UNDERSTANDING THE GENETICS OF FLOWER PRODUCTION IN *PETUNIA*

By

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ABSTRACT

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Petunia (*Petunia* × *hybrida)* has ranked among the top three bedding plants sold in the United States since 1994 and was the top seller in 2013-2015. Understanding the genetics underlying traits related to flowering capacity will be useful to improve commercially available cultivars by decreasing flowering time and improving crop quality. Quantitative trait loci (QTL) mapping can facilitate understanding of the genetic control of flower production and development of marker-assisted breeding strategies to improve breeding efficiencies. In this study, an F_7 *P. axillaris* × *P. exserta* recombinant inbred line population was phenotyped for several crop timing and quality related traits at three greenhouse temperature regimes and at four field locations. Total flower bud and branch number were positively correlated under two of the three greenhouse temperatures. Flowering performance was positively correlated with vigor and plant height at all field locations. All of these traits exhibited transgressive segregation in the population, indicating that they are under polygenic control, and have high broad-sense heritabilities, collectively suggesting that this population should be useful for identification of QTL associated with these traits. A total of 68 and 79 QTL for five greenhouse and seven field traits were identified using composite interval mapping. Thirty of these QTL were detected either at multiple temperatures, years, or locations suggesting that they are robust and useful for marker development. The identification and characterization of QTL underlying flower component traits in different environments provides new insight into the genetic and environmental control of flowering-related traits in petunia.

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CHAPTER 1
LITERATURE REVIEW

Introduction

As the top ranking bedding plant sold in the United States, petunia had a wholesale value estimated at $29 million in Michigan alone in 2015 (USDA-NASS, 2016). With such economic importance within the greenhouse industry, it is not surprising that much focus has been centered on the potential for using petunia for breeding and genetics research. Additionally, growers have indicated a desire to improve commercially available cultivars by decreasing flowering time and improving crop quality (Warner, personal communication).

Petunia background

Petunia spp. are native to South America, ranging mostly from south-eastern Brazil through Uruguay, Paraguay, Bolivia, and Argentina (Sink, 1984; Stehmann et al., 2009; Wijsman, 1982). There are 14 recognized species – *Petunia altiplana, P. axillaris, P. bajeensis, P. bonjardinensis, P. exserta, P. inflata, P. integrifolia, P. interior, P. mantiqueirensis, P. occidentalis, P. reizii, P saxicola, P. scheideana,* and *P. secreta* – however up to 30 subspecies were designated at one point (Gerats and Vandenbussche, 2005; Rijpkema et al., 2006; Sink, 1984; Stehmann et al., 2009). *Petunia axillaris* is characterized by its white corolla and tall habit (Ando et al., 2001; Griesbach, 2007; Stehmann et al., 2009). The plants are pollinated at night by sphingid hawkmoths that are attracted to the scent produced by the flowers (Ando et al., 2001; Stehmann et al., 2009). The distinct floral characteristics of *P. exserta* separate it from the other species. The flowers are uniquely red with a star shaped morphology (Stehmann et al., 2009);. Similar to *P. axillaris,* it has an erect habit and yellow pollen (Lorenz-Lemke et al., 2006; Stehmann et al., 2009). While most *Petunia* species are pollinated by insects, *P. exserta* are the only species pollinated by hummingbirds (Lorenz-Lemke et al., 2006; Stehmann et al., 2009). *P.*

exserta is endemic to a localized region in Serra do Sudeste in Southern Brazil (Stehmann et al., 2009). The natural habitats where *P. exserta* is found are rocky sand stone towers and typically under shade conditions (Lorenz-Lemke et al., 2006; Stehmann et al., 2009). These unique growing conditions make *P. exserta* a valuable species for research because it is reflective of the growing conditions that are challenging for commercial growers in Michigan, i.e., low light.

Flower capacity

For ornamental annuals, flower capacity includes traits such as duration of bloom time, the total number of flowers, floral coverage, and flower longevity, which are important traits that promote season-long garden appeal for consumers.

Genetic control of flower capacity

Extending flowering duration is an important component in a plant's flower capacity, which in some instances can be achieved through early flowering plants (Rafferty et al., 2016). For example, a negative correlation between flower time and cut-flower yield in gerbera (*Gerbera hybrida*) plants suggest that late flowering plants resulted in less flower production (Rafferty et al., 2016; Yu et al., 1993). Across many species, flower induction appears to result from production of a mobile protein (Ayre, 2010; Notaguchi et al., 2008; Romanov, 2012) first identified in *Arabidopsis* as the product of the flowering time gene *FLOWERING LOCUS T* (*FT*) (Corbesier et al., 2007; Notaguchi et al., 2008).

Multiple studies have shown that *FT* function is conserved across species. For example, *Populus tremula* × *tremuloides* (aspen) trees overexpressing the *Populus trichoarpa FT* (*PtFT1*) initiated flower formation 50-80% earlier than wild-type plants (Böhlenius et al., 2006). The

translocation of the Hd3a protein, a rice ortholog of *FT*, through the vascular tissue to the apex meristem acted as a signal for flower induction in rice (Tamaki et al., 2007). Additionally, suppression of *Hd3a* expression in rice increased days to flower and decreased number of branches while over-expression increased branching (Tsuji et al., 2015). Furthermore, increased *FT* expression in *Brachypodium distachyon* restored the flowering phenotype within *PHYTOCHROME C (PHYC*) mutants, which previously exhibited delayed flowering (Woods et al., 2014).

Light is a major factor in regulating flower induction and flowering time. For example, the transcription factor gene *CONSTANS* (*CO*) positively regulates *FT* expression through the photoperiod-dependent pathway (Kardailsky et al., 1999; Kobayashi et al., 1999; Samach et al., 2000; Valverde et al., 2004). Additionally, the photosensory light receptors phytochrome and cryptochrome regulate *CO* and *LEAFY* (*LFY*), a floral meristem identity gene, to influence flowering time (Lin, 2000). Cryptochrome works congruently with phytochrome to induce early flowering in *Arabidopsis* grown under blue-light by suppressing red-light-dependent inhibitors of flowering (Guo et al., 1998). Similar to cryptochrome, the *PHYTOCHROME-DEPENDENT LATE-FLOWERING* gene was identified in *Arabidopsis*, which suppresses late flowering inhibitors and accelerates flowering time through regulation of CO protein stability (Endo et al., 2013). In contrast, *PHYC* mutants exhibited extreme delayed flowering compared to wild type *B. distachyon* plants, indicating a regulatory role for *PHYC* in flower timing (Woods et al., 2014). However, *PHYC* may be essential for early flowering in barley and wheat under long days even though *PHYC* has been indicated as a flowering repressor in *Arabidopsis* and rice (Chen et al., 2014; Nishida et al., 2013).

While the mechanism of floral induction has been intensively studied, much remains unknown about the mechanisms controlling quantitative floral traits such as flower number. Quantitative traits are controlled by multiple genes that can have small to moderate effects on the traits. A few studies have identified genes influencing flower number. For example, the MADS box gene *JOINTLESS* encodes for MADS box proteins that revert the inflorescence meristem to vegetative growth, which reduces flower number in tomato *jointless* mutants (Mao et al., 2000; Szymkowiak and Irish, 2006). Similarly, the tomato *BLIND* (*BL*) gene encodes for a *MYB* transcription factor, which terminates shoot growth after formation of the first inflorescence and, therefore, effectively eliminates formation of new branches and flowers (Schmitz et al., 2001). Additionally, overexpression of a different MADS box gene, *SIMBP11*, increased flower number in tomato, which could partially be attributed to the increase in branch number (Guo et al., 2017). Surprisingly, the *SIMBP11* transgenic plants also showed a 2–3-fold increase in *BL* expression, suggesting that the *SIMBP11* gene may positively regulate *BL* through a feedback regulation mechanism (Guo et al., 2017). These studies support flower number as a quantitative trait and multiple gene networks may work together to regulate the increased and decreased flower number phenotypes. Additional studies are needed to gain a complete understanding of the genetic control for flower number.

Environmental control of flower capacity

Environmental parameters, such as irradiance, temperature, and nutrition, have been demonstrated to impact traits related to flower capacity. For example, an increase in the average photosynthetic daily light integral and average daily temperature were correlated with a decrease in time to flower in petunia (Blanchard et al., 2011; Warner, 2010). Increasing daily light

integral from 2 to 18 mol m^{-2} per day or increasing temperature from 8 to 20°C increased total bud and flower number for *Primula vulgaris* (Karlsson, 2002). However, increasing daily light integral will eventually result in a threshold where no additional gain is observed for decreasing flowering time (Blanchard et al., 2011). Additionally, decreased flowering time in petunia was achieved through increasing irradiance levels from 200 to 500 $\mu mol \cdot m^{-2} \cdot s^{-1}$ and through the use of night-interruption lighting to simulate long photoperiods compared to growth under 9-hour short days (Shimai, 2001; Warner, 2010). Supplying 90 $\mu mol \cdot m^{-2} \cdot s^{-1}$ of supplemental lighting towards the end of petunia seedling production decreased days to flower; however there was a negative trade-off with shoot mass (Oh et al., 2010). Additional studies have shown that decreasing time to flower in petunia may negatively impact plant quality by decreasing branching and flower number at first flowering (Warner and Walworth, 2010).

Temperature has been shown to differentially impact flower production across species. Increasing temperature resulted in greater flower number for multiple crops including *Aeschynanthus speciosus* (basket plant; Welander, 1984), *Chrysanthemum morifolium* (chrysanthemum; Carvalho et al., 2005), *Cyclamen persicum* (cyclamen; Karlsson and Werner, 2001; Oh et al., 2008), *Peperomia caperata* (peperomia; Brøndum and Friis, 1990), *Primula vulgaris* (primrose; Karlsson, 2002), and *Sandersonia aurantiaca* (Christmas bells; Catley et al., 2002). Conversely, a decrease in flower number with increased temperature has been reported in *Platycodon grandifloras* (balloon flower; Park et al., 1998), *Coreopsis grandiflora* (large-flowered tickseed), *Leucanthemum ×superbum* (Shasta Daisy), *Rudbeckia fulgida* (orange coneflower; Yuan et al., 1998), *Antirrhinum majus* (snapdragon), *Calendula officinalis* (calendula), *Impatiens walleriana* (impatiens), *Mimulus × hybrida* (Monkey flower), and *Torenia fournieri* (wishbone flower; Warner and Erwin, 2005a).

Temperature is the primary factor influencing plant development rate and, thus, time to flower for herbaceous annuals. Increasing temperature within a species-specific range decreases time to flower if plants are grown under otherwise inductive conditions (such as an appropriate photoperiod) (Blanchard et al., 2011). Optimal temperatures for flowering time have been documented in many species, such as *Centradenia inaequilateralis* (Friis and Christensen, 1989). Moreover, flower bud abortion was observed in *Arabidopsis* and *Pelargonium* × *domesticum* (geranium) plants grown at higher temperatures (Powell and Bunt, 1978; Warner and Erwin, 2005b). Multiple studies have reported that plant quality decreased with increasing average daily temperature, which may be attributed to plants receiving less cumulative light when grown at higher temperature due to faster development rate (Blanchard and Runkle, 2011; Mathieu et al., 2014; Mattson and Erwin, 2003; Niu et al., 2001; Vaid et al., 2014).

Components of flower capacity

Plant architecture influences flower capacity

A major factor contributing to flower capacity is plant morphology, which can influence inflorescence and flower production. For example, a positive correlation between branch number and flower bud number was reported in two out of three interspecific petunia F_2 populations (Warner and Walworth, 2010). Additionally, plant architecture is an important factor for increasing rice grain yield by reducing tiller numbers and increasing panicle branch number (Jiao et al., 2010). The recessive *aberrant panicle organization 1* mutant in rice reduced the number of primary branches and spikelets, resulting in an approximate 70% reduction in flower number (Ikeda et al., 2005). Reduced height genes (*Rht*) have been used in bread wheat (*Triticum aestivum*) breeding to regulate plant height, peduncle length, and ear length to increase

yield (Chen et al., 2015; Rebetzke and Richards, 2000). Plant morphology is influenced by both genetic and environmental factors, including light, water availability, and nutrient status (Dierig and Crafts-Brandner, 2011; Ewart, 1984; Folta and Childers, 2008; Heuvelink, 1989; Martín-Trillo et al., 2011; Snowden and Napoli, 2003).

Environmental and cultural control of branching

Plant nutrition can affect plant architecture, which can have downstream effects on flower production. Nitrogen and phosphorus deficiency resulted in a decrease in shoot branching in wheat (Yoneyama et al., 2012). Limiting nitrate availability demonstrated a reduction in shoot branching in *Arabidopsis* (de Jong et al., 2014). Inadequate nutrient levels can also influence phytohormones to affect plant development by signaling the release of auxin or strigolactone in sub-optimal conditions (de Jong et al., 2014; Yaish et al., 2010). Increasing nitrogen availability in rice (*Oryza sativa*) increased branching in conjunction with cytokinin application, but decreased branching in conjunction with auxin (Xu et al., 2015).

Additional environmental factors that affect branching in ornamental plants include water availability, light, and temperature. Temporary water restriction of previously irrigated rose bush (*Rosa hybrida*) for 7-35 days increased axillary bud outgrowth by 14-23%, however flower shoot number was unaffected (Demotes-Mainard et al., 2013). In the same study, rose plants grown under low light intensity (91 $\mu mol \cdot m^{-2} \cdot s^{-1}$) in growth chambers for an average of 16 days increased lateral outgrowth by 25% and flower shoot number increased by 35% compared to plants grown under high light intensity (580 $\mu mol \cdot m^{-2} \cdot s^{-1}$). In a different study, mechanical stimulation of young rose plants during propagation was an effective method to obtain compact potted rose with twice as many branches compared to unstimulated plants (Morel et al., 2012).

Branch number is similarly affected by light quantity and quality. For example in *Salvia exserta* (salvia), an increase in irradiance from 250 or 400 μmol·m^{-2}·s^{-1} to 870 or 1040 μmol·m^{-2}·s^{-1} produced a threefold increase in branch number but no differences were observed between 870 and 1040 μmol·m^{-2}·s^{-1} (Mata and Botto, 2011). Additionally, salvia plants grown under a photoselective film to provide a red to far-red ratio of 5.7 doubled the number of lateral branches compared to control plants. In the same study, increasing temperature had an opposing effect on branch number compared to increasing light levels. For example, lateral shoot number decreased with an increase in temperature from 17 to 26°C, though flowering time was also decreased (Mata and Botto, 2011).

Phytohormone control of branching

Phytohormones such as strigolactones and auxins have been implicated in the suppression of axillary branch outgrowth in *Arabidopsis*, pea, and chrysanthemum (*Dendranthema grandiflorum*) (Dun et al., 2013; Gomez-Roldan et al., 2008; Hayward et al., 2009; Liang et al., 2010; Ongaro and Leyser, 2008; Rameau, 2010). Originally recognized in the rhizosphere of parasitic plants and having a role in developing symbiotic relationships to mycorrhizal fungi, strigolactones have also been identified in non-parasitic plants such as pea, petunia, and *Arabidopsis* and play a role in shoot branching (Gomez-Roldan et al., 2008; Rameau, 2010). Strigolactones are carotenoid-derived phytohormones that are synthesized in plant roots and transported acropetally to inhibit axillary bud outgrowth (Gomez-Roldan et al., 2008; Rameau, 2010; Umehara et al., 2008). The presence of strigolactones inhibited shoot branching in garden pea (*Pisum sativum*) (Gomez-Roldan et al., 2008), chrysanthemum (Liang et al., 2010), rice (*Oryza sativa*) and *Arabidopsis* (Umehara et al., 2008). Strigolactone transport is

regulated by the ATP-binding cassette transporter PDR1 to increase branching in petunia (Kretzschmar et al., 2012). The *Arabidopsis MORE AXILLARY GROWTH1* (*MAX1)* and *MAX2* mutants have been identified to increase shoot branching through suppression of strigolactone synthesis and perception (Booker et al., 2005; Drummond et al., 2012; Stirnberg et al., 2002). The petunia ortholog of the AtMAX2 protein, *PhMAX2A*, was shown to similarly control branching, indicating functional conservation across species (Drummond et al., 2012).

Auxin controls apical dominance and indirectly suppresses axillary bud outgrowth. Removal of the apical stem, a source of auxin, activated dormant buds to initiate branch development in *Arabidopsis* (Domagalska and Leyser, 2011; Ongaro and Leyser, 2008). Additionally, auxin has been shown to upregulate strigolactone synthesis by promoting the carotenoid cleavage dioxygenases *MAX3* and *MAX4* expression levels in *Arabidopsis* (Hayward et al., 2009). Likewise, strigolactone regulates auxin flow and transport through a positive feedback loop (Bennett et al., 2006; Crawford et al., 2010; Müller and Leyser, 2011).

In addition to strigolactone and auxin, cytokinin affects branching in plants. Similar to strigolacone, cytokinin is synthesized in the roots as well as the stem and transported through the xylem to axillary buds; however, unlike strigolactone and auxin, cytokinin acts directly in the buds to promote outgrowth (Cline, 1991; Müller and Leyser, 2011). Increasing levels of cytokinin results in greater axillary growth and shoot branching (Yaish et al., 2010). Additionally, application of synthetic cytokinin with gibberellin stimulated lateral branch outgrowth and provided significant synergistic effect on bud outgrowth and formation in *Jatropha curcas* (Ni et al., 2015).

Cytokinin interacts with auxin to influence plant architecture. Auxin is shown to repress cytokinin biosynthesis leading to repressed shoot outgrowth (Nordstöm et al., 2004). A model

for the interaction of these two phytohormones describes the presence of auxin repressing the synthesis of cytokinin, but when the shoot apex was removed, decreased auxin levels led to increased cytokinin synthesis and increased axillary bud growth (Shimizu-Sato et al., 2009). Additionally, exogenous application of cytokinin can trigger shoot outgrowth even in the presence of auxin (Chatfield et al., 2000; Faiss et al., 1997; Wickson and Thimann, 1958). While exogenous application of auxin upregulates strigolactone production, cytokinin synthesis was downregulated at nodal sites in pea (Tanaka et al., 2006). Concurrently, cytokinins have been shown to upregulate auxin biosynthesis and increase auxin levels in the stem, which suggests cytokinin has a role in auxin transport (Jones et al., 2010; Li and Bangerth, 2003, 1992).

Cytokinin synthesis is driven by isopentenyl transferase (*ipt*) enzymes (Barry et al., 1984). Transgenic tobacco overexpressing the *ipt* gene exhibited an increased axillary bud outgrowth phenotype as a result of endogenous cytokinin accumulation (Medford et al., 1989). Within petunia, the *Sho* (*Shooting*) mutant encodes for isopentyl transferase, which catalyzes the formation of bioactive cytokinins and increased shoot induction (Zubko et al., 2002). Additionally, application of nitrogen and auxin increased expression of OsIPT genes and inhibited cytokinin biosynthesis (Liu et al., 2011).

Different environmental stimuli may upregulate or downregulate the expression of branching genes and affect plant morphology depending on the species (Doust, 2007; Wang et al., 2010; Wen et al., 2015). Low phosphate levels or application of exogenous auxin up-regulated *DgD14* — a strigolactone receptor in chrysanthemum — while exogenous strigolactone application down-regulated *DgD14* expression to reduce the average number of branches (Wen et al., 2015). Applications of nitrogen and cytokinin downregulated the

expression of the rice branching gene, *FINE CULM 1*, whereas exogenous auxin and strigolactone treatments upregulated *FINE CULM 1* expression levels (Xu et al., 2015).

Specifically within petunia, there are multiple genes influencing branching that are affected by environmental stimuli. For example, overexpression of an auxin regulated gene known as *EXPANSIN*, which encodes for cell wall metabolism and expansion proteins, led to increased axillary branching in transgenic *P. hybrida* (Dal Santo et al., 2011). Additionally, both phosphorus and light quality treatments significantly altered the expression of two *TCP* genes – a family of transcription factors associated with cell proliferation, signaling pathways, and bud outgrowth – to suppress branching in petunia (Cubas et al., 1999; Drummond et al., 2015). Regulation of these branching genes can have important downstream effects on flower number.

Quantitative trait mapping

Many important traits such as yield and crop quality are regulated by many genes with low effects and are considered quantitative. Specific regions within plant genomes associated with a quantitative trait are known as quantitative trait loci (QTL) (Collard et al., 2005). Individual progeny of an F_2 population were used to develop near homozygous recombinant inbred lines (RIL) through inbreeding. In addition to homozygosity, another advantage of a RIL population is the greater opportunities for recombination events to break closely linked markers through multiple rounds of meiosis (Alonso-Blanco et al., 1998). QTL mapping uses statistical analysis to detect chromosomal regions associated with quantitative phenotypic variation and detect correlations between phenotypes and genetic markers in segregating populations like F_2 and RIL (Asíns, 2002; Mackay et al., 2009; Tanksley, 1993). QTL analysis has been utilized to understand the genetic control of branching, yield, and pest resistance traits in agronomic crops

for improving new cultivars (Chakrabarti et al., 2014; Collard and Mackill, 2008; He et al., 2014; Shirasawa and Hirakawa, 2013; Yue et al., 2015; Zhang et al., 2010). However, QTL studies are limited for ornamental crops due to a lack of genetic maps and molecular markers (Byrne, 2007). But advances in molecular techniques and lower genotyping costs have improved access to generate large numbers of genetic markers needed for molecular studies in ornamental crops (Debener, 2012; Yagi, 2015). For example, 39 QTL were identified for ten floral traits including eight QTL for flower number in an interspecific F_2 population of the perennial plant, *Aquilegia* (Zhu et al., 2014). Genome wide association analysis and single marker analysis of variance in chrysanthemum identified eleven markers associated with four strigolactone pathway genes *BRC1*, *CCD7*, *CCD8*, and *MAX2* (Klie et al., 2016).

Compared to other ornamental crops, petunia has been used as a model genetic system since the 1950's due to its relatively low chromosome number (2n=14), ease of cultivation (sexual or asexual), inbreeding potential, large genetic variability, and available genomic resources (Bossolini et al., 2011; Ganga et al., 2011; Gerats and Vandenbussche, 2005; Strommer et al., 2009). The first petunia gene map published in 1984 by Cornu presented 60 loci, which was updated to 200 markers with the introduction of restriction fragment length polymorphisms (RFLP) and later amplified fragment length polymorphism (AFLP) markers (Strommer et al., 2009). Improvements in technology with a variety of genetic markers and genotyping-by-sequencing approach has contributed to the development of available draft genomes of *P. axillaris* and *P. inflata*, a close relative of *P. integrifolia* (Collard et al., 2005; Sims et al., 2012). A genetic linkage map of a *P. integrifolia* × *P. axillaris* F_2 population was created using simple sequence repeat (SSR) molecular markers mined from publically available

P. axillaris transcripts and cleaved amplified polymorphic sequence markers (Tychonievich et al., 2013; Vallejo et al., 2015).

