length,

and leaf width. The number of nodes to flowering was weakly associated with yield (r=-0.20, Chapter 7). Percent emergence was positively correlated with yield (r=0.44; Chapter 7). Increases in seed dormancy also appeared to decrease yield. Based on the above data, it is likely that increasing lodging resistance and increasing stem diameter, which is associated with lodging resistance, will increase yield.

CHAPTER SIX: A MODEL FOR SEED SIZE AND YIELD

Introduction

Mathematical modeling can explain why there is so little correlation found between seed yield and seed size in the Delta x RER population. Actual yield as defined here is the weight of seed harvested minus the weight of seed planted. The weight of seed planted is an important characteristic in peas because seeding rates are ~170 kg/ha (152 lb/A) (Perry Miller personal communication, Spies et al. 2010). Actual yield is a better measure of productivity than the common measurement of grain yield at harvest. According to the National Agricultural Statistics Service (NASS), the average grain yield at harvest in Montana for the years 2001-2016 is 1735 kg/ha (1548 lb/A).

Given seeding rates of for pea of 170kg/ha (152 lb/A) and an average crop yield of 1735 kg/ha (1548 lb/A), the ratio between harvested grain and seeds planted (harvest ratio) is approximately 10.2:1. Chickpeas also have a similar harvest ratio. This ratio is very low compared to most other crops. Camelina, for example, has a harvest ratio of ~600:1 (assuming 3 lb of seed planted for a harvested yield of 1800 lb/A), and wheat has a harvest ratio of ~40:1 (assuming a seeding rate of 70 lb/A and 50-bushel grain yield). Based on the harvest ratio of pea and an estimated germination rate of 80%, we can estimate that most peas grown in Montana produce only 12-13 seeds per plant (~ 2 pods), with 1 peduncle per plant.

The harvest ratio determines the overall efficiency of planting a crop. In theory, increasing the harvest ratio will increase efficiency. There are several mechanisms

whereby harvest ratio could be increased. Firstly, seeding rate could be decreased. However, yield is often highly dependent on seeding rate. Secondly, seed size could be decreased, which would reduce the weight of seed planted, but yield and seed size are possibly correlated. Thirdly, the seeding rate could be reduced, but varietal differences in optimum seeding rate could be exploited. It has been shown that branching varieties of peas achieve optimum yield potential at lower seeding rates (Spies et al. 2010), and the branching habit appears to decrease lodging susceptibility (Chapter 4). Branched varieties also may be more competitive with weeds (Spies et al. 2011), which are a consistent problem in pulse crops.

The ability of a plant of a given variety to produce a specific amount of photosynthate is fixed in a given environment. Grain yield is associated with harvest index, which is the ratio of grain yield to plant biomass. This source-sink relationship in peas is dependent on seed size, pod length, plant height, leaf size, stem width, stem wall thickness, leaf thickness, stipule size, and other factors. In this chapter a mathematical model was created to examine the effect of pod length and seed size on the proportion of photosynthates available for proportioning into seed and pod material. All other parameters of harvest index were assumed to be fixed, and only pod size and seed size were varied. Mathematical models predicting the ideotype for pod length and seed size were developed in pea.

Materials and Methods

A Model for the Ideal Pod Length

The formula for the surface area of a cylinder ($SA=2\pi r^2 + 2\pi rh$) allows the prediction of total pod surface area per plant when pod length is varied. Yield, seed size, and all other parameters are held constant. It is assumed that pea pods have the basic shape of a cylinder. Seed size (r in the formula) is held constant and only pod length is varied. Yield is also held constant at a fixed value. Since seed number is fixed, the number of pods per plant will vary with various pod lengths. For example, if a 2.5 cm pod holds 5 seeds that are each 0.5 cm in diameter and the yield potential is fixed at 100 seeds in the model, the plant will produce 20 pods. The surface area of the twenty pods based on the formula for the surface area of a cylinder is 188.4 cm^2. Changing the pod length to 5 cm will increase the number of seeds per pod, and only 10 pods will be needed to have the same yield potential. The surface area of the 10 pods is 172.7 cm^2. The pod surface was compared between long-podded and short-podded pea to determine the ideotype for pod size.

A Model for the Ideal Seed Size

While the ideal pod length can be predicted with a few simple equations, the model to predict the ideal seed size is far more complicated. There are approximately ten assumptions required to make the model work. The core of the ideal seed size model is the equation for actual yield (yield per acre - seed planted), but 22 different equations are also required to increase the accuracy of the model.

Assumptions of the Model

- This model assumes that the density of a dry pea seed is 1.176 g/cm3, which was empirically determined in this study from a single sample of dry pea seed.
- This model assumes the density of a dry pea pod is approximately 1.05g/cm3 which was empirically determined from a single sample of pea pods.
- This model assumes that seed weight (which also influences pod size and pod number when the yield potential is held constant) is the only characteristic of a plant that varies in this model. This model is designed to determine the ideal 100 seed weight for a specific yield potential.
- This model assumes that the amount of photosynthate available to produce either pods or seeds is fixed.
- This model assumes that the amount of photosynthate available to produce either pods or seeds is equal. It is likely less photosynthate is required to produce a pound of pods vs a pound of seed because seeds are often enriched in nutrient content and green pods and seeds are photosynthetic. If that is the case, the ideal 100 seed weight would be lower than the seed weight predicted in this model
- This model does not account for the weight of the peduncle, which attaches the pod to the stem. The peduncle generally is fairly short and light and it was empirically determined from a small dataset to weigh approximately 10% of the weight of the pods and seed. Each peduncle can support 2 pods. Generally, only 1-2 peduncles are produced per plant in Montana.
- In this model pods and peduncle are not photosynthetic and do not contribute to photosynthate. If pods do contribute significantly to photosynthate then the ideal seed size would be smaller than the seed size predicted in this model.
- This model assumes that the number of pods per plant and seed number can vary at no cost to the plant. In reality, usually only 1-2 pods are produced per node. Thus more nodes have to be produced in order to produce pods and seed. If producing more nodes significantly drains photosynthate, then the ideal seed size would be larger than predicted in this model.
- This model assumes that pea seed and seed harvested from the field have an equal cost to the grower, but this is not the case. However, in many cases, growers do use their own seed.
- This model assumes that the formulas for the surface area and volume of a cylinder are a good approximation of the shape of a pea pod. This model does not take into account the portion of the tip and base of the pod not occupied by seed.

The model for seed size uses the following equations: Each one of the parameters is a column title in the seed size model and explains the underlying calculations occurring in Figure 21.

Seed planted per acre= ((seeding rate/m^2)/(sq. feet per m^2))*(square feet in an acre).

Seed cost multiplier (cost of seed planted per lb/ value of harvested grain per lb).

Mass per seed (Mass)= 100 seed weight/100. This can be varied or held constant at a specific value in order to determine the ideal seed weight.

Volume of a seed=(Mass)/(seed density) Seed density is approximately 1.176g/cm^3.

Radius of a seed (r) = The radius of a seed is found by using the formula for the volume of a sphere (volume=4/3πr^3) and solving for the radius. Therefore r={(Volume)/((4/3)*π)}^(1/3).

Seed diameter (Seed Diam)=2*radius

Pod Length = This can be varied in the model or held constant at a specific value. Pod length is generally about 6cm in peas, but this varies depending on the variety. This parameter affects seeds per pod, and pod number.

Pod Width=Seed diameter +pod wall thickness*2. Pod wall thickness was determined empirically to be approximately 0.194 mm on average.

Number of seeds per pod=dry pod length/dry seed diameter.

Photosynthates= This is the total amount of sugars and other nutrients available for partitioning into seeds, seed testa, and pods (in units of grams per plant). This parameter is held constant at an arbitrarily determined value. This model assumes that pea plant sizes, leaf number, and other factors are the same. Only seed size and a few other yield parameters are varied. The percentage of photosynthates dedicated to seeds or pods will vary depending on seed size and pod number. It is quite often that pea plants have enough energy to create about 20g of pod wall material, seeds, and seed testa, based on empirical data. Photosynthate (g)= (mass per seed*((length of pod/ radius of seed)*pod number)) + (pod number *(πr$_1$^{2}h- πr^{2}h)*pod density in g/cm2). In this case h=pod length, r1=pod width, and r=seed diameter. This equation can be partitioned into two components where (mass per seed*((length of pod/ radius of seed)*pod number)) is the seed yield component and (pod number *(πr$_1$^{2}h- πr^{2}h)*pod density in g/cm2) is the pod weight component. The percentage of photosynthates being directed toward the seed or pods for any seed weight can be determined by dividing the seed yield component or pod weight component by the total weight of photosynthates.

Pod number based on amount of photosynthate produced (Pod #) = This the number of pods per plant. It is important to note that to produce the same amount of photosynthate per plant, pod number must vary when seed size varies. Pod number can be determined with the following formula. Pod # = photosynthates/ {(mass per seed*(h /seed diameter))+((πr$_1$^{2}h- πr^{2}h)*pod density)}. In this case h=pod length, r$_1$=half of the pod width, and r=seed radius. Photosynthates are held constant at an arbitrary value (10 g), and the weight of photosynthates is divided by the weight of a pod at a given seed size.

Amount of photosynthate allocated to seed= This is number of grams of photosynthate that is allocated to the seed and not allocated to the pods. The amount of photosynthate allocated to the seed is dependent on seed size and pod length. The formula for the amount of photosynthate allocated to seed=Mass*(Pod L/Seed Diam)*Pod#.

% Allocated to Seed=(Photosynthate allocated to seed/Photosynthate)*100

Expected yield potential= This will vary depending on the environment and location. According to the National Agricultural Statistics Service the average yield in Montana during the 15-year period between 2001 and 2016 was 1735 kg/ha (1548 lb per acre).

Expected weight of yield components= This is the total weight of pods, seed, and seed testa produced by a specific plant. The relative proportion of pods, seeds, and seed testa is dependent on seed diameter, and the total amount of pod material required to achieve a specific yield potential decreases with increases in seed size. In general, the weight of seed is 85% of the weight of the total weight of all the yield components, so the expected yield in Montana (1548 lb/A) is divided by .85. The expected weight of yield components is held constant in the seed size model, but the number is dependent on expected yield.

Yield at harvest= the expected yield at harvest of seed given a specific seed weight. Yield at harvest = (% Allocated to seed/100) *Expected weight of yield components. Expected yield at harvest increases with increasing seed weight because less photosynthate is going to pod weight.

Actual seed yield= Yield at harvest minus the weight of seed planted. This is essentially parabolic. It reaches a high point at the ideal seed weight and then starts to decline.

Volume of seed that is occupied by the testa = This is part of the volume of a seed that is composed of the seed coat. The seed coat (testa) is the outer surface of the seed, and it is often stripped from the seed during processing. It was empirically determined in this study that the seed testa has an average thickness of 0.0125cm. The volume can be found by determining the volume of a seed and subtracting the volume of the seed without its testa. Volume of testa= $4/3\pi r_1^3$- $4/3\pi r^3$. In this case r_1=seed radius, and r=seed radius minus testa thickness.

Mass of testa=Volume of testa*seed density. It is possible that the testa may be more or less dense than a whole seed, but it sinks in water, indicating that it has a density over 1 g/cm^3.

Actual seed yield minus testa= seed mass - mass of testa

Number of g of seed= the number of grams of photosynthate dedicated to the seed without its testa.

Percent of photosynthates going to seed without its testa= (number of g of seed/Photosynthates) *100

Yield minus the weight of seed testa= Expected weight of yield components*Percent of photosynthates going to seed without its testa.

Weight of seeds planted with varying 100 seed weights= (Total seeds per acre*Mass per seed) *the number of grams per pound (there are 453.59237 g per lb).

Results

A model (Figure 20) was successfully created to predict the ideotype for pod length. This model shows the relationship between pod surface area per plant and pod length within the normal range in pea.
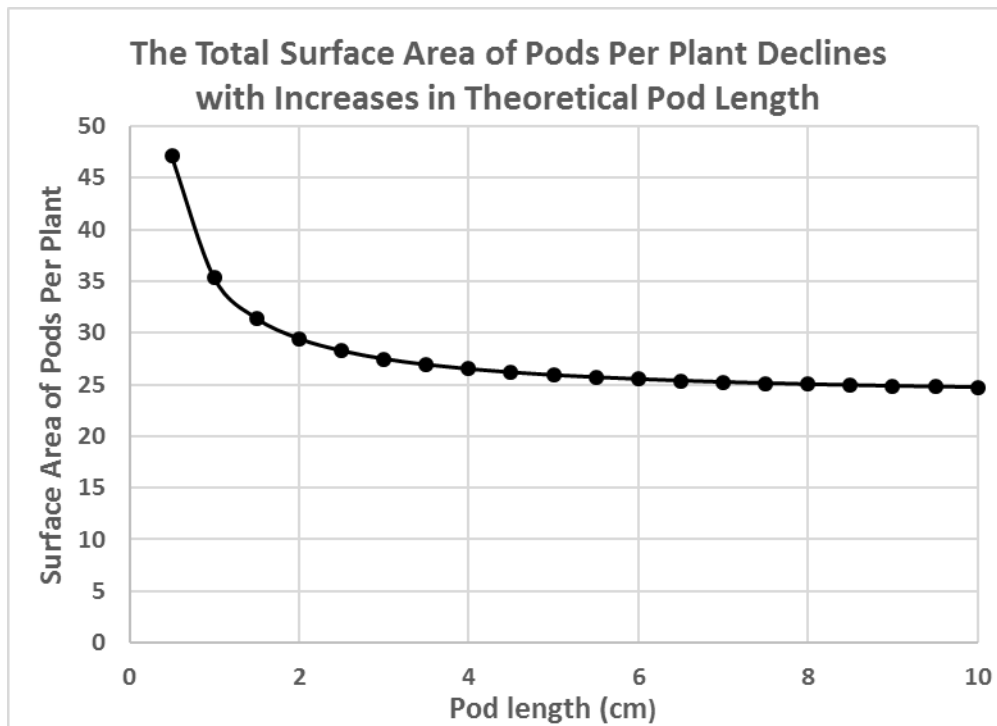
Figure 20: Pod length and pod surface area (assuming a seed yield per plant of 15 seeds and a 0.5 cm seed diameter).

The final model for seed size is below (the pod length model is on the first sheet of the spreadsheet). Right click on the file. Click on worksheet object. Click on open, then click on view, and zoom to 100%. The model is also available at Zenodo (Smitchger 2017a). Follow instructions in the model. Change yield potential, pod length, or seeding density to a single value in all the cells in any green column and observe the effect on ideal seed size.

