Mol Breeding (2015) 35:2
DOI 10.1007/s11032-015-0218-4

Identification of QTL for crop timing and quality traits in an interspecific *Petunia* population

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Received: 16 July 2014 / Accepted: 1 December 2014 / Published online: 15 January 2015

Abstract Genetic mapping in ornamental plant species has lagged behind crop plants from other sectors of agriculture. Here, we describe the generation of a genetic linkage map for the important herbaceous ornamental crop petunia and the identification of QTL for several key crop timing and quality traits, including plant development rate, days to flower and flower number. An F_2 population derived from a cross between the progenitor species of cultivated petunia, *P. integrifolia* × *P. axillaris*, exhibited transgressive segregation for a broad panel of crop timing and quality traits. A genetic linkage map comprised of 75 simple sequence repeat and six cleaved amplified polymorphic sequence markers spanning 359.1 cM across seven linkage groups was developed and utilized to identify 24 QTL for ten crop timing and quality traits. These included QTL explaining 26.3, 25.9, 26.2 and 43 % of the observed phenotypic variation for flower length, branch number, internode length and the number of flower buds on the primary shoot, respectively. These data provide a foundation for understanding the genetic control of critical traits and identify molecular markers with potential utility to facilitate gene discovery in petunia.

Keywords *Petunia axillaris* · *Petunia integrifolia* · Simple sequence repeat markers · Linkage mapping · Quantitative trait locus

Introduction

The cultivated petunia, *Petunia* × *hybrida*, is derived from a hybrid created in the mid-1800s between *P. axillaris* (with both self-compatible and self-incompatible genotypes) and *P. integrifolia* (a self-incompatible species) (all $2n = 14$; Stehmann et al. 2009). Commercially, petunias are produced as an annual bedding crop, grown in greenhouses in the late winter and early spring for sale to consumers in full bloom. In cold climates like the northern USA and Europe, a major cost of petunia production is greenhouse heating (Bartok 2001). The total cost of heating for a crop of petunias depends on the heating costs per day, and the number of days required to bring the crop into flower and a marketable size. Lowering greenhouse temperatures can reduce heating costs on a per-day basis, but also reduces plant development rates, resulting in longer cropping time, and potentially higher overall

Electronic supplementary material The online version of this article (doi:10.1007/s11032-015-0218-4) contains supplementary material, which is available to authorized users.

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heating and labor costs for crop production (Blanchard and Runkle 2011). Petunia varieties that reach flowering earlier by some combination of higher leaf initiation rate (development rate) and/or initiating flower buds after producing fewer vegetative nodes would have a shorter cropping time and be more economically and environmentally sustainable to produce. We previously determined that specific accessions of *P. integrifolia* and *P. axillaris* have faster development rates than a broad panel of *P.* × *hybrida* cultivars (Warner and Walworth 2010).

Wild *Petunia* species also exhibit other novel traits including unique plant architecture (Ando and Hashimoto 1993) that may be desirable to introgress into cultivated lines. The differences between wild and domesticated petunia could be due to the genetics of the specific individuals used to create the original hybrid, loss of genes during a domestication genetic bottleneck, inadvertent selection during cultivation or a combination of all three, as commonly seen during domestication (Tanksley and McCouch 1997; Meyer and Purugganan 2013). Regardless of the underlying mechanisms, wild petunia species could be useful in diversifying and improving the genetics of cultivated petunia to develop cultivars with desirable ornamental and growth traits. Interspecific hybridization between *Petunia* spp. is generally successful, though with varying degrees of fertility (Watanabe et al. 1996, 2001; Griesbach 2007). Several interspecific hybrid populations of petunia have been produced and evaluated (Mather and Edwardes 1943; Stuurman et al. 2004; Galliot et al. 2006; Bossolini et al. 2011), but the emphasis of these studies has been on floral traits rather than growth and development traits.

The first linkage maps of *Petunia* were constructed using morphological markers (Vlaming et al. 1984) which are fundamentally limited in number and restrict high-resolution mapping. These early maps, however, provided the initial framework and determined the naming of the chromosomes from one to seven. Subsequent linkage maps based on restriction fragment length polymorphisms (RFLPs) and amplified fragment length polymorphisms (AFLPs) were developed (Gerats et al. 1995; Strommer et al. 2000, 2002; Galliot et al. 2006) in both interspecific populations and crosses within *P. hybrida*. Though these markers are more abundant than morphological markers, these maps still lack fine resolution, and these marker types are not well suited to marker-assisted selection. More recently, linkage maps incorporating simple sequence repeat (SSR) markers have been generated for two interspecific *Petunia* populations (Bossolini et al. 2011). SSRs are more advantageous for marker-assisted selection, as they are co-dominant and highly reproducible.