Modern commercial cultivars of *P.* × *hybrida* are believed to originate from *P. axillaris* and *P. integrifolia* (Strommer et al., 2009). Petunia wild species exhibited significant variation in development rate compared to commercial cultivars, indicating a loss of alleles for this trait during traditional breeding programs (Warner and Walworth, 2010). Three interspecific populations utilizing *P. integrifolia*, *P. axillaris*, *P. exserta,* and *P.* × *hybrida* were evaluated by Warner and Walworth (2010). All populations exhibited transgressive segregation and variability for many crop timing and quality traits. Variation in crop quality traits (e.g. flower diameter, flower length, leaf developmental rate, node below first flower, and leaf length) among different *Petunia* species and commercial cultivars suggests that wild species can contribute genetic diversity to commercial cultivars (Vallejo et al., 2015; Warner and Walworth, 2010). A subsequent study of a separate F_2 *P. integrifolia* × *P. axillaris* population identified two major QTL (>25%VE) that explained 42% and 25% of the variation for flower bud number on the main stem and branch number, respectively (Vallejo et al., 2015).

These previous studies were conducted on F_2 populations, which can exhibit high heterozygosity. Therefore, two interspecific F_7 recombinant inbred line (RILs) populations were developed through single seed descent from the F_2 populations: *P. integrifolia* × *P. axillaris* and *P. axillaris* × *P. exserta*. The F_7 *P. integrifolia* × *P. axillaris* RILs were phenotyped for crop timing and quality traits at four different temperature ranges (Lin, 2014). This population exhibited similar transgressive segregation shown by the F_2 population across all temperature ranges. A bi-modal distribution for difference in days to flower between short- and long-day conditions was observed (Lin, 2014). In addition to greenhouse studies of these two F_7

populations, they were evaluated for field performance, including flowering intensity, at seven different locations throughout the United States during the summer of 2014. Genetic linkage maps of both RIL populations were created using single nucleotide polymorphism (SNP) markers obtained from tunable genotyping by sequencing (Guo et al., 2017).

Thesis goals and objectives

Goals

The goal of this study was to understand the genetic and environmental controls of flower number in *Petunia* through phenotyping an interspecific F_7 RIL population derived from a cross between *P. axillaris* and *P. exserta* under different environments to genetically map and identify QTL for crop quality traits.

Objectives

1. Phenotype the interspecific *P. axillaris* × *P. exserta* F_7 RIL population with 171 lines at three temperature regimes for crop timing and quality traits.
2. Identify potential QTL for flower number and branching traits at first flowering and compare to the QTL identified in the F_2 population.
3. Identify potential QTL for flower intensity and quality traits from different field locations and compare to flower number at first flowering.

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CHAPTER 2

EFFECT OF TEMPERATURE AND PHOTOPERIOD ON FLOWERING CHARACTERISTICS OF A *PETUNIA* RECOMBINANT INBRED LINE POPULATION

Introduction

Petunia (*Petunia* × *hybrida*) ranked first among annual bedding plants sold in the United States in 2015, with a total wholesale value of $133 million in the 15 states surveyed (USDA-NASS, 2016). The ease of cultivation and propagation for petunia has supported its role as a model plant for plant biology research. Flowering is an important ornamental trait that can improve plant aesthetics and market value. Plant architecture can greatly influence the intensity of flower production (Elitzur et al., 2009; Guo et al., 2017; Huang et al., 2017; Mauro-Herrera and Doust, 2016). Previous studies have been conducted to understand the control of flowering time, flower development and flower color (Adams et al., 1998; Chaïlakhyan, 1975; Guo et al., 1998; Holton et al., 1993; Powell and Bunt, 1978; van der Krol and Chua, 1993; Van Houwelingen et al., 1998; Yin et al., 1996). However, the environmental and genetic interaction for many crop timing and quality traits related to flowering are not well characterized.

Breeding programs for ornamental crops have traditionally focused on flower color (Tornielli et al., 2009). However, there has been an increasing interest in understanding how genetic and environmental factors such as day length and temperature influence plant architecture, morphology, and development rate (Blanchard et al., 2011; Doust, 2007; Snowden and Napoli, 2003; Vallejo et al., 2015). *Physaria fendleri* plants grown at extreme average daily temperatures (8.4 and 33.4°C) had 63% fewer branches compared to plants grown at average moderate temperatures (15.4 and 25.1°C) (Dierig and Crafts-Brandner, 2011). However, *Eustoma grandiflorum* plants had increased branch number when grown under a 10 h photoperiod compared to a 20 h photoperiod (Islam et al., 2005). Plants that produce more branches have a greater capacity to produce flower buds. However, branching is also a complex trait that is impacted by both genetic and environmental factors, including plant hormones, light

quality and quantity, and nutrition (de Jong et al., 2014; Finlayson et al., 2010; Gomez-Roldan et al., 2008; Li et al., 2013; Mata and Botto, 2011).

Three independent loci impacting apical dominance have been identified in petunia and the mutants were designated as *decreased apical dominance* (*dad1*, *dad2*, and *dad3*) (Napoli and Ruehle, 1996). These mutants exhibit more branching and compactness compared to wild-type plants (Napoli, 1996), whereas *sympodial* (*sym*) mutants have delayed axillary branching compared with wild-type (Napoli and Ruehle, 1996). The *dad2-1* and *dad3* petunia mutants showed increased branch number under a short day photoperiod. Conversely, no differences were observed for *dad1-1* or the double mutant *dad1-1 sym1* (Snowden and Napoli, 2003), suggesting an interaction between photoperiod and the genetic control for branching. Similar relationships have been identified in pea (*Pisum sativum*) and *Arabidopsis* (Beveridge et al., 2003; Stirnberg et al., 2002).

The effect of photoperiod and temperature on total flower bud and branch number varies by species and even cultivar (Craig and Runkle, 2016; Vaid and Runkle, 2015; Warner, 2010). In a study of four *Petunia* spp., an increase in total branch number at first flowering was observed for three of the four species, whereas an increase in total bud number was observed for two of the four species, when grown under a short day photoperiod (Warner, 2010). However, *Cyclamen persicum* ‘Metis Purple Flame’ produced the most flower buds when grown under a 16-h photoperiod and the lowest under an 8-h photoperiod, regardless of temperature (Oh et al., 2008). In contrast, increased flower bud number was observed in two *Hydrangea macrophylla* cultivars grown at a moderate temperature (17°C) compared to a high temperature (24°C) regardless of photoperiod (Nordli et al., 2011). Many studies have evaluated the effect of temperature alone or in conjunction with photoperiod or phytohormones on branching and

flowering (Dierig and Crafts-Brandner, 2011; Seiler, 1998; Su et al., 2001; Torres and Lopez, 2011; Vlahos et al., 1992). However, there is a lack of information regarding the genetic basis for variation in the temperature sensitivity of these traits. Identification of genetic controls that can lead to increased branching or flower number at varying temperatures and photoperiods will be beneficial for breeders and growers to develop new cultivars that produce more flowers.

Wild species may be a useful source of genetic variation for petunia breeding by introgression of alleles that may have been lost during selection for other desirable traits (Vallejo et al., 2015; Warner and Walworth, 2010). In many crops, including rice, tomato, potato, maize, ground nut, and wheat, wild relatives have been used successfully as a source of alleles to improve traits such as pest and disease resistance (Hajjar and Hodgkin, 2007). Increased genetic diversity can lead to innovative cultivars such as apples with red flesh and strawberries with enlarged fruit size (Stegmeir et al., 2010; van Nocker et al., 2012). Wild type alleles have contributed to variation in flower color as well as plant morphology in a wide array of ornamental crops such as *Lilium*, *Fuchsia*, *Papaver somniferum, Passiflora, Iris, Polianthes tuberosa, Hemerocallis, Ranunculus, Kalanchoe,* and *Paeonia* (Barba-Gonzalez et al., 2013; Cao et al., 2013; Costa et al., 2007; Izumikawa et al., 2007; Nesi et al., 2014; Saikia and Gupta, 2014; Shi et al., 2015; Silva et al., 2014; Talluri and Murray, 2014; Yang et al., 2013).

Previous studies in our lab have shown that some accessions of wild petunia species exhibited significantly faster development rate at varying temperatures compared to a panel of modern cultivars (Warner and Walworth, 2010). The study also showed that the interspecific *P. exserta* × *P. axillaris* F_2 generation had significant differences for node number, flower diameter, branch number, and leaf length compared to parental lines, suggesting they may be a useful source of genetic variation to improve commercial cultivars. The objective of this study was to

characterize phenotypic variation in an F_7 *P. axillaris* × *P. exserta* recombinant inbred line (RIL) population for (a) crop timing and quality traits in response to temperature and (b) quality traits in response to environment.

Materials and methods

Greenhouse experiment

Seeds of 171 F_7 *P. axillaris* (PI 667515) × *P. exserta* (OPGC943) RILs and the two parents (Figure 2-1) were sown on November 5, 2014 and again on November 20, 2015 in 288-cell plug trays filled with 50% vermiculite and 50% soil-less media (70% peat moss, 21% perlite, 9% vermiculite [v/v]; Suremix, Michigan Grower Products Inc., Galesburg, MI, USA). The 171 lines were chosen from a population of 200 lines that were previously phenotyped in the field and genotyped using a genotyping-by-sequencing method (Guo et al., 2017). A total of 29 lines were removed for greenhouse testing due to heterozygous and missing genotypes. Seed trays were covered with clear dome lids and kept in a growth chamber at 23°C and 50% relative humidity under a 10-h photoperiod (provided by fluorescent lamps) for germination. Dome lids were removed when 75% of the seeds had germinated within a tray. Seedlings were thinned to one plant per cell as needed. When seedlings had developed two true leaves, the temperature was lowered to 20°C. Plugs were removed as necessary to provide additional growing space.

After 3 weeks in the growth chamber, the trays were moved to the Plant Science Greenhouses at Michigan State University (East Lansing, MI) under ambient light. On December 2, 2014 and December 15, 2015, 12 plants per RIL and per each parent were transplanted into 10-cm diameter round pots with the soilless media mix described above and moved into treatments.

Four treatments, each consisting of three replications per RIL and per each parent were arranged in a randomized complete block design. Treatment temperatures were constant 14, 17, or 20°C under a 16-h photoperiod, and 20°C under a 9-h photoperiod. Actual average weekly temperatures are presented in Figure 2-2. All plants received supplemental lighting (~67-124 ± 18 µmol m^{-2} s^{-1} of photosynthetically active radiation from 0600-2200 HR for the 16-h photoperiod, and from 0800-1700 HR for the 9-h photoperiod) provided by high-pressure sodium lamps. Blackout cloth was closed from 1700-0800 HR to achieve the 9-h photoperiod. Initially, plants were grown pot-tight and were subsequently spaced 10 – 20 cm 14, 21, and 27 days after initiation of treatments (DAT) at 20, 17 and 14°C, respectively. Plants were overhead irrigated as needed with deionized water containing a water-soluble fertilizer (125 ppm N, 30 ppm P, 145 ppm K; MSU Orchid RO Water Special 13-3-15; GreenCare Fertilizers, Inc., Kankakee, IL).

Greenhouse data collection

In 2014, the number of nodes on the primary shoot (Nodes) were counted 0, 14 and 28 DAT for plants grown under the 16-h photoperiod. Nodes were counted 0, 14, 21 and 28 DAT in 2015. Day 0 started on December 6-8, 2014 and December 19-21, 2015 depending on treatment. Development rate (DRate) was calculated as the increase in node number per unit time and expressed in nodes d^{-1}. Plants at 20°C treatment exhibited signs of reproductive growth at 28 DAT. Therefore, DRate data is only presented for 14 DAT.

The following data were collected for each plant under the 16-h photoperiod when the first flower opened on the main stem: date of anthesis (DTA), total number of open flowers and flower buds (bud length > 3mm) (FlBud), number of flower buds along the main stem (FlBudPS), number of flower buds on the apical meristem (FlBudAS), total branch number

(lateral stem > 5cm) (Branch), number of branches with flower buds (FlBranch), diameter of first open flower (FlDiam), node number below first open flower (excluding node of first flower) (Nodes), height to node of first open flower (as measured from media line) (HghtFl), and length and width of the third leaf below the first flower (LLeng and LWid). Internode length was calculated as the average distance between nodes (cm) (Internode). Additional data were collected from plants that had the first open flower on a lateral branch: date of first open flower on a lateral shoot (DTA2), node number below the flowering lateral branch (NBL), node number below the open flower on the lateral branch (NodesLB), and diameter of open flower on lateral branch (FlDiam2) (Table 2-1).

For plants grown under the 9-h photoperiod, the following data were collected: DTA, FlDiam, and location of open flower (main stem or lateral branch) (Side). For plants with the first open flower on the main stem, node number below the first open flower on the main stem (Nodes) was collected. For plants with the first open flower on a lateral branch, node number below the flowering lateral branch (NBL) and node number below the first open flower (NodesLB) were collected.

Field experiment

In 2014, 200 RILs from this population were evaluated at four field locations representing four distinct climates throughout the United States (Table 2-2). The seeds for each line were sown and the plants were grown on site. Each line was transplanted after 7 to 8 weeks of cultivation in the greenhouses. At each location, three replicates were transplanted into the field with the exception of the Huntersville, NC location, which only had two replicates. The plants were irrigated and fertilized according to standard practices at each trialing site. After ca.

12 weeks in the field, the plants were evaluated for percentage canopy coverage with flowers (Flow), plant vigor (scale 1-9, 1 = low vigor and 9 = high vigor; Vigor), plant compactness (scale 1-9, 1 = not compact, 9 = very compact; Comp), plant height (measured from the soil line to the top of the plant canopy; Height), and plant maximum width (measured as the widest length of the plant canopy; MaxWid) at all locations (Table 2-1). In addition, flower color retention (scale 1-9, 1 = very faded, 9 = not faded; ColorRet) was assessed at Huntersville, NC; Gilroy, CA; and Buellton, CA and plant minimum width (measured as the shortest length of the plant canopy; MinWid) was calculated at Huntersville, NC and Buellton, CA.

Data analysis

Data were analyzed using Statistical Analysis Software v9.4 (SAS Institute, Cary, NC). Analyses were performed using ANOVA to compare the mean parental lines to RIL population. Broad-sense heritability (H^2) was calculated for all evaluated traits as described by Fehr (1987). The equation was based on the variance component and calculated using the expected mean squares for each source $H^2 = \frac{\sigma_g^2}{\sigma_g^2+\sigma_\varepsilon^2}$ where σ_g^2 is the variance of the genotype effect and σ_ε^2 is the variance of the environmental effect. The variance of the environmental effect was calculated as $\sigma_\varepsilon^2 = \frac{\sigma_{gy}^2}{y} + \frac{\sigma_{gt}^2}{t} + \frac{\sigma_{gty}^2}{ty} + \frac{\sigma_e^2}{rty}$ where σ_{gy}^2 is the variance among the genotype by year, σ_{gt}^2 is the variance among the genotype by temperature, σ_{gty}^2 is the variance among genotype by temperature and year, σ_e^2 is the residual, y is the number of years in the study, t is the number of temperature treatments, and r is the number of replicates.

Broad-sense heritability was calculated at individual temperature treatments using the above equation, however the variance of the environmental effect was calculated as $\sigma_\varepsilon^2 = \frac{\sigma_{gy}^2}{y} + \frac{\sigma_e^2}{ry}$ and terms as described above.

For plants phenotyped in the field, broad-sense heritability was calculated at all locations using the same H^2 equation. However, the variance of the environmental effect was calculated as $\sigma_\varepsilon^2 = \frac{\sigma_{gl}^2}{l} + \frac{\sigma_e^2}{rl}$ where σ_{gl}^2 is the variance among the locations, σ_e^2 is the residual, l is the number of locations in the study, and r is the number of replicates. At individual locations, broad-sense heritability was estimated using $\sigma_\varepsilon^2 = \frac{\sigma_e^2}{r}$ with terms as described above.

Results

Greenhouse results

The population exhibited transgressive segregation for all flowering traits at each temperature, which indicates the presence of extreme phenotypes compared to the parental lines (Table 2-3; Figure 2-3 – 2-5). The percentage of plants exhibiting first flower opening on a lateral shoot increased eightfold as temperature decreased from 20 to 14°C (Table 2-4).

Development rate was calculated for each RIL 14 days after treatment initiation. The average DRate was 0.25, 0.38, and 0.42 nodes d^{-1} for plants grown at 14, 17, and 20°C, respectively, which represents a 68% increase with increasing temperature (Table 2-3). Development rate was negatively correlated with DTA and positively correlated with Nodes

even though DTA was also positively correlated with Nodes at all temperatures (Table 2-5). Development rate was positively correlated with Branch at 14°C however it was negatively correlated with FlBud.

Petunia exserta had the earliest flowering time of the two parents at all temperatures (Table 2-3). Six out of the 171 RILs flowered earlier than either parent at all three temperatures in both years (AE_11, AE_20, AE_230, AE_301, AE_315, and AE_81). However, there were 67 more lines that flowered earlier than both parents in 2015 (data not shown). Average DTA for the population was 68, 49, and 43 d at 14, 17, and 20°C, respectively. DTA was positively correlated with FlBud and FlDiam at all temperatures, however it was only positively correlated with Branch at 17 and 20°C (Table 2-4). Additionally, DTA was positively correlated with FlBudAS at all temperatures but negatively correlated to FlBudPS at 17 and 20°C.

Total flower bud number was positively correlated with FlBudAS and FlDiam at all temperatures and positively correlated with Branch at 17 and 20°C while negatively correlated at 14°C (Table 2-5). Mean FlBud was 36, 28, and 23 at 14, 17, and 20°C respectively, which represents a 36% decrease in flower number with increased temperature (Table 2-3). FlBud was also significantly correlated with DTA at all temperatures. Branch was not significantly different across temperatures. *Petunia axillaris* had higher FlBud at all temperature and higher Branch at 20°C and FlBudAS at 17°C compared to *P.exserta*.

Photoperiod results

Average DRate increased with increased photoperiod however DTA and NodesLB decreased (Table 2-10). DTA was negatively correlated to DRate at 16-h photoperiod but positively correlated at 9-h photoperiod (Table 2-11). DTA was also positively correlated to

FlDiam and NodesLB at both photoperiods. NodesLB was positively correlated to DRate at both photoperiods and only positively correlated to FlDiam at 9-h photoperiod.

Correlation of greenhouse and field performance

Flower performance in the greenhouse was determined by a combination of the number of flowering branches and the number of flowers on each branch. These traits can potentially be used to predict flower performance for plants grown within the field. Within the sixteen earliest flowering RILs at all temperatures and years, three RILs (AE126, AE18, and AE275) were among the highest performing (rank: >90%) lines for Flow. Additionally for the RILs with the highest flower bud number, two RILs (AE126 and AE17) were among the highest performing lines for Flow. In contrast, the RILs with the greatest number of branches were not found among the highest performing lines in the field. Lines AE126 and AE17 could be used as parents in a testcross to evaluate potential contributions in a breeding program to predict field flower performance using flowering time and flower number at first flowering.

Field results

Field performance for the *P. axillaris* × *P. exserta* RIL population resulted in a wide range of variation depending on location, with a majority of the traits displaying transgressive segregation (Figure 2-6 – 2-9). The RILs grown at Buellton, CA had the highest mean Flow at 54% while plants grown at Huntersville, NC had the lowest at 41% (Table 2-6). Flow was positively correlated to all traits at all locations except for ColorRet (Table 2-7). Similarly, Vigor was positively correlated to all traits except Comp at Bellefonte, PA and Huntersville, NC and ColorRet at Buellton, CA and Gilroy, CA.

Broad-sense heritability estimates

For traits measured in the greenhouse experiments, broad-sense heritability was relatively high (Table 2-8). Similar heritability results were seen across the different temperature treatments for all traits excluding DRate, which was 46% lower at 14°C than 17 or 20°C. With the exception of DRate, FlBudPS, FlBranch, and FlBudAS, all traits had high heritability (>0.7) across the temperature treatments.

Broad-sense heritability estimates for field traits were moderately high with the exception of Comp, MinWid, and ColorRet (Table 2-9). Heritability estimates were high for all traits at Bellefonte, PA and Huntersville, NC; moderate at Buellton, CA; and low at Gilroy, CA. Flow heritability was high at Bellefonte, PA and Huntersville, NC (H^2 = 0.97 and 0.86, respectively), moderate at Buellton, CA (H^2 = 0.65), and low at Gilroy, CA (H^2 = 0.29).

Discussion

The long-term goal of this research is to elucidate the genetic controls of flowering in petunia. The ability of plants to be floriferous is one of the major concerns for ornamental breeders because this impacts consumer purchases. Flower production can be increased by increasing the number of apical buds on a plant or increasing the number of buds on lateral branches. Increasing branch number can potentially increase flower bud number by providing additional axillary buds. Many factors can impact the characteristics of these traits including genetics and environmental conditions. The objective of this study was to characterize phenotypic variation for crop timing and quality traits in response to various environmental conditions for an F_7 *P. axillaris* × *P. exserta* RIL population. The transgressive segregation of

the population in different environments indicate that multiple genes contribute to total flower production (Figure 2-3 – 2-9). Additionally, the sub-traits contributing to total flower production also appear to be under polygenic control.

In this study, increasing temperature or photoperiod accelerated time to flower (Table 2-3 – 2-10). However, early flowering plants at the highest temperature (20°C) had the lowest Branch and FlBud. These plants tended to be taller and have larger leaves but similar node number to plants grown at lower temperatures. Our results are similar to the previous description of interspecific petunia F_2 populations, where early flowering lines had reduced crop quality at first flowering (Warner and Walworth, 2010). Decreased plant quality, such as low branch number and long internode length at high average daily temperatures, have been reported for various crops including osteospermum (*Osteospermum ecklonis*), nemesia (*Nemesia foetans*), heliotrope (*Heliotropium arborescens*), stock (*Matthiola incana*), snapdragon (*Antirrhinum majus*), geranium (*Pelargonium* × *hortorum*), flowering tobacco (*Nicotiana alata*), cupflower (*Nierembergia caerulea*) (Vaid et al., 2014), trailing petunias (Adams et al., 1997), and salvia and marigold (Moccaldi and Runkle, 2007).

Effect of temperature on branch number

Temperature significantly contributed to branch number in this population; as temperature increased branch number decreased (Table 2-3). A previous study for fifteen ornamental annuals grown under two different daily light integrals and different temperatures found varying responses for branch number (Vaid et al., 2014). For example, as temperature increased from 16 to 26°C, branch number decreased by approximately 43 and 45% in snapdragon and diascia (*Diascia barberae*), respectively, while branch number increased by

approximately 23 and 25% in torenia (*Torenia fournieri*) and heliotrope, respectively (Vaid et al., 2014). A reduction in branch number at higher temperatures was reported for fuchsia (*Fuchsia* × *hybrida*) (Erwin et al., 1991) and balloon flower (*Platycodon grandifloras*) (Park et al., 1998). The petunia cultivar 'Snow Cloud' showed decreased branch number with an increase in temperature, regardless of irradiance level (Kaczperski et al., 1991). In contrast, a study by Warner and Erwin (2006) with twelve pansy (*Viola* × *wittrockiana*) cultivars showed variable reduction in branch number with increasing temperature depending on cultivar. However, other studies found that temperature did not have an effect on branch number for the petunia cultivars 'Wave Purple Classic' and 'Wave Purple Improved' (Vaid and Runkle, 2015) and four pansy cultivars (Mattson and Erwin, 2003). These contrasting results support the interaction of genetics and environment to influence plant architecture.