% Allocated to Seed=(Photosynthate allocated to seed/Photosynthate)*100

Expected yield potential= This will vary depending on the environment and location. According to the National Agricultural Statistics Service the average yield in Montana during the 15 year period between 2001 and 2016 was 1548 lbs per acre. Yield potential has a dramatic effect on the ideal seed size. Smaller expected yields indicat

Expected weight of yield components= This is the total weight of pods, seed, and seed testa produced by a specific plant. The relative proportion of pods, seeds, and seed testa is dependent on seed diameter, and the total amount of pod material required to achieve a specific yield potential decreases with increases in seed size. In

Yield at harvest= the expected yield at harvest of seed given a specific seed weight. Yield at harvest=(% allocated to seed/100)*Expected weight of yield components. Expected yield at harvest increases with increasing seed weight because less photosynthate is going to pod weight.

Actual seed yield= Yield at harvest minus the weight of seed planted. This is essentially parabolic. It reaches a high point at the ideal seed weight and then it starts to decline.

Volume of seed that is occupied by the testa = This is part of the volume of a seed that is composed of the seed coat. The seed coat (testa) is the outer surface of the seed, and it is often stripped from the seed during processing. It has an average thickness of .0125cm. The volume can be found by determining the volume of a seed and

Mass of testa=Volume of testa*seed density

Actual seed yield minus testa=Mass-mass of testa

Number of g of seed= the number of grams of photosynthate dedicated to the seed without its testa. Number of g of seed=Number of seeds per pod*pod number*actual seed yield minus testa

Percent of photosynthates going to seed without its testa= (number of g of seed/Photosynthates) *100

Yield minus the weight of seed testa= Expected weight of yield components*Percent of photosynthates going to seed without its testa.

Weight of seeds planted with varying 100 seed weights= (Total seeds per acre*Mass per seed)*the number of grams per pound =453.59237.

Assumptions of the model:

This model assumes that seed weight (which also influences pod size and pod number) is the only characteristic of a plant that varies. Therefore this model is designed to determine the ideal 100 seed weight for a specific yield potential.

This model assumes that the amount of photosynthate available to produce either pods or seeds is fixed since other characteristics of a plant are assumed to not vary.

This model assumes that the amount of photosynthate available to produce either pods or seeds is equal. In reality, it probably requires less energy to produce a pound of pods than it does a pound of seed. If that is the case, the ideal 100 seed weight would be lower than predicted in this model

This model does not account for the weight of the peduncle, which attaches the pod to the stem. The peduncle generally is fairly short and light and it weighs approximately 10% of the weight of the pods. Each peduncle can support 2 pods.

This model assumes that the pods and peduncle are not photosynthetic. In this model they do not contribute to photosynthate

This model assumes that the number of pods per plant and seed number can vary.

This model assumes that pea seed costs as much as harvested seeds, but this is not likely to be the case. However, in many cases, growers do use their own seed.

This model assumes that pea pods are shaped like a cylinder. This model does not account for the actual shape of the pod. The formula for the volume of a cylinder are expected to be a fairly good approximation of the shape of a pea pod.

The green columns can be modified. Do not modify the gray, pink or yellow columns. The pink column is Hundred seed weight, which increases in each row. The Yellow columns are the result columns.

Change every green cell in any of the green columns to a specific value, and observe the effect on the ideal seed size.

Figure 21: Model to determine the ideal seed size for peas in Montana

The model in Figure 22 predicts the ideal 100 seed weight to be 11.5 g based on a yield potential of 1735 kg/ha (1548 lb/A) and a seeding rate of 80 seeds/m^2. The ideal seed size is at the vertex of the curve.

Figure 22:The ideal seed size for pea in Montana.

The model in Figure 23 predicts the ideal 100 seed weight with the testa removed to be

~17 g based on a yield potential of 1548 lb/A and a seeding rate of 80 seeds/m^2. The ideal

seed size is at the vertex of the curve.
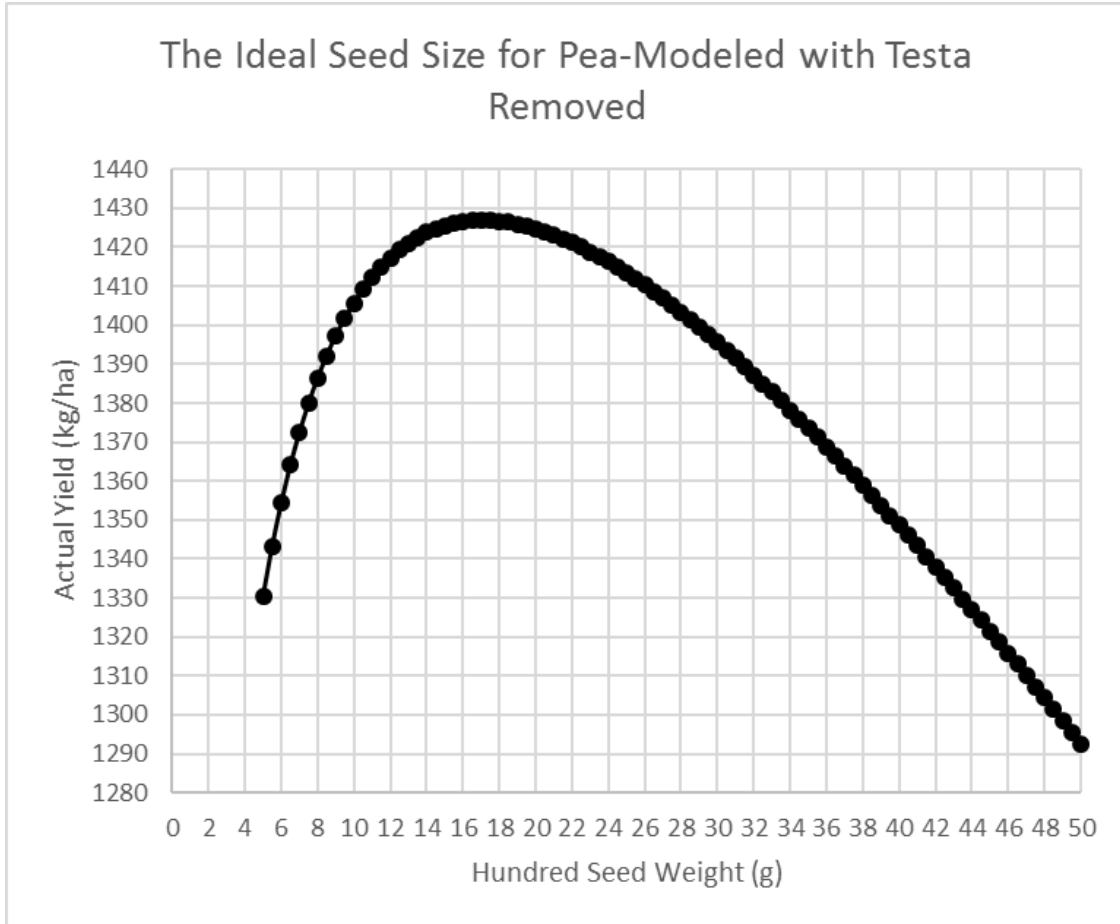
Figure 23: The ideal seed size for pea-modeled with seed testa removed.
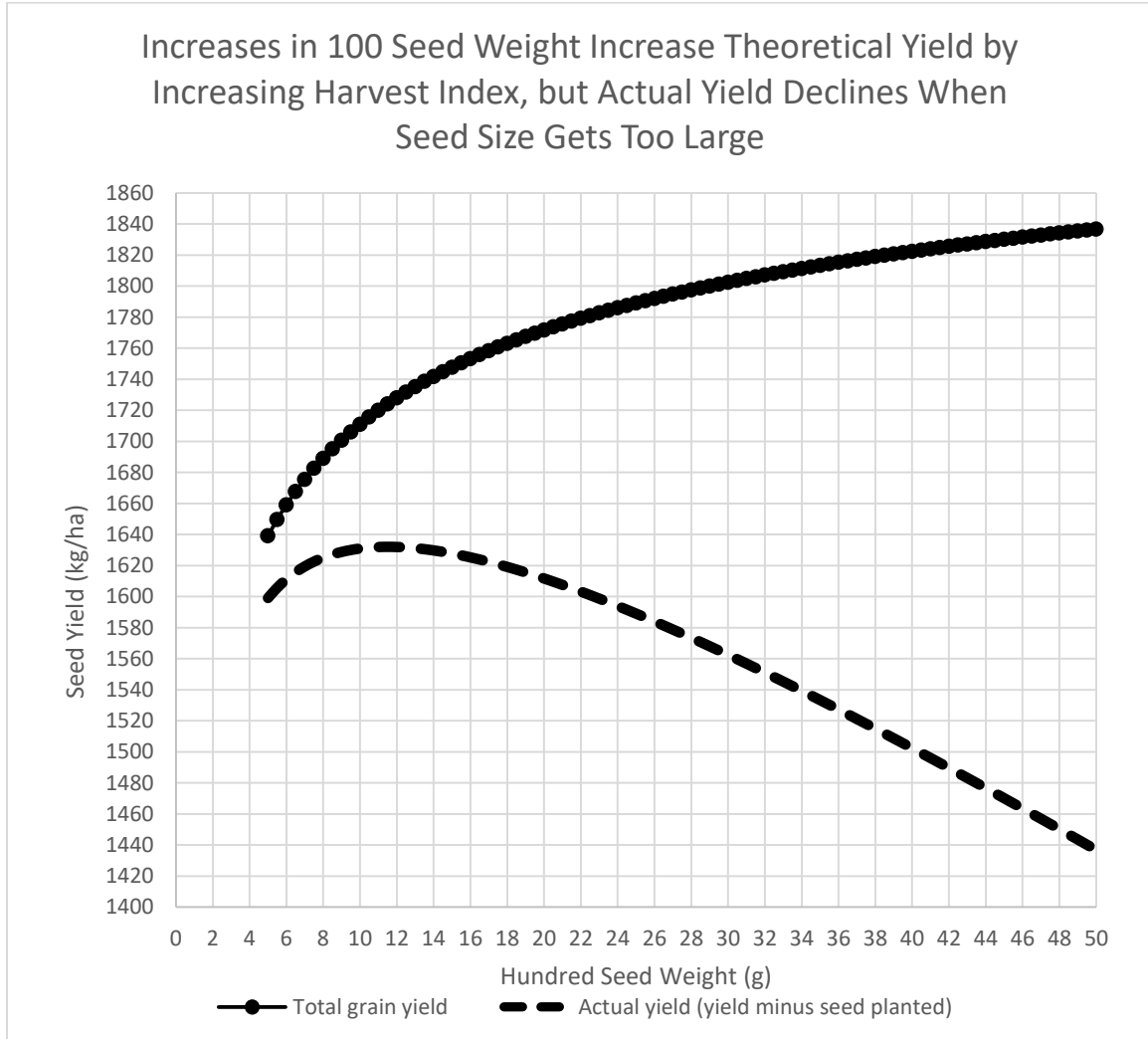
Figure 24: The contrast between the weight of the grain harvested and actual yield (grain harvested minus seed planted).

Discussion

Based on the model, yield varies only slightly when a given seed weight is near the vertex of the curve. Therefore, the ideal 100 seed weight is actually better estimated as a range from 8.5 to 16 g. It should be noted that some of the parameters used in this model such as seed density, pod density, pod wall thickness, testa thickness, and testa density could be refined in this model to better reflect their true values. It is also likely

that these values vary considerably in different genotypes. Moreover, the contribution of pods to photosynthates should be estimated, and this model should be validated with more empirical data. However, this model is expected to be a reasonable approximation of the ideal seed size.

A change in seed density does not change the outcome of the model, and a change in pod wall thickness, pod density, testa thickness, and testa density also have a rather weak effect on the overall model, with a doubling in testa thickness increasing the ideal 100 seed weight by only 4 g. Seeding rate and expected grain yield have the most important effects on the model. Halving the seeding rate will increase the ideal 100 seed weight from 11.5 g. to 20 g. Doubling grain yield from 1548 lb A (1735 kg/ha) to 3098 lb/A will also increase the ideal 100 seed weight from 11.5 g. to 20 g.

The Ideotype for Pod Length

It should be noted that increasing pod length also increases the number of seeds per pod, reducing the number of pods needed per plant to reach a given yield potential. Increases in harvest index are the major mechanism by which increases in pod length increase theoretical yield. This model predicts longer pods are a more efficient use of plant material than shorter pods. This effect is predicted to be the same in chickpeas, lentils and other legumes. This result is due to the fact that pod ends require plant material. Shorter pods with fewer seeds per pod require more pod ends to reach the same yield potential as a longer podded variety. As a result, the surface area of pod material for a given yield potential declines with increased pod length. However, it is important to

note that the plant may not always have enough photosynthate to create longer pods. In peas, average pod length is approximately 6 cm based on data collected in this study, but this number will differ in various germplasm. It is important to note that the decrease in surface area of the pod material follows an exponential decay curve, with each 1cm increase in pod length decreasing pod surface area by less than a previous 1cm increase in pod length. The ideal pod length would need to be determined empirically, but this model indicates that the ideotype of pea is a plant with longer pods since it would be a more efficient use of plant material. However, the current average length of pods (6cm) is already nearly ideal. Therefore, pod length should not be an important target for pea breeders. However, increasing pod length in lentil and chickpea germplasm would likely increase crop yield.

The Ideotype for Seed Size in Pea

It is important to note that the ideal seed size varies depending on the yield potential. Higher yielding areas have a larger ideal seed size because there is a greater return on each seed planted. The predicted ideal seed size is far smaller than the seed size of most commercial varieties. This result may be due to breeders selecting for yield rather than actual yield, which will decrease the overall efficiency of agricultural systems rather than having the desired effect of increasing profitability. Larger seed sizes could also be more attractive to consumers. However, for many of the end uses of pea such as pea flour and split peas, seed size is likely irrelevant. This analysis indicates that actual yields,

which are adjusted for the effect of seed size, should be reported in extension publications, rather than grain yield per acre.

Yield potential has a dramatic effect on the ideal seed size. Smaller expected yields indicate that a smaller seed size is ideal, but higher yielding areas require peas with larger seed sizes to achieve their highest actual yield. Based on the average yield for Montana over the past 15 years (1735 kg/ha; 1548 lb/A), this model predicts the ideal seed size is approximately 11.5 g. per hundred seeds (Figure 22). This is nearly two times lower than the seed weights of most commercial lines (Mohammed and Chen 2016). Higher yielding areas have a higher ideal seed size than lower yielding areas. The predicted ideal seed size is much smaller than the current seed size of commercial varieties, but it is important to note that the difference in actual yield between a variety with the ideal seed size and a pea variety with the seed size of most commercial lines is only approximately 30 lb/A (34 kg/ha). While this is relatively small on a per acre basis, it could potentially reduce net profit of growers in Montana by 1.74 million dollars given a price of $0.22/kg ($0.10/lb) and an area of production of ~235,000 ha (580,000 acres).

The Ideal Seed Size Modeled with Testa Removed

This model assumes that the only variable is seed size, but it does not account for the fact that the seed testa is often peeled from the seed after harvest. A model can be produced to account for the weight of the testa because the weight of the testa per unit of mass will vary depending on the size of the seed. It has been shown empirically in this study that the average testa thickness is approximately 0.125 mm (this estimate was based

on a small dataset). The model accounting for testa thickness indicates that the ideal

hundred seed weight is 17 g at the average yield potential in Montana. The increase in

ideal seed weight when the testa is removed is due to the fact that larger seeds have a

smaller surface area per unit of mass than smaller seeds.