The genetic control of many critical crop timing and production traits in petunia is poorly understood. Identification of QTL underlying these traits, and the impending availability of two *Petunia spp.* genomes (Sims et al. 2012), will facilitate the identification of candidate genes regulating these traits. Markers also allow breeders to characterize the linkage between traits so that individuals with a desirable linkage phase can be selected to minimize linkage drag and identify unrelated heterotic groups for the development of F_1 hybrids (Lande and Thompson 1990; Mohan et al. 1997).

The objectives of this study were to: (1) generate and characterize a segregating interspecific hybrid population between the progenitor species of the cultivated petunia to evaluate the genetic variability encompassed by these species and (2) develop a genetic linkage map and identify QTL for traits effecting growth and development rate and additional morphological traits of interest. A genetic map based on an interspecific F_2 population derived from a cross between *P. integrifolia* and *P. axillaris* was developed that contained 81 genetic markers and spanned approximately 359 cM. This F_2 population exhibited wide phenotypic variation and facilitated the identification of 24 QTL for several important crop timing and quality traits including those associated with morphology and development rate. This study illustrates the potential for utilizing natural variation present in wild petunia species for crop improvement and gene discovery.

Materials and methods

Population development and phenotypic data

Petunia integrifolia × *P. axillaris* F_1 individuals produced by Warner and Walworth (2010) were grown in a greenhouse at 20 °C under a 16-h photoperiod achieved by ambient light supplemented with high-pressure sodium lighting (50 μmol m^{-2} s^{-1}) from 0600 to 2200 h. Due to observed self-incompatability (data not shown) in the *P. integrifolia* ×

P. axillaris F_1 plants, F_2 seed was generated by cross-pollinating between several different F_1 individuals.

Seed of the parental species, F_1 and F_2 generations, were sown in 288-cell plug trays filled with soilless medium (Suremix, Michigan Grower Products Inc., Galesburg MI) and placed in a greenhouse at 23 °C under intermittent overhead mist. After 14 days, seedling trays were moved to a greenhouse at 20 °C under a 16-h photoperiod. Five weeks after seed sow, 212 *P. integrifolia* × *P. axillaris* F_2 individuals, and ten seedlings each of the parental species and F_1, were transplanted into 10-cm-diameter pots (450 mL) filled with the same soilless medium and moved to a greenhouse at 17 °C under a 16-h photoperiod achieved as described above, and the number of nodes (leaves >1 cm in length) on the main stem were determined.

Node counts were repeated every 7 days for 28 days. Development rate (in nodes day^{-1}) was then calculated as the increase in node number divided by the number of days in between counts. The 28-day average development rate is reported. On the day of anthesis of the first flower on the main stem, leaf number below the first flower bud, flower diameter, flower length, number of visible flower buds on main stem, total number of visible flower buds, plant height, height to the flowering node and axillary branch number were determined for each individual. Pearson's correlation coefficients between measured traits were calculated using SPSS (Version 22.0 for Windows; IBM, Armonk, NY). Broad-sense heritability estimates were calculated as previously described (Warner and Walworth 2010).

Molecular marker development and linkage map construction

We previously identified 591 SSRs from publically available *P. axillaris* EST sequences (Tychonievich et al. 2013) and 103 SSRs derived from *P.* × *hybrida*. Of those, 132 *P. axillaris*-derived SSRs and 33 *P.* × *hybrida*-derived SSRs were polymorphic between *P. axillaris* and *P. integrifolia*. From these, 21 SSRs derived from *P.* × *hybrida* and 92 SSRs derived from *P. axillaris* were used to genotype 164 individuals from the F_2 population. PCR conditions, visualization and marker scoring were as described by Tychonievich et al. (2013). In addition to the SSRs, the population was genotyped with eleven cleaved amplified polymorphic sequence (CAPS) markers that were resolved with restriction enzymes as previously described by Bossolini et al. (2011).

A genetic linkage map was generated with JoinMap 4.0 (Van Ooijen 2006) using the Kosambi mapping function (Kosambi 1944). Linkage parameters were set to a recombination fraction of 0.30, logarithmic odds (LOD) score of 3.0 and error detection ratio of five percent. Markers with more than fifty missing individuals were removed. Linkage group (chromosome) number assignment was inferred from previous mapping studies (Strommer et al. 2000, 2009; Bossolini et al. 2011).