The variation for branching may be due to varying hormone levels at different temperatures (Bajguz and Hayat, 2009; Fujii and Saka, 2001; Thomashow, 1999). For example, abscisic acid concentration increased by 60% in grape skin when grown at 20°C compared to plants grown in 30°C (Yamane et al., 2006). Additionally, cytokinin content in wheat (*Triticum aestivum* 'Stephens') kernels was reduced by 50% for plants placed at a 35°C treatment compared to plants grown in 25°C (Banowetz et al., 1999). While auxin and cytokinin have been known to control shoot branching (Ongaro and Leyser, 2008), gibberellins have been implicated to stimulate lateral branch development in sweet cherry trees (Elfving et al., 2011) and *Jatropha curcas* (Ni et al., 2015). Similarly, foliar spray applications of Fascination – a cytokinin and gibberellic acid plant growth regulator – increased branching for nine ornamental annual plants (Lieth and Dodge, 2005). An upregulation of major biosynthetic gibberellin genes were observed in *Arabidopsis* hypocotyls grown at 29°C compared to 20°C (Stavang et al.,

2009). In contrast, low temperatures can reduce bioactive gibberellins and inhibit stem growth and elongation through the presence of gibberellin 2-oxidases (*GA2ox*) (Martin et al., 1999; Sakamoto et al., 2004; Thomas et al., 1999). *GA2ox* is stimulated by the activation of the C-repeat/drought-responsive element binding factor (*CBF1/DREB1b*) (Achard et al., 2008). *CBF1* belongs to a network of a cold-responsive genes that are active at low temperatures such as 4°C to induce freezing tolerance (Achard et al., 2008). However, induction of cold-responsive genes has been reported at temperatures as high as 19°C in Arabidopsis mutants (Ishitani et al., 1998). Also, tolerance to low temperature has been observed in tomato (*Solanum lycopersicum*) plants with over-expression of *Arabidopsis CBF1* genes when grown at 10°C (Zhang et al., 2011).

Effect of temperature on flower number

In this study, FlBud decreased by 36% as temperature increased from 14 to 20°C (Table 2-3). Similar response to temperature was seen in other species, such as in balloon flower, where flower bud number was reduced by half in response to a temperature increase from 14 to 30°C (Park et al., 1998). For evening primrose (*Oenothera fruticosa*), a change in temperature from 15 to 30°C decreased flower and bud numbers from 170 to 30 (Clough et al., 2001). A reduction of flower number by 75% in orange coneflower (*Rudbeckia fulgida*), 55% in Shasta daisy (*Leucanthemum* × *superbum*), and 80% in tickseed (*Coreopsis grandiflora*) was observed as temperature increased from 16 to 26°C (Yuan et al., 1998). In other ornamental crops, a 10°C decrease in temperature resulted in an increase of flower number: 202% in cup flower (*Nierembergia caerulea*), 76% in diascia, 85% in globe amaranth (*Gomphrena globose*), 101% in pot marigold (*Calendula officinalis*), and 95% in stock (Vaid et al., 2014).

The relationship between temperature and flower bud number is species-specific. For example, flower number for three petunia cultivars decreased, whereas flower number for one impatiens cultivar increased and flower number for four pansy cultivars was not affected by increasing temperature 12 to 24 °C (Mattson and Erwin, 2003). A linear relationship between flower number and temperature has been observed in begonia (*Begonia x tuberhybrida*) (Djurhuus, 1985), Christmas Bells (*Sandersonia aurantiaca*) (Catley et al., 2002), chrysanthemum (*Chrysanthemum morifolium*) (Carvalho et al., 2005), cyclamen (*Cyclamen persicum*) (Karlsson and Werner, 2001), and hot water plant (*Achimenes*) (Vlahos et al., 1992). Additionally, the magnitude of change for flower bud number is cultivar dependent. For example, as temperature increased from 17 to 25°C, a 62% increase in flower number was reported in the *Achimenes* cultivar 'Flamenco' whereas flower number increased over 200% for the same temperature fluctuation for 'Hilda' and 'Rosenelfe' (Vlahos et al., 1992). Similarly, as temperature increased from 20 to 30°C, the reduction in flower bud number of 12 pansy cultivars ranged from 20 to 77% (Warner and Erwin, 2006). Also, flower number in the petunia cultivar 'Wave Purple' decreased by 3 whereas in 'Dream Rose' flower number decreased by 11 as temperature increased from 12 to 24°C (Mattson and Erwin, 2003). In this study, the increase of flower bud number ranged from 1 to 300% depending on the RIL (Figure 2-10) as temperature decreased from 20 to 14°C.

The flowering signal gene named *FLOWERING LOCUS T* (*FT*) contributes multiple roles in plant and flower development such as influencing flowering time and flower induction (Pin and Nilsson, 2012). Tomato plants with heterozygous loss-of-function alleles for *SINGLE FLOWER TRUSS* (*SFT*), the ortholog of *Arabidopsis FT*, increased total inflorescences and flowers per inflorescence (Krieger et al., 2010). The variation in flower number at various

temperatures in this study may be controlled by the regulation of differentially expressed genes within the *FT* pathway. Although the major pathway responsible for *FT* upregulation is the photoperiodic pathway (Balasubramanian et al., 2006; Turck et al., 2008), temperature activation of *FT* independent of the photoperiodic pathways has been shown in Arabidopsis (Balasubramanian et al., 2006). High temperature removes H2A.Z-nucleosomes – temperature signal mediators (Kumar and Wigge, 2010) – which provides the basic helix-loop-helix transcription factor *PHYTOCHROME INTERACTING FACTOR 4 (PIF4*) with chromatin access for binding to *FT* promoter near transcriptional start site to activate *FT* expression (Kumar et al., 2012). Even though *PIF4* transcription is active at 12°C, low temperature can inhibit *PIF4* activity due to instability of PIF4 proteins at low temperatures (Kumar et al., 2012). Additionally, repression of *PIF4* activity can be induced by DELLA proteins through hindering *PIF4* DNA binding ability (Feng et al., 2008; Lucas et al., 2008). However, gibberellin can trigger the degradation of DELLA protein (Davière and Achard, 2013; Silverstone et al., 2001; Sun and Gubler, 2004; Thomas and Sun, 2004) and release *PIF4* activity. Future studies will be performed to identify genomic regions associated with flower number and different genes and genetic pathways.

Effect of photoperiod

Relative to plants grown under the 16-h photoperiod, plants grown under the 9-h photoperiod were later flowering but had more branches at first flowering. However, low plant quality such as elongated growth habit was observed for these lines (QCC, personal observation). Similar plant response to photoperiod was observed for evening primrose (Clough et al., 2001) and trailing petunia (Adams et al., 1997). Though, temperature is a better determinant of

development rate compared to photoperiod (Ritchie and Nesmith, 1991; Tan et al., 2000). Higher development rates in plants grown under 16-h photoperiod (Table 2-10) is possibly due to higher average plant temperature, which increases plant respiration and in turn increase photosynthesis in non-limiting environments. The increased flowering time under the 9-h photoperiod could be attributed to flower bud abortion, which has been previously observed in petunia cultivars (Shimai, 2001; Vaid and Runkle, 2015). Alternatively, flower development may depend on long days for some cultivars. A short-day photoperiod can contribute to flower bud abortion by decreasing photosynthesis, which impacts the availability of carbohydrates for floral development (Marcelis et al., 2004; Opik and Rolfe, 2005). Molecular analysis of petunia flower buds indicate that the genes involved with the metabolic pathway for carbohydrate metabolism are differentially expressed in normal and abortive flowers (Yue et al., 2013).

Conclusions

The transgressive segregation of the population for all the evaluated traits in the greenhouse and the field (Figure 2-3 – 2-9) indicate that these traits are under polygenic control. Therefore, the use of molecular markers may improve breeding efficiency for these traits (Collard and Mackill, 2008). Marker assisted breeding continues to advance for ornamental crops as the technology becomes more cost-effective (Byrne, 2007; Riek and Debener, 2010) and increased genomic information becomes available (Arens et al., 2012). For ornamental crops, molecular markers are often used to assess the genetic relationships between species and cultivars (Huylenbroeck and Laere, 2010; Kuligowska et al., 2016, 2015). The earliest known utilization of marker assisted selection have been through developing disease resistant rose cultivars (Debener et al., 2003; Linde et al., 2006). More recently, molecular markers are being

developed to identify leaf color in ornamental kale (*Brassica oleracea*) (Ren et al., 2015; Zhu et al., 2016), floral morphology in passionflower (*Passiflora*) (Melo et al., 2016), dwarf morphology in crape myrtle (*Lagerstroemia*) (Ye et al., 2016), and reblooming habit in daylilies (*Hemerocallis*) (Zhu et al., 2015). Within petunia, molecular markers are being employed for association with double flower trait (Liu et al., 2016), drought resistance (Tao et al., 2014); developmental and quality traits (Guo et al., 2015; Vallejo et al., 2015). The high heritability for the traits observed in this population indicates a strong genetic component. Future studies using this population in QTL mapping will be useful to better understand the genetic control of these traits.

APPENDIX

Table 2-1. List of traits measured

Trait Abbreviation	Trait Description
DRate	development rate after 14 days
DTA	date of anthesis for the first open flower on main stem
FlBud	total visible flowers and buds number
FlBudPS	the number of visible flower buds on the main stem
Branch	total branch number (branches >5cm)
FlBranch	the number of branches with flower buds
FlDiam	the diameter of the first flower (cm)
Nodes	the node number below first flower
HghtFl	the height of the main stem to the node of the first flower (cm)
LLeng	the length of the third leaf below node of first flower
LWid	the width of the third leaf below node of first flower
Internode	the average distance between nodes (cm)
FlBudAS	the number of visible flower buds on the apical shoot
DTA2	date of anthesis for the first open flower on a lateral shoot
NBL	the number of nodes/branches below the first flowering lateral shoot
NodesLB	the node number below the first flower on a lateral shoot
FlDiam2	the diameter of the first flower on a lateral shoot (cm)
Flow	floral coverage of plant canopy (%)
Vigor	plant vigor (1-9)
Comp	plant compactness (1-9)
Height	plant height (cm)
MaxWid	plant maximum width (cm)
MinWid	plant minimum width (cm)
ColorRet	flower color retention (1-9)

Table 2-2. Field experiment locations for *P. axillaris* × *P. exserta* F_7 recombinant inbred line population in 2014

Location	Climate[z]	Collaborator
Bellefonte, PA	Warm summer continental	Garden Genetics
Gilroy, CA	Mediterranean	Syngenta
Huntersville, NC	Humid subtropical	Metrolina Greenhouses
Buellton, CA	Semiarid	Ball Horticultural Company

[z] Based on the Köppen-Geiger climate classification system (Peel et al., 2007)

Table 2-3. Descriptive statistics at different temperature treatments for twelve developmental traits analyzed in *P. axillaris* × *P. exserta* F_7 recombinant inbred line population in 2014 and 2015

Trait[z]	n[y]	Mean	Sd	Min	Max	PA	PE	t-value
All temperatures								
DRate	3068	0.35	0.11	0.00	0.79	0.41	0.37	1.74
DTA	2990	53.61	13.09	31.00	95.00	60.44	52.28	9.84**[x]
FlBud	2990	28.93	14.43	1.00	95.00	41.50	30.50	5.67**
FlBudPS	1468	8.32	2.66	2.00	21.00	7.22	9.67	-2.67*
Branch	2990	9.88	2.40	2.00	25.00	11.06	10.67	1.03
FlBranch	2990	8.42	2.47	0.00	21.00	10.11	9.17	2.62*
FlDiam	2990	5.19	0.71	2.50	8.00	5.36	4.67	4.42**
Nodes	2993	16.76	2.56	10.00	30.00	25.56	15.28	28.55**
HghtFl	2990	20.43	5.89	3.50	49.00	23.06	16.11	11.89**
Internode	2988	1.23	0.33	0.21	2.54	0.91	1.06	-4.4**
LLeng	2958	11.39	2.26	3.50	19.50	7.69	12.08	-6.83**
LWid	2958	5.42	1.22	1.50	15.00	3.36	6.19	-11.4**
FlBudAS	2988	4.50	1.76	0.00	12.00	5.33	4.11	3.39*
Temperature 14°C								
DRate	1022	0.25	0.09	0.00	0.50	0.31	0.27	1.09
DTA	1000	68.38	9.39	46.00	95.00	74.83	65.33	7.07*
FlBud	1000	36.19	14.76	8.00	95.00	51.83	41.17	3.95*
FlBudPS	495	8.06	2.37	3.00	17.00	7.67	10.33	-2.22
Branch	1000	11.27	2.35	5.00	21.00	12.67	12.67	0.00
FlBranch	1000	9.67	2.20	1.00	21.00	12.00	10.33	2.50
FlDiam	1000	5.50	0.66	3.50	8.00	5.67	5.25	5.00*
Nodes	1000	16.98	2.61	10.00	30.00	27.50	15.67	17.75**
HghtFl	1000	19.98	6.15	6.50	49.00	20.00	17.08	2.88*
Internode	1000	1.19	0.35	0.32	2.45	0.73	1.10	-5.83*
LLeng	991	10.74	2.18	4.00	18.50	5.67	11.75	-6.13*
LWid	991	4.97	1.09	1.50	9.00	2.67	5.92	-6.89*
FlBudAS	1000	4.71	1.55	0.00	11.00	5.83	4.83	1.34

Table 2-3 (cont'd)

Trait[z]	n[y]	Mean	Sd	Min	Max	PA	PE	t-value
Temperature 17°C								
DRate	1026	0.38	0.07	0.00	0.79	0.44	0.39	1.96
DTA	1000	48.96	6.82	37.00	74.00	56.33	48.50	4.27*
FlBud	1000	27.88	12.17	1.00	76.00	42.33	28.17	3.21*
FlBudPS	491	8.13	2.56	2.00	19.00	7.67	9.33	-0.76
Branch	1000	9.63	2.14	3.00	25.00	11.00	10.50	0.90
FlBranch	1000	8.30	2.20	0.00	17.00	10.33	9.50	1.25
FlDiam	1000	5.18	0.66	2.50	7.50	5.17	4.50	2.00
Nodes	999	16.56	2.44	11.00	28.00	24.83	14.83	14.55**
HghtFl	1000	20.00	5.58	6.00	38.00	24.58	16.75	6.93*
Internode	999	1.21	0.31	0.38	2.38	0.99	1.13	-2.36
LLeng	984	11.40	2.13	4.00	17.50	8.50	12.17	-3.90*
LWid	984	5.42	1.15	1.50	15.00	3.75	6.42	-8.88**
FlBudAS	999	4.49	1.73	0.00	12.00	6.33	3.83	3.35*
Temperature 20°C								
DRate	1020	0.42	0.07	0.07	0.64	0.48	0.45	0.51
DTA	990	43.39	5.89	31.00	63.00	50.17	43.00	7.07*
FlBud	990	22.66	12.87	2.00	84.00	30.33	22.17	3.06*
FlBudPS	482	8.78	2.98	3.00	21.00	6.33	9.33	-2.60
Branch	990	8.74	1.98	2.00	19.00	9.50	8.83	2.83*
FlBranch	990	7.26	2.39	0.00	19.00	8.00	7.67	0.63
FlDiam	990	4.89	0.67	2.50	7.00	5.25	4.25	3.10*
Nodes	994	16.73	2.60	12.00	30.00	24.33	15.33	18.00**
HghtFl	990	21.32	5.83	3.50	39.00	24.58	14.50	11.54**
Internode	989	1.28	0.34	0.21	2.54	1.02	0.95	1.26
LLeng	983	12.03	2.28	3.50	19.50	8.92	12.33	-2.51
LWid	983	5.87	1.25	1.50	15.00	3.67	6.25	-5.24*
FlBudAS	989	4.29	1.96	0.00	11.00	3.83	3.67	0.71

[z] Trait abbreviations: as defined in Table 2-1

[y] n=sample number, Mean=population average, Sd=sample standard deviation, Min=minimum sample value, Max=maximum sample value, PA = average for *P. axillaris*, PE = average for *P. exserta*, t-value = ANOVA results comparing RILs to parental lines

[x] * and ** indicate significance at $P < 0.05$ and 0.001, respectively

Table 2-4. The number of plants flowering on a lateral shoot before the apical stem at different temperatures and photoperiods in *P. axillaris* × *P. exserta* F_7 recombinant inbred line population in 2014 and 2015

Treatment	2014	2015
Temperature (°C)		
14	121 (24%)	123 (25%)
17	41 (8%)	48 (10%)
20	8 (2%)	21 (4%)
Photoperiod (hr)		
9	455 (91%)	420 (89%)
16	8 (2%)	21 (4%)

Table 2-5. Pearson's correlation coefficients at different temperature treatments for traits measured in *P. axillaris* × *P. exserta* F_7 recombinant inbred line population in 2014 and 2015

Trait[z]	DRate	DTA	FlBud	FlBudPS	Branch	FlBranch	FlDiam	Nodes	HghtFl	Internode	LLeng	LWid
All temperatures												
DTA	-0.78**[y]											
FlBud	-0.45**	0.64**										
FlBudPS	0.11**	-0.12**	0.26**									
Branch	-0.22**	0.34**	0.28**	-0.05								
FlBranch	-0.36**	0.52**	0.67**	-0.04	0.65**							
FlDiam	-0.32**	0.36**	0.38**	-0.05	0.08**	0.20**						
Nodes	0.20**	0.13**	0.11**	0.13**	0.18**	0.03	0.05*					
HghtFl	0.08**	-0.06*	-0.03	0.30**	0.28**	0.02	0.06*	0.37**				
Internode	-0.03	-0.12**	-0.08**	0.23**	0.19**	0.02	0.04	-0.14**	0.86**			
LLeng	0.03	-0.14**	-0.25**	-0.14**	-0.01	-0.07**	-0.17**	-0.41**	-0.04	0.18**		
LWid	0.09**	-0.21**	-0.27**	0.04	0.03	-0.07**	-0.27**	-0.32**	0.13**	0.31**	0.78**	
FlBudAS	-0.27**	0.39**	0.72**	0.53**	0.16**	0.55**	0.21**	-0.02	0.11**	0.15**	-0.01	0.03
Temperature 14°C												
DTA	-0.64**											
FlBud	-0.44**	0.62**										
FlBudPS	-0.04	0.07	0.31**									
Branch	0.26**	-0.23**	-0.1*	-0.09*								
FlBranch	-0.21**	0.27**	0.45**	-0.09*	0.51**							
FlDiam	-0.19**	0.10*	0.31**	0.08	-0.18**	-0.06						
Nodes	0.33**	0.11**	0.12**	0.16**	0.14**	0	-0.02					
HghtFl	0.14**	-0.14**	-0.12**	0.35**	0.41**	0.05	0.12**	0.29**				
Internode	-0.04	-0.18**	-0.17**	0.25**	0.35**	0.08*	0.14**	-0.18**	0.88**			
LLeng	-0.15**	0.03	-0.26**	-0.13*	0.07*	-0.01	-0.15**	-0.56**	-0.14**	0.13**		
LWid	-0.11**	-0.06	-0.30**	0.06	0.18**	0.04	-0.24**	-0.51**	0.04	0.29**	0.80**	
FlBudAS	-0.48**	0.46**	0.65**	0.57**	-0.07*	0.41**	0.18**	-0.04	0.06	0.11**	-0.04	-0.03

Table 2-5 (cont'd)

Trait[z]	DRate	DTA	FlBud	FlBudPS	Branch	FlBranch	FlDiam	Nodes	HghtFl	Internode	LLeng	LWid
Temperature 17°C												
DTA	-0.50**											
FlBud	-0.21**	0.64**										
FlBudPS	0.13*	-0.25**	0.31**									
Branch	-0.03	0.13**	0.22**	-0.01**								
FlBranch	-0.18**	0.50**	0.66**	-0.01	0.60**							
FlDiam	-0.12**	0.22**	0.29**	-0.18**	-0.06	0.12**						
Nodes	0.35**	0.13**	0.12**	0.05	0.16**	0.01	0.06					
HghtFl	0.03	-0.02	-0.08*	0.26**	0.27**	-0.05	0.08*	0.43**				
Internode	-0.18**	-0.08*	-0.13**	0.23**	0.21**	-0.04	0.06	-0.1*	0.85**			
LLeng	-0.26**	0.11**	-0.22**	-0.24**	0.09*	0	-0.05	-0.43**	-0.08*	0.16**		
LWid	-0.23**	0.04	-0.27**	-0.08	0.18**	0	-0.18**	-0.31**	0.06	0.25**	0.77**	
FlBudAS	-0.26**	0.63**	0.77**	0.46**	0.22**	0.60**	0.19**	0.05	0.01	0.01	-0.02	-0.03
Temperature 20°C												
DTA	-0.29**											
FlBud	-0.08*	0.66**										
FlBudPS	0.07	-0.13*	0.41**									
Branch	0.07*	0.21**	0.36**	0.03								
FlBranch	0.01	0.51**	0.75**	0.06	0.64**							
FlDiam	-0.07*	0.16**	0.25**	0.06	0	0.12**						
Nodes	0.30**	0.24**	0.10**	0.13*	0.26**	0.05	0.09*					
HghtFl	-0.09*	0.28**	0.23**	0.23**	0.35**	0.18**	0.10*	0.41**				
Internode	-0.27**	0.16**	0.19**	0.15**	0.21**	0.16**	0.05	-0.17**	0.82**			
LLeng	-0.13**	0.08*	-0.08*	-0.14*	0.13**	0.05	-0.09*	-0.30**	0.03	0.21**		
LWid	-0.18**	0.16**	0.01	0.02	0.19**	0.10**	-0.14**	-0.23**	0.19**	0.33**	0.74**	
FlBudAS	-0.13**	0.64**	0.83**	0.53**	0.26**	0.65**	0.20**	-0.05	0.27**	0.32**	0.06	0.18**

[z] Trait abbreviations: as defined in Table 2-1

[y] * and ** indicate significance at $P < 0.05$ and 0.001, respectively

Table 2-6. Descriptive statistics at different locations for crop quality traits analyzed in *P. axillaris* × *P. exserta* F_7 recombinant inbred line population in 2014

Trait[z]	n[y]	Mean	Sd	Min	Max
All locations					
Flow	2110	49.18	23.27	0	90
Vigor	2115	5.10	1.88	1	9
Comp	2115	3.45	1.73	1	9
Height	2115	43.54	12.64	3	79
MaxWid	2115	107.80	30.96	7	213
MinWid	1015	84.90	24.75	8	170
ColorRet	1221	4.95	1.99	1	9
Buellton, CA					
Flow	591	54.37	22.55	0	90
Vigor	595	4.34	1.22	1	8
Comp	595	4.14	1.11	1	8
Height	595	40.16	8.80	13	78
MaxWid	595	98.72	24.65	13	163
MinWid	569	82.75	23.81	8	170
ColorRet	440	4.88	1.92	1	9
Gilroy, CA					
Flow	538	52.92	25.81	0	90
Vigor	539	4.87	1.44	1	9
Comp	539	4.12	1.08	1	7
Height	539	42.87	9.86	13	69
MaxWid	539	105.94	27.39	10	213
MinWid	446	87.63	25.68	11	163
ColorRet	331	3.98	1.87	1	8
Bellefonte, PA					
Flow	584	45.99	20.74	0	80
Vigor	584	5.52	2.18	1	9
Comp	584	3.49	2.09	1	9
Height	584	46.42	15.42	3	79
MaxWid	584	116.11	34.02	8	176
ColorRet	429	5.72	1.86	1	9
Huntersville, NC					
Flow	397	41.11	21.22	0	80
Vigor	397	5.92	2.21	1	9
Comp	397	1.46	0.83	1	5
Height	397	45.26	14.86	12	79
MaxWid	397	111.71	35.16	7	196