Conclusions

The ideal 100 seed weight predicted in this model (11.5 g.) is much lower than the

seed weight of current cultivars grown in Montana (Mohammed and Chen 2016). It is

likely that plant breeders have selected for higher harvested grain yield and have not

considered the effect of seed size on actual yield.

Based on this model, selecting for individuals with larger seed sizes will increase

yield due to increased harvest index, but it will also increase the cost of seed. This model

assumes that the price of seed peas is the same price as the cost of peas harvested out of

the field. In reality, this is not the case, foundation seed often costs far more than

common seed, indicating smaller seed sizes would be better.

This analysis indicates that breeders should not be selecting specifically for loci

that increase seed size because the correlation between seed size and yield is fairly weak

based on empirical from the Delta x RER population and other studies. Pea breeders

should also focus on higher actual yield (yield minus planted seed weight) rather than

yield, because actual yield reflects the true benefit to the grower, assuming that there is

no premium for seed size. Additionally, extension publications in peas and other pulses

should be reporting actual yield per acre rather than grain yield per acre, when no

premium for larger seed exists. Processors who remove the pea seed testa during processing should offer premium prices for larger seeded peas, if the testa is not utilized in other processes.

The ideotype for pea seed size in Montana has not been entirely defined. It is likely, based on this analysis, that lower yielding areas of Montana could greatly benefit from a highly branched variety that could be seeded at a lower seeding rate. Varieties with higher basal branching achieve optimum yield at lower seeding rates (Spies et al. 2010), indicating that efficiency could be increased. The seed size for irrigated and higher yielding areas is already nearly ideal. Therefore, smaller seeded, branched varieties should only be a focus in low yielding areas of Montana. It should be noted that a similar analysis would likely be valid in other crops with a low harvest ratio, such as chickpea, if no premium for seed size exists. Defining the ideotype for seed size in peas and other crops would allow crop breeders to increase the efficiency of cropping systems and promote food security across the world.

CHAPTER SEVEN: THE EXPECTED VALUE OF THE QTLS AND TRAITS TO PEA

BREEDING PROGRAMS

The QTL analysis in this study was layered, in which all QTL analyses were overlaid over other QTL analyses. Examining traits individually to uncover the number of QTL controlling favorable traits could be potentially misleading if pleiotropy exists. Since the goal of this study was to discover alleles necessary for marker assisted selection and genomic selection in field pea, pleiotropic effects of each QTL were considered

Pleiotropic Effects and the Expected Value of Lodge III-1 (*Le*) to Breeding Programs

Since the *Le* locus affects the synthesis of the plant hormone gibberellin, which has profound impacts on plant growth, including control of flowering and germination (Hedden and Sponsel 2015), the Lodge III-1 QTL has pleiotropic effects on ten different traits measured in this study. QTL for all traits except yield were centered around the *Le* locus, with flanking markers being less significant, indicating that *Le* may be the gene responsible for these effects. The effect on yield may be due to *Np*, within the QTL, but whether other effects are due to nearby genes has not been determined by this study. Both height and leaf length were strongly correlated. It is likely that the increased level of gibberellins present in tall genotypes simultaneously increase both the elongation of the stem and the leaf.

Table 48: Putative pleiotropic effects of Lodge III-1 measured at the Le marker. Delta was the dwarf parent and RER was the tall parent. The analysis was conducted on all 254 lines.

Trait	p-value	% variation explained	Effect	mean Delta (dwarf)	mean RER (tall)
Height	1.08×10^{-85}	78.90%	25.5 cm	38.40 cm	63.89 cm
Lodging	7.53×10^{-39}	49.90%	20.4%	26.22%	46.65%
Leaf length	5.83×10^{-37}	47.90%	2.93 cm	14.24 cm	17.17 cm
Stem diameter	3.64×10^{-24}	34.20%	0.27 mm	2.24 mm	1.97 mm
Number of flowering nodes	5.53×10^{-6}	8.20%	0.82 nodes	4.21 nodes	5.03 nodes
Branch number	4.6×10^{-5}	6.50%	0.35 brnch.	2.8 brnch.	2.45 brnch.
Total node number	1.29×10^{-4}	5.80%	1.34 nodes	21.71 nodes	23.05 nodes
Side Branch diameter	2.4×10^{-4}	5.40%	.1 mm	2.4 mm	2.3 mm
Epicotyl diameter	0.020	1.80%	.06 mm	1.74 mm	1.68 mm
Yield*	0.022	2.10%	301.5 kg/ha	3625.9 kg/ha	3324.4 kg/ha

*=may be due to *Np* rather than *Le*.

The Expected Value of the Lodge III-1 QTL in Dry Pea Breeding Programs

Based on the strong effect on lodging, it appears that only the dwarf allele of *Le* has a high value to pea breeding programs. It is likely that plant breeders would like to select against tall varieties due to their propensity to lodge heavily. It would be fairly easy to develop a KASP or CAPS marker for *Le* since the gene and the genetic sequence causing the phenotype is known. The advantage of such a marker would be the ability to distinguish between homozygous tall and heterozygous lines during MAS in early generations. However, it is easy to determine which lines have the tall or dwarf allele morphologically at a very young age, and observation is likely to be a more practical method of selecting dwarf cultivars than using a genetic marker.

Pleiotropic Effects and the Expected Value of the Lodge I-1 QTL in Breeding Programs.

Mutations that affect auxin levels often have a major impact on the plant since auxin levels coordinate the function of many biological pathways. Research by DeMason and Chawla (2004) and the empirical data collected in this study support a model whereby reduced auxin levels caused by the *af* allele result in decreased stem diameter. The combination of data for main stem diameter and compressed main stem thickness support a model whereby main stem diameter is decreased in *af* lines due to decreases in compressed main stem thickness. Auxin has been documented to stimulate ethylene production (Suttle 2003), which influences stem thickening. The classical acid-growth hypothesis also indicates that auxin influences H^+-ATPases, which pump H+ out of the cell wall, loosening cellulose microfibrils in the cell wall and facilitating cell expansion, indicating that auxins may affect stem width/elasticity of cell walls.

Table 49: Putative pleiotropic effects of Lodge I-1 associated with *Af* (analyzed on all 254 lines at the *af* locus)

Trait	p-value	% variation explained	Effect	mean Delta (semi-leafless)	mean RER (wild type leaf)
Main stem diameter	2.085×10^{-14}	20.90%	0.21 mm	1.98 mm	2.19 mm
Compressed main stem diameter	7.56×10^{-14}	20.10%	0.12 mm	0.82 mm	0.94 mm
Side Branch diameter	2.60×10^{-8}	11.70%	0.16 mm	2.26 mm	2.42 mm
Epicotyl diameter	9.59×10^{-8}	10.90%	0.12 mm	1.64 mm	1.76 mm
% Lodging	1.268×10^{-5}	7.40%	7.8%	32.30%	40.10%
Stem flexion	1.35×10^{-5}	7.90%	0.06 mm	0.21 mm	0.27 mm
Yield	0.0013	4.10%	417 kg/ha	3226 kg/ha	3643 kg/ha
Compressed branch diameter	0.006	3.00%	0.04 mm	0.79 mm	0.83 mm
Yield per plant	0.009	2.70%	0.82 g/plant	8.62 g	7.80 g
Nodes to first flower	0.0091	2.70%	0.91 nodes	18.32 nodes	17.41 nodes

The Expected Value of the *Afila* Locus in Pea Breeding

It is likely that plant breeders would like to select against *Af* varieties due to their propensity to lodge. However, in this trial *Af* lines had 12.9% higher yield than *af* lines. This result indicates that if lodging resistance can be found by another mechanism, *Af* lines might increase yield. A tradeoff between higher yielding *Af* cultivars and lower yielding *af* cultivars might be made by mixing the two cultivars prior to seeding. This has been previously shown to increase yield (Schouls and Langelan 1994). However, the effect on lodging would have to be assessed. Currently only the *af* allele has utility in a dry pea breeding program.

It would be possible to create a genetic marker for the *Af* locus by looking at candidate genes in the lower part of LG I, but the gene is not currently known. The advantage of such a marker would be to distinguish between homozygous *Af* and heterozygous lines during MAS in early generations. However, it is easy to determine which lines are homozygous for the *af* allele by observing the seed leaf of the seedling, and observation is likely to be a more practical method of selecting *af* cultivars than using a genetic marker. While it initially appeared to be a homeotic gene that only affected leaf development, this study indicates that the Lodge I-1 QTL associated with the *Af* locus has pleiotropic effects on a number of traits. Understanding these results will give a greater understanding of the mechanism of gene action for *Af*.

Pleiotropic Effects of Lodge II-1 (Putatively the *A* Locus)

The Lodge II-1 QTL has pleiotropic effects on ten different traits measured in this study. In addition to the traits measured in this study, white flower lines have been shown to have a pleiotropic effect on digestibility, because indigestible tannin-protein complexes form in colored flowered lines (Hejdysz et al. 2015). QTL for % emergence, % lodging, and yield were centered around the *A* locus, with flanking markers being less significant, indicating that *A* may be the gene responsible for these effects. Other effects were not centered at the *A* locus and may be due to nearby genes within the QTL, such as *Lf*, a gene which controls the length of the juvenile phase (Murfet and Reid 1993). The entire section of LG II below *A* was significant for nodes to first flower and total node number. However, the lodging QTL was centered at *A*, and flanking markers were less significant.

There is moderate evidence to indicate that the Lodge II-1 QTL increases stem diameter and side branch diameter. This effect may be explained by the effect on seed dormancy and emergence. White flowered lines may grow larger stems because they have more time and resources during the growing season, when compared to *A* lines which generally emerge late. This effect is likely why *a* lines had higher yield and yield per plant.

Table 50: Putative pleiotropic effects of the Lodge II-1 QTL (analyzed on all 254 lines at the *A* locus unless noted)

Trait	p-value	% Variation explained	Effect	Delta (mean) (*a*)	RER (mean) (*A*)
% Emergence	8.92×10^{-12} (all)	17.50%	9.82%	88.94%	79.12%
Nodes to first flower	3.49×10^{-10} (all)	14.90%	2 nodes	16.8 nodes	18.8 nodes
Small plot yield	1.04×10^{-7} (all)	11.10%	614 lbs/A	3437 lbs/A	2823 lbs/A
Maximum nodes per plant	0.000023 (all)	6.20%	1.34 nodes	21.73 nodes	23.07 nodes
% Lodging	0.00118 (all)	5.40%	7.0%	33.41%	39.44%
Number of flowering nodes*	0.0008 (all) / 0.0002 (dwf)	4.6% (all) / 11.9% (dwf)	0.61 (all) / 0.79 (dwf)	4.94 nd. (all) / 4.51 nd. (dwf)	4.33 nd. (all) / 3.72 nd. (dwf)
Main stem diameter*	0.008 (all) / 0.0012 (dwf)	2.9% (all) / 10.8% (dwf)	0.07 mm (all) / 0.14 mm (dwf)	2.14 mm (all) / 2.29 mm (dwf)	2.07 mm (all) / 2.15 mm (dwf)
Side Branch diameter*	0.034 (all) / 0.0002 (dwf)	1.8% (all) / 12.4% (dwf)	0.07mm (all) / 0.17mm (dwf)	2.38 mm (all) / 2.48 mm (dwf)	2.32 mm (all) / 2.31 mm (dwf)
Seed dormancy	0.0036 (dwf)	8.60%	0.56 points	1.86 points	2.42 points
Yield per plant	0.005 (all)	3.20%	0.88 g.	8.78 g	7.90 g

*due to the effect of Le= analyses measured the effect on all 255 lines and also on a subset of 94 dwarf genotypes.

The Expected Value of the Lodge II-1 QTL in Breeding Programs

Lodge II-1, centered at *A*, has a number of desirable effects, namely increasing yield, emergence, and lodging resistance. This QTL appears to have a high level of utility in dry pea breeding programs. Based on the effects of the QTL, the A locus may be a good candidate gene for this QTL. In contrast to the other lodging phenotypes, which are likely caused by mutations in genes related to plant hormones, the white/colored flower gene is a bHLH transcription factor that has a G to A transition mutation in a splice donor site, leading to a mis-spliced m-RNA with a premature stop codon, resulting in a truncated protein (Hellens et al. 2010). The sequence of the mutation causing the gene is known (Hellens et al. 2010), and a perfect marker for marker assisted selection could be developed.

Lodge III-2. Putative Pleiotropic Effects of the *Hr/Rms1/M* Region

The Lodge III-2 QTL has pleiotropic effects on 19 different traits measured in this study, including six stem strength traits, four maturity time traits, plant height, basal and aerial branching, leaf length, emergence, yield, and seed size. QTL for all traits except branch diameter, yield, and yield per plant were centered around the *Hr* locus, with flanking markers being less significant, indicating that *Hr* may be the gene responsible for these effects. Other effects may be due to nearby genes, such as *Rms1* or *M*, within the QTL, but whether other effects are due to nearby genes has not been determined by this study. The following results are based on data averaged across all site-years, unless otherwise noted.

Table 51: Putative pleiotropic effects of the Lodge III-2 QTL (analyzed on all 254 lines at the *Hr* locus, unless noted)

Trait	p-value	% Variation explained	Effect	Delta (mean)	RER (mean)
Maturity time (1=early, 4=late)	8.47×10^{-65}	75.8%	1.05 points	1.28 points	2.33 points
Total node number	7.57×10^{-27}	43.8%	3.80 nodes	20.22 nodes	24.01 nodes
Compressed side branch thickness	4.56×10^{-26}	42.4%	.14 mm	.73 mm	.87 mm
Nodes to first flower	1.88×10^{-21}	36.0%	3.46 nodes	15.81 nodes	19.27 nodes
Aerial branching	1.71×10^{-16}	29.6%	1.05 points	1.38 points	2.43 points
Epicotyl diameter	1.79×10^{-15}	26.9%	.21 mm	1.59 mm	1.80 mm
stem flexion	2.53×10^{-14}	26.6%	.11 mm	.29 mm	.17 mm
Basal branch number	2.43×10^{-12}	21.5%	.64 branch	2.25 branch	2.89 branch
100 Seed weight	9.28×10^{-11}	18.9%	2.71 g.	17.21 g.	14.50 g.
Yield per plant (Measured at the A55 marker)	3.84×10^{-6}	20.6%	2.06 g/plant	7.67 g/plant	9.73 g/plant
Plant height	0.00003	8.2%	8.26 cm	46.28 cm	54.54 cm
Side Branch diameter	0.00009	6.3%	.11 mm	2.29 mm	2.40 mm
% Emergence	0.00012	7.1%	5.62 cm	87.8%	82.1%
Small plot yield (Measured at the A55 marker)	0.00025	13.5%	755 kg/ha	3486 kg/ha	4241 kg/ha
Main stem diameter	0.00030	6.1%	.12 mm	2.17 mm	2.05 mm
% Lodging	0.00600	3.6%	5.72%	39.4%	33.7%
Leaf length (tall phenotype only)#	0.00700	7.2%	.81 mm	17.66 mm	16.85 mm
Number of flowering nodes	0.034	2.2%	.44 nodes	4.40 nodes	4.84 nodes
Compressed main stem thickness	int	int	int	int	int

Int=Interaction, there is strong evidence to indicate that this trait is affected, but the effect was not consistent.

may be merely a secondary effect of increased basal branching.

