Chapter 12
The Effect of Photoperiod on Flowering Time, Plant Architecture, and Biomass in Setaria

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Abstract The effect of photoperiods of 8 h (8:16 light:dark), 12 h (12:12), and 16 h (16:8) on flowering time, plant architecture, and biomass production were investigated in an RIL population derived from a cross between domesticated foxtail millet (*Setaria italica*) and its wild progenitor green foxtail (*S. viridis*). Flowering time, height, and biomass were found to be highly and positively correlated in all three photoperiod regimes. Branching, however, is weakly and variably associated with the other three traits. After the effects of variation in daily radiation and temperature were removed, ANOVA analyses of *Photoperiod* and *RIL* (genotype) found both factors and their interaction significant for all traits, with *RIL* and *Photoperiod * RIL* also explaining large amounts of variation. However, while *Photoperiod* by itself explained much of the variation in flowering time and in branching, it explained little of that for height and biomass. Regions were identified where all three trials identify QTL in the same genomic regions as well as QTL found in either the 8 and 12 h trials or the 12 and 16 h trials. This pattern may be evidence for differences in regulation between shorter and longer photoperiods. Comparison of QTL with previous greenhouse and field trials finds several overlapping QTL and multiple independent QTL. A well-supported QTL region on chromosome IV has been shown previously to contain a number of genes in the CONSTANS—FT pathway, and these results suggest that this pathway is conserved across photoperiods. Further genetic analysis of the multiple non-overlapping QTL regions between the photoperiod trials will be necessary to narrow down a list of candidate genes responsible for differences in flowering time and architecture between photoperiods.

Keywords Flowering time • Setaria • Photoperiod • Branching • Height • Biomass • QTL analysis • Foxtail millet • Green foxtail

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12.1 Introduction

The potential of Setaria as a model system is primarily based on its attributes for genetic analysis, particularly the small diploid genome, small physical stature, C_4 photosynthetic capability, transformability, and a growing list of genetic and genomic resources (Bennetzen et al. 2012; Doust et al. 2009; Li and Brutnell 2011). Although there are other model grasses, including rice (the first sequenced grass genome) and Brachypodium (a wild grass in the pooid clade related to wheat, barley, and rye), Setaria has the advantage of being a C_4 grass in the panicoid clade, close to maize, sorghum, switchgrass, and pearl millet. In addition, there is substantial genetic and phenotypic differentiation amongst wild populations of green foxtail (*S. viridis*) as well as genetic changes associated with domestication in its domesticated variant, foxtail millet (*S. italica*). The potential of Setaria to be a new model system is especially significant because there is a high efficiency callus transformation system (Van Eck and Swartwood 2015) (Chap. 20), as well as recent reports on the success of spike dip transformation with *Agrobacterium*, which is the first for any grass system (Saha and Blumwald 2016) (Chap. 21).

The use of Setaria as a model for biofuel grasses has prompted interest in the genetic regulation of, and correlation between, traits such as flowering time, plant architecture, and biomass (Mauro-Herrera and Doust 2016; Mauro-Herrera et al. 2013; Doust et al. 2004). The wide latitudinal spread of *S. viridis* from high to subtropical latitudes in both hemispheres suggests that changes in photoperiod may have significant effects on these traits. In addition, Setaria appears to differ from other model grass systems in that the center of diversity of green foxtail and the domestication of foxtail millet from green foxtail appears to have occurred at a relatively high latitude (Jia et al. 2013) (Chaps 2, 3, and 4), raising the possibility that photoperiodic control of flowering in Setaria may not conform to the model that has emerged from rice, a species that evolved and was domesticated in the tropics (Vaughan et al. 2008). Photoperiodic control of flowering in pooid crops, such as wheat, barley, and the model species Brachypodium, differs from that in Setaria, because they require a vernalization response to achieve competency to flower (Higgins et al. 2010), a strategy not known in Setaria or other panicoid grasses.