QTL analysis was performed using composite interval mapping (CIM) of the QTL Cartographer v2.5 software (Wang et al. 2012) and confirmed utilizing the CIM function of R/qtl package version 1.21-2 (Broman et al. 2003). For QTL Cartographer, model 6 forward–backward stepwise regression was used, and five markers were selected automatically as cofactors. A walk speed of 2 cM and empirically derived LOD thresholds at a 0.05 probability based on 1,000 permutations were used to identify QTL. Other parameters were used in default settings. For R/qtl, QTL genotype probability was calculated using the calc.genoprob function with a step size of 1 cM and Kosambi's mapping function. The cim function was then used with five marker covariates and a window size of 20 with Kosambi's mapping function. The results of these two analyses were very similar (Supplemental Fig. S1), and therefore, the results from QTL Cartographer are presented. The QTL map was generated using MapChart software (Voorrips 2002).

Results

Phenotypic data

The *P. integrifolia* × *P. axillaris* population showed a wide range of variation including transgressive segregation for all traits measured (Fig. 1), even for traits for which the parental species values were similar, such as the number of nodes below the first flower (Fig. 1c) and the number of axillary branches (Fig. 1d). For the critical crop timing traits of node number below the first flower and development rate, the population showed a three-fold and fourfold range of values, respectively.