The Expected Value of the Lodge III-2 QTL in Breeding Programs

The underlying gene causing this QTL is currently unknown. While lines possessing the *Hr* gene had higher crop yields than lines with the *hr* gene, *Hr* was not the most significant marker for yield in the QTL. The increase in yield may be due to the increased basal branching found in the RER derived lines, which might be advantageous under the lower seeding rates used in this trial. However, in 2016, with a low commercial seeding rate of 60 plants/m^2, this QTL still explained 20% of the variation in yield in this trial, indicating that seeding rate was not a likely factor. The RER allele also decreased

lodging and affected 100 seed weight, with lines with the Delta allele having higher seed weights than RER derived lines. The increased yield in lines with the RER derived QTL is surprising because lines with the RER derived allele had lower emergence than lines with the Delta derived allele. The green bridge effect indicates that diseases and disease vectors will move from senescing plants to plants that are still green, indicating that later maturing RIL lines would have higher disease pressure in this population. Later maturing RILs may have been more susceptible to vectors of pea seed-borne mosaic virus, resulting in lower emergence in the following year.

If the *Hr* gene is responsible for the positive effects of this locus, it might be difficult to use this gene in breeding due to its strong increase in maturity time. A genotype by environment interaction is likely to be expected because late maturing varieties might be subject to drought in dry areas and frost in many areas of Canada. Recently, this gene was proposed to correspond with *ELF3* in Arabidopsis, a gene involved in circadian cycle signaling (Klein et al. 2014). It is likely that the *Hr* gene strongly influences the levels of a plant phytohormone such as ethylene. *ELF3* in Arabidopsis, the ortholog of *Hr*, has been shown to cause interactions in ethylene signaling and abscisic acid signaling, and *ELF3* is a multi-functional regulatory molecule (Sakuraba et al. 2014, 2016). Abscicic acid partially regulates strigolactones, which are a branching inhibitor. Strigolactones and cytokinins, which are mediated by auxin, act antagonistically in the buds to control bud outgrowth in pea (Brewer et al. 2009, Cheng et al. 2013). A number of markers have been developed for *ELF3* (Weller et al. 2012). It

might be wise to create a KASP marker for this locus since the sequence of the gene would likely work well using that technology. The sequence of the gene is known.

It is possible that this yield QTL is controlled by *Rms1* due to the effects on branching. The lines with the RER allele had pronounced basal and aerial branching, which influences the number of pods per plant due to the increased amount of pod bearing branches. Highly branched varieties also require lower seeding rates than less branched varieties (Spies et al. 2010), which would decrease the cost of planted seed. It is probable that RER lines were higher yielding because the number of pods was increased due to greater numbers of basal branches rather than due to an increase in seed weight. It is striking that lines with increased yield and height had better lodging resistance.

This RER derived allele of this QTL decreases lodging, increases height, significantly increases yield per plant, but decreases 100 seed weight. Overall this indicates that the RER derived allele may have a high level of utility, which may warrant further investigation.

Pleiotropic Effects of Lodge III-3 (Near SSR-AD73).

The Lodge III-3 QTL has pleiotropic effects on 9 different traits measured in this study. There was not a well-defined peak for the QTLs for the 9 traits, but rather, a number of markers were nearly equally significant in the 59-82 cM region on LG III. The main effects appeared to be centered in the region between markers SSR-AB111 at 66.1 cM and CDC-27 at 73.9 cM. There is weak evidence to indicate that SSR-AD73 is the

best marker for the QTL. The following results are based on data averaged across all site-

years, unless otherwise noted.

Table 52: Putative pleiotropic effects of Lodge III-3 (analyzed across all 254 lines unless
noted)

Trait	p-value	% Variation explained	Effect	Delta (mean)	RER (mean)
Compressed main stem thickness	4.79×10^{-6}	8.7%	.07 mm	.92 mm	.85 mm
100 Seed weight	0.00002	7.7%	1.73 g.	16.7 g	15.0 g
Compressed side branch thickness	0.00190	4.1%	.04 mm	.83 mm	.79 mm
Main stem diameter	0.00200	4.1%	.09 mm	2.15 mm	2.06 mm
Small plot yield (Dwarf only*)	0.00220	9.7%	634 kg/ha	4116 kg/ha	3481 kg/ha
Side Branch diameter	0.01600	2.5%	.07 mm	2.39 mm	2.32 mm
Yield per plant (Dwarf only*)	0.02500	5.3%	1.04 g	9.06 g	8.02 g
% Lodging (all 255 lines)	0.04400	1.7%	3.86%	34.3%	38.1%
Epicotyl diameter	0.04970	1.7%	.05 mm	1.74 mm	1.69 mm

* only dwarf lines analyzed, no effect across all genotypes

The Value of the Lodge III-3 QTL in Breeding Programs

The Delta derived allele appears to primarily increase stem diameter and

compressed stem diameter, reducing lodging as a result. There also appears to be a

positive effect on yield and seed size. Therefore, it appears that the Delta derived allele

has usefulness in breeding programs. However, determining the underlying gene

responsible for this QTL may be difficult.

Pleiotropic Effects of Lodge I-2.

The Lodge I-2 QTL has pleiotropic effects on seven different traits measured in

this study. QTL for all traits except yield were centered around the A7384 and A7380

markers. The following results are based on data averaged across all site-years, unless

otherwise noted.

Table 53: Putative pleiotropic effects of Lodge I-2 (analyzed on 94 dwarf RILs at the
A7384 marker)

Trait	p-value	% Variation explained	Effect	Delta (mean)	RER (mean)
Maturity time (1=early, 4=late)	0.00065	11.7%	.38 points	2.01 points	1.63 points
% Lodging (two site years)	0.0096/.0008	7.1/12.0%	7.4/9.8%	19.7/18.0%	27.0/27.8%
Side Branch diameter	0.00400	8.7%	.14 mm	2.47 mm	2.33 mm
Nodes to first flower	0.00760	7.4%	1.4 nodes	18.13 nodes	16.73 nodes
Total node number	0.00800	7.3%	1.35 nodes	22.22 nodes	20.87 nodes
Small plot yield*	0.03100	4.8%	449 kg/ha	4044 kg/ha	3596 kg/ha
Yield per plant	0.04900	4.1%	.92 g	9.03 g	8.11 g

* = the effect for small plot yield was not centered within the QTL

The Value of Lodge I-2 in Breeding Programs

The Delta derived allele of Lodge I-2 may increase maturity time, side branch diameter,

yield, and yield per plant. This allele could be valuable in pea breeding programs.

However, the pleiotropic effects on maturity time need to be considered when assessing

the value of this allele.

Pleiotropic Effects of Lodge II-2

The Lodge III-2 QTL may influence 10 different traits measured in this study.

QTL for these traits were not always centered near the A7258 marker linked to lodging

resistance. The entire region between Lodge II-2 and the *A* locus was important for nodes

to 1st flower, number of flowering nodes, total node number, yield per ha, yield per plant,

and stem flexion. However, interval mapping consistently indicated that there were two

separate QTL in the region for each of these traits. The QTL for main stem diameter, side

branch diameter, and % lodging clearly appeared to be separate QTL. Other effects may

be due to nearby genes, such as *Lf*, within the QTL. The following results are based on

data averaged across all site-years, unless otherwise noted.

Table 54: Putative pleiotropic effects of the Lodge II-2 QTL (analyzed on 94 dwarf lines
at the A7258 marker)

Trait	p-value	% Variation explained	Effect	Delta (mean)	RER (mean)
Nodes to first flower	7.5×10^{-7}	23.6%	2.47 nodes	16.33 nodes	18.80 nodes
Number of flowering nodes	0.0013	10.9%	.77 nodes	4.5 nodes	3.73 nodes
Total node number	0.0018	10.2%	1.59 nodes	20.83 nodes	22.42 nodes
Small plot yield	0.0044	8.5%	610 kg/ha	4060 kg/ha	3449 kg/ha
Yield per plant	0.0063	7.9%	1.29 g	9.07 g	7.78 g
Main stem diameter	0.0076	7.6%	.12 mm	2.28 mm	2.16 mm
Side Branch diameter	0.011	6.9%	.12 mm	2.45 mm	2.33 mm
Seed dormancy (1=no dormancy, 3=dormant)	0.017	6.1%	.47 points	1.93 points	2.40 points
% Lodging	0.022	5.6%	4.94%	23.2%	28.2%
stem flexion	0.032	5.2%	.04 mm	.24 mm	.20 mm

The Value of the Lodge II-2 Allele in Breeding Programs

The Delta derived allele appeared to reduce lodging, increase yield, and decrease

maturity time. It appears that the Delta derived allele has a high level of utility in

breeding programs.

Pleiotropic Effects of Lodge II-3 (Putatively the *Mo* Locus)

The Lodge II-3 QTL had weak pleiotropic effects on five different traits measured

in this study. *Mo* influences resistance to several viruses (Choi et al. 2012). The QTLs for

yield, number of flowering nodes, and side branch diameter were centered around the *Mo*

locus, with flanking markers being less significant, indicating that *Mo* is a candidate gene

for these effects. It is possible that the difference in viral infection between the resistant

and susceptible lines is responsible for the pleiotropic effect of this locus.

Table 55: Putative pleiotropic effects of the Lodge II-3 (analyzed on 94 lines at the *Mo* locus)

Trait	p-value	% Variation explained	Effect	Delta (mean)	RER (mean)
Small plot yield	0.00270	11.8%	704 kg/ha	4217 kg/ha	3513 kg/ha
Nodes to first flower	0.00450	10.6%	1.63 nodes	16.33 nodes	17.96 nodes
% Lodging	0.02647	6.0%	6.28%	23.2%	28.2%
Number of flowering nodes	0.033	5.4%	0.530	4.41 nodes	3.88 nodes
Side Branch diameter	0.09200	3.5%	.09 mm	2.43 mm	2.34 mm

<u>The Value of the Lodge II-3 QTL in Breeding Programs</u>

The Lodge II-3 QTL, centered at the *Mo* locus, may increase yield and reduce

lodging by reducing viral infection. Although ELIZA testing indicated that no pea seed-

borne mosaic virus was present, the current test does not detect all isolates of the virus

and it is likely that pea seed-borne mosaic virus was present based on the symptoms

observed in the field. This Delta derived allele appears to have strong utility in Montana.

<u>Putative Pleiotropic Effects and Utility of Lodge VI-1</u>

Averaged over all site-years the effects of this QTL appeared to be centered near

the A1580 SNP marker; however, better markers for this QTL should be developed

because the precise center of the QTL was not clear due to poor marker quality of

flanking loci.

Table 56: Putative pleiotropic effects of Lodge VI-1 (analyzed on 94 dwarf RILs at the A1580 marker)

Trait	p-value	% Variation explained	Effect	Delta (mean)	RER (mean)
100 Seed weight	0.0012	11.8%	2.08 g.	17.18 g	15.10 g
Leaf width (sub-af)*	0.0013	27.90%	2.06	9.86 cm	7.80 cm
Leaf length	0.0049	9.0%	1 cm	14.8 cm	13.8 cm
Side Branch diameter	0.016	6.80%	.12 mm	2.47 mm	2.35 mm
Number of flowering nodes	0.025	5.9%	.55 nodes	4.34 nodes	3.79 nodes
% Lodging	0.134	2.6%	3.5%	23.8%	27.3%

* [sub-af= only ~35 *afila* lines used for the analysis]

Pleiotropic Effects of the Tsw1.1 Seed Weight QTL

This locus affected a number of other traits in addition to 100 seed weight, indicating that this gene is a major QTL with multiple pleiotropic effects. The QTL for all traits appeared to be centered near the A6724 marker located at 3.4 cM on LG I, however a 7.2 cM gap occurred below this marker. Indicating that the actual gene could be above the A6724 marker.

Table 57: Putative pleiotropic effects of the Tsw 1.1 seed weight QTL (analyzed on 94 dwarf RILs at the A6724 marker)

Trait	p-value	% Variation explained	Effect	Delta (mean)	RER (mean)
100 Seed weight	7.88×10^{-12}	39.2%	3.84 g.	18.08 g.	14.21 g.
Leaf length	5.11×10^{-6}	20.0%	1.41 mm	14.94 mm	13.53 mm
Side Branch diameter	0.00007	15.5%	.19 mm	2.50 mm	2.31 mm
stem flexion	0.0002	13.7%	.07 mm	.26 mm	.19 mm
Main stem diameter	0.0005	12.1%	.15 mm	2.31 mm	2.16 mm
Comp. side branch thickness	0.0012	10.6%	.06 mm	.82 mm	.76 mm
Aerial branching	0.0035	9.0%	0.580	1.60 points	2.18 points
Basal branch number	0.0055	8.0%	0.4 stems	2.6 stems	3.00 stems
Internode length	0.025	5.2%	.18 cm	1.90 cm	1.72 cm
Compressed main stem diameter	0.039	4.4%	.05 mm	.93 mm	.88 mm

The Value of the Tsw 1.1 Locus in Pea Breeding Programs

The main trait of importance is seed weight. As mentioned previously (Chapter 5), it appears that this QTL is the same QTL found in five previous studies assuming the correct orientation of the linkage group (Timmerman-Vaughan et al 1996 and 2005, Irzykowska and Wolko 2004, Gondo et al. 2007, Krajewski et al. 2012, Ferrari et al. 2016). There was weak evidence to indicate an effect on lodging. The effect on lodging was consistent across all six site-years with individuals with the RER derived allele outperforming the Delta allele. However, when averaged across all site-years the effect was very weak (p=0.149). Lines with the RER derived allele had only 1.91% less lodging than individuals with the Delta allele. The Delta allele had increased seed weight and stem diameter, but the mean yield for the Delta derived and RER derived allele was nearly identical. At this point it appears that this trait has weak utility, however, it should be noted that decreasing 100 seed weight by 3.84 g (the effect by the RER derived allele) would also increase actual yield by 30.7 kg/ha (27.4 lb/A), assuming a seeding rate of 80 seeds/m^2. This would increase actual yield (grain yield minus seed planted) by 1.96% based on the average reported yield in Montana during 2001-2016 (1735 kg/ha; 1548 lb/A).