[z] Trait abbreviations: as defined in Table 2-1

[y] n=sample number, Mean=population average, Sd=sample standard deviation, Min=minimum sample value, Max=maximum sample value

[x] * and ** indicate significance at $P < 0.05$ and 0.001, respectively

Table 2-7. Pearson's correlation coefficients at different locations for crop quality traits analyzed in *P. axillaris* × *P. exserta* F_7 recombinant inbred line population in 2014

Trait[z]	Flow	Vigor	Comp	Height	MaxWid	MinWid
All locations						
Vigor	0.49**[y]					
Comp	0.12**	-0.32**				
Height	0.34**	0.65**	-0.21**			
MaxWid	0.42**	0.73**	-0.32**	0.62**		
MinWid	0.42**	0.76**	0.18**	0.45**	0.82**	
ColorRet	-0.19**	0.07*	-0.16**	0.14**	-0.03	-0.17**
Buellton, CA						
Vigor	0.43**					
Comp	0.34**	0.23**				
Height	0.11*	0.45**	0.26**			
MaxWid	0.36**	0.76**	0.08*	0.43**		
MinWid	0.38**	0.74**	0.10*	0.40**	0.81**	
ColorRet	-0.15*	-0.17**	-0.01	0.12*	-0.20**	-0.15*
Gilroy, CA						
Vigor	0.61**					
Comp	0.50**	0.42**				
Height	0.40**	0.55**	0.52**			
MaxWid	0.44**	0.76**	0.26**	0.52**		
MinWid	0.47**	0.77**	0.29**	0.49**	0.83**	
ColorRet	-0.14**	-0.11	-0.07	0.03	-0.15*	-0.14*
Bellefonte, PA						
Vigor	0.69**					
Comp	-0.37**	-0.51**				
Height	0.55**	0.76**	-0.47**			
MaxWid	0.66**	0.76**	-0.68**	0.70**		
ColorRet	-0.09	0.11*	0.04	0.07	-0.11*	
Huntersville, NC						
Vigor	0.74**					
Comp	-0.34**	-0.47**				
Height	0.50**	0.59**	-0.44**			
MaxWid	0.48**	0.64**	-0.56**	0.64**		

[z] Trait abbreviations: as defined in Table 2-1

[y] * and ** indicate significance at $P < 0.05$ and 0.001, respectively

Table 2-8. Broad-sense heritability estimates at different temperatures for *P. axillaris* × *P. exserta* F_7 recombinant inbred line population in 2014 and 2015

Trait[z]	H^{2y}			
	All temperature	**14°C**	**17°C**	**20°C**
DRate	0.56	0.28	0.52	0.50
DTA	0.82	0.72	0.74	0.72
FlBud	0.83	0.71	0.77	0.74
FlBudPS	0.72	0.69	0.56	0.57
Branch	0.86	0.79	0.74	0.70
FlBranch	0.63	0.63	0.50	0.51
FlDiam	0.90	0.83	0.80	0.75
Nodes	0.96	0.93	0.93	0.92
HghtFl	0.94	0.88	0.88	0.87
Internode	0.94	0.88	0.88	0.84
LLeng	0.86	0.84	0.83	0.81
LWid	0.87	0.86	0.84	0.78
FlBudAS	0.87	0.72	0.74	0.64

[z] Trait abbreviations: as defined in Table 2-1

[y] Broad sense heritability – measure of phenotypic variance attributable to genetic differences among genotype as calculated as $V_G/(V_G + V_E)$ where V_G is the among-genotype variance component from ANOVA and V_E is the residual variance component from ANOVA.

Table 2-9. Broad-sense heritability estimates at different locations for *P. axillaris* × *P. exserta* F_7 recombinant inbred line population in 2014

Trait[z]	H^{2y}				
	All location	**Buellton, CA**	**Gilroy, CA**	**Bellefonte, PA**	**Huntersville, NC**
Flow	0.75	0.65	0.29	0.97	0.86
Vigor	0.77	0.77	0.55	0.96	0.88
Comp	0.02	0.60	0.35	0.93	0.90
Height	0.79	0.79	0.58	0.92	0.76
MaxWid	0.75	0.76	0.53	0.95	0.85
MinWid	0.68	0.72	0.40	.	.
ColorRet	0.79	0.71	0.67	0.99	.

[z] Trait abbreviations: as defined in Table 2-1

[y] Broad sense heritability – measure of phenotypic variance attributable to genetic differences among genotype as calculated as $V_G/(V_G + V_E)$ where V_G is the among-genotype variance component from ANOVA and V_E is the residual variance component from ANOVA.

Table 2-10. Descriptive statistics at different photoperiod treatments for traits analyzed in *P. axillaris* × *P. exserta* F_7 recombinant inbred line population in 2014 and 2015

Trait[z]	n[y]	Mean	Sd	Min	Max	PA	PE	t-value
All photoperiod								
DRate	1522	0.40	0.07	0.07	0.64	0.47	0.42	.
DTA	1955	59.15	17.73	31	105	64.09	60.83	2.32
FlDiam	1955	4.90	0.64	2.5	7	5.14	4.58	2.98*[x]
NodesLB	1958	19.04	6.23	2	58	25.36	17.33	2.55*
NBL	942	3.51	2.92	0	20	1.80	3.17	.
9 h photoperiod								
DRate	502	0.36	0.07	0.07	0.57	0.45	0.36	.
DTA	964	75.40	9.02	50	105	80.80	78.67	0.12
FlDiam	964	4.93	0.61	3	7	5.00	4.92	1.52
NodesLB	964	21.69	7.62	2	58	26.60	19.33	0.74
NBL	912	3.59	2.93	0	20	1.80	3.17	-2.29
16 h photoperiod								
DRate	1020	0.42	0.07	0.07	0.64	0.48	0.45	0.5
DTA	991	43.35	5.86	31	63	50.17	43.00	7.07*
FlDiam	991	4.88	0.66	2.5	7	5.25	4.25	3.10*
NodesLB	994	16.47	2.59	7	28	24.33	15.33	18.00**
NBL	30	0.83	0.75	0	2	.	.	.

[z] Trait abbreviations: as defined in Table 2-1

[y] n=sample number, Mean=population average, Sd=sample standard deviation, Min=minimum sample value, Max=maximum sample value, PA = average for *P. axillaris*, PE = average for *P. exserta*, t-value = ANOVA results for RILs, temperature treatments, and years

[x] * and ** indicate significance at $P < 0.05$ and 0.001, respectively

Table 2-11. Pearson's correlation coefficients at different photoperiod treatments for traits measured in *P. axillaris* × *P. exserta* F_7 recombinant inbred line population in 2014 and 2015

Trait[z]	DRate	DTA	FlDiam	NodesLB
All photoperiod				
DTA	-0.38**[y]			
FlDiam	-0.02	0.10**		
NodesLB	0.01	0.59**	0.14**	
NBL	0.01	0.12**	-0.04	0.02
9hr photoperiod				
DTA	0.11*			
FlDiam	0.06	0.15**		
NodesLB	0.24**	0.64**	0.21**	
NBL	0.05	0.05	-0.03	-0.01
16hr photoperiod				
DTA	-0.28**			
FlDiam	-0.06	0.14**		
NodesLB	0.30**	0.18**	0.02	
NBL	0.05	0.44*	-0.32	-0.06

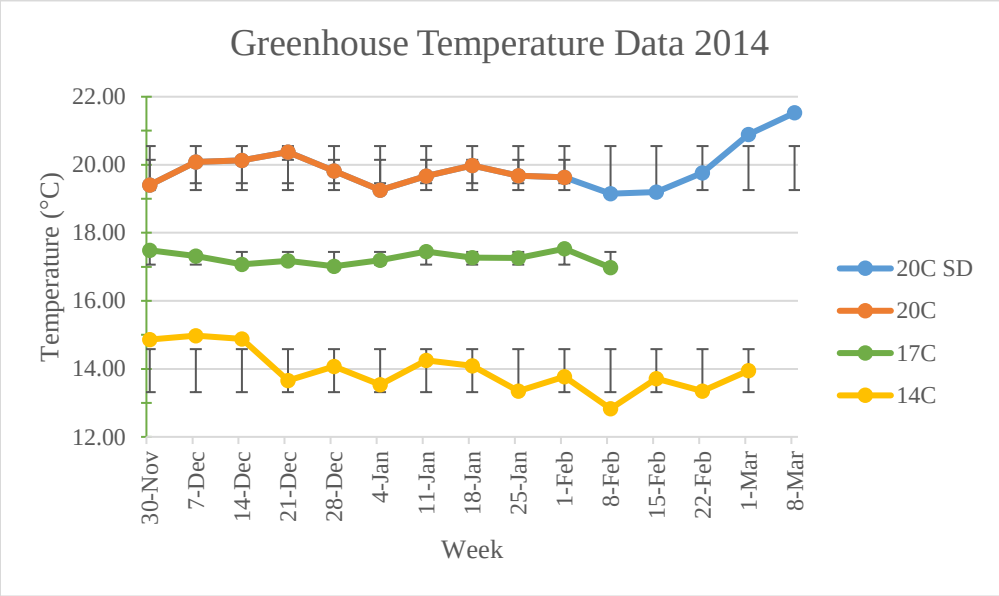
[z] Trait abbreviations: as defined in Table 2-1

[y] * and ** indicate significance at $P < 0.05$ and 0.001, respectively

Figure 2-1. Parental lines used for creating the *P. axillaris* × *P. exserta* F_7 recombinant inbred line population

A *P. axillaris* (PI 667515) **B** *P. exserta* (OPGC943)

Figure 2-2. Weekly temperature (mean ± standard deviation) data for all treatments.



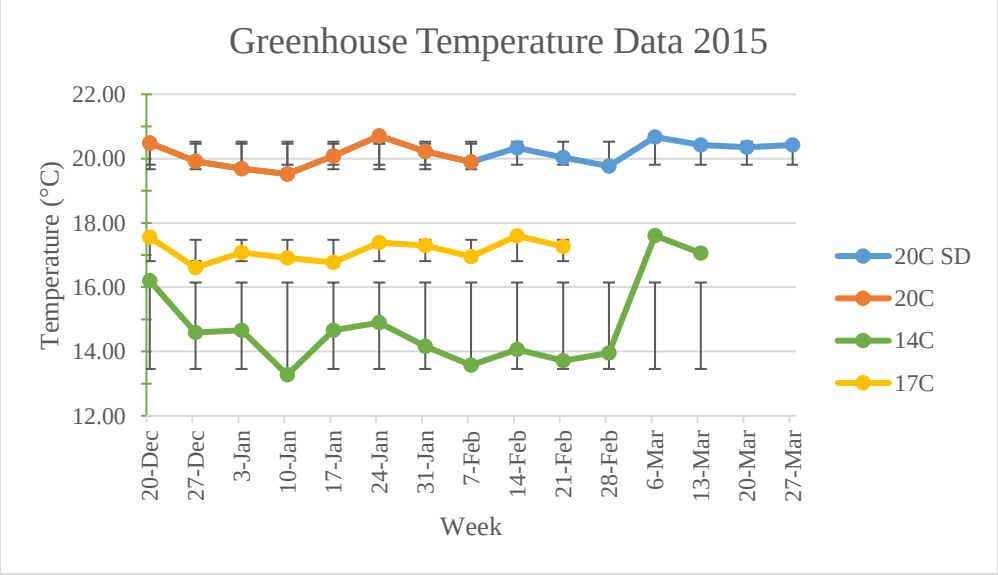


Figure 2-3. Frequency distribution for crop timing and quality traits for *P. axillaris* × *P. exserta* F_7 recombinant inbred line population at 14°C evaluated in 2014 and 2015

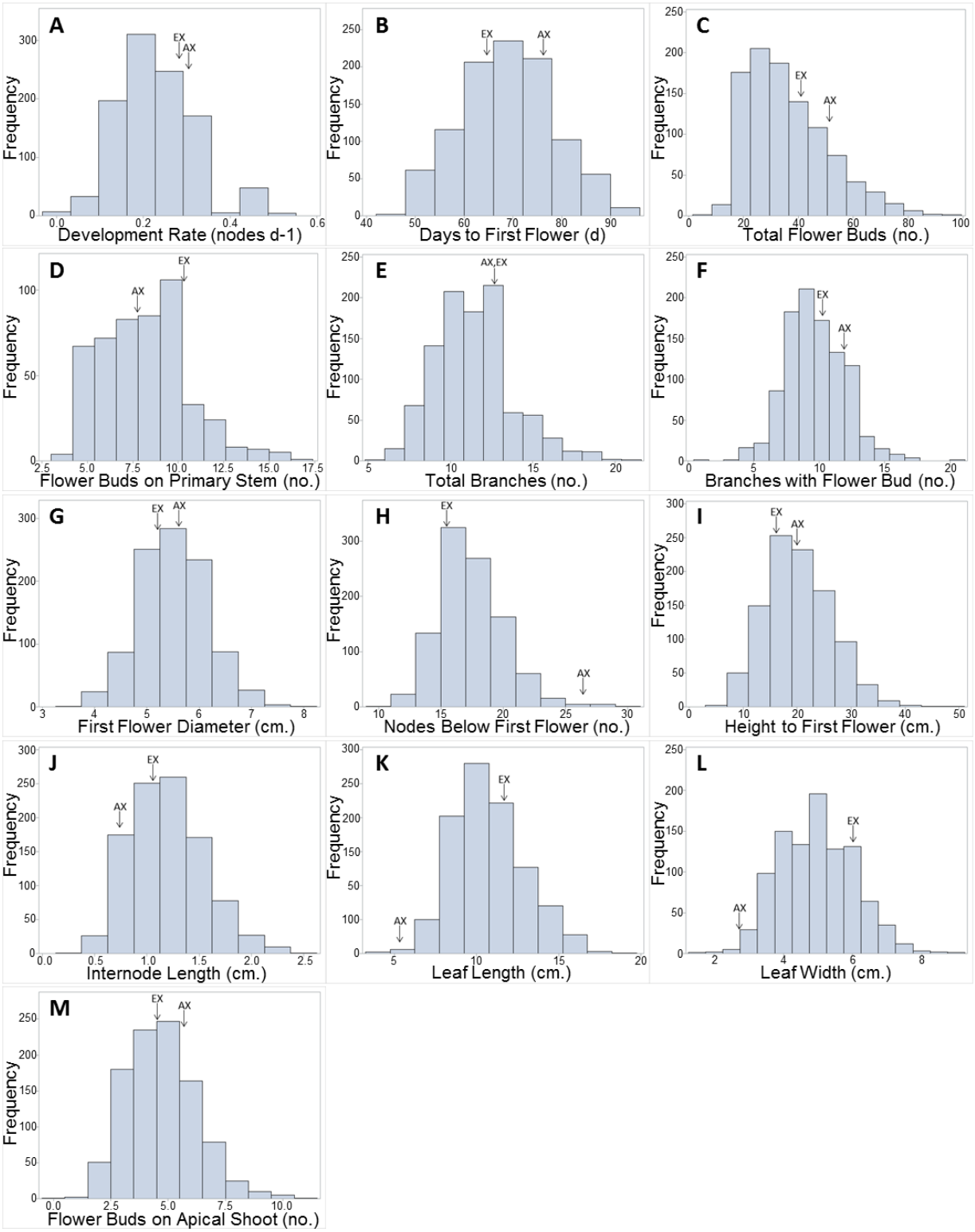


Figure 2-3 (cont'd)

A development rate at 14 days after transplant **B** days to first flower bud opening **C** total flower and bud number **D** number of flower buds on the main stem **E** total branch number **F** total number of branches with flower buds **G** diameter of the first flower **H** node number below first flower **I** plant height to the flowering node **J** length of the third leaf below node of first flower **K** width of the third leaf below node of first flower **L** number of flower buds on the apical shoot. AX = Mean for *P. axillaris*. EX = Mean for *P. exserta*.

Figure 2-4. Frequency distribution for crop timing and quality traits for *P. axillaris* × *P. exserta* F_7 recombinant inbred line population at 17°C evaluated in 2014 and 2015

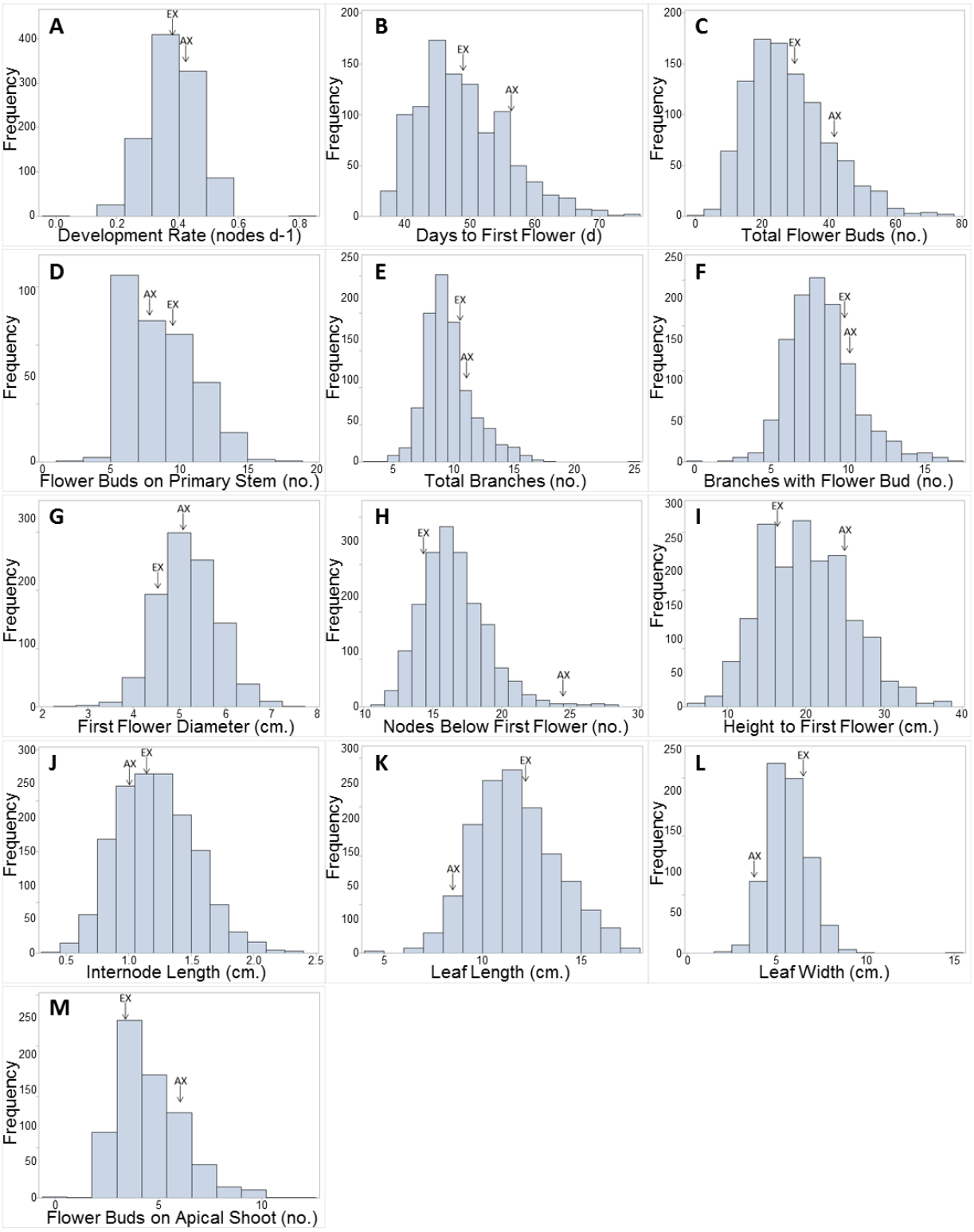


Figure 2-4 (cont'd)

A development rate at 14 days after transplant **B** days to first flower bud opening **C** total flower and bud number **D** number of flower buds on the main stem **E** total branch number **F** total number of branches with flower buds **G** diameter of the first flower **H** node number below first flower **I** plant height to the flowering node **J** length of the third leaf below node of first flower **K** width of the third leaf below node of first flower **L** number of flower buds on the apical shoot. AX = Mean for *P. axillaris*. EX = Mean for *P. exserta*.