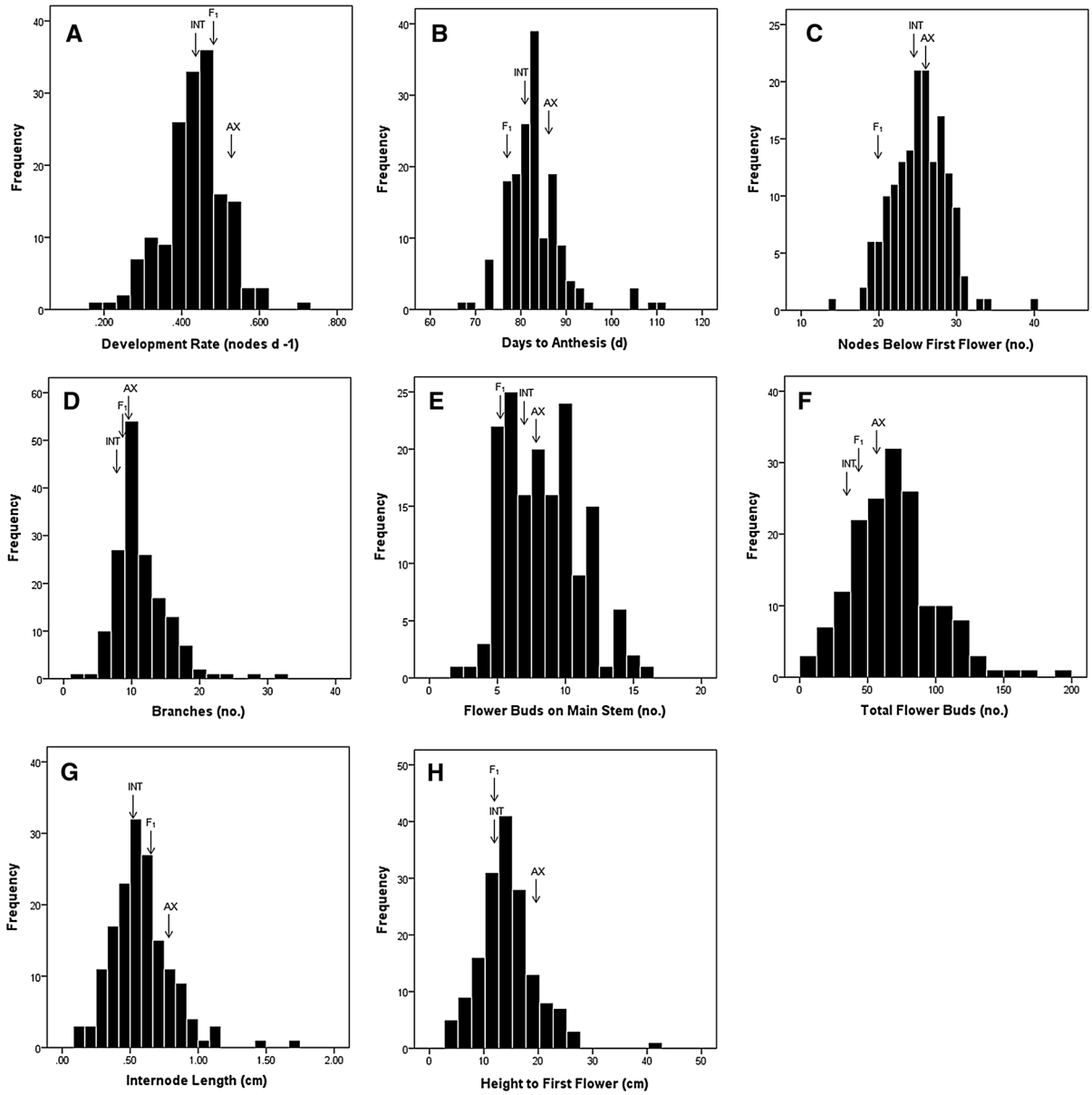

Fig. 1 Frequency distributions for crop timing and quality traits **a** development rate, **b** days to anthesis of the first flower, **c** number of nodes below the first flower, **d** number of axillary branches, **e** number of flower buds on the main stem, **f** total number of flower buds when the first flower opened, **g** average internode length and **h** plant height to the flowering node for a *P. integrifolia* × *P. axillaris* F_2 population

Direct measurement of days to flower was less variable, with less than a twofold range of values. In addition to the measured traits, we observed ca. ten individuals with irregular white variegation on the leaves (data not shown), and individuals with deformed growth and extreme dwarfism which did not survive to flowering and were removed from the study.

Among crop timing traits, development rate was strongly and positively correlated with node number below the first flower, but negatively correlated with days to flower (Table 1; Supplemental Fig. S2), while node number below the first flower was correlated positively with days to flower. There were also correlations between crop timing traits and crop quality traits. Development rate, node number below the first

Table 1 Pearson's correlation coefficients for traits measured in a *P. integrifolia* × *P. axillaris* F_2 population

Trait[a]	DRate	DTA	Nodes	Branch	FlBudPS	FlBud	FlDiam	FlLeng	Height	HghtFl	Internode
DTA	−0.508**[b]										
Nodes	0.250**	0.473**									
Branch	0.095	0.296**	0.445**								
FlBudPS	0.001	0.019	0.071	−0.020							
FlBud	0.189*	0.163*	0.415**	0.595**	0.166*						
FlDiam	−0.257**	0.133	−0.171*	−0.180*	0.049	−0.101					
FlLeng	0.125	0.012	0.095	0.214**	0.153	0.179*	0.061				
Height	−0.091	0.114	−0.30	0.098	0.350**	0.064	0.335**	0.449**			
HghtFl	−0.052	0.167*	0.061	0.127	0.314**	0.153	0.295**	0.414**	0.918**		
Internode	−0.165*	0.003	−0.308**	−0.048	0.262**	−0.17	0.348**	0.354**	0.876**	0.920**	
BrLeng	0.089	0.024	0.072	0.308**	−0.191*	0.311**	0.156*	0.166*	0.332**	0.346**	0.306**

[a] Trait abbreviations: development rate in nodes day^{-1} (DRate), days to anthesis of the first flower (DTA), number of nodes below the first flower (Nodes), number of lateral branches (Branch), number of flower buds on the primary stem (FlBudPS), total number of flower buds (FlBud) diameter of the first open flower (FlDiam), length of the first open flower (FlLeng), plant height (Height), height to the first flower (HghtFl), internode length (Internode) and length of the longest lateral branch (BrLeng)

[b] * and ** indicate significance at $P < 0.05$ and 0.01, respectively

flower and days to flower were all correlated positively with total flower bud number but uncorrelated with flower bud number on the primary shoot. The growth-related traits flower diameter, flower length, plant height, internode length and axillary branch length were all correlated positively with each other, with the exception of flower diameter and flower length, which were uncorrelated (Table 1). Broad-sense heritability estimates were high for most traits (Table 2), with the exception of days to anthesis ($H^2 = 0.402$).

Linkage map construction

A linkage map of 81 markers on seven linkage groups spanning 359.1 cM was developed (Suplemental Table S1; Fig. 2). The average linkage group was 51.3 cM long, while the longest was 73.5 cM (chromosome 2), and the shortest 33.6 cM (chromosome 1). There was an average of 4.78 cM between markers. Chromosomes 4, 6 and 7 showed segregation ratios significantly distorted from the expected 1:2:1 ratio for a F_2 hybrid population. On all three chromosomes, all markers showing Chi-squared values significantly deviated from the expected segregation ratios were due to a lack of markers homozygous for *P. integrifolia* alleles, and a corresponding excess of markers heterozygous or homozygous for *P. axillaris* alleles. Distorted segregation ratios have previously been observed in interspecific hybrid *Petunia* maps (Bossolini et al. 2011).