The Utility of a LG IV Branch Diameter Locus

There is a fairly strong QTL for maturity time and other traits between the AGAT locus and the SSR-A9 locus on LG 4A. The exact position of the QTL could not be determined from the mapping data. This allele was significant for lodging in only one

site-year (p=.005, 2016 Moccasin) where it decreased lodging by 7.5% in lines with the

Delta allele when compared to the RER derived allele (19.2 vs 26.7% respectively). As

expected for a QTL that influences height, this QTL also affected leaf length. The QTL

for side branch diameter is centered near the A9 locus. The QTL for other traits is

centered slightly below the A9 locus.

Table 58: Putative pleiotropic effects of a LG IV branch diameter locus

Trait	p-value	% Variation explained	Effect	Delta (mean)	RER (mean)
Side Branch diameter (A9)*	0.0023	9.4%	.14 mm	2.48 mm	2.34 mm
Plant height	0.004	8.4%	3.98 cm	40.7 cm	36.7 cm
Maturity time (1=early, 4=late)	0.007	7.6%	.30 points	1.95 points	1.65 points
Leaf length	0.007	7.2%	.84 cm	14.64 cm	13.78 cm
Seed dormancy (1=no dormancy, 3=dormant)	0.029	5.0%	0.43 points	2.32 points	1.89 points

* p-value was .005 for % lodging during one site year in Moccasin, not important other site years.

Utility of the LG IV Branch Diameter Locus

This locus appeared to have no effect on yield. The positive effect on lodging and

plant height appear to indicate that the Delta derived allele may have a minor level of

utility if an increase in height is desired. However, the associated effect on maturity time

will have to be considered if yield and maturity time are correlated in a specific region.

Correlation Matrices for Various Traits.

Previously, the pleiotropic effects of specific QTL were examined. The following

analysis takes a broader view and looks at the pleiotropic effects of specific traits.

Pleiotropic effects are common between important crop traits, often making it difficult to

select for an important agronomic trait without having an adverse effect on another trait.

This study examined pleiotropy between various important agronomic traits in dry peas, examining whether there was a correlation between various traits. This analysis will allow breeders to assess the effects that might occur if a specific trait was selected for or against. Correlation is an effect size, and we can describe the strength of the correlation using the guide where 0.00-0.19 is "very weak", 0.20-0.39 is "weak", 0.40-0.59 is "moderate", 0.60-0.79 is "strong", and 0.80-1.0 is "very strong" (Evans 1996). By using the psych package in R, correlation matrices were generated to explore these pleiotropic relationships. Correlation matrices are used to determine whether quantitative traits are associated. In the below correlation matrices a number of traits were assessed. Correlation matrices for each year can be found in Appendix B.
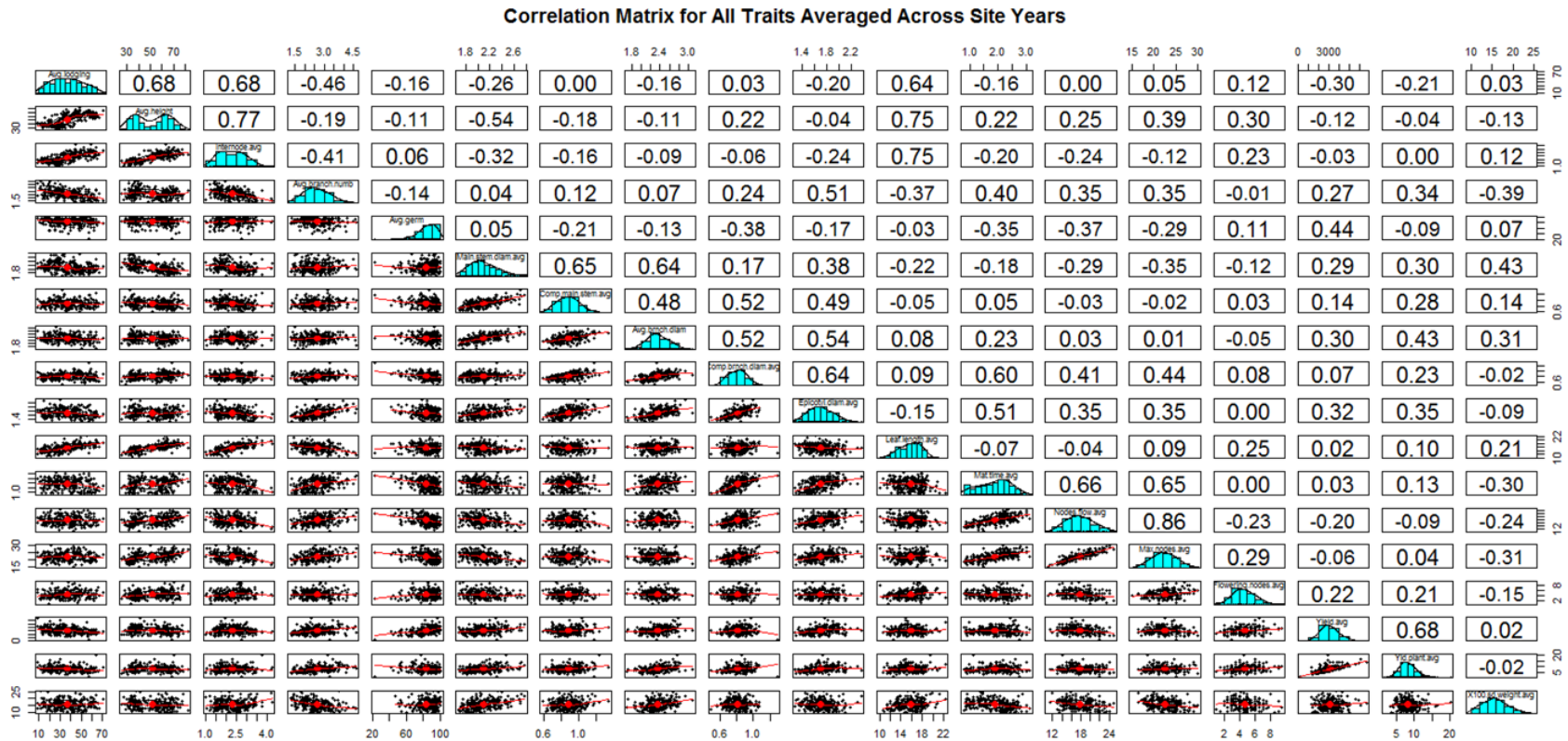
Figure 25: Correlation matrix for all traits averaged over site-years. A correlation matrix is a visual presentation of the correlations between a number of different quantitative variables. The quantitative traits are represented by the histograms on the diagonal. Scatterplots for each trait are shown below the diagonal, and correlations (r) are above the diagonal. The matrix is read by finding at any two traits on the diagonal and looking at the correlations in the column or row above or to the right of it, respectively. Scatterplots are shown in the row to the left or column below each trait on the diagonal. For example, traits 1 and 3 have a correlation of 0.68 (correlation shown in row 1 column 3, scatterplot in row 3, column 1). Traits 2 and 4 have a correlation of -0.19. (scatterplot in row 4 column 2).

Legend (Traits from left to right along the diagonal): Avg.Lodging=% lodging averaged over all site-years. Avg.height= plant height averaged across all site-years. Internode.avg= Average internode length across all site-years. Avg.branch.numb= average branch number across all site-years. Avg.germ=average % germination across all site-years. Main.stem.diam.avg= Average main stem diameter across all site-years. Comp.main.stem.avg= compressed main stem thickness averaged across all site-years. Avg.branch.diam= basal branch diameter averaged across all site-years. Comp.branch.diam.avg= Compressed branch diameter averaged across all site-years. Epicotyl.diam.avg=Epicotyl diameter averaged across all site-years. Leaf.length.avg=leaf length averaged across all site-years. Mat.time.avg=maturity time rated on a 1-4 scale and averaged across all site-years. Nodes.flow.avg= The average number of nodes to flowering across all site-years. Max. nodes.avg= The maximum number of nodes prior to senescence averaged across site-years. Flowering.nodes.avg= the average number of flowering nodes. Yield.avg= plot yield averaged across site-years. Yld.plant.avg= the average yield per plant averaged across site-years. X100.sd.weight.avg= 100 seed weight averaged over site-years.

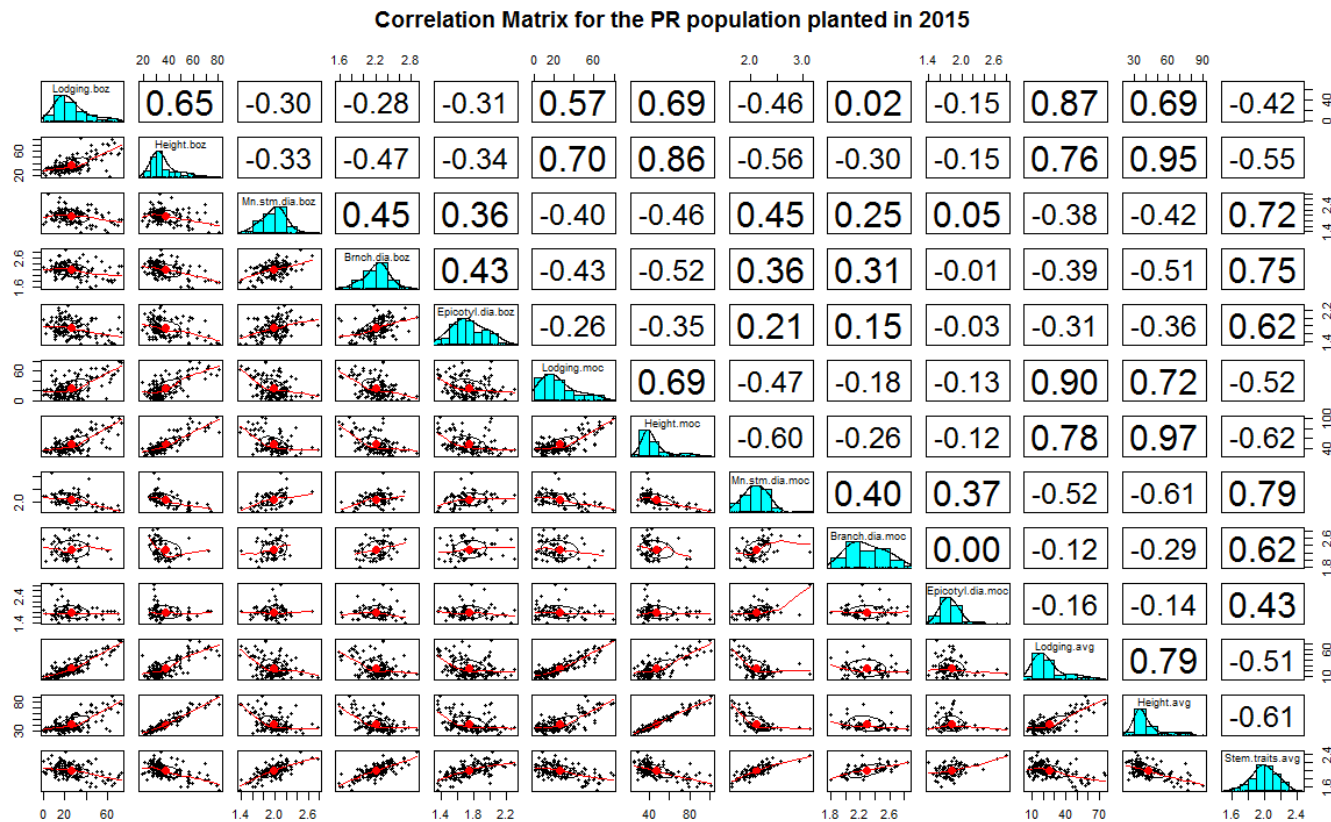**Correlation Matrix for the PR population planted in 2015**

Figure 26: Correlation matrix for the PR population grown at Bozeman and Moccasin, MT during 2015. A correlation matrix is a visual presentation of the correlations between a number of different quantitative variables. The quantitative traits are represented by the histograms on the diagonal. Scatterplots for each trait are shown below the diagonal, and correlations (r) are above the diagonal. The matrix is read by finding at any two traits on the diagonal and looking at the correlations in the column or row above or to the right of it, respectively. Scatterplots are shown in the row to the left or column below each trait on the diagonal. For example, traits 1 and 3 have a correlation of -0.30 (correlation shown in row 1 column 3, scatterplot in row 3, column 1). Traits 2 and 4 have a correlation of -0.47. (scatterplot in row 4 column 2).

Legend (Traits from left to right along the diagonal): Lodging.boz=% lodging at the Bozeman, MT site in 2015. Height.boz=the length of the main stem/plant height at the Bozeman, MT site in 2015. Mn.stm.dia.boz= main stem diameter Bozeman 2015. Brnch.diam.15=Branch diameter Bozeman 2015. Epicotyl.dia.15=Epicotyl diameter at the Bozeman location. Lodging.moc=% lodging at the Moccasin, MT site in 2015. Height.moc=the length of the main stem/plant height at the Moccasin, MT site in 2015. Mn.stm.dia.moc= main stem diameter Moccasin 2015. Brnch.diam.moc=Branch diameter Moccasin 2015. Epicotyl.dia.moc=Epicotyl diameter at the Moccasin location. Lodging.avg=Lodging averaged across both sites. Height.avg=Height averaged across both locations. Stem.traits.avg=all stem diameter traits averaged across locations.

Correlations with Plant Height

Stem length (plant height) and internode length were strongly and positively associated with lodging when averaged across all site-years, indicating that selecting for taller plants in a breeding program may also increase lodging susceptibility. The same effect was seen in the PR population. It is important to note that much of the variation in plant height in the Delta x RER population was due to *Le*, which has been shown in this population to decrease stem diameter and increase plant height. As a result, there was a moderate and negative correlation between main stem diameter and height. If height genes did not concurrently decrease stem diameter and increase plant height, less of an effect on lodging would likely occur. If an inference can be drawn by the effect of *Le*, gibberellin dependent plant height genes may increase height at the expense of decreasing stem diameter. Nearly all height mutants in pea affect gibberellins in some way (Murfet and Reid 1993)

Height was strongly and positively correlated with leaf length, and weakly associated with flowering time, indicating that selecting for height would increase leaf length and lengthen the days to flowering. However, the effect on flowering may be

caused by *Hr*, which is associated with height. Branch number was weakly and positively associated with plant height, indicating that taller varieties have fewer basal branches, possibly because taller plants have reduced light penetration into the canopy and branching is dependent on environment.