Figure 2-5. Frequency distribution for crop timing and quality traits for *P. axillaris* × *P. exserta* F_7 recombinant inbred line population at 20°C evaluated in 2014 and 2015

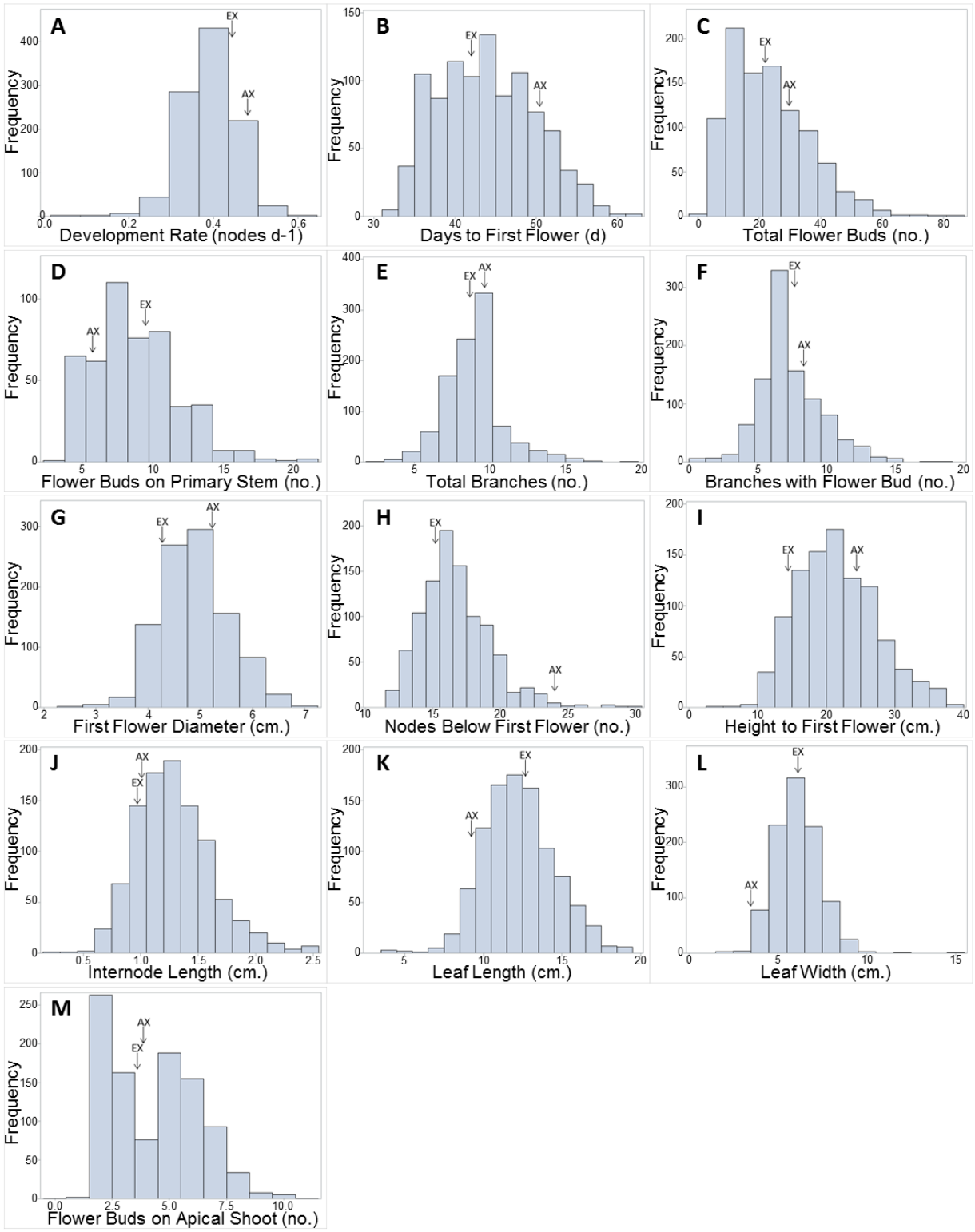
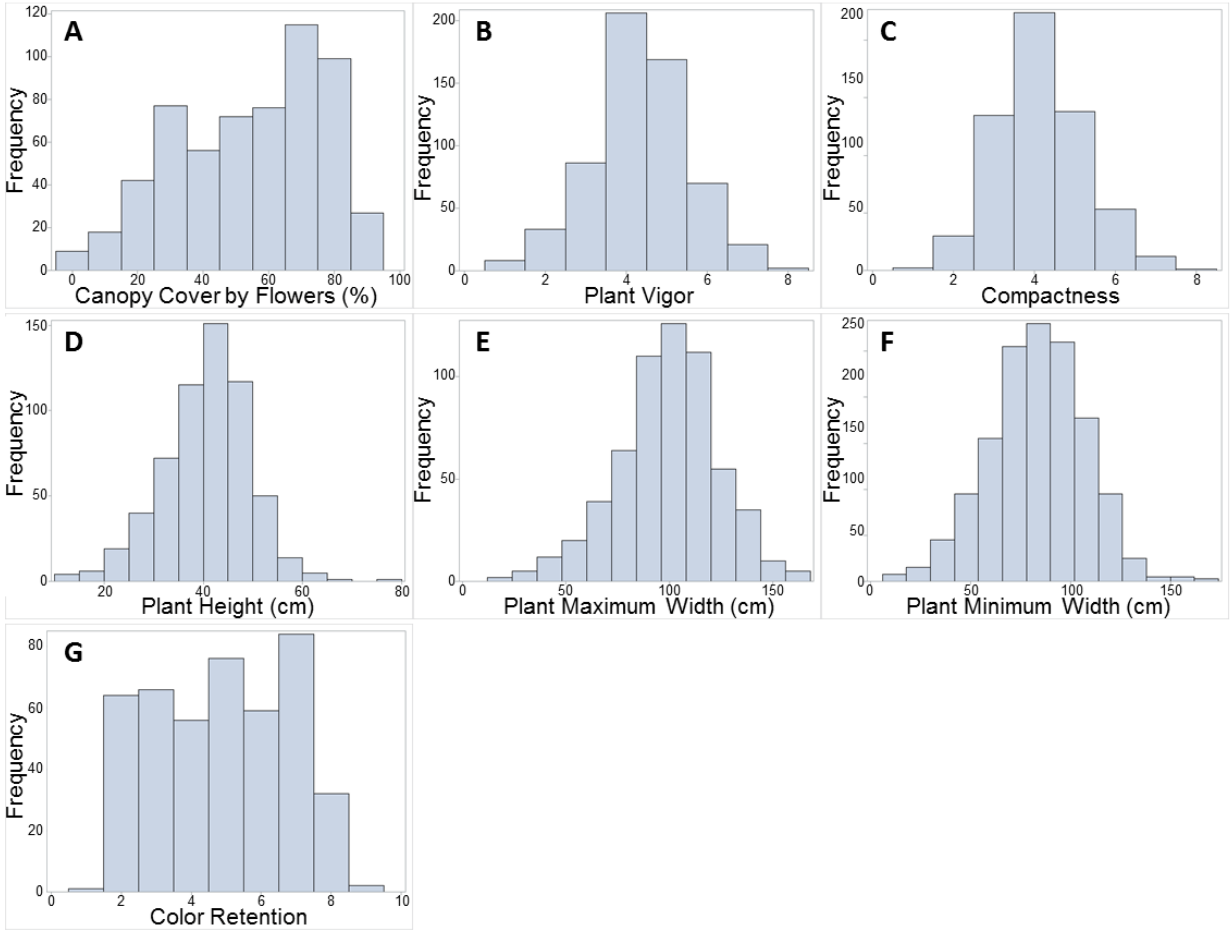


Figure 2-5 (cont'd)

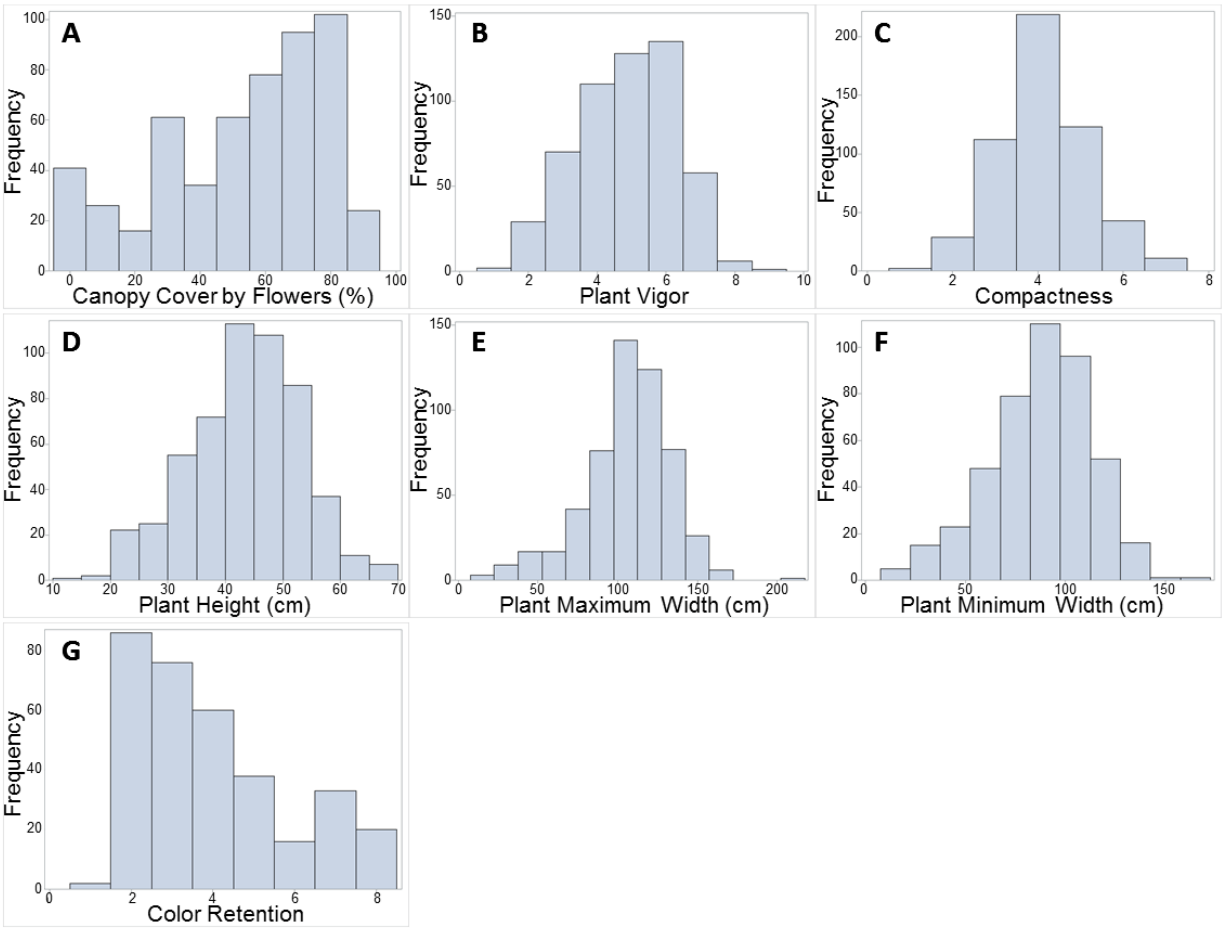
A development rate at 14 days after transplant **B** days to first flower bud opening **C** total flower and bud number **D** number of flower buds on the main stem **E** total branch number **F** total number of branches with flower buds **G** diameter of the first flower **H** node number below first flower **I** plant height to the flowering node **J** length of the third leaf below node of first flower **K** width of the third leaf below node of first flower **L** number of flower buds on the apical shoot. AX = Mean for *P. axillaris*. EX = Mean for *P. exserta*.

Figure 2-6. Frequency distribution for crop quality traits for *P. axillaris* × *P. exserta* F_7 recombinant inbred line population at Ball in Buelton, CA evaluated in 2014



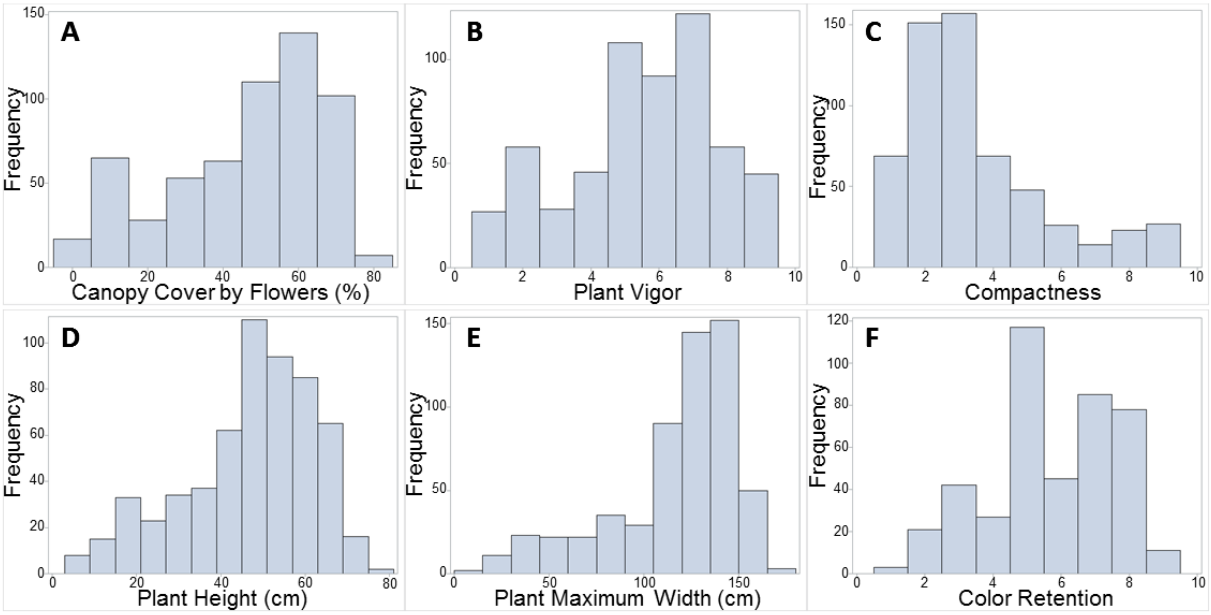
A percentage of plants flowering **B** plant vigor **C** plant compactness **D** plant height **E** plant maximum width **F** plant minimum width **G** flower color retention

Figure 2-7. Frequency distribution for crop quality traits for *P. axillaris* × *P. exserta* F_7 recombinant inbred line population at Syngenta in Gilroy, CA evaluated in 2014



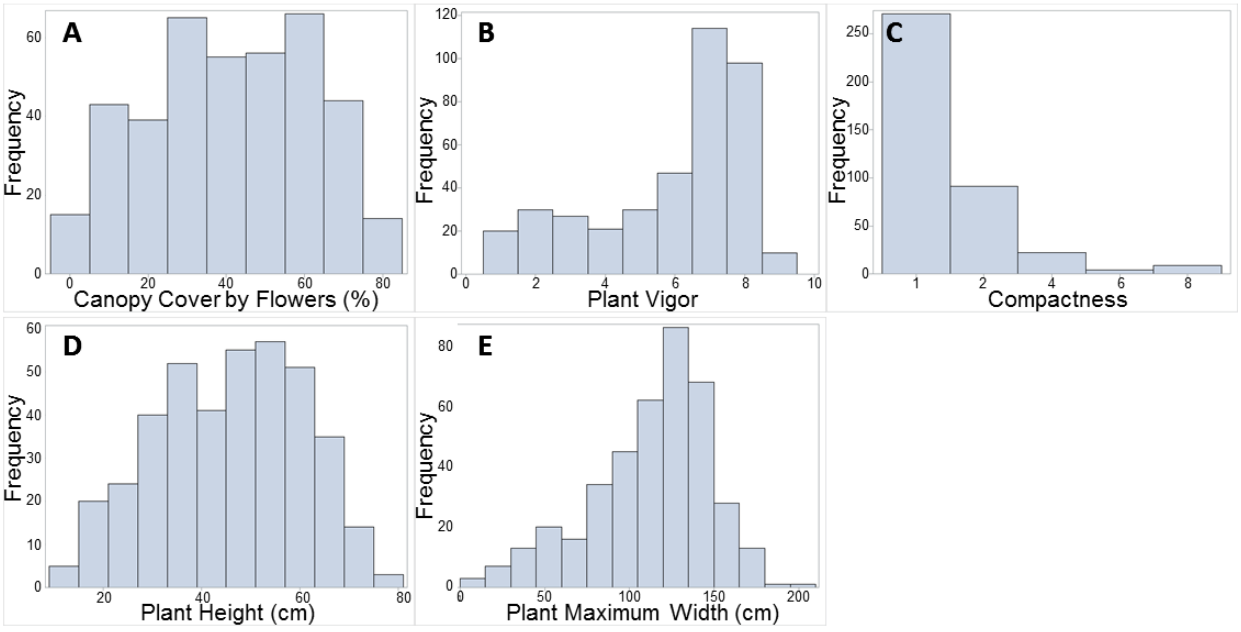
A percentage of plants flowering **B** plant vigor **C** plant compactness **D** plant height **E** plant maximum width **F** plant minimum width **G** flower color retention

Figure 2-8. Frequency distribution for crop quality traits for *P. axillaris* × *P. exserta* F_7 recombinant inbred line population at Garden Genetics in Bellfonte, PA evaluated in 2014



A percentage of plants flowering **B** plant vigor **C** plant compactness **D** plant height **E** plant maximum width **F** flower color retention

Figure 2-9. Frequency distribution for crop quality traits for *P. axillaris* × *P. exserta* F_7 recombinant inbred line population at Metrolina Greenhouses in Huntersville, NC evaluated in 2014



A percentage of plants flowering **B** plant vigor **C** plant compactness **D** plant height **E** plant maximum width

Figure 2-10. Percent increase in the number of flower buds as temperature decreased from 20 to 14°C

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CHAPTER 3

QUANTITATIVE TRAIT LOCI IDENTIFICATION AND EVALUATION FOR FLOWERING AND BRANCHING TRAITIS IN A *PETUNIA* RECOMBINANT INBRED LINE POPULATION

Introduction

As part of a $4.4 billion floriculture industry million (USDA-NASS, 2016), new petunia cultivars are released annually to add new characteristics or improve plant performance. In 2017, over 50 new cultivars were released from nine breeding companies. Although new cultivars are introduced to the market, improvements to current varieties are still needed for many traits such as increased flowering capacity. The flowering capacity of ornamental plants include traits such as the total number of flowers, flower longevity, and duration of bloom time. Each of these traits are important characteristics for breeders to consider.

Multiple component traits related to plant architecture can influence the total number of flowers per plant. For example, the number of branches and the number of flowers per branch both contribute to each plant's flowering intensity. An increase in branch number can provide additional inflorescence meristems for floral bud initiation. In our study of an F_7 interspecific *Petunia axillaris* × *P. exserta* population with 171 recombinant inbred lines (RILs), a positive correlation between branch number and flower number was detected at multiple temperatures (Chapter 2).

Some of the genes impacting shoot development have been identified and are closely linked with floral development. For example, the *BLIND* gene encodes a *Myb* transcription factor that controls lateral meristem initiation and leads to a reduction in the number of lateral shoots and flowers per inflorescence in tomato (Schmitz et al., 2001). Additionally, mutations in the rice gene *OsSPL14* (*SQUAMOSA PROMOTER BINDING PROTEIN-LIKE 14*) increased primary branch numbers in the panicles leading to an increase in grain number (Miura et al., 2010). However, the genetic control and environmental interactions influencing the component traits and flower number remains elusive.

Breeding for field flower performance can be time consuming and resource heavy when evaluating large numbers of progeny. Identifying greenhouse traits that are predictive of field performance can reduce the number of lines for field testing and, therefore, reduce cost. The F_7 RIL population described above was developed through single seed descent from an F_2 population (Warner and Walworth, 2010) to characterize phenotypic variation and identify potential genetic interactions between crop quality traits. This population has been genotyped using single-nucleotide polymorphism (SNP) markers generated from genotyping-by-sequencing (Guo et al., 2017). Flower number and its component traits were characterized at three temperature treatments in the winter of 2014 and 2015 (Chapter 2). Additionally, the population was phenotyped for quality traits in the summer of 2014 at four field locations. The phenotypic data showed positive correlation between total flower bud number and branching at two temperature treatments. Additionally, flower performance at the field trials showed positive correlation with vigor and height at all locations and with compactness at two of the four locations. The segregation within this population indicated that these traits are under polygenic control. Broad-sense heritabilities for total flower bud number, branch number, and flowering percentage were high for all temperature and location treatments with the exception of flowering percentage at Gilroy, CA (Chapter 2). The high heritability for these traits indicate that this population is suitable for genetic mapping and identification of novel genetic components regulating these traits.

Identification of major quantitative trait loci (QTL) for the component traits and the understanding of their genetic control can facilitate the development of marker-assisted breeding strategies to improve breeding efficiency for improved and novel cultivars (Collard and Mackill, 2008; Shirasawa and Hirakawa, 2013). These genetic regions associated with traits of interest

would also be useful for candidate gene identification. These studies will continue to advance our understanding of the genes controlling these traits and their impact on flower capacity. The objectives of this study were to identify QTL associated with (a) flower component traits at three greenhouse temperatures and (b) quality traits at four field environments using the interspecific *P. axillaris* × *P. exserta* F_7 RIL population.

Materials and methods

Phenotypic data

An F_7 *P. axillaris* × *P. exserta* RIL population of 171 lines was phenotyped in 2014 and 2015 at the Plant Science Greenhouses at Michigan State University (East Lansing, MI,) as described in Chapter 2. Additionally, 200 RILs from this F_7 population were evaluated in 2014 at four field locations representing four distinct climates throughout the United States (Table 3-1). The following phenotypic data (see Chapter 2 for details) were collected and analyzed from the greenhouse trials: total number of open flowers and flower buds at first flowering (bud length > 3mm) (FlBud), number of flower buds on the apical stem (FlBudAS), total branch number (lateral stem > 5cm) (Branch), number of branches with flower buds (FlBranch), and flower buds on the main stem (FlBudPS) (Table 3-2). The following data were collected and analyzed from the field trials: flowering percentage (Flow), plant vigor (Vigor), compactness (Comp), maximum plant width (MaxWid), plant height (Height), minimum plant width (MinWid), and flower color retention (ColorRet).

Linkage map construction

The population was genotyped as described by Guo et al. (2017) using 188 RILs and the two parental lines. A total of 6,291 single nucleotide polymorphisms (SNPs) were converted into 368 bins based on recombination breakpoints (Xu, 2013). A genetic linkage map was generated using JoinMap 4.0 (Van Ooijen, 2006) with the bin markers. Bins with a similarity value of 1.00 were removed from the calculations. The bin markers were placed into individual linkage groups using the LOD (logarithm of the odds) thresholds from 2.0 to 10.0 and linkage groups were determined using LOD thresholds of 4.0 to 6.0. Marker order and map distance were calculated using the regression module with the Kosambi mapping function (Kosambi, 1943). The mapping parameters were set to a recombination frequency of 0.30, a LOD score of 3.00, and a goodness-of-fit jump threshold of 5. The linkage groups were oriented and assigned chromosome (Chr) numbers according to a previous study (Bossolini et al., 2011).

QTL mapping

After removal for lines with missing data or high heterozygosity for this F_7 population, 158 and 163 RILs contained both genotypic and phenotypic data for the greenhouse and field trials, respectively. These RILs and a total of 356 bin markers were used for QTL mapping. Analysis for QTL was performed using the composite interval mapping (CIM) Model 6 algorithm in QTL Cartographer v2.5 software (Wang et al., 2012). The forward-backward regression method was used with five control markers. The control parameters were set to a window size of 10.0 cM, a walk speed of 1.0 cM, and marker probability threshold was defined at 0.05. A LOD threshold determined by 1000 permutations at an error rate of 5% for each trait were used to identify significant QTL. LOD values for each QTL were calculated from the

likelihood-ratio (LR) statistics. The proportion of total phenotypic variation explained (%VE) by each QTL was estimated using R^2 values. QTL for the same trait with overlapping confidence interval that were detected in two or more temperatures/years or in multiple locations were considered the same QTL and were denoted as robust QTL (rQTL). QTL were visualized using MapChart v2.2 software (Voorrips, 2002) using a subset of markers to facilitate visualization. Markers were filtered for visualization with the criteria that markers must be a minimum of 1 cM apart. For visualization, the shaded rectangles represented the range of potential peak positions and the line segments represented the combined overlapping confidence interval at 1-LOD value for the QTL. QTL names were determined by denoting "q" for QTL, followed by the QTL abbreviation, the chromosome where the QTL was detected, and the order within the chromosome.

Results

Linkage map

A total of 356 out of 368 bins were mapped to seven Chrs (Figure 3-1). The linkage map contained an average of 51 bins per Chr (Table 3-3). Chr 5 had the fewest bin markers with 23, while Chr 3 had the most with 92 bin markers. The Chrs ranged from 15.7 to 75.8 cM with an average genetic distance of 38.6 cM per Chr. The average marker interval ranged from 0.4 to 1.4 cM. The linkage map spanned a total genetic distance of 270.1 cM, contained 87% of the total SNP markers, and had an average of 0.8 cM between markers. The markers had good coverage of the genome except for a small region on Chr 7 which contained the biggest gap at 15.1 cM.

QTL for greenhouse traits at three temperatures

Cumulatively, 15 QTL were detected for FlBud on Chrs 1-4 (Table 3-4) and six of these were rQTL. The rQTL *qFB.1.1* was detected in five environmental settings and explained a combined phenotypic variation of 41.3%. Two rQTL (*qFB.4.1* and *qFB.4.2*) on Chr 4 were detected in four of the six environments and explained more than 25% of the phenotypic variation. The additive effects for the QTL for FlBud ranged from 1.2 to 4.5. *P. exserta* contributed the beneficial alleles for two rQTL but *P. axillaris* contributed the beneficial allele for the remaining QTL, including four rQTL.