Table 2 Broad-sense heritability (H^2) estimates for crop timing and quality traits in a *Petunia integrifolia* × *P. axillaris* F_2 population

Trait[a]	H^2
DRate	0.766
DTA	0.403
Nodes	0.761
Branch	0.898
FlBudPS	0.533
FlBud	0.841
FlDiam	0.757
FlLeng	0.721
Height	0.830
HghtFl	0.776
Internode	0.667
BrLeng	0.727

[a] Trait abbreviations: development rate in nodes day^{-1} (DRate), days to anthesis of the first flower (DTA), number of nodes below the first flower (Nodes), number of lateral branches (Branch), number of flower buds on the primary stem (FlBudPS), total number of flower buds (FlBud) diameter of the first open flower (FlDiam), length of the first open flower (FlLeng), plant height (Height), height to the first flower (HghtFl), internode length (Internode) and length of the longest lateral branch (BrLeng)

Fig. 2 Genetic linkage map of a *P. integrifolia* × *P. axillaris* F_2 population and locations of QTL for development rate (DR), days to flower (DTA), flower diameter (FD), flower length (FL), plant height (HGT), branch number (BR), branch length (BL), nodes below the first flower (NBF), internode length (INT), flower bud number on the primary shoot (FBP) and total flower bud number (FB)

QTL analysis

QTL were identified for all measured crop timing and quality traits except for days to flower (Fig. 2; Table 3; Supplemental Fig S1.). Three QTL for development rate, identified on chromosome 1, 2 and 5, cumulatively explained 34 % of the observed variation. *Petunia axillaris* contributed the favorable alleles for development rate on chromosomes 1 and 2, while *P. integrifolia* contributed the favorable allele of the greatest overall effect [18 % variation explained (VE)], located on chromosome 5. Two QTL were identified for the number of nodes below the first flower, with the QTL on chromosome 4 (*NBF4.1*) explaining 21 % of the variation.

Several major QTL (>25 % VE) were identified for traits relating to crop quality (Table 3), including flower length (*FL4.1*), branch length (*BL6.1*), branch number (*BR1.1*), internode length (*INT5.1*) and a QTL for the number of flower buds on the primary shoot (*FBP6.1*) explaining 43 % of the observed variation. For those QTL, *P. axillaris* contributed the favorable alleles for flower length, branch number and number of flower buds on the primary shoot, and *P. integrifolia* contributed favorable alleles for branch length and internode length (shorter internodes are desirable).

Discussion

Utilization of genetic mapping in ornamental crops for traits of horticultural importance has lagged behind