Correlations between Branching, Lodging, and Yield

Branch number was moderately and negatively associated with lodging when averaged across site-years, and the same effect was seen across all site-years, indicating that selecting for increased basal branching in a breeding program would reduce lodging. Increasing branch number likely decreases lodging by increasing the number of tendrils per plant, allowing the branches to tie together much better with other plants. The exception to this rule was the Tsw 1.1 locus which concurrently reduced stem diameter and increased branching. Increasing branching (increasing tendril number per plant) unsurprisingly has the same effect on lodging as the *afila* locus, which increases tendril number per plant. However, there is also a moderate and positive correlation between epicotyl diameter and branching, indicating that branched varieties have a much larger basal structure and stronger basal strength. In 2013, it was noted that branched lines tended to have a much stronger basal strength when subjectively rated (data not shown), but data on basal strength was not collected in subsequent years. As mentioned in the materials and methods, seeding rates were purposely kept low in order to aid lodging. The positive correlation between yield and branching is likely due to the propensity of branching varieties to collect more sunlight when seeding rates are low. However, branch

number and yield were correlated in Bozeman in 2016, when the seeding rate was 60 plants per square meter, indicating that branching increased yield regardless of seeding rate, butt has also been shown previously that branched varieties do achieve optimum yield at lower seeding rates (Spies et al. 2010). Branch number was negatively associated with 100 seed weight due to the effect of the Tsw 1.1 locus, which strongly decreases seed size and increases branching. This single locus is also likely to be the reason why main stem diameter and side branch diameter are associated with 100 seed weight. Leaf length was weakly and negatively correlated to branch number, indicating that leaf length decreases with increasing branch number. More competition for light (due to greater numbers of side branches) may decrease leaf length.

Stem Diameter Traits

Across all site-years, basal main stem diameter, basal side branch diameter, and epicotyl diameter were weakly or very weakly negatively associated with lodging, indicating that selecting for increased stem diameter would increase resistance to lodging in a breeding program. Compressed stem thickness and compressed side branch thickness had no apparent effect on lodging, indicating that selecting for these traits in a breeding program would have no effect on lodging. Stem diameter traits and compressed stem thickness traits were shown to be moderately correlated with each other, indicating that selection for increased main stem diameter in a breeding program would likely increase compressed stem thickness, side branch diameter, and epicotyl diameter. Lines with the RER derived allele of *Hr/Rms1/M*, which have narrower main stems and thicker side

branches, are the exception to this rule. Compressed branch diameter and epicotyl diameter were moderately and positively associated with later maturity. It is likely that increasing flowering time gives plants more time to develop larger stems and branches. Selecting for increased epicotyl and compressed branch diameter may also select for increased flowering time.

Yield

While there was not an association between maturity time and yield when averaged across all site-years, there was a negative and weak association between yield and the number of nodes to flowering, indicating that increasing the number of nodes to flowering will decrease yield. It is likely that drier locations than Bozeman would have a larger association between plot yield and nodes to flowering. There was a weak and negative association between maturity time traits and germination, indicating that lowered germination will increase the maturity time of the crop, possible because more water and other resources are available to the remaining plants. As expected, yield and yield per plant were moderately correlated. Averaged across all site-years, germination was moderately correlated with yield. The number of flowering nodes was weakly and positively associated with plant height and yield. Across all site-years, plot yield was weakly and negatively associated with lodging.

The PR Population

The PR population was a separate population that was received from the University of Saskatchewan, which was planted in two locations in 2015. Lodging and height had a similar correlation in both locations, but surprisingly, lodging in Moccassin was only moderately correlated with lodging in Bozeman, indicating that there was probably a great deal of environmental variation between the two locations. Stem diameter traits were weakly or moderately negatively correlated with both plant height and lodging. This significant result indicated taller plants had smaller stem diameters, even though *Le* was not segregating in this population. Stem diameter was negatively associated with lodging, indicating that lodging decreased when stem diameter increased.

These results confirmed the effects observed in the Delta x RER population, indicating that the results of this study were consistent in at least two RIL populations. No other RIL populations were studied.

Importance of the Correlation Matrices

A great deal of breeding effort goes into selecting for traits such as disease resistance, lodging resistance, and other traits. While the results of this study cannot be applied to all other studies in pea genetics. The results of these correlation matrices can be used to understand how selecting for a single trait could undesirably affect another trait, causing problems in a breeding program. While a number of QTL studies find QTLs for a specific trait, this study indicated the specific mechanism by which each QTL functions, allowing a better understanding of the utility of a specific trait or QTL.

APPENDICES

APPENDIX A

DNA EXTRACTION, INITIAL GENETIC MAP, AND DATA

FOR STEM FLEXION

DNA Extraction Procedure for PCR Analysis

Note: You must proceed with the extraction until the DNA is stable. You can let the DNA sit after precipitation with ethanol, while it is drying, and in the Tris EDTA.

How to Make up DNA Extraction Buffer Stock Solution for 200 ml stock solution

1. Add 144 ml of distilled water to 28 ml of 5 M NaCl to 20 ml of 1 M Tris-HCL (PH 8.0).
2. Then add 8 ml of 0.25 M EDTA or 4 ml of 0.5 M EDTA + 4 ml of water.

DNA extraction

1. Turn on the block heater to the high setting (65C) and fill the wells half full of water.
2. Make up the CTAB extraction buffer. For ten samples measure out 10 ml of DNA extraction buffer in a small graduated cylinder. Add 0.1 grams of CTAB. Dissolve the CTAB in the Extraction buffer using a hot plate and a stir stick then add 40 µl of mercapto-ethanol.
3. Do not forget to add the mercapto-ethanol.
4. Label a set of 1.5 ml micro-centrifuge tubes for the number of samples you need to process.
5. Pipet 100 µl of a chloroform/ Isoamyl alchohol mixture into the labeled 1.5 ml microcentrifuge tube. The mixture is made by mixing 24 ml of Chloroform with 1 ml of isoamyl alchohol in a graduated cylinder. This makes a 24: I solution.
6. Take each leaf sample and place it in a small mortar. Pipet in 0.9 ml of the CTAB extraction buffer that you made earlier and grind the leaf sample until the leaf sample has been fully macerated.
7. Pour the slurry into the labeled 1.5 ml micro-centrifuge tubes, while leaving as much solid matter in the crucible as possible.
8. Heat the tubes for 20-30 minutes at 65C in the block heater.
9. While you are waiting, clean out the crucibles and misc glassware. And label the 1.5 ml centrifuge tubes for step 12. Make sure the line number and letter are written on both the side and top of the tube
10. Add enough chloroform/lsoamyl alchohol to fill the tube most of the way (this is generally about 0.5ml)
11. Shake the contents of the tube vigorously by hand to form an emulsion and then centrifuge on the microfuge at room temperature (8,000 to 10,000 rpm) for 10 minutes to separate the phases.
12. The aqueous upper phase is transferred to a clean, labeled micro-centrifuge tube. There should be 0.5 to 0.8 ml of aqueous phase at this point.
13. Precipitate the DNA by adding 0.8 to 1 ml of ice cold 95% ethanol. Gently invert the tubes to mix the two phases (this is a good stopping point). The extraction generally works better if you leave the tubes in the refrigerator overnight.
14. Centrifuge the contents in the refrigerator for 12-15 min at 12,000 rpm. Keep tabs down.

15. Carefully pour out the supernatant. Note the white pellet of DNA at the bottom of the tube and set the tubes in order of least DNA to most DNA. The amount of DNA extracted will determine the amount of reagent added in step 18. Add 0.7 ml of 0.2 M Sodium Acetate in 75% ethanol and allow the tube to sit for 5 minutes.
16. Pour off the Sodium acetate and quickly pipet 0.4 ml of 0.01 M Ammonium Acetate into the tubes to rinse out the sodium. After you are done pipetting, quickly pour it off. If you let the DNA sit in the Ammonium Acetate, it will dissolve the DNA.
17. Invert the tubes onto a dry paper towel, and let the DNA air dry for no less than 3 hours.
18. Dissolve the pellet of DNA in 50 to 100 µl of Tris EDTA (pH 8.0) and put in the fridge OVERNIGHT to await a PCR. The amount you add depends on the amount of DNA extracted. Use a larger volume for the largest pellets of DNA.
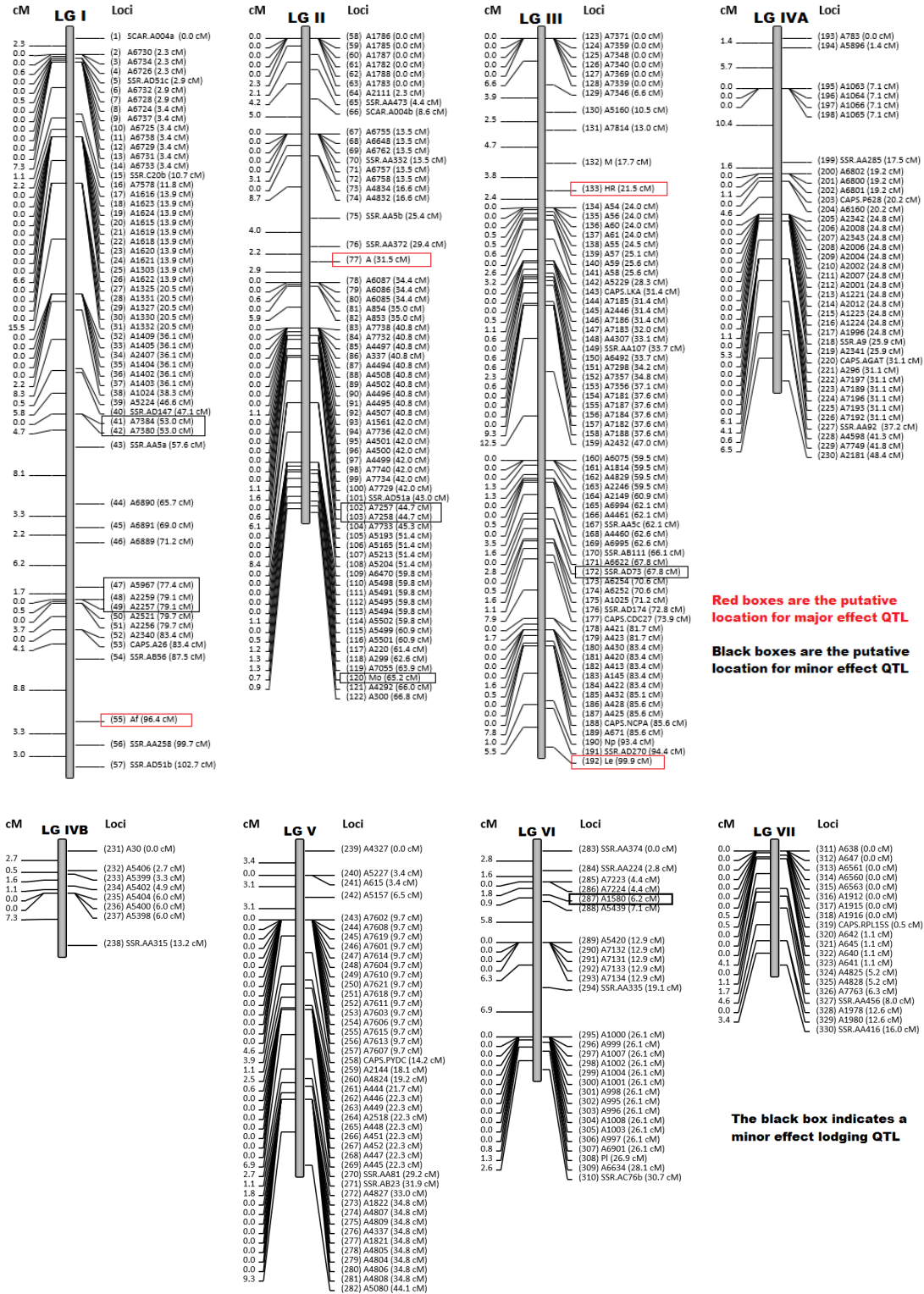
The Map with All 330 Genetic Markers

Figure 27: The genetic map with all 330 genetic markers

Data for Stem Flexion

In this study compressed stem diameter and stem flexion were strongly correlated. Therefore, only data on compressed stem diameter is presented in chapter 3. QTLs for stem flexion are presented here. Stem flexion was assessed by subtracting the relaxed stem diameter after being compressed from the compressed thickness value.

Table 59:QTLs discovered for stem flexion

QTL Name and/or Closest Genetic Marker	LG	Lines used (1)	% Variation (single site year) (2)	p-value (Unadusted, single site year) (3)	False Discovery Rate (Benjamini-Hochberg) (4)	Source of Desirable allele (5)	Effect (mm) (6)
Major QTL (strong evidence). Listed in order of importance (based on p-value)							
Lodge III-2 *(Hr/Rms1/M)*	III	all	32.20%	1.38×10^{-17}	$\sim 1 \times 10^{-15}$	RER	.28 mm
Lodge I-1 *(Afila)*	I	all	6.70%	0.00013	0.00112	RER	.12 mm
Putative Tsw 1.1 (A6724)	I	dwf	13.00%	0.00040	0.00792	Delta	.16 mm
Putative QTL (weak evidence). Listed in order of importance based on p-value.							
A4832 (near *A*)	II	dwf	7.60%	0.0080	0.1435	Delta	.13 mm

(1) Lines used [all= statistical analysis on all 255 lines]; [dwf= analysis used only 94 dwarf lines]

(2) % Variation is the percentage of variation explained by the QTL.

(3) P-value (Unadjusted)=this p-value is not adjusted for multiple comparisons.

(4) False Discovery Rate=The expected proportion of false positives after correcting for multiple comparisons.

(5) Source of desirable allele= The parental allele that increases stem flexion.

(6) The effect size is the mean difference in stem flexion between the two genotypes.