For the FlBud component traits FlBudAS and FlBudPS, 14 and seven QTL were detected, respectively (Table 3-4). For FlBudAS, QTL were detected at all Chr except on Chr 5. The QTL for FlBudPS were detected on Chr 2, 3, 4, and 6. There were four rQTL for FlBudAS but no rQTL was detected for FlBudPS. The rQTL *qFBA.4.1* and *qFBA.4.*4 for FlBudAS co-localized to the same regions on Chr 4 as the rQTL *qFB.4.1* and *qFB.4.2* for FlBud, respectively, (Table 3-4; Figure 3-2). Additionally, two rQTL for FlBudAS explained more than 25% of the phenotypic variation, whereas none of the QTL for FlBudPS explained more than 10%. The additive effects ranged from 0.2 to 0.5 and 0.5 to 0.8 for FlBudAS and FlBudPS, respectively. For FlBudAS *P. exserta* contributed the beneficial alleles for three QTL, however, *P. axillaris* contributed the beneficial alleles for the remaining QTL including the four rQTL. Additionally, *P. axillaris* contributed the beneficial alleles for five of the seven QTL for FlBudPS.

A total of 17 and 15 QTL were detected for Branch and FlBranch, respectively (Table 3-4). There were two rQTL for Branch, and both were detected on Chr 3 (Table 3-4; Figure 3-2). The rQTL *qBR.3.3* explained a combined phenotypic variation of 72.3% and had the greatest additive effect. Another QTL detected on Chr 3 explained 33.4% of the variation and

had the second greatest additive effect of 1.3 but was only detected at one temperature in 2014. *P. axillaris* contributed the beneficial alleles for six QTL while *P. exserta* contributed the beneficial allele for the remaining QTL including the two rQTL. Three rQTL were detected for FlBranch on Chr 1, 4, and 5, respectively (Table 3-4; Figure 3-2). None of the QTL for FlBranch explained more than 25% of the phenotypic variation, but six explained 10-20%. Additionally, the QTL for FlBranch have additive effects ranging from 0.3 to 0.8 and the beneficial alleles were equally contributed by *P. axillaris* and *P. exserta*.

A total of 15 rQTL were detected for four traits on Chr 1-6 (Table 3-4; Figure 3-2). Six of the rQTL were detected on Chr 4, whereas only one rQTL was detected on Chr 5 and 6. Three rQTL detected for FlBud, FlBudAS, and FlBranch co-localized to a 5 cM region, whereas the three rQTL detected for FlBud, and FlBudAS co-localized to a region approximately 3 cM wide on Chr 4. Additionally, two rQTL detected for FlBud and FlBranch co-localized to a 1 cM region on Chr 1.

QTL for field data at four locations

A total of 79 QTL were detected for the seven traits grown at the four field locations with an average of 11 QTL per trait (Table 3-5). The most QTL detected were for Height with 16 QTL while only six QTL were detected for MinWid, which can at least partially be attributed to only having data for two of the four locations.

None of the QTL for Flow explained more than 25% of phenotypic variation, but the two QTL explaining the greatest phenotypic variation of 23.8% and 17.6% also had the greatest additive effect of 10.3 and 10.8, respectively (Table 3-5). A total of four rQTL for Flow were detected on Chr 1, 2 and 4 (Table 3-5; Figure 3-3). *P. exserta* contributed the beneficial allele

for each of the rQTL for Flow on Chr 1 and 2, whereas *P. axillaris* contributed the beneficial allele for the two rQTL on Chr 4.

Only one rQTL was detected for Vigor and explained a combined phenotypic variation of 24.3% and was located on Chr 4 (Table 3-5; Figure 3-3). The QTL for Vigor, *qVIG.2.2*, explained 30.7% of the variation and had the greatest additive effect of 1.2. *Petunia exserta* contributed the beneficial allele for this major QTL, and *P. axillaris* contributed the beneficial allele for two other major QTL. However, each of the three major QTL were only detected at one location.

There were two rQTL detected for Height and explained a combined phenotypic variation of 40.8% and 41.6% (Table 3-5; Figure 3-3). Both rQTL were located on Chr 2 and had beneficial alleles from *P. exserta*. For MaxWid, four rQTL were detected. Three of these rQTL were located on Chr4 and had beneficial alleles from *P. axillaris*. One rQTL *qMAX.2.1* was detected on Chr 2, explained the greatest phenotypic variation of 36.9%, and had the greatest additive effect of 20.8 for MaxWid. *Petunia exserta* contributed the beneficial allele for this rQTL and also for the major QTL *qMAX.2.2,* which explained 31.3% of the variation and had the second greatest additive effect of 20.0. Additionally, *P. exserta* contributed more beneficial alleles for the QTL for Flow, Comp, Height, and all the QTL for ColorRet, whereas, *P. axillaris* contributed more beneficial alleles for the QTL for Vigor, MaxWid, and all the QTL for MinWid.

The 15 rQTL for the six field traits in four locations were detected on all chromosomes except for Chr 5 and 6 (Table 3-5; Figure 3-3). Chr 2 and 4 both had six rQTL, whereas Chr 1, 3, and 7 each had 1 rQTL. On Chr 2, four of the six rQTL for each trait – Flow, Compact,

MaxWid, and Height – co-localized to a 0.4 cM region. Comparatively, the six rQTL on Chr 4 were spread across three regions with two co-localizing rQTL for two traits at each region.

Comparison of rQTL for greenhouse and field data

Co-localizing rQTL for greenhouse and field traits were only detected on Chr 4 (Figure 3-4). A 5 cM region contained four rQTL for one field trait, MaxWid, and three greenhouse traits, FlBud, FlBudAS, and FlBranch. Another is a 2.1 cM region containing five rQTL for two greenhouse, FlBud and FlBudAS, and two field traits, Vigor and MaxWid. No rQTL for greenhouse traits co-localized with Flow.

Discussion

The flowering capacity of each plant is a product of multiple traits including the number of branches and the number of flowers per branch. Dissecting the genetic control of these traits is a challenging task because a single genotype may exhibit a wide range of phenotypic variation in differing environments (Chapter 2). The complex interaction between genotype and environment is compounded by multiple genes that could be in linkage within the genetic region associated with the trait (Darvasi and Pisanté-Shalom, 2002). The aim of this study was to understand the associations between phenotype and genotype in a *P. axillaris* × *P. exserta* F_7 RIL population in multiple field and controlled temperature environments.

QTL identified for greenhouse traits

The flowering capacity of an F_7 petunia RIL population was examined using QTL analysis for flower component traits (e.g. total branch number, number of flowers on the apical

stem, etc.). Flowering traits are quantitative and influenced by environmental factors such as temperature and photoperiod (Chapter 1). A total of 68 putative QTL for five greenhouse traits were identified on all Chr for three temperatures in two years with 15 rQTL detected on Chr 1-6 (Table 3-4; Figure 3-2). The rQTL co-localized within one region spanning 1 cM on Chr 1 and within two regions each spanning 5 cM and 3 cM on Chr 4, respectively (Figure 3-2). The significant correlations among these traits, with the exception of flower bud on the primary stem (Chapter 2), and the similar QTL regions suggest that the traits are tightly linked. The genetic controls for some traits can have downstream effects on related traits. For example, the alleles on Chr 4 influencing total flower number would be associated with the number of flowers on the apical stem.

In this study, the QTL for branch number were distributed across six of the seven Chr with two rQTL detected on Chr 3 (Table 3-4; Figure 3-2). These QTL can be influential markers for improving branch number due to their robustness across multiple temperatures and years. Additionally, these QTL could potentially be linked to auxin and cytokinin biosynthetic genes as phytohormones contribute to the regulation of branching (Shimizu-Sato et al., 2009). Auxin maintains shoot apical dominance and indirectly represses axillary outgrowth by downregulating cytokinin biosynthesis (Eklöf et al., 1997; Hall and Hillman, 1975; Nordström et al., 2004; Thimann and Skoog, 1934). In contrast, cytokinin promotes axillary bud outgrowth even in the presence of auxin at certain developmental stages (Wickson and Thimann, 1958).

Whereas previous research implicates auxin and cytokinin in branching control (Müller and Leyser, 2011), potential genes within the QTL regions may also belong to the strigolactone pathway genes. Strigolactones are carotenoid-derived plant metabolites and have also been identified as inhibitors of bud outgrowth and shoot formation (Drummond et al., 2012; Gomez-

Roldan et al., 2008; Kretzschmar et al., 2012; Umehara et al., 2008; Waldie et al., 2014). In chrysanthemum, phenotypic variation for shoot branching has been associated with allelic variation of branching genes in the strigolactone pathway (Klie et al., 2016). Orthologs of these branching genes such as *MORE AXILLARY BRANCHING (MAX), CAROTENOID CLEAVAGE DIOXYGENASE7 (CCD7),* and *TCP* have been identified in petunia, including *MAX2B, CCD7,* and *TCP1-TCP3* (Drummond et al., 2015, 2012, 2009). The QTL identified in this study were not linked to *CCD7*. However, a BLAST search indicated that *MAX2B* is on scaffold 384 of the *P. axillaris* genome (Bombarely et al., 2016). This scaffold contains a marker (AE_bin_301_62_14_156_2_2) flanking the QTL *qFBN.5.1* for flower branch number. Additionally, *TCP1* is located on scaffold 86, which contains four markers AE_bin_89_94_49_1, AE_bin_222_330_313_2, AE_bin_222_330_313_1, AE_bin_223_81 flanking four QTL for branch number (*qBR.1.2*), flowering branch number (*qFBN.1.2*), flower number (*qFB.4.2*), and flower number on the apical stem (*qFBA.4.4*). Future efforts to fine map the candidate region on Chr 3 will help develop a more thorough understanding of the quantitative mechanism for branching regulation in petunia.

An average of 14 QTL were detected for each trait (Table 3-4). For flower bud-related QTL, *P. axillaris* contributed more beneficial alleles, whereas *P. exserta* contributed more beneficial alleles for branching-related QTL. Both parents contributed favorable alleles for all traits. These results support incorporating wild species into breeding programs to introgress alleles that may have been lost during breeding (Tychonievich et al., 2013) for flower component traits.

Association of QTL for greenhouse & field traits

The rQTL for percentage of canopy flower coverage in the field trials did not co-localize to rQTL for flower component traits in the greenhouse trials (Figure 3-2 – Figure 3-4). However, two rQTL *qVIG.4.5* and *qMAX.4.6* for plant vigor and canopy maximum width on Chr 4 were detected within a 3 cM region to two flower number and one flower number on the apical stem rQTL (Table 3-4; Table 3-5; Figure 3-4). Additionally on Chr 4, a 3 cM region contained one rQTL *qMAX.4.3* for canopy maximum width which co-localized with one rQTL each for flower bud number, flower bud on the apical stem, and flowering branch number. These regions would be useful for candidate gene discovery. Furthermore, all of the rQTL in these regions had beneficial alleles contributed by *P. axillaris* suggesting that *P. axillaris* can provide alleles that influence greenhouse traits and may be predictive of some field traits. However, the inability to detect strong effect QTL in different settings can stem from additional environmental cues that are difficult to control and account for in field trials (Weinig et al., 2002). Additionally, plant developmental stage can impact QTL detection for the same traits (Zhang et al., 2010). For example, soybean QTL expression for flower number varied across different developmental stages (Zhang et al., 2010).

Lines AE126 and AE17 were identified as the highest performing lines for flowering time, flower bud number, and floral coverage (Chapter 2). Both of these lines have the same alleles as the beneficial alleles for five rQTL, *qFB.1.1*, *qFB.2.2*, *qFB.4.1*, *qFB.4.2*, *qFB.4.3*, for flower bud number. Additionally, AE126 has the same beneficial allele for the floral coverage rQTL *qFP.1.2*. These results suggest that line AE126 could be useful in a breeding program to predict field flower performance using flowering time and flower number at first flowering.

Relationship with other traits/crops

Four QTL including one rQTL for total branch number and flowering branch number were detected on the same chromosome as the previously identified QTL for branch number in a *P. integrifolia* × *P. axillaris* F_2 population (Vallejo et al., 2015). Additionally, four QTL including one rQTL for flower bud on the apical stem and flower bud on the primary stem were detected on the same chromosome as the previously identified QTL for flower buds on the main stem in the F_2 population. The QTL for total number of flower bud on the primary stem on Chr 6 (*FBP6.1*) and total branch number on Chr 1 (*BR1.1*) explained 43 and 26% of the variation, respectively, in the F_2 population (Vallejo et al., 2015). Conversely, in this study, the QTL for these traits that were detected on the same Chr explained only 6% – 11% of the variation (Table 3-4). Additionally in the F_7 population, two major QTL for each trait flower number and flower number on the apical stem were detected on Chr 4 and one major QTL on Chr 3 and one on Chr 4 for total branch number. Whereas the QTL (*FB1.1*) for flower number on Chr 1 in the *P. integrifolia* × *P. axillaris* population was not a major QTL and only explained 13% of the variation, but it had a large additive effect (17.78 flower number) with beneficial alleles from *P. axillaris* (Vallejo et al., 2015). While the largest additive effect for any total flower number QTL in the *P. axillaris* × *P. exserta* population was also inherited from *P. axillaris*, the effect was much lower at 4.5. Additionally, in the F_7 population, *P. exserta* contributed the beneficial alleles for the QTL on Chr 1 for flower number, which indicates that both parents can provide beneficial alleles for this trait.

One rQTL for flower number co-localized to the same region as the rQTL for flower branch number on Chr 4 (Figure 3-2). Similarly, QTL for spikelets per panicle and primary branch number co-localized in rice (Balkunde et al., 2013). One of the four candidate genes

found within the QTL-containing region was a putative expressed nitrilase gene, which converts indole-3-acetonitrile (IAN) to indole-3-acetic acid (IAA) through hydrolysis (Kobayashi et al., 1993). IAA is one of the most abundant plant auxins, which controls plant growth and development through cell elongation and cell division (Teale et al., 2006). There are multiple pathways leading to IAA synthesis including many precursor and intermediary hormones (Strader and Bartel, 2008). In Arabidopsis, the *YUCCA* and *CYP79B* pathways increase IAN production, leading to increased levels of auxin (Zhao et al., 2002). In petunia, *FLOOZY* (*FZY*) has been identified as an ortholog of the *YUCCA* gene (Tobeña-Santamaria et al., 2002), which suggests that petunia shares the IAN-dependent IAA biosynthetic pathway. However, the QTL identified in the greenhouse trials did not co-localize with the markers within the scaffold for the *FZY* gene. The inability to identify QTL linked with the *FZY* gene in this study could be related to inadequate variation for this gene and/or gaps in the genome coverage (Bossolini et al., 2011; Guo et al., 2015). The use of a near-isogenic line population with different parents could detect association to the *FZY* gene that was undetectable using this RIL population (Keurentjes et al., 2007). Future studies that utilize saturated molecular maps may increase the power to detect QTL and aid candidate gene identification. Additionally, marker identification and development can be incorporated into breeding programs for marker-assisted selection.

Association of flower component QTL to known flowering genes

Flower number is an important trait that influences the aesthetic value of ornamental plants. Desirable flower characteristics include increased flower number and repeat or continuous blooming. However, quantitative analysis and candidate gene identification for these traits have not been comprehensively studied in ornamental crops. In this study, 15 QTL were

detected for flower number with both parents contributing favorable alleles (Table 3-4). In a study with two soybean RIL populations sharing one parent, two QTL were detected for pod number in one of the populations (W. Liu et al., 2011). The marker associated with one of the QTL was linked to the flowering maturity gene loci E1 (Funatsuki et al., 2005). Soybean maturity loci are major genes that control variation in flowering time through the photoperiodic pathway (Bernard, 1971; Xia et al., 2012) and influence branching and growth habit (Zhai et al., 2014). Although maturity genes have not been identified in petunia, the results from this study can facilitate future efforts for gene identification via reverse genetics (Berenschot and Quecini, 2014).

Conclusion

The current study of flower production and its component traits has provided new insight into its complex genetic control. The analyses of greenhouse flower production and component traits revealed that these traits are co-dependent due to their phenotypic correlation and QTL co-localization. Genomic regions of interest for flower production traits in the greenhouse and field performance were identified on Chr 2 and 4. Evaluation and validation of these QTL in distinct genetic backgrounds will be necessary to further evaluate the potential importance of these regions. Future studies to fine map candidate regions can identify genes controlling flower component traits and provide markers for improving flower production.

APPENDIX

Table 3-1. Field experiment locations for *P. axillaris* × *P. exserta* F_7 recombinant inbred line population in 2014

Location	Climate[z]	Collaborator	Abbreviation
Bellefonte, PA	Warm summer continental	Garden Genetics	PA
Gilroy, CA	Mediterranean	Syngenta	CA1
Huntersville, NC	Humid subtropical	Metrolina Greenhouses	NC
Beullton, CA	Semiarid	Ball Horticultural Company	CA2

[z] Based on the Köppen-Geiger climate classification system (Peel et al., 2007)

Table 3-2. List of traits measured and used for QTL analysis

Trait Description	Abbreviation	QTL Abbreviation
Greenhouse Traits		
Total visible flowers and buds number	FlBud	FB
Total branch number (branches >5cm)	Branch	BR
The number of branches with flower buds	FlBranch	FBN
The number of visible flower buds on the apical shoot	FlBudAS	FBA
The number of visible flower buds on the main stem	FlBudPS	FBP
Field Traits		
Percentage of canopy flower cover (%)	Flow	FP
Plant vigor (1-9)	Vigor	VIG
Plant compactness (1-9)	Compact	COM
Plant height (cm)	Height	HGT
Plant maximum width (cm)	MaxWid	MAX
Plant minimum width (cm)	MinWid	MIN
Flower color retention (1-9)	ColorRet	CR

Table 3-3. Summary of genetic linkage map for *P. axillaris* × *P. exserta* F_7 recombinant inbred line population

Chr	Bins (No.)	Length (cM)	SNP (No.)	Average marker density (cM)
1	39	15.75	1032	0.40
2	64	33.95	1106	0.53
3	92	75.80	945	0.82
4	51	46.09	782	0.90
5	23	21.38	525	0.93
6	62	43.03	624	0.69
7	25	34.08	465	1.36
Total	**356**	**270.08**	**5479**	**0.76**

Table 3-4. Summary of QTL identified at three greenhouse temperatures for the *P. axillaris* × *P. exserta* F_7 recombinant inbred line population in 2014 and 2015

Trait[z]	QTL	Chr	Nearest marker	Environment	Posit. (cM)	Interval (cM)[y]	LOD[x]	LOD threshold[w]	α[v]	%VE[u]
FlBud	qFB.1.1	1	AE_bin_72_5	2014-20C	5.71	4.3-5.9	5.93	2.56	-1.81	10.63
				2015-14C	5.71	3.5-5.8	4.20	2.65	-3.06	7.29
			AE_bin_71_9	2014-17C	5.91	4.1-8.1	6.61	2.50	-2.02	11.64
				2015-17C	5.91	4.4-6.9	3.14	2.63	-2.21	5.32
				2015-20C	5.91	5.8-8.2	4.30	2.55	-2.33	6.42
	qFB.2.1	2	AE_bin_63_260_253_1	2014-14C	5.71	5.6-5.9	4.15	2.43	3.41	9.94
	qFB.2.2	2	AE_bin_3_202_229_2	2015-17C	32.41	31.7-32.6	3.91	2.63	-2.94	6.74
			AE_bin_3_202_229_1	2015-14C	32.61	31.7-33.6	4.85	2.65	-4.25	8.44
	qFB.3.1	3	AE_bin_103_2	2015-20C	3.41	1.6-5.7	3.81	2.55	2.10	5.73
			AE_bin_104_1	2015-14C	4.21	2.8-5.2	3.04	2.65	2.54	5.22
	qFB.3.2	3	AE_bin_105_7	2014-17C	13.81	7.3-18.8	3.32	2.50	1.42	5.54
	qFB.3.3	3	AE_bin_107_1	2014-17C	23.11	19.8-23.4	3.15	2.50	1.35	5.22
	qFB.3.4	3	AE_bin_110_2	2014-14C	28.21	24.4-32.3	4.07	2.43	2.53	9.71
	qFB.3.5	3	AE_bin_115_2	2014-20C	35.91	34.5-38.5	2.63	2.56	1.15	4.46
	qFB.3.6	3	AE_bin_120_1	2014-20C	42.71	41.7-46.1	4.01	2.56	1.41	6.64
	qFB.3.7	3	AE_bin_143_2	2015-17C	62.71	61.9-66.4	2.65	2.63	1.90	4.53
	qFB.3.8	3	AE_bin_159_15	2015-14C	74.11	73.9-74.3	3.23	2.65	2.68	5.63
	qFB.4.1	4	AE_bin_231_1	2014-14C	16.21	14.9-18.1	5.58	2.43	2.82	12.35
				2014-17C	16.21	15.2-17.5	7.83	2.50	2.47	18.00
			AE_bin_198_1	2015-20C	17.21	15.7-18.3	12.05	2.55	4.54	27.15
			AE_bin_202_1	2015-14C	21.21	18.1-22.6	5.03	2.65	3.50	9.07
	qFB.4.2	4	AE_bin_207_2	2015-17C	25.01	23.7-25.1	8.79	2.63	3.71	16.30
			AE_bin_224_5	2014-14C	25.41	25.1-25.9	6.22	2.43	2.88	12.43
			AE_bin_208_7	2014-20C	25.71	25.6-26.2	14.43	2.56	2.99	28.75
			AE_bin_222_330_313_2	2014-17C	26.21	26.1-26.6	11.14	2.50	2.76	21.17
	qFB.4.3	4	AE_bin_226_1	2015-14C	27.11	27.0-28.9	5.22	2.65	3.61	9.44
				2015-20C	27.11	27.0-27.2	9.65	2.55	4.04	15.94
	qFB.4.4	4	AE_bin_216_1	2014-14C	41.01	38.8-43.5	3.08	2.43	2.07	6.44

Table 3-4 (cont'd)