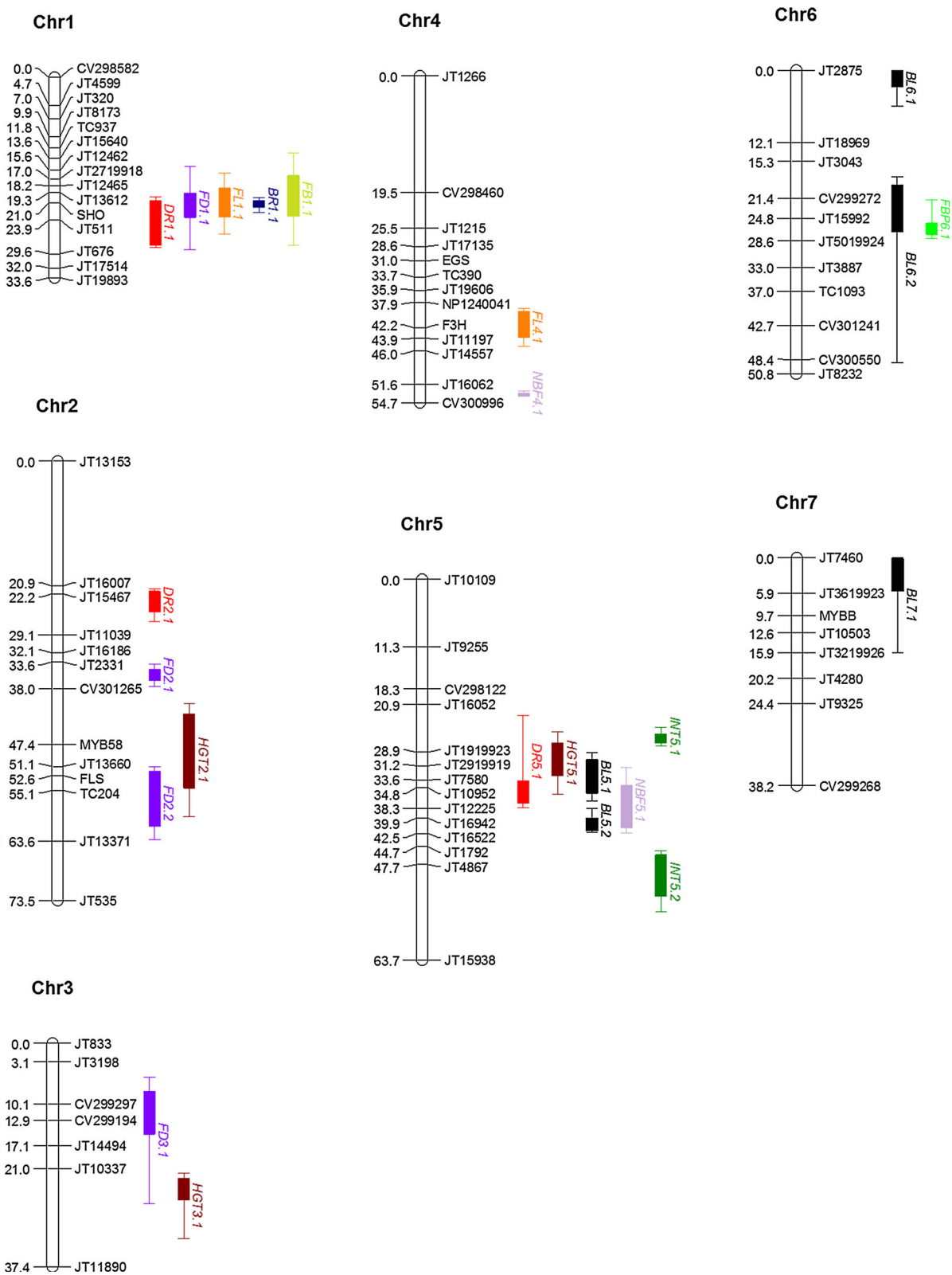
Chr1
0.0 CV298582
4.7 JT4599
7.0 JT320
9.9 JT8173
11.8 TC937
13.6 JT15640
15.6 JT12462
17.0 JT2719918
18.2 JT12465
19.3 JT13612
21.0 SHO
23.9 JT511
29.6 JT676
32.0 JT17514
33.6 JT19893
DR1.1
FD1.1
FL1.1
BR1.1
FB1.1
Chr4
0.0 JT1266
19.5 CV298460
25.5 JT1215
28.6 JT17135
31.0 EGS
33.7 TC390
35.9 JT19606
37.9 NP1240041
42.2 F3H
43.9 JT11197
46.0 JT14557
51.6 JT16062
54.7 CV300996
FL4.1
NBF4.1
Chr6
0.0 JT2875
12.1 JT18969
15.3 JT3043
21.4 CV299272
24.8 JT15992
28.6 JT5019924
33.0 JT3887
37.0 TC1093
42.7 CV301241
48.4 CV300550
50.8 JT8232
BL6.1
FBP6.1
BL6.2
Chr2
0.0 JT13153
20.9 JT16007
22.2 JT15467
29.1 JT11039
32.1 JT16186
33.6 JT2331
38.0 CV301265
47.4 MYB58
51.1 JT13660
52.6 FLS
55.1 TC204
63.6 JT13371
73.5 JT535
DR2.1
FD2.1
HGT2.1
FD2.2
Chr5
0.0 JT10109
11.3 JT9255
18.3 CV298122
20.9 JT16052
28.9 JT1919923
31.2 JT2919919
33.6 JT7580
34.8 JT10952
38.3 JT12225
39.9 JT16942
42.5 JT16522
44.7 JT1792
47.7 JT4867
63.7 JT15938
DR5.1
HGT5.1
BL5.1
BL5.2
NBF5.1
INT5.1
INT5.2
Chr7
0.0 JT7460
5.9 JT3619923
9.7 MYBB
12.6 JT10503
15.9 JT3219926
20.2 JT4280
24.4 JT9325
38.2 CV299268
BL7.1
Chr3
0.0 JT833
3.1 JT3198
10.1 CV299297
12.9 CV299194
17.1 JT14494
21.0 JT10337
37.4 JT11890
FD3.1
HGT3.1

Table 3 Summary of QTL identified in a *P. integrifolia* × *P. axillaris* F_2 population