APPENDIX B:

CORRELATION MATRICES
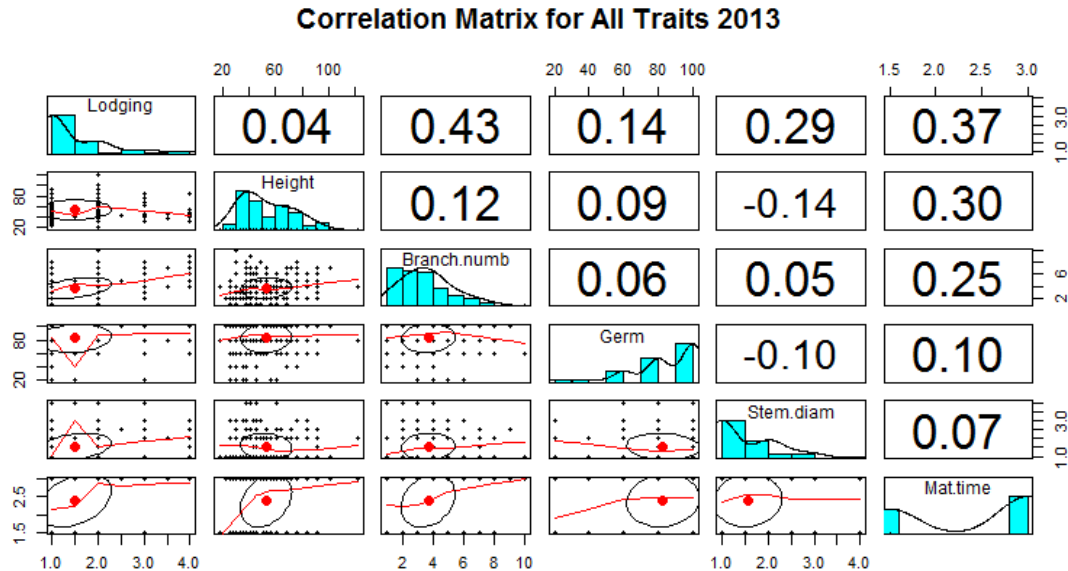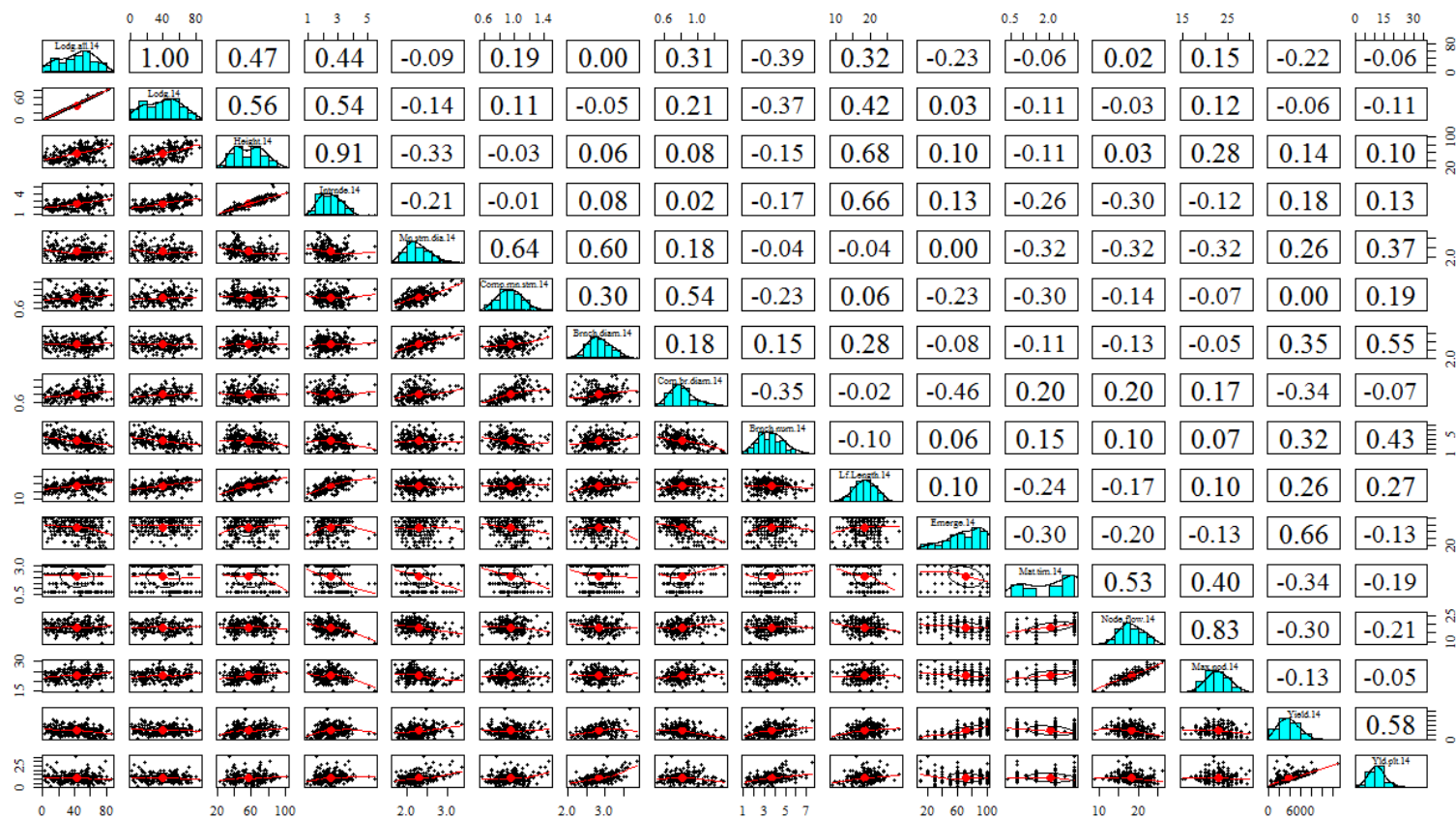
Correlation Matrix for All Traits 2013

Figure 28: Correlation matrix for all traits in 2013. A correlation matrix is a visual presentation of the correlations between a number of different quantitative variables. The quantitative traits are represented by the histograms on the diagonal. Scatterplots for each trait are shown below the diagonal, and correlations (r) are above the diagonal. The matrix is read by finding at any two traits on the diagonal and looking at the correlations in the column or row above or to the right of it, respectively. Scatterplots are shown in the row to the left or column below each trait. For example, traits 1 and 3 have a correlation of 0.43 (correlation shown in row 1 column 3, scatterplot in row 3, column 1). Traits 2 and 4 have a correlation of 0.09 (scatterplot in row 4 column 2).

The traits are as follows from left to right: Lodging=Lodging on a 1-4 scale. It is important to note that lodging was rated on a 1-4 scale only in 2013, where 1 indicated high levels of lodging and 4 indicated no lodging. Height=the length of the stem=plant height. Branch.numb=basal branch number including the main stem. Germ=% germination. Stem.diam=stem diameter rated on a 1-4 scale where 1 is very narrow and 4 is large. Mat.time=maturity time rated on a 1-4 scale.

<u>2013</u>

There was a moderate correlation between the lodging resistance and branch

number. As branch number increased, lodging decreased. There was a very weak

correlation between germination and lodging, indicating that lodging decreased when

germination increased, a result which was seen in most subsequent years. There was also a weak correlation between stem diameter and lodging, indicating that as stem diameter increased lodging decreased, a result which was seen in subsequent years. There was a weak correlation between maturity time and lodging, but it is important to note that this effect is likely a result of the rating method since many later maturing lines were rated prior to full maturity in 2013.

Figure 29: Correlation matrix for all traits in 2014. A correlation matrix is a visual presentation of the correlations between a number of different quantitative variables. The quantitative traits are represented by the histograms on the diagonal. Scatterplots for each trait are shown below the diagonal, and correlations (r) are above the diagonal. The matrix is read by finding at any two traits on the diagonal and looking at the correlations in the column or row above or to the right of it, respectively. Scatterplots are shown in the row to the left or column below each trait. For example, traits 1 and 3 have a correlation of 0.47 (correlation shown in row 1 column 3, scatterplot in row 3, column 1). Traits 2 and 4 have a correlation of 0.54 (scatterplot in row 4 column 2).

Legend (Traits from left to right along the diagonal): Lodging.all=% Lodging with poorly germinating lines included. Lodging1= Lodging with poorly germinating lines excluded. Height1=the length of the stem=plant height. Internode.length=internode length. Main.stm.diam= main stem diameter. Comp.main.stem= compressed main stem thickness. Branch.diam=Branch diameter. Comp.brnch.diam=compressed branch diameter. Branch.numb.1=basal branch number. Leaf.length=Length of the compound leaf including tendrils. Emerge.14=% Emergence. Mat.time.1=maturity time rated on a 1-4 scale. Nodes.1st.flow= nodes to first flower. Max.nodes=The maximum number of nodes attained by the plant prior to senescence. Yield.lb.Acre=Yield in pounds per acre. Yld.plant=Yield per plant.

2014

Lodging was weakly and positively correlated with compressed stem thickness in 2014, indicating that thicker stems increase lodging, possibly due to the increased weight of the stems. This is expected based on the stress equation and the equation for stem area, which predict that increasing stem wall thickness would decrease stress but also increase stem area, which would increase load. Plant height and internode length were moderately and positively correlated with lodging, indicating that selecting for taller cultivars would also increase lodging. Plant height and internode length strongly influenced leaf length, indicating that selecting for increased leaf length would also select for increased plant height. Leaf length was positively associated with lodging because it is strongly influenced by plant height. Selectable markers would be useful to select for genes that increase leaf length without increasing height since this trait is weakly associated with yield. Yield and yield per plant were weakly to moderately positively associated with stem and branch diameter, indicating that selecting for larger branch diameters would have a positive effect on yield. Yield was strongly and positively correlated with percent germination and yield per plant. As expected, maturity time, nodes to flowering, and

nodes to senescence were all moderately or very strongly correlated. Main stem diameter was weakly and negatively correlated with maturity time traits, indicating that as maturity time increased, main stem diameter decreased. Later maturing lines had narrower stems.
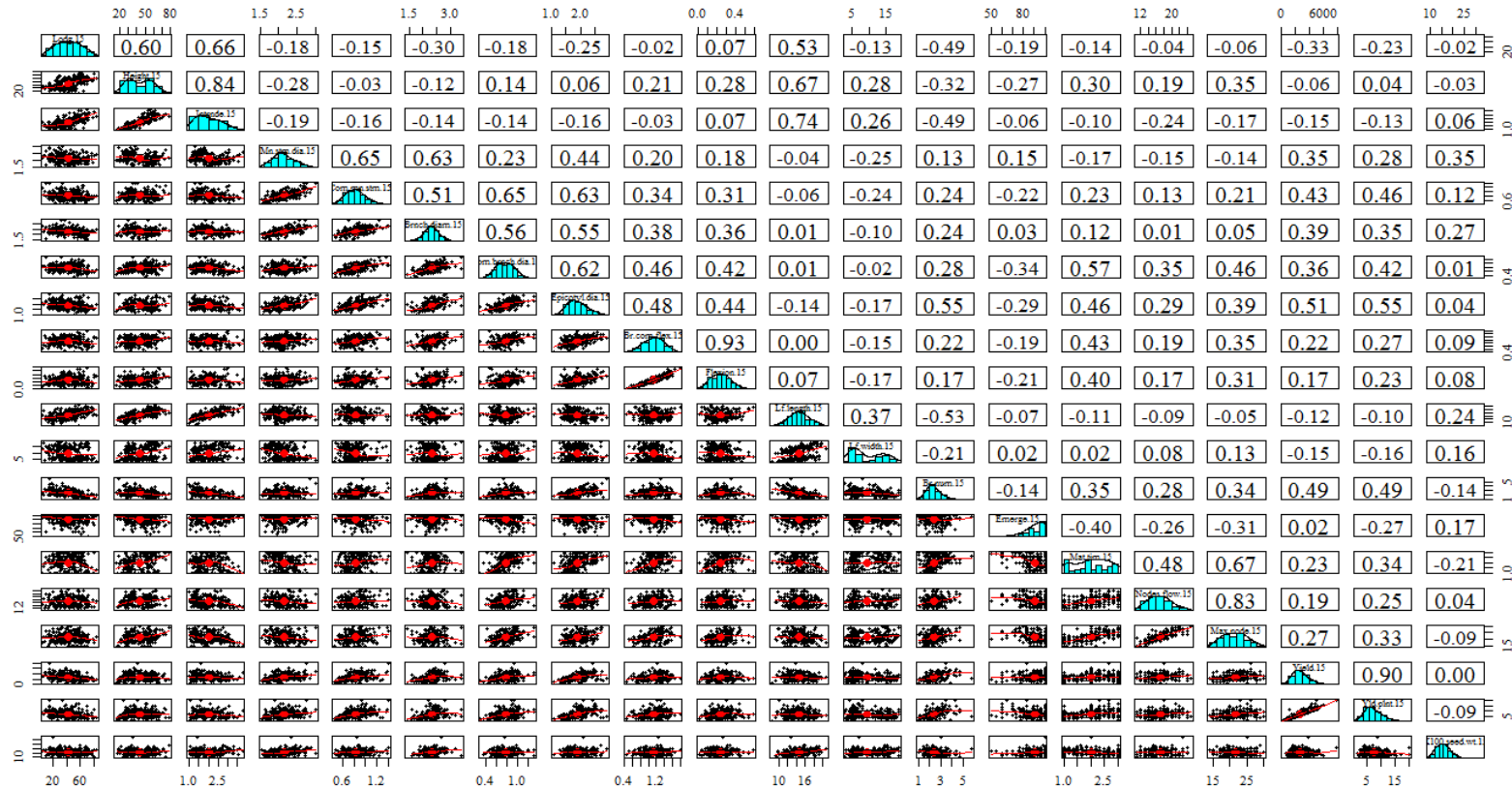
Figure 30: Correlation matrix for Bozeman in 2015. A correlation matrix is a visual presentation of the correlations between a number of different quantitative variables. The quantitative traits are represented by the histograms on the diagonal. Scatterplots for each trait are shown below the diagonal, and correlations (r) are above the diagonal. The matrix is read by finding at any two traits on the diagonal and looking at the correlations in the column or row above or to the right of it, respectively. Scatterplots are shown in the row to the left or column below each trait. For example, traits 1 and 3 have a correlation of 0.66 (correlation shown in row 1 column 3, scatterplot in row 3, column 1). Traits 2 and 4 have a correlation of -0.28 (scatterplot in row 4 column 2).

Legend (Traits from left to right along the diagonal): Lodg.15=% Lodging. Height.15=the length of the main stem/plant height. Intrnde.15=internode length. Mn.stm.dia.15= main stem diameter. Com.mn.stm.15= compressed main stem thickness. Brnch.diam.15=Branch diameter. Com.brnch.dia.15=compressed branch diameter. Epicotyl.dia.15=Epicotyl diameter. Br.com.flex.15= compressed branch diameter measured when there was no crushing pressure on the stem, when the stem had a chance to rebound from crushing. Flexion.15 = The amount the stem rebounded after crushing, which was determined by subtracting compressed branch diameter from Br.com.flex.15. Lf.length.15=Length of the compound leaf including tendrils. Lf.width.15=width of the compound leaf, measured by stretching out the tendrils. Br.num.15=basal branch number. Germ.15=% germination. Mat.tim.15=maturity time rated on a 1-4 scale. Nodes.flow.15= nodes to first flower. Max.node.15=The maximum number of nodes attained by the plant prior to senescence. Yield.15 =Yield in pounds per acre. Yld.plnt.15=Yield per plant. 100.seed.wt.15=100 seed weight.

Bozeman 2015

Lodging was weakly and negatively correlated with compressed stem thickness. This was opposite the effect seen in 2014, indicating that this trait does not reliably predict lodging. Plant height and internode length were strongly and positively correlated with lodging, which was the same result as in 2014. Plant height and internode length strongly increased leaf length. Leaf width was weakly associated with plant height. Stem diameter was weakly and negatively associated with lodging for the second year in row. There was a moderate negative correlation between branch number and lodging for the second year in a row, but branch number was positively correlated with yield for the second year in a row. Yield per plant was moderately and positively correlated with stem and compressed stem thickness traits, which was similar to the result seen in 2014. Yield was very strongly and positively correlated with yield per plant. As expected, maturity time, nodes to flowering, and nodes to senescence were all moderately or very strongly correlated.