Trait[z]	QTL	Chr	Nearest marker	Environment	Posit. (cM)	Interval (cM)[y]	LOD[x]	LOD threshold[w]	α[v]	%VE[u]
FlBudAS	qFBA.1.1	1	AE_bin_74_1	2014-20C	8.01	7.3-8.7	3.24	2.68	-0.15	5.02
	qFBA.2.1	2	AE_bin_63_260_253_2	2015-20C	5.61	5.5-5.9	3.17	2.52	0.30	5.74
			AE_bin_63_260_253_1	2015-17C	5.71	5.6-6.0	6.83	2.67	0.48	13.26
	qFBA.2.2	2	AE_bin_2_2	2014-14C	31.71	29.7-32.6	2.96	2.63	-0.22	6.18
	qFBA.3.1	3	AE_bin_115_2	2014-20C	35.91	34-38.5	3.35	2.68	0.15	5.20
	qFBA.3.2	3	AE_bin_120_1	2014-20C	42.71	39.1-45.8	3.16	2.68	0.15	4.91
	qFBA.4.1	4	AE_bin_231_1	2014-17C	16.21	15.1-17.5	6.51	2.37	0.38	18.93
				2015-14C	16.21	15.7-18.3	3.29	2.56	0.28	9.68
				2015-20C	16.21	15.1-18.1	6.46	2.52	0.36	16.12
			AE_bin_198_1	2014-20C	18.21	16.5-18.7	11.96	2.68	0.34	25.38
	qFBA.4.2	4	AE_bin_207_2	2015-17C	25.01	24.1-25.2	7.81	2.67	0.36	13.45
	qFBA.4.3	4	AE_bin_210_117_218_4_1_1	2014-20C	25.91	25.7-25.9	18.08	2.68	0.40	34.94
	qFBA.4.4	4	AE_bin_223_81	2014-17C	26.41	26.2-26.9	11.56	2.37	0.46	27.17
			AE_bin_229_48	2015-14C	26.61	26.5-26.8	7.02	2.56	0.42	16.41
	qFBA.4.5	4	AE_bin_226_1	2015-20C	27.11	27.0-27.2	11.94	2.52	0.47	25.18
	qFBA.4.6	4	AE_bin_220_5	2015-17C	30.81	29-31.7	6.98	2.67	0.34	12.16
	qFBA.6.1	6	AE_bin_272_3_88_2	2015-14C	33.51	33-33.8	2.68	2.56	0.24	5.97
			AE_bin_272_3_88_1	2014-14C	33.71	33.6-34.0	4.87	2.63	0.32	10.19
	qFBA.6.2	6	AE_bin_253_20	2015-14C	35.01	34.5-35.1	2.76	2.56	0.23	6.13
	qFBA.7.1	7	AE_bin_316_1	2014-14C	21.11	19.0-22.3	3.46	2.63	-0.19	7.20
FlBudPS	qFBP.2.1	2	AE_bin_3_202_229_2	2015-14C	32.41	31.4-32.6	4.37	2.61	-0.78	9.83
	qFBP.3.1	3	AE_bin_161_14_267_2	2015-17C	74.71	74.1-75.5	2.98	2.50	-0.47	5.95
	qFBP.4.1	4	AE_bin_231_1	2015-17C	15.21	14.4-18.0	3.40	2.50	0.53	7.11
	qFBP.4.2	4	AE_bin_229_48	2015-14C	26.61	26.4-26.9	2.76	2.61	0.50	6.42
	qFBP.4.3	4	AE_bin_226_1	2015-17C	27.11	27.0-28.2	4.52	2.50	0.62	9.29
	qFBP.6.1	6	AE_bin_247_3	2015-14C	27.11	24.6-27.9	2.70	2.61	0.50	5.92
	qFBP.6.2	6	AE_bin_252_5	2015-17C	35.41	35.3-36.4	3.85	2.50	0.60	8.18

Table 3-4 (cont'd)

Trait[z]	QTL	Chr	Nearest marker	Environment	Posit. (cM)	Interval (cM)[y]	LOD[x]	LOD threshold[w]	α[v]	%VE[u]
	qBR.1.1	1	AE_bin_88_525_28_2	2014-14C	11.01	11.0-11.4	7.52	2.71	0.80	11.00
	qBR.1.2	1	AE_bin_89_94_49_1	2015-14C	11.81	11.7-15.3	5.36	2.61	0.49	9.07
	qBR.2.1	2	AE_bin_4_1	2014-14C	30.61	27.0-31.7	2.86	2.71	-0.62	4.17
	qBR.3.1	3	AE_bin_142_3	2015-14C	61.61	61.3-62.5	8.58	2.61	-0.66	16.61
	qBR.3.2	3	AE_bin_147_3	2014-14C	65.21	64.5-65.5	11.42	2.71	-1.09	23.62
				2014-20C	65.21	64.1-65.7	4.90	2.65	-0.44	9.14
				2015-17C	65.21	64.5-65.7	3.74	2.52	-0.49	8.69
				2015-20C	65.21	64.5-65.7	3.06	2.50	-0.42	5.64
	qBR.3.3	3	AE_bin_165_1	2014-14C	70.31	69.7-71.3	18.45	2.71	-1.35	35.97
				2015-20C	70.31	69.7-70.9	3.68	2.50	-0.46	7.63
			AE_bin_166_17	2014-17C	72.31	71.3-72.5	3.87	2.60	-0.48	7.50
Branch			AE_bin_173_12	2015-14C	72.71	72.4-72.9	10.75	2.61	-0.73	20.15
	qBR.3.4	3	AE_bin_182_17_237_2	2014-14C	74.01	73.9-74.3	17.63	2.71	-1.32	33.43
	qBR.3.5	3	AE_bin_154_3	2015-17C	75.01	74.7-75.3	6.23	2.52	-0.62	14.05
	qBR.4.1	4	AE_bin_198_1	2015-17C	17.21	16.8-18.3	3.44	2.52	0.49	8.66
	qBR.4.2	4	AE_bin_210_117_218_4_2_2	2015-20C	26.11	26.0-26.2	10.16	2.50	0.73	20.48
	qBR.4.3	4	AE_bin_221_1	2014-20C	27.01	26.7-27.0	11.20	2.65	0.74	25.81
	qBR.4.4	4	AE_bin_220_5	2015-17C	29.81	27.1-33.4	3.12	2.52	0.43	6.78
	qBR.5.1	5	AE_bin_290_8	2015-14C	10.31	9.1-12.0	4.44	2.61	-0.44	7.55
	qBR.5.2	5	AE_bin_303_4	2014-14C	13.41	13.3-16.9	3.54	2.71	-0.51	4.91
	qBR.6.1	6	AE_bin_232_4	2015-14C	0.01	0-2.7	3.21	2.61	-0.40	5.62
	qBR.6.2	6	AE_bin_248_11_176_1	2015-14C	27.91	26.0-28.5	3.19	2.61	0.41	5.38
	qBR.6.3	6	AE_bin_273_3	2015-14C	33.11	32.4-33.6	3.83	2.61	0.44	6.42

Table 3-4 (cont'd)

Trait[z]	QTL	Chr	Nearest marker	Environment	Posit. (cM)	Interval (cM)[y]	LOD[x]	LOD threshold[w]	α[v]	%VE[u]
FlBranch	qFBN.1.1	1	AE_bin_70_1	2014-17C	4.91	3.3-5.1	3.84	2.49	-0.46	9.39
			AE_bin_69_2	2014-20C	5.81	4.6-5.9	3.18	2.60	-0.30	6.38
	qFBN.1.2	1	AE_bin_89_94_49_1	2015-14C	11.81	11.7-15.3	5.24	2.55	0.48	8.86
	qFBN.3.1	3	AE_bin_142_3	2015-14C	61.61	61.3-62.5	8.41	2.55	-0.65	16.32
	qFBN.3.2	3	AE_bin_147_3	2015-17C	65.21	64.5-65.7	4.20	2.68	-0.48	9.83
	qFBN.3.3	3	AE_bin_165_1	2015-17C	70.31	68.5-71.6	6.87	2.68	-0.63	17.02
	qFBN.3.4	3	AE_bin_173_12	2015-14C	72.71	72.4-72.9	10.68	2.55	-0.72	20.02
	qFBN.3.5	3	AE_bin_154_3	2015-17C	75.01	74.7-75.3	7.70	2.68	-0.64	17.19
	qFBN.4.1	4	AE_bin_198_1	2014-20C	17.21	16.8-18.7	3.11	2.60	0.37	9.86
				2015-20C	17.21	15.8-18.2	9.09	2.55	0.77	23.11
	qFBN.4.2	4	AE_bin_200_2	2015-17C	20.11	18.3-22.0	2.93	2.68	0.41	6.80
	qFBN.4.3	4	AE_bin_210_117_218_4_2_2	2015-20C	26.11	26.0-26.2	9.38	2.55	0.71	20.42
	qFBN.4.4	4	AE_bin_221_1	2014-20C	27.01	26.6-27.8	2.83	2.60	0.32	7.52
	qFBN.5.1	5	AE_bin_287_1	2015-17C	6.01	0-9.4	2.88	2.68	-0.38	6.19
			AE_bin_290_8	2015-14C	10.31	9.1-12.0	4.50	2.55	-0.44	7.67
			AE_bin_292_38	2014-14C	10.91	10.4-12.3	3.66	2.43	-0.46	8.66
			AE_bin_293_10	2015-17C	11.61	9.4-13.3	2.96	2.68	-0.37	5.98
			AE_bin_302_49	2014-17C	12.61	10.3-13.3	3.67	2.49	-0.31	8.38
			AE_bin_295_2	2014-20C	14.01	13.3-18.9	2.70	2.60	-0.29	6.07
	qFBN.6.1	6	AE_bin_232_4	2015-14C	0.01	0-2.7	3.21	2.55	-0.40	5.64
	qFBN.6.2	6	AE_bin_248_11_176_1	2015-14C	27.91	26.0-28.5	3.29	2.55	0.42	5.56
	qFBN.6.3	6	AE_bin_273_3	2015-14C	33.11	32.4-33.6	3.93	2.55	0.45	6.60

[z] Trait abbreviations: as defined in Table 3-2
[y] Combine overlapping confidence interval as determined by 1-LOD values
[x] LOD values calculated from likelihood-ratio statistics
[w] LOD threshold determined at 0.05 probability based on 1,000 permutations
[v] Additive effect of QTL, positive values indicate beneficial alleles from *P. axillaris*
[u] Percentage of variation explained by QTL estimated using R^2 statistics

Table 3-5. Summary of QTL identified at four field locations for the *P. axillaris* × *P. exserta* F_7 recombinant inbred line population in 2014

Trait[z]	QTL	Chr	Nearest marker	Loc.	Posit. (cM)	Interval (cM)[y]	LOD[x]	LOD threshold[w]	α[v]	%VE[u]
Flow	qFP.1.1	1	AE_bin_78_1	CA1	8.81	6.8-9.1	2.76	2.66	-4.47	6.07
	qFP.1.2	1	AE_bin_95_2	CA2	13.31	13.3-15.3	7.36	2.44	-6.65	14.20
			AE_bin_95_2	NC	14.31	12.6-15.3	2.99	2.59	-4.85	5.88
	qFP.2.1	2	AE_bin_58_12	NC	19.51	19.4-19.7	11.45	2.59	-10.27	23.81
			AE_bin_61_3	PA	19.61	19.4-19.7	8.56	2.63	-10.97	18.95
	qFP.2.2	2	AE_bin_52_48	CA2	20.21	19.9-21.2	2.94	2.44	-4.22	5.21
	qFP.2.3	2	AE_bin_4_1	NC	27.71	25.7-28.8	5.17	2.59	-10.77	17.56
	qFP.3.1	3	AE_bin_112_12	NC	31.41	27.9-33.8	2.84	2.59	5.07	4.92
	qFP.4.1	4	AE_bin_187_4	PA	4.21	0-6.2	4.31	2.63	6.28	8.91
			AE_bin_187_4	CA2	5.21	1.5-7.3	5.80	2.44	6.00	11.37
	qFP.4.2	4	AE_bin_195_4	NC	11.91	10.6-14.3	3.07	2.59	4.76	5.65
			AE_bin_197_3	CA2	14.31	12.8-14.5	4.81	2.44	5.35	9.02
	qFP.4.3	4	AE_bin_207_2	CA1	25.01	24.1-25.4	3.04	2.66	5.10	6.77
	qFP.6.1	6	AE_bin_236_1	CA2	10.11	8.1-13.2	3.04	2.44	-4.39	5.53
Vigor	qVIG.2.1	2	AE_bin_61_3	PA	19.61	19.4-19.7	12.56	2.74	-1.09	22.34
	qVIG.2.2	2	AE_bin_52_48	NC	20.01	19.9-20.5	17.41	2.65	-1.22	30.72
	qVIG.2.3	2	AE_bin_44_4	PA	21.61	21.1-21.8	12.82	2.74	-1.10	22.61
	qVIG.2.4	2	AE_bin_4_1	PA	28.71	26.4-30.5	5.17	2.74	-1.15	15.47
	qVIG.3.1	3	AE_bin_128_16_310_2	CA1	48.51	48.1-48.9	2.59	2.54	0.22	4.19
	qVIG.4.1	4	AE_bin_184_2	NC	0.01	0-1.0	8.68	2.65	0.79	13.85
	qVIG.4.2	4	AE_bin_187_4	CA2	4.21	2.6-6.6	11.28	2.65	0.55	26.01
	qVIG.4.3	4	AE_bin_197_3	CA2	14.31	12.2-14.4	11.79	2.65	0.56	27.08
	qVIG.4.4	4	AE_bin_198_1	CA1	17.21	15.2-18.3	6.27	2.54	0.44	14.05
	qVIG.4.5	4	AE_bin_229_48	CA1	26.61	26.4-26.9	10.86	2.54	0.51	19.71
				PA	26.61	26.4-27.1	3.05	2.74	0.51	4.62
	qVIG.4.6	4	AE_bin_201_1	CA2	27.81	27.4-29.9	5.19	2.65	0.37	9.37
	qVIG.6.1	6	AE_bin_239_2	NC	21.01	15.3-23.4	3.81	2.65	-0.65	5.41
	qVIG.6.2	6	AE_bin_252_5	NC	35.31	35.1-35.5	3.81	2.65	0.68	5.48
	qVIG.7.1	7	AE_bin_320_2	CA1	28.21	24.6-29.2	2.69	2.54	0.24	5.09

Table 3-5 (cont'd)

Trait[z]	QTL	Chr	Nearest marker	Loc.	Posit. (cM)	Interval (cM)[y]	LOD[x]	LOD threshold[w]	α[v]	%VE[u]
Compact	qCOMP.1.1	1	AE_bin_74_1	CA1	8.01	6.3-8.8	2.63	2.52	-0.18	5.28
	qCOMP.1.2	1	AE_bin_91_6	CA2	11.51	10.9-11.6	8.25	2.48	-0.32	14.04
	qCOMP.1.3	1	AE_bin_95_2	CA1	14.31	12.6-15.3	2.69	2.52	-0.19	5.91
	qCOMP.2.1	2	AE_bin_6_1	CA2	0.01	0-3.4	2.59	2.48	-0.20	4.23
	qCOMP.2.2	2	AE_bin_16_7	CA2	10.21	9.5-10.8	6.50	2.48	-0.32	12.92
	qCOMP.2.3	2	AE_bin_31_10	NC	18.21	18.0-19.0	6.65	2.49	0.34	15.28
	qCOMP.2.4	2	AE_bin_55_14	CA2	19.41	19.3-19.4	8.19	2.48	-0.36	13.71
			AE_bin_58_12	PA	19.51	19.3-19.6	7.42	2.68	0.81	14.84
	qCOMP.2.5	2	AE_bin_28_3	NC	24.31	23.9-26.7	7.31	2.49	0.36	16.67
			AE_bin_4_1	PA	28.71	26.3-29.3	3.20	2.68	1.05	14.70
	qCOMP.2.6	2	AE_bin_3_202_229_1	CA1	32.61	31.7-33.6	3.61	2.52	-0.30	7.17
	qCOMP.4.1	4	AE_bin_206_4	PA	24.11	23.3-24.9	5.59	2.68	-0.67	10.81
	qCOMP.5.1	5	AE_bin_290_8	PA	10.31	8.4-11.6	2.72	2.68	-0.45	5.06
	qCOMP.5.2	5	AE_bin_295_2	CA1	15.01	13.9-17.6	4.50	2.52	0.24	9.62
	qCOMP.7.1	7	AE_bin_315_1	CA2	22.41	20.5-24.1	3.18	2.48	-0.19	4.88
ColorRet	qCR.2.1	2	AE_bin_15_11	PA	9.81	8.9-10.5	4.00	2.68	-0.65	9.55
	qCR.2.2	2	AE_bin_50_8	PA	19.21	19.1-19.3	7.10	2.68	-0.95	15.95
	qCR.2.3	2	AE_bin_4_1	PA	27.71	25.2-28.7	3.74	2.68	-1.10	14.13
	qCR.3.1	3	AE_bin_165_1	PA	69.71	68.0-70.3	3.26	2.68	-0.53	7.87
	qCR.3.2	3	AE_bin_163_1	CA2	71.61	71.2-72.1	4.11	2.59	-0.58	9.50
	qCR.3.3	3	AE_bin_180_16	CA2	73.41	73.1-73.6	4.71	2.59	-0.61	10.72
			AE_bin_177_199_237_1	CA1	73.51	73.3-73.7	6.20	2.66	-0.72	14.87
	qCR.6.1	6	AE_bin_244_6	PA	25.61	23.4-26.6	4.59	2.68	-0.66	9.90
	qCR.6.2	6	AE_bin_263_50_86_2	PA	32.01	30.6-32.3	3.94	2.68	-0.59	8.30
	qCR.7.1	7	AE_bin_318_1	PA	20.21	20.1-22.1	4.34	2.68	-0.57	9.58
			AE_bin_316_1	CA2	21.11	20.8-22.4	5.92	2.59	-0.68	15.97
	qCR.7.2	7	AE_bin_324_45	PA	30.21	29.4-30.8	4.87	2.68	-0.60	10.33
	qCR.7.3	7	AE_bin_329_7	CA2	32.01	31.1-33.0	5.97	2.59	-0.65	14.46

Table 3-5 (cont'd)

Trait[z]	QTL	Chr	Nearest marker	Loc.	Posit. (cM)	Interval (cM)[y]	LOD[x]	LOD threshold[w]	α[v]	%VE[u]
MaxWid	qMAX.1.1	1	AE_bin_64_2	CA1	0.01	0-1.2	3.83	2.58	5.46	6.82
	qMAX.1.2	1	AE_bin_82_5	CA1	11.31	11.0-11.5	4.90	2.58	6.13	8.49
	qMAX.2.1	2	AE_bin_55_14	NC	19.41	19.3-19.4	17.23	2.54	-20.75	36.88
			AE_bin_58_12	PA	19.51	19.3-19.7	11.62	2.71	-15.60	19.27
	qMAX.2.2	2	AE_bin_27_2	NC	24.11	23.4-24.3	13.88	2.54	-20.03	31.34
	qMAX.4.1	4	AE_bin_185_1	CA2	1.71	0.3-7.2	5.03	2.68	6.97	8.51
				PA	1.71	0.8-3.7	6.46	2.71	11.47	9.73
	qMAX.4.2	4	AE_bin_193_1	CA2	11.11	10.9-11.2	10.16	2.68	10.17	22.27
	qMAX.4.3	4	AE_bin_231_1	CA1	16.21	15.1-17.4	8.96	2.58	9.34	19.43
			AE_bin_198_1	CA2	17.21	15.9-21	5.97	2.68	8.22	12.08
			AE_bin_199_3	PA	18.71	17.9-21.2	4.46	2.71	9.29	6.52
	qMAX.4.4	4	AE_bin_207_2	CA1	25.01	24.6-25.6	10.47	2.58	9.45	19.44
	qMAX.4.5	4	AE_bin_210_117_218_4_2_1	CA2	26.01	25.9-26.1	5.51	2.68	7.28	9.68
	qMAX.4.6	4	AE_bin_229_48	CA1	26.61	26.4-26.9	10.94	2.58	9.56	20.08
			AE_bin_226_1	PA	27.01	26.4-27.5	4.54	2.71	9.15	6.65
MinWid	qMIN.4.1	4	AE_bin_187_4	CA2	5.21	4.9-7.3	5.84	2.65	7.40	11.26
	qMIN.4.2	4	AE_bin_231_1	CA1	16.21	16.0-17.5	8.02	2.63	9.43	20.64
	qMIN.4.3	4	AE_bin_200_2	CA2	19.11	18.7-20.8	7.44	2.65	8.09	13.61
	qMIN.4.4	4	AE_bin_207_2	CA1	25.01	24.9-25.3	10.67	2.63	9.99	23.37
	qMIN.4.5	4	AE_bin_229_48	CA1	26.61	26.4-27.4	10.66	2.63	10.00	23.31
	qMIN.7.1	7	AE_bin_218_4	CA1	33.01	31.0-34.0	3.26	2.63	5.48	6.84

Table 3-5 (cont'd)

Trait[z]	QTL	Chr	Nearest marker	Loc.	Posit. (cM)	Interval (cM)[y]	LOD[x]	LOD threshold[w]	α[v]	%VE[u]
	qHGT.2.1	2	AE_bin_7_5	PA	2.91	0-4.9	2.73	2.61	-4.20	6.85
	qHGT.2.2	2	AE_bin_17_3	PA	11.01	10.8-11.4	5.73	2.61	-5.45	11.27
	qHGT.2.3	2	AE_bin_30_1	NC	17.91	16.8-18.2	6.96	2.84	-5.98	14.89
	qHGT.2.4	2	AE_bin_50_8	PA	19.21	18.7-19.4	14.01	2.61	-7.92	24.56
			AE_bin_61_3	NC	19.61	19.3-19.9	7.89	2.84	-6.02	16.23
	qHGT.2.5	2	AE_bin_49_8	CA1	20.61	20.2-20.7	4.04	2.60	-2.21	7.48
	qHGT.2.6	2	AE_bin_44_4	PA	21.61	21.1-21.8	14.88	2.61	-8.13	25.71
			AE_bin_43_2	CA2	21.81	21.6-21.9	9.96	2.67	-3.20	15.92
Height	qHGT.2.7	2	AE_bin_27_2	NC	24.11	22.8-24.3	6.74	2.84	-5.95	14.33
	qHGT.2.8	2	AE_bin_4_1	PA	28.71	26.9-31.3	8.80	2.61	-9.78	23.59
	qHGT.2.9	2	AE_bin_3_202_229_2	CA2	32.41	32.2-32.6	6.88	2.67	-3.54	11.38
	qHGT.4.1	4	AE_bin_187_4	PA	4.21	4.0-6.7	3.89	2.61	4.83	9.19
	qHGT.4.2	4	AE_bin_206_4	CA2	24.11	23.3-24.8	6.27	2.67	2.52	8.72
	qHGT.4.3	4	AE_bin_229_48	CA1	26.61	26.4-27.1	5.59	2.60	2.49	9.39
	qHGT.5.1	5	AE_bin_288_1	CA1	7.01	5.0-8.4	4.29	2.60	2.24	8.26
	qHGT.5.2	5	AE_bin_299_1	CA2	13.31	12.9-13.4	7.21	2.67	2.51	10.41
	qHGT.7.1	7	AE_bin_314_2	CA2	22.91	22.1-24.5	2.84	2.67	-1.47	3.53

[z] Trait abbreviations: as defined in Table 3-2
[y] Combine overlapping confidence interval as determined by 1-LOD values
[x] LOD values calculated from the likelihood-ratio statistics
[w] LOD threshold determined at 0.05 probability based on 1,000 permutations
[v] Additive effect of QTL, positive values indicate beneficial alleles from *P. axillaris*
[u] Percentage of variation explained by QTL estimated using R^2 statistics

Figure 3-1. Linkage map for the *P. axillaris* × *P. exserta* F_7 recombinant inbred line population