Trait[a]	LOD threshold	QTL	LG	Nearest marker	Position (cM)	LOD	a[b]	%VE[c]
Drate	3.31	*DR1.1*	1	SHO	21.01	4.24	0.03	8.70
		DR2.1	2	JT15467	22.20	3.81	0.02	7.45
		DR5.1	5	JT10952	34.77	8.70	−0.06	18.06
FlDiam	4.19	*FD1.1*	1	SHO	21.01	6.62	−3.13	12.64
		FD2.1	2	JT2331	35.58	5.89	4.39	14.22
		FD2.2	2	FLS	54.64	4.21	2.88	7.82
		FD3.1	3	CV299297	12.06	5.37	3.49	10.78
FlLeng	3.13	*FL1.1*	1	SHO	21.01	7.96	2.87	12.33
		FL4.1	4	NP1240041	41.91	11.93	4.38	26.25
Height	4.93	*HGT2.1*	2	CV301265	46.05	6.79	3.77	15.63
		HGT3.1	3	JT10337	22.98	5.19	0.87	11.90
		HGT5.1	5	JT1919923	30.94	11.79	4.97	23.88
BrLeng	3.43	*BL5.1*	5	JT2919919	31.22	4.94	3.88	12.19
		BL5.2	5	JT16942	41.90	3.51	4.55	10.46
		BL6.1	6	JT2875	2.01	4.34	8.69	31.08
		BL6.2	6	CV299272	24.25	4.30	−4.98	10.65
		BL7.1	7	JT3887	4.01	4.05	−7.51	12.73
Branch	8.01	*BR1.1*	1	SHO	21.01	12.86	4.14	25.91
FlBudPS	3.27	*FBP6.1*	6	JT15992	28.15	18.58	2.09	42.99
FlBud	3.80	*FB1.1*	1	JT12465	18.25	5.86	17.78	13.37
Nodes	3.44	*NBF4.1*	4	JT16062	53.59	8.85	2.90	21.28
		NBF5.1	5	JT16942	39.90	3.90	−2.16	7.11
Internode	6.30	*INT5.1*	5	JT16052	26.96	11.16	0.21	26.19
		INT5.2	5	JT1792	46.70	6.53	0.22	14.10

[a] Trait abbreviations: development rate in nodes day^{-1} (DRate), days to anthesis of the first flower (DTA), diameter of the first open flower (FlDiam; mm), length of the first open flower (FlLeng; mm), plant height (Height; cm), number of lateral branches (Branch), number of flower buds on the primary stem (FlBudPS), total number of flower buds (FlBud), number of nodes below the first flower (Nodes) and average internode length (Internode; cm)

[b] Additive effect of the *P. axillaris* allele (units are provided in the trait abbreviations summary)

[c] Percentage of variation explained

other crop sectors, with most effort confined to rose (Debener and Mattiesch 1999; Dugo et al. 2005) and *Lilium* (Abe et al. 2002; Shahin et al. 2011). While there have been some genetic mapping efforts in petunia, these studies have largely focused on self-incompatibility (Wang et al. 2003) and traits related to pollination syndrome, including flower size, nectar volume and floral scent production (Stuurman et al. 2004; Galliot et al. 2006; Klahre et al. 2011). Here, we report the first QTL identified for several important crop timing and quality traits for petunia, utilizing an interspecific hybrid population derived from the progenitor species of cultivated petunia.

Unlike many food crops, which have been under artificial selection for thousands of years and have a set of the 'domestication syndrome' traits critical for successful use as a crop (Hammer 1984), most ornamental crops, like petunia (dating to ca. the 1830's), have been more recently domesticated. The close relationship between domesticated and wild individuals reduces the chances of significant negative linkage, and wild relatives of petunia have been successfully used to introgress novel traits into petunia (Griesbach 2007). Similarly, unlike food crops, where breeders often have to generate varieties that fit a narrow range of flavor and processing requirements,

novelty is highly desirable for ornamental crops and is often a significant focus of breeding programs. Therefore, desirable alleles identified in this population could be introgressed into elite petunia breeding lines.

All measured traits exhibited population distributions that suggest these traits are under polygenic control, and a high degree of transgressive segregation was observed (Fig. 1). Our previous results identified that the *P. integrifolia* and *P. axillaris* accessions used to generate the F_2 population described herein exhibited faster development rates than a panel of grandiflora-type *P.* × *hybrida* cultivars (Warner and Walworth, 2010). Twenty-two of the F_2 population plants had faster development rates than the faster parent (*P. axillaris*; Fig. 1a), suggesting further improvement over a number of existing commercial cultivars. There were several correlations among the crop timing traits development rate, node number below the first flower and days to flower (Table 2). Development rate was strongly correlated negatively with days to flower. These traits were not significantly correlated in the interspecific hybrid F_2 populations *P. exserta* × *P. axillaris*, *P.* × *hybrida* × *P. axillaris* or *P. axillaris* × *P. integrifolia* (the reciprocal cross of the population described here), although the population sizes were considerably smaller (Warner and Walworth 2010). Node number below the first flower and days to flower were correlated positively, similar to results for the *P. axillaris* × *P. integrifolia* population (Warner and Walworth 2010).