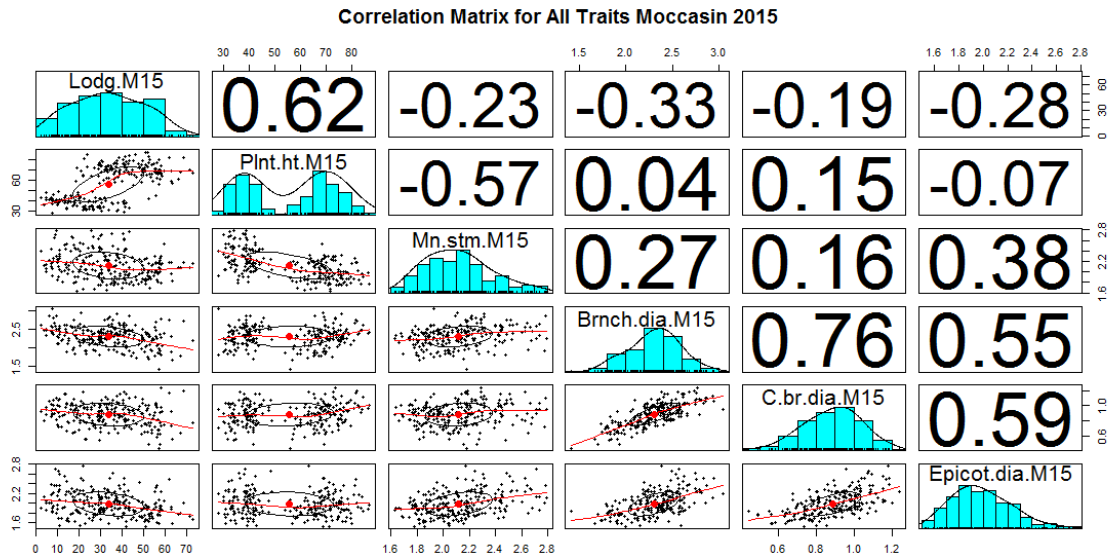**Correlation Matrix for All Traits Moccasin 2015**

Figure 31: Correlation matrix for all traits at Moccasin in 2015. A correlation matrix is a visual presentation of the correlations between a number of different quantitative variables. The quantitative traits are represented by the histograms on the diagonal. Scatterplots for each trait are shown below the diagonal, and correlations (r) are above the diagonal. The matrix is read by finding at any two traits on the diagonal and looking at the correlations in the column or row above or to the right of it, respectively. Scatterplots are shown in the row to the left or column below each trait. For example, traits 1 and 3 have a correlation of -0.23 (correlation shown in row 1 column 3, scatterplot in row 3, column 1). Traits 2 and 4 have a correlation of 0.04 (scatterplot in row 4 column 2).

Legend (Traits from left to right along the diagonal): Lodg.M15=% Lodging at the Moccasin, MT location in 2015. Plnt.ht.M15=the length of the main stem/plant height. Mn.stm.M15= main stem diameter. Brnch.dia.M15=Branch diameter. C.br.dia.M15=compressed branch diameter. Epicot.dia.M15=Epicotyl diameter.

<u>Moccasin 2015</u>

Although fewer traits were recorded, the effects were the same as in previous

years. Plant height increased lodging, and stem diameter traits were weakly and

negatively correlated with lodging. Branch diameter was strongly correlated with

compressed branch diameter, continuing a trend which was seen in other site-years and indicating that increases in side branch diameter also increase compressed side branch thickness.
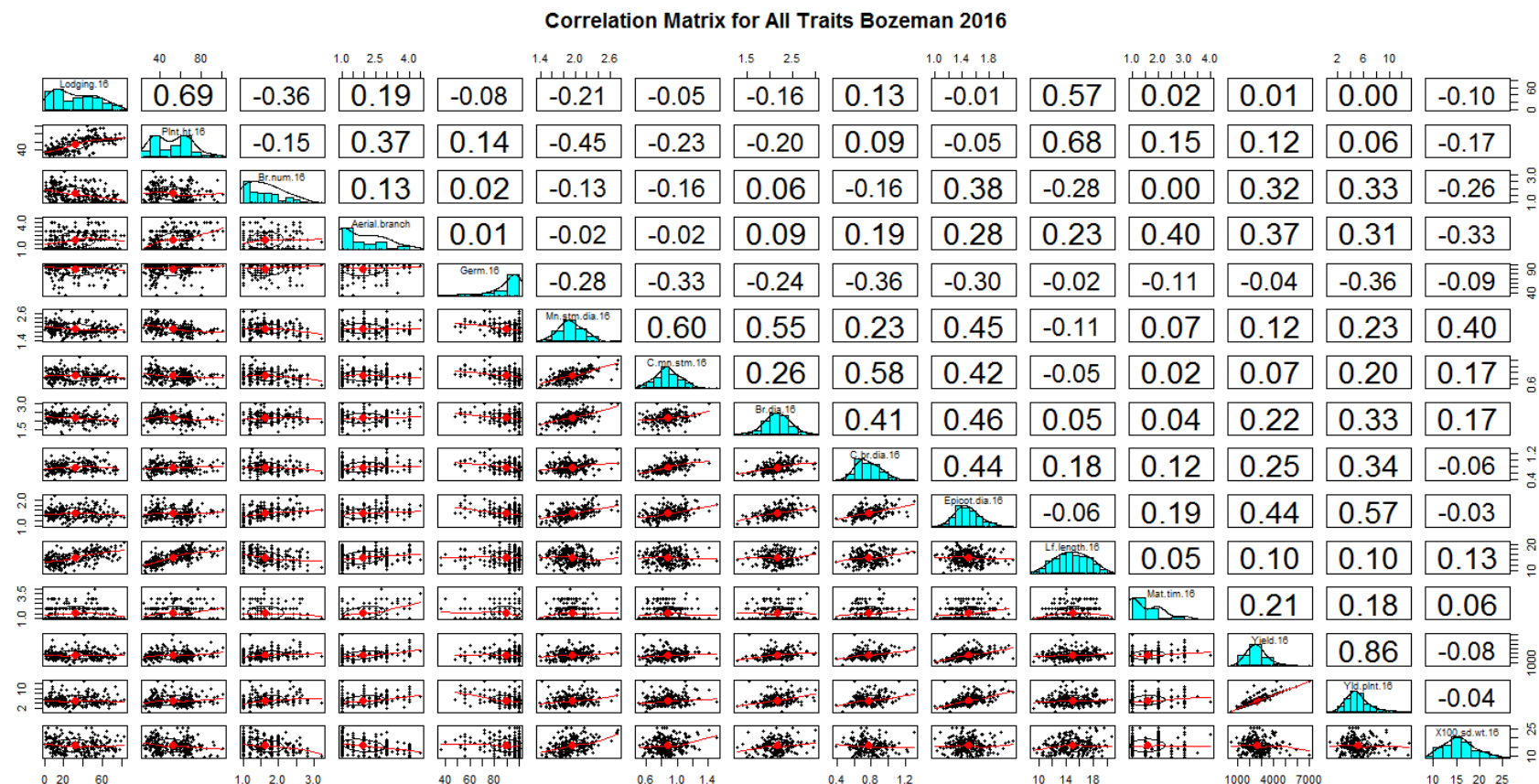
Figure 32: Correlation matrix for all traits at Bozeman in 2016. A correlation matrix is a visual presentation of the correlations between a number of different quantitative variables. The quantitative traits are represented by the histograms on the diagonal. Scatterplots for each trait are shown below the diagonal, and correlations (r) are above the diagonal. The matrix is read by finding at any two traits on the diagonal and looking at the correlations in the column or row above or to the right of it, respectively. Scatterplots are shown in the row to the left or column below each trait. For example, traits 1 and 3 have a correlation of -0.36 (correlation shown in row 1 column 3, scatterplot in row 3, column 1). Traits 2 and 4 have a correlation of 0.37 (correlation shown in row 2 column 4, scatterplot in row 4 column 2).

Legend (Traits from left to right along the diagonal): Lodging.16=% lodging Bozeman 2016. Plnt.ht.16=the length of the main stem/plant height. Brnch.num.16= Branch number. Aerial.branch=Aerial branching in the upper part of the stem rated on a 1-4 scale with 1 having no aerial branching and 4 having an excessive amount. Germ.16=% Germination. Mn.stm.dia.16= main stem diameter. C.mn.stm.16= compressed main stem thickness. Br.dia.16= branch diameter. C.br.dia.16= Compressed branch diameter. Epicotyl.dia.16=Epicotyl diameter. Lf.length.16=leaf length. Mat.tim.16=maturity time rated on a 1-4 scale. Yield.16= plot yield. X100.sd.wt.16= 100 seed weight

<u>Bozeman 2016</u>

Lodging was very weakly correlated with compressed stem thickness traits, but stem diameter traits were negatively correlated with lodging for the 4th site-year in a row, showing that they are a better predictor of lodging than compressed stem thickness. Plant height was strongly and positively correlated with lodging, which was the same result for all site-years. Plant height strongly increased leaf length. Aerial branching was positively correlated with lodging, but also positively associated with plant height. Similar to the effect of branching, aerial branching had a weakly positive effect on yield, indicating that a highly branched phenotype might be desirable to increase crop yield. Aerial branching was also moderately and positively associated with maturity time. Branch number was negatively and moderately associated with lodging, which was seen in all previous site-years. Yield and Yield per plant were moderately and positively correlated with stem and compressed stem thickness traits, especially stem diameter, which was similar to the results seen in previous site-years. Yield was very strongly and positively correlated with yield per plant

With the exception of one site-year, maturity time had no effect on lodging or was negatively correlated, indicating that lodging decreased with increased maturity time.

However, it is possible that this is an artifact of sampling. Plants in Moccasin were rated for lodging over the course of a single day. Due to differing maturity times, it is likely that younger, greener plants had less lodging than plants that matured earlier. In any case, the effect was weak. All the other effects were similar to the effects seen in other site-years, indicating that they were consistent. Stem diameter traits were weakly and positively associated with lodging, which was seen all other site-years. Branch number was negatively associated with lodging, and leaf length and height were both positively and moderately associated with lodging.
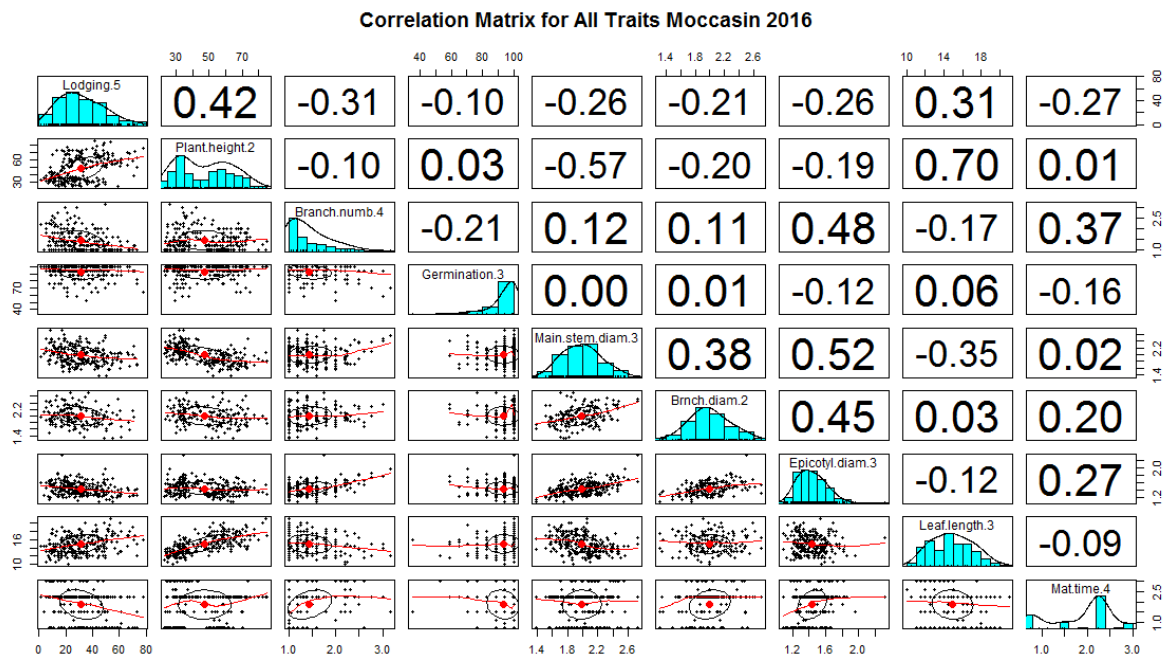

Figure 33: Correlation matrix for all traits at Moccasin in 2016. A correlation matrix is a visual presentation of the correlations between a number of different quantitative variables. The quantitative traits are represented by the histograms on the diagonal. Scatterplots for each trait are shown below the diagonal, and correlations (r) are above the diagonal. The matrix is read by finding any two traits on the diagonal and looking at the correlations in the column or row above or to the right of each trait, respectively. Scatterplots are shown in the row to the left or column below each trait. For example, traits 1 and 3 have a correlation of -0.31 (correlation shown in row 1 column 3, scatterplot in row 3, column 1). Traits 2 and 4 have a correlation of 0.03 (correlation shown in row 2 column 4, scatterplot in row 4 column 2).

Legend (Traits from left to right along the diagonal): Lodging.5=% lodging Moccasin 2016. Plant.height.2=the length of the main stem/plant height. Branch.numb.4= Branch number. Germination.3=% Germination. Main.stem.diam.3= main stem diameter. Brnch.diam.2= branch diameter. Epicotyl.diam.3=Epicotyl diameter. Leaf.length.3=leaf length. Mat.time.4=maturity time rated on a 1-4 scale.

Moccasin 2016

As expected, lodging was positively correlated with plant height. Lodging was negatively correlated with branch number, all stem diameter traits, and maturity time. The effect on maturity time may have been due to the rating method. In contrast to the Bozeman location, where all lines were rated for lodging as they matured, lines at Moccasin were rated only once. Earlier maturing lines likely showed poorer lodging tolerance merely because they were rated a few weeks after senescence rather than at the time of maturity. Crop standability declines after senescence due to the effects of rain, wind, and decomposition, indicating that growers should harvest their pea crop immediately after senescence in order to facilitate harvest.

APPENDIX C

THE DATA FILES USED FOR QTL MAPPING

Data Files Used for QTL Mapping

The Excel spreedsheet with all data with 196 markers for the Delta x RER population is available at Zenodo (Smitchger 2017b).

The Excel spreadsheet with all data for 330 markers is available at Zenodo (Smitchger 2017c).

The Excel spreadsheet with all data for the 330 markers and including sequence data can be found at Zenodo (Smitchger 2017d).

The Excel spreadsheet with all data for all SNP markers, including sequence is available at Zenodo (Smitchger 2017e).

The data from the PR population for plant height, stem diameter, and lodging for both the Bozeman and Moccasin dataset for 2015 is available at Zenodo (Smitchger 2017 f).

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Conflict of Interest Statement

The author declares that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.