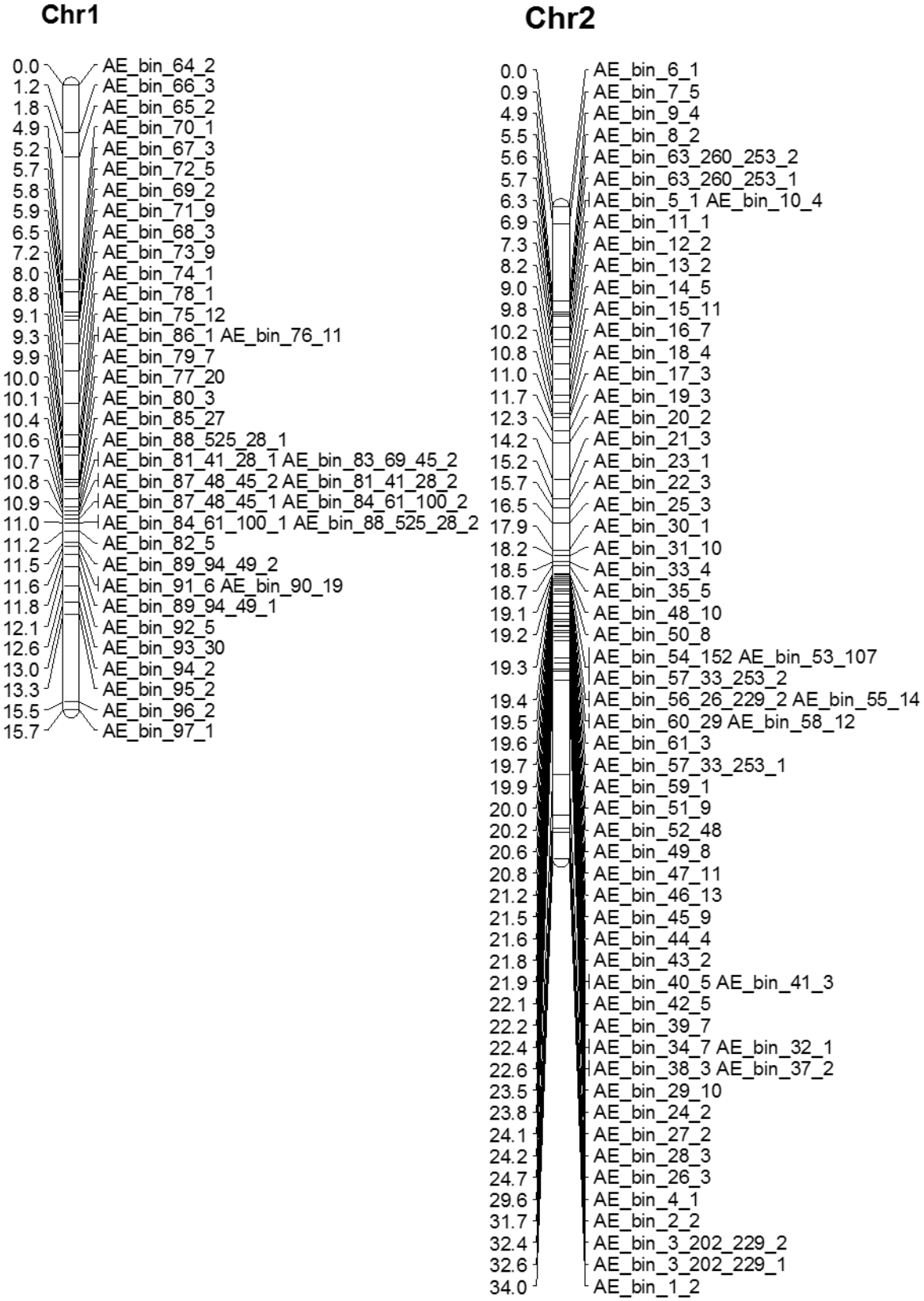


Figure 3-1 (cont'd)

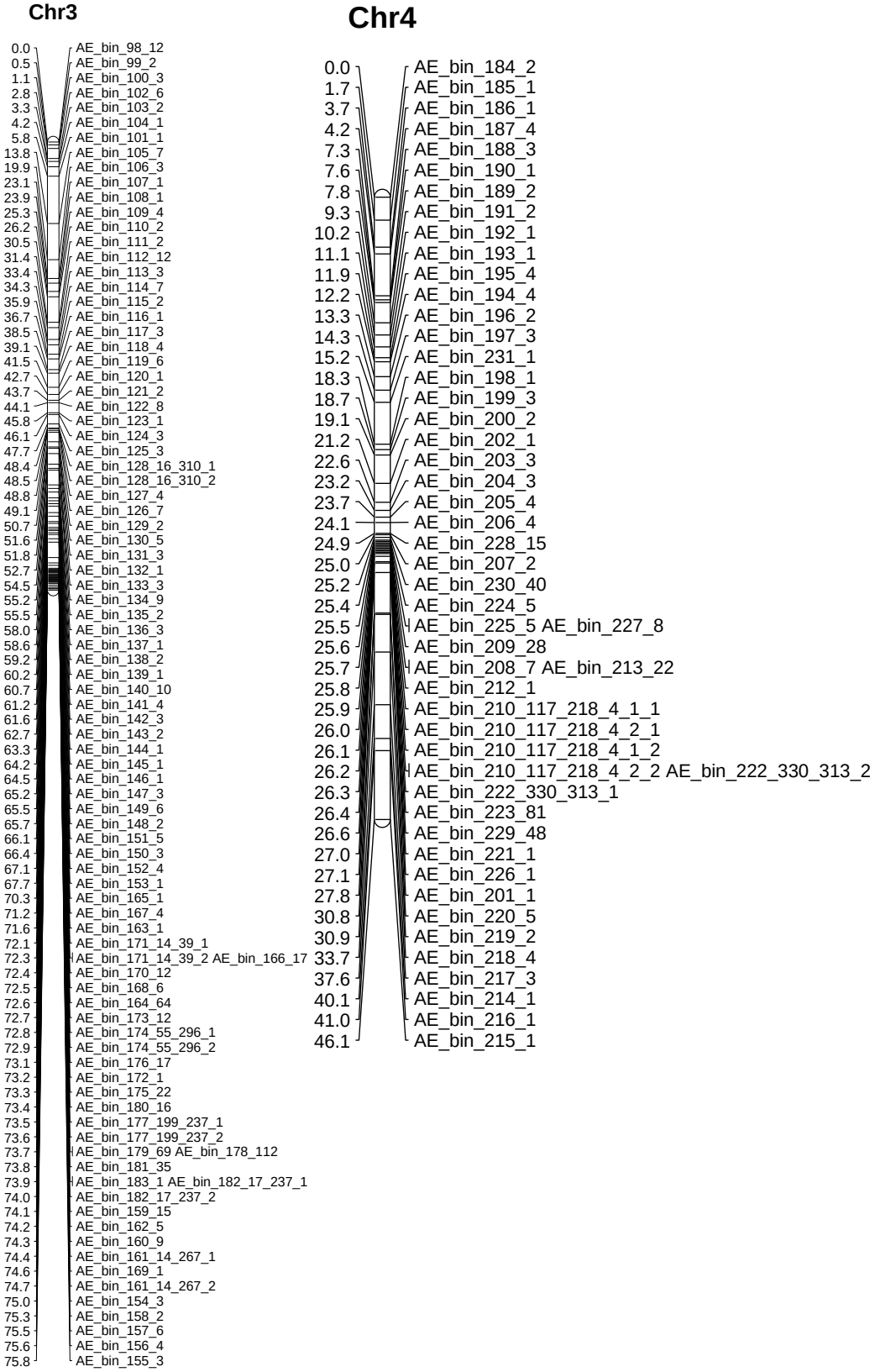


Figure 3-1 (cont'd)

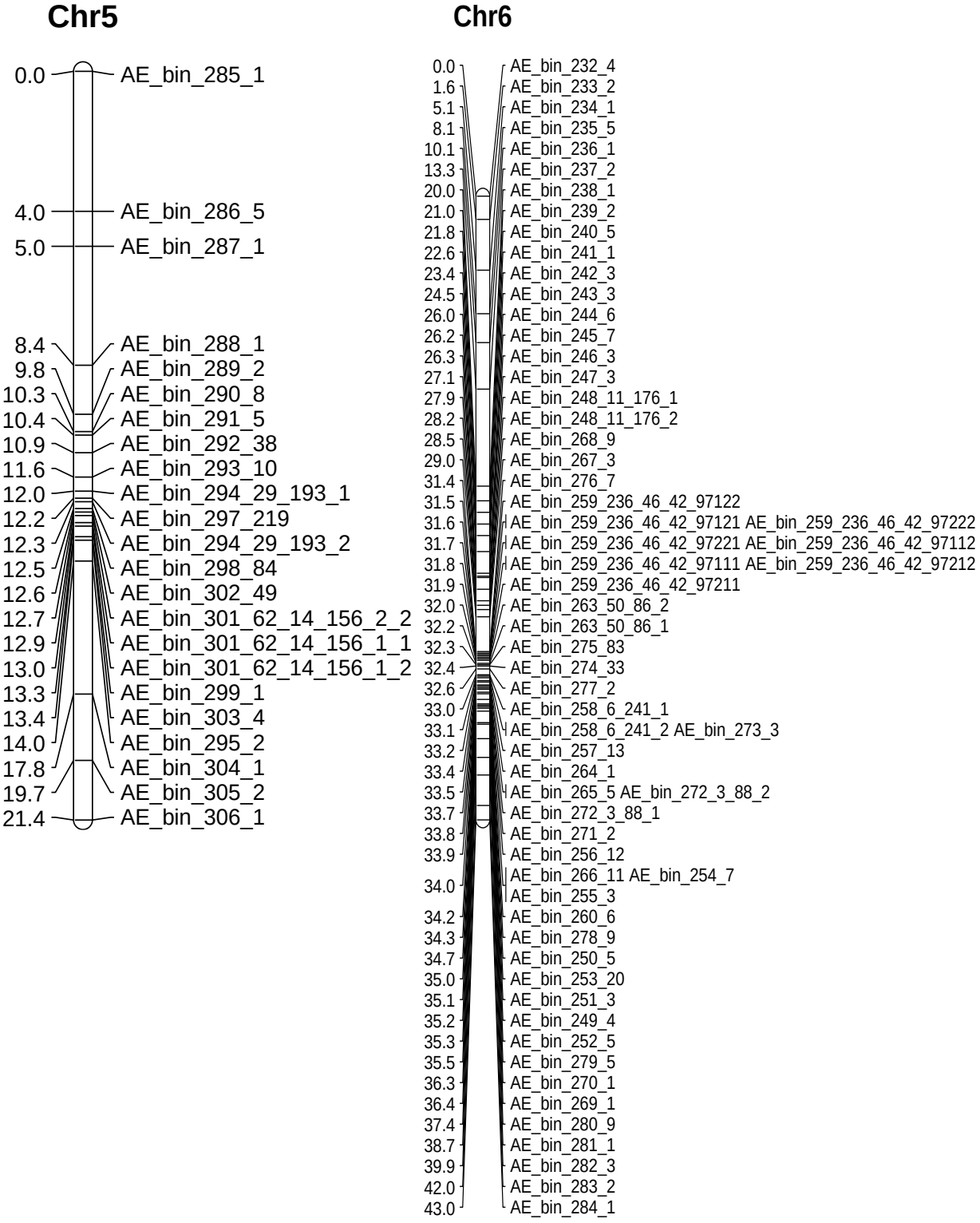


Figure 3-1 (cont'd)

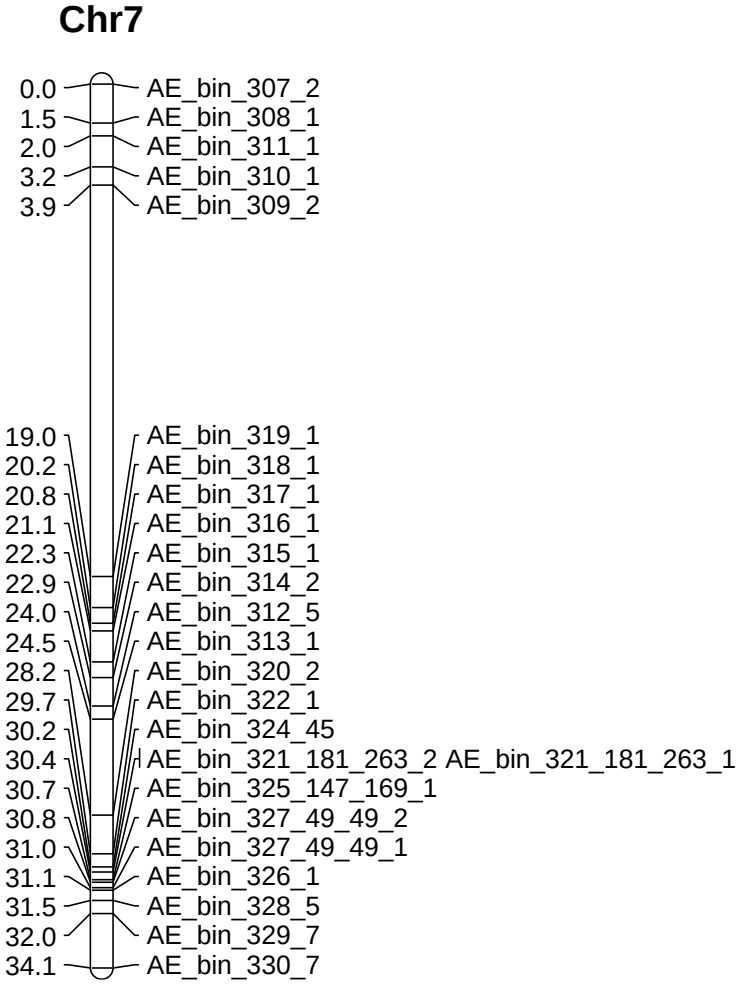


Figure 3-2. Summary of rQTL for greenhouse traits at three temperatures in a *P. axillaris* × *P. exserta* F_7 recombinant inbred line population in 2014 and 2015. Note that only a subset of bin markers are included to ease visualization. The shaded rectangle represents the range of peak positions and the line segments represent the combined confidence interval at 1-LOD value.

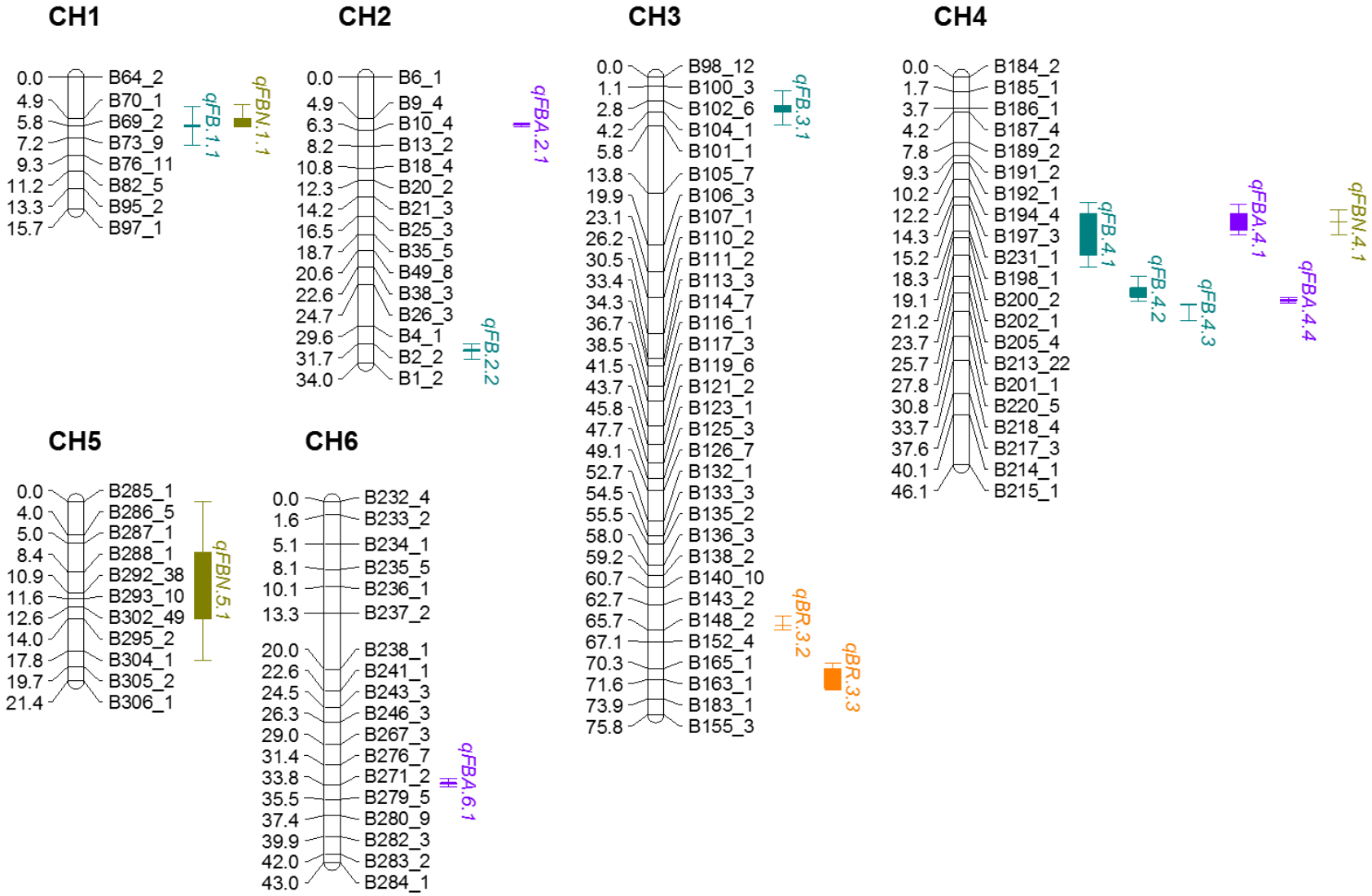


Figure 3-3. Summary of rQTL for field traits at four locations in a *P. axillaris* × *P. exserta* F_7 recombinant inbred line population in 2014. Note that only a subset of bin markers are included to ease visualization. The shaded rectangle represents the range of peak positions and the line segments represent the combined confidence interval at 1-LOD value.

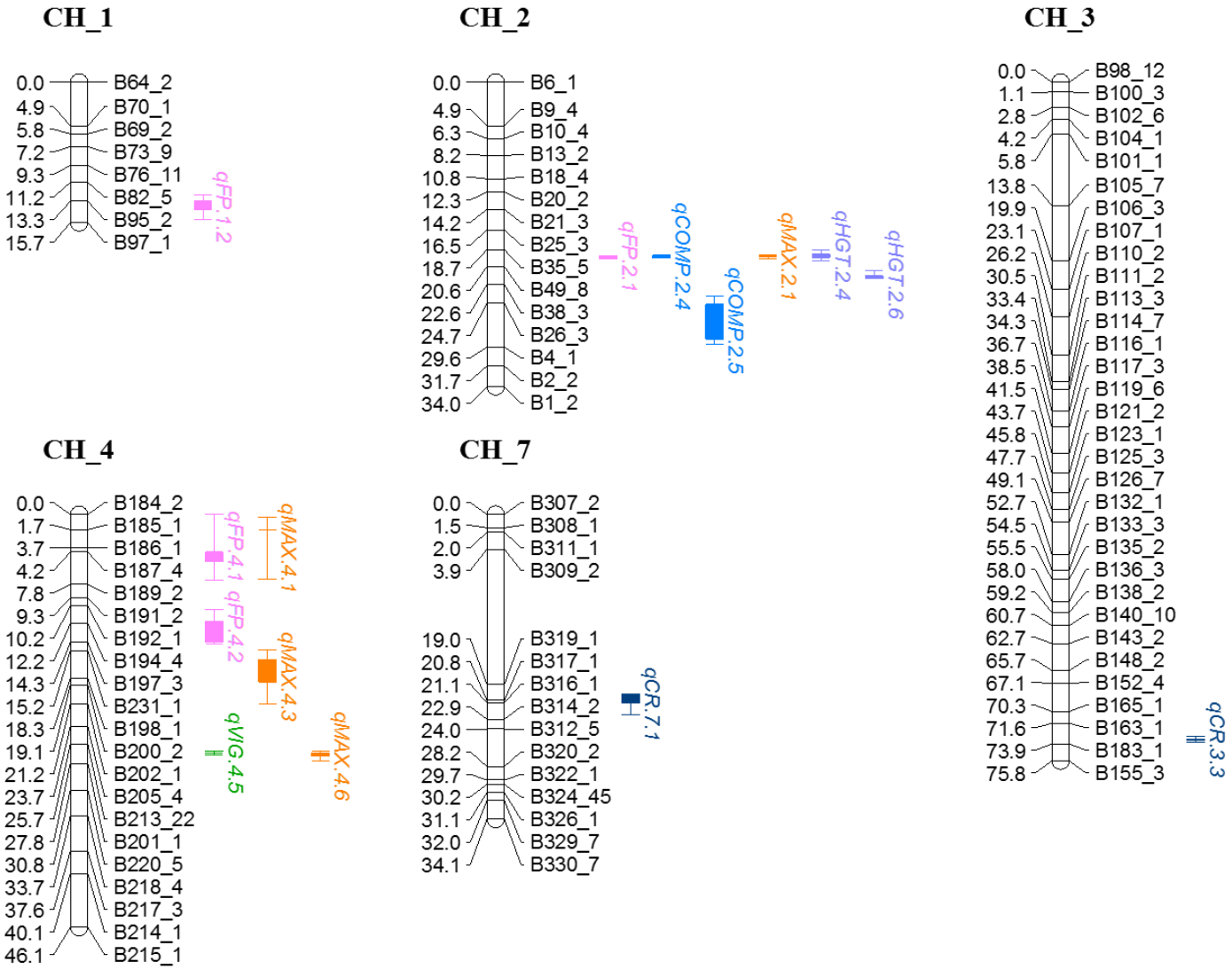
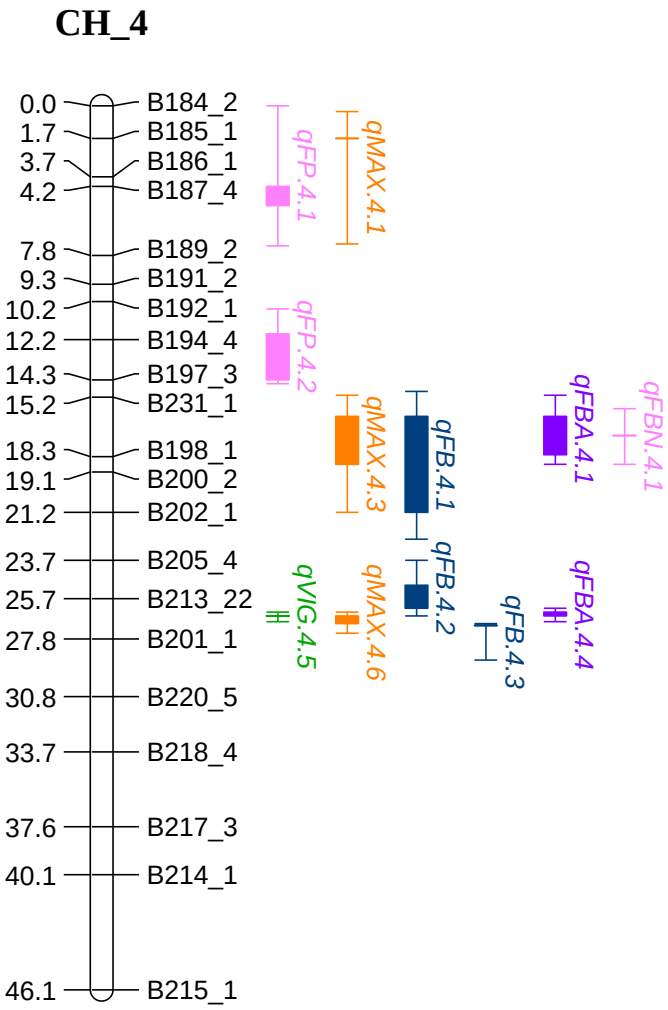


Figure 3-4. Summary of rQTL on Chromosome 4 for greenhouse and field traits in a *P. axillaris* × *P. exserta* F_7 recombinant inbred line population in 2014 and 2015. Note that only a subset of bin markers are included to ease visualization. The shaded rectangle represents the range of peak positions and the line segments represent the combined confidence interval at 1-LOD value.



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