The total map distance reported here (359.1 cM) is similar to the 404.6 cM map generated for a *P. axillaris* × *P. inflata* (until recently, classified as a subspecies of *P. integrifolia*) BC_1 population using AFLP markers (Galliot et al. 2006). Linkage maps of petunia have consistently resulted in short linkage groups due to a low frequency of recombination, though the severity of the effect varies by population and chromosome (Strommer et al. 2002). A test cross population generated by crossing an F_1 hybrid between two *P. axillaris* subspecies to an inbred *P.* × *hybrida* line produced a genetic linkage map with a total map distance of only 2.4 cM (Galliot et al. 2006). Ten Hoopen et al. (1996) used four independent T-DNA insertion lines, each with an insertion mapping to <1 cM from the flavanol synthase gene *Fl* on chromosome 2, and florescence in situ hybridization (FISH) to provide physical evidence of suppressed recombination in petunia. Despite the tight genetic linkage between the T-DNAs and *Fl*, the four insertions physically mapped to locations spanning ca. two-thirds of chromosome 2. The *Rm1* (recombination modulator 1) locus affects recombination rates in petunia (Cornu et al. 1989; Robert et al. 1991). The issue of suppressed recombination is a significant problem for breeders. A breeder working with a low recombining population faces increased linkage drag, as large sections of a chromosome are inherited together, making it more difficult to introgress useful traits from wild relatives without also introducing potentially undesirable traits into elite varieties.

Several QTL that could be very useful for gene discovery in petunia were identified in this study. The only trait presented here that has previously been mapped in *Petunia* is flower diameter. Utilizing a *P. axillaris* × *P. inflata* BC_1 population, Galliot et al. (2006) identified QTL for flower diameter on chromosomes 2, 3, 4 and 5, while the current study identified QTL on chromosomes 1, 2 and 3 (Table 3). At least one QTL was identified for each of the traits evaluated (Table 3; Fig. 2) except days to anthesis, and QTL explaining >25 % of the observed variation were identified for several plant quality traits. Of particular note are a QTL on chromosome 6 which explains 42 % of the variation of number of flower buds on the main stem and a QTL explaining 25 % of the variation in branch number on chromosome 1 which together could be used to select for more floriferous, highly branched individuals.

Three QTL for development rate were identified, though these three loci collectively explained only 34 % of the observed variation, with *DR5.1* having the largest effect (18 % VE). Therefore, it seems likely that multiple loci of small effect combine to control this trait. Several genes impacting development rate have been identified in other species. Mutations in *PLASTOCHRON1* and *PLASTOCHRON2* in rice (Miyoshi et al. 2004; Kawakatsu et al. 2006), *TERMINAL EAR1* in maize (Veit et al. 1998) and *ALTERED MERISTEM PROGRAM1* (Helliwell et al. 2001) in arabidopsis increase the leaf initiation rate, while mutations in *PHYTOCHROME B* (Reed et al. 1993) and *SERRATE* (Prigge and Wagner 2001) decrease leaf initiation rate. The influence of naturally occurring allelic differences at these loci on development rate has not been evaluated. In addition, QTL for several traits (development rate, flower diameter,

flower length and branch number) co-localized on chromosome 1 around a CAPS marker for the isopentenyl transferase gene *SHO*, with a QTL for flower bud number also overlapping this marker. This gene encodes an enzyme catalyzing cytokinin biosynthesis in petunia, and was originally identified in an activation-tagged line exhibiting increased lateral shoot production (Zubko et al. 2002). This region may exert an influence on these growth and development traits through a general impact on cytokinin synthesis. Additional work is needed to fine map this region to determine whether *SHO* or other genes in the region are responsible for these effects and also to determine whether known regulators of development rate co-localize with the identified QTL.

This study has identified variation in key quality and timing traits in an important ornamental crop species. However, the QTL described here were identified from a single, non-replicated population. Future efforts will focus on validation and refinement of these QTL in an F_7 population of recombinant inbred lines (RILs) that are derived from the F_2 population described herein. The use of the RIL population will facilitate replicated trials under different environments to determine the robustness of the QTL and ascertain their utility for improvement of petunia quality traits.

Acknowledgments We thank Mike Olrich for technical assistance. This research was supported by the US Dept. of Agriculture (USDA) Specialty Crop Block Grant Program and the USDA Specialty Crop Research Initiative (award 2011-01508). R. M .W. and C. S. B. are supported in part by Michigan AgBioResearch and through USDA National Institute of Food and Agriculture, Hatch project numbers MICL02121 and MICL02265.

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