In this chapter, the response of Setaria to changes in photoperiod is explored, using a recombinant inbred line (RIL) population derived from a cross between domesticated foxtail millet (*Setaria italica*) and its wild progenitor green foxtail (*S. viridis*) (Bennetzen et al. 2012). Much of what is known about the response of grasses to differences in photoperiod is from studies in rice, where there are two photoperiod-dependent pathways; one of these is homologous to that found in Arabidopsis and other land plants, while the other appears to be confined to grasses (Mauro-Herrera et al. 2013). The first pathway involves the key regulator CONSTANS, which positively regulates FT in Arabidopsis but whose ortholog, *HD1*, in rice negatively regulates the FT co-orthologs, *HD3A* and *RFT1*, active under short-day and long-day conditions, respectively (Hayama et al. 2003; Izawa et al. 2002; Song et al. 2010; Komiya et al. 2009). It is not known whether Setaria exhibits a long and short day signaling pathway in the same way that rice does,

although there are three co-orthologs of FT in the Setaria genome (Bennetzen et al. 2012). In contrast to rice, the functional FT homolog in maize (*ZCN8*) and its equivalent in sorghum are in a different clade of PEBP proteins (Lazakis et al. 2011; Meng et al. 2011; Wolabu et al. 2016). RNA-seq and qRT-PCR data from *S. viridis* (unpublished) suggests that one of the Setaria co-orthologs of FT as well as a Setaria homolog of *ZCN8* are expressed at the same time during the transition of the vegetative shoot apical meristem to an inflorescence meristem.

The second photoperiod-controlled flowering time pathway identified in rice involves the negative regulators *GHD7* and *EHD1*, which work together to precisely determine the length of photoperiod that will induce flowering. Homologs of these two genes have been identified as involved in flowering time regulation in maize and sorghum, suggesting that the *GHD7-EHD1* pathway is a grass-specific flowering time pathway (Hung et al. 2012; Murphy et al. 2011, 2014; Yang et al. 2014). Orthologs of *GHD7* and *EHD1* have been identified in the Setaria genome but not functionally tested.

The effect of photoperiod on traits that interact with flowering time, such as biomass accumulation and plant architecture, have been little studied in most panicoid grasses. However, information on the effect of photoperiod on plant growth is important for Setaria as a model system because it has not been selected for photoperiod insensitivity, unlike modern cultivars of maize and sorghum. A common growth chamber strategy is to grow it under a 12:12 h light:dark photoperiod regime, as will be seen in other investigations presented in this book (Chaps 10, 11, 13, 14, 18–21). Such a strategy minimizes the effect of environmental variation on phenotype and encourages rapid flowering and fast cycling of generations—important criteria for a model system. However, field grown *S. viridis* and *S. italica* are rarely grown under less than 14 h light, and may be grown in as much as 16 h light in higher latitudes. In photoperiod-sensitive plants, such as Setaria, these differences might be expected to produce differences in both flowering time and plant growth traits.

To investigate these questions, we have grown a RIL population derived from a cross between *Setaria italica* (foxtail millet) and *S. viridis* (green millet) in three different photoperiod regimes (8:16, 12:12, and 16:8 h light:dark), while minimizing variation in other environmental variables. We report here on a QTL analysis of variation in flowering time, plant architecture, and biomass under these photoperiod regimes and compare results with previously published analyses using the same RIL population in greenhouse and field environments (Mauro-Herrera and Doust 2016; Mauro-Herrera et al. 2013).

12.2 Materials and Methods

12.2.1 Plant Materials, Experimental Design, and Phenotyping

A total of 182 F_7 RILs from an interspecific cross between *S. italica* accession B100×*S. viridis* accession A10 (Bennetzen et al. 2012) were evaluated for flowering time, plant height, total branching, and biomass at flowering in a walk-in growth

chamber at Oklahoma State University (Stillwater, OK). Three trials were undertaken, at photoperiod ratios (light:dark) of 8:16, 12:12, and 16:8. The chamber was kept at 30 % humidity and day and night temperatures were 28 and 22 °C, respectively. Two other variables, besides photoperiod duration, varied between trials. These were amount of daily radiation received (directly related to photoperiod duration) and temperature (as the combination of different day lengths and the difference in day and night temperatures led to differences in the average temperature of each trial). The effects of daily radiation and temperature cannot be separated in this study, and their values were 8.64 E (Einstein = mol m^{-2} s^{-1}) and 24 °C in the 8 h trial, 12.96 E and 25 °C in the 12 h trial, and 17.28 E and 26 °C in the 16 h trial. Illumination from full spectrum fluorescent tubes averaged 300 μmol m^{-2} s^{-1}. Three replicate pots of each RIL were grown in each experiment, with each pot having a single plant. Pots were randomized, and plants were spaced 8.5 cm apart. Pot volume was approximately 215 cm^3, and pots were filled with Metro-Mix 366 (Sun Gro Horticulture Canada Ltd). Plants were irrigated as needed with an aqueous complete fertilizer mix (Jack's mix: Nitrogen, Phosphorous and Potassium (20-20-20), JR Peters, PA).

12.2.2 Phenotypic Measurement

We used days to heading as the measurement of flowering, with plants recorded as flowering when the inflorescence on the main culm was first visible in the sheath of the flag leaf (Mauro-Herrera et al. 2013). Culm height (height of the main stem of the grass plant) was measured from the base of the plant to the ligule (leaf collar) of the flag leaf on the main culm. Total branches comprised both tillers (at base of plant) and any aerial branches. Total aboveground biomass was measured by drying whole plants for at least 1–2 weeks in a plant drier, and then weighing after removing the roots.

12.2.3 Statistical Analyses

Traits were tested for normality and transformed where appropriate. Relationships between traits were explored by bivariate Pearson phenotypic correlations, using both original variables (transformed where necessary) and with effect of RIL removed (by using the residuals obtained from an ANOVA for each trait with RIL as the independent variable). Boxplots of each parent and for the combined RIL population were made for each trait. Trait differences between photoperiods were analyzed using ANOVA. Because it is likely that days to flowering is affected by both photoperiod (measured by a plant as the length of darkness in each 24 h period) and carbon gain (directly related to hours of light and temperature), trait values were first regressed against the total amount of illumination each plant received until flowering, and residuals used in the ANOVA analyses. The model fitted for all ANOVA analyses consisted of two factors, Photoperiod (fixed) and RIL (random).

Partial eta squared values were calculated to estimate proportion of trait variance explained by each factor or interaction. All analyses were performed with SPSS version 21 (IBM SPSS, Armonk, NY).

12.2.4 QTL Analyses

For QTL analyses, we used the previously published 684 marker genetic map (Mauro-Herrera et al. 2013). QTL Cartographer Unix version 1.16 (Basten et al. 1994, 2002) was used for QTL analyses with the composite interval mapping (CIM) method, a genome scan interval of 1 cm, a window size of 10, and the forward and backward regression method (Jansen and Stam 1994; Zeng 1994). QTL analyses were conducted for each trait in each photoperiod trial, as well as a joint analysis for each trait across all three trials. The joint analysis measured both main effect QTL detected across trials as well as QTL that had a significant genotype by trial interaction. LOD threshold values were estimated via 1000 permutations (Churchill and Doerge 1994; Doerge and Churchill 1996). Comparisons amongst the growth chamber trials and between growth chamber and previous greenhouse and field trials were conducted by comparing overlap between QTLs for each trait, especially with respect to the position of the maximum LOD values.

12.3 Results

12.3.1 Phenotypic Variation

Trait distributions were tested for normality, and biomass and branch number were square root transformed to improve the normality of their distributions. Transformed trait values were used for these two traits in subsequent analyses.

In all three trials, *S. viridis* flowered before *S. italica*, with most of the RILs flowering at intermediate times (Fig. 12.1). Flowering time in the RILs was skewed towards that of the earlier flowering *S. viridis* plants. Flowering time of the *S. italica* plants was especially long and variable in the 16 h trial (Fig. 12.1). There was little transgressive segregation for flowering time. *S. viridis* plants were shorter than *S. italica* plants at flowering, and the RILs in general had plant heights skewed towards *S. viridis* (Fig. 12.1). However, there was substantial transgressive segregation for height in the RIL population at all photoperiods, with greatest transgressive segregation in the 16 h trial. Transgressive variation was also seen for total branch number in all trials, and the *S. viridis* parent always had more branches than the *S. italica* parent (Fig. 12.1). In the 8 and 12 h trials, the *S. italica* plants did not produce any tillers or aerial branches at all. *Setaria viridis* always had less biomass than *S. italica*, and the biomass of the RIL lines was skewed towards *S. viridis* (Fig. 12.1). There was some transgressive segregation for biomass, especially in the 16 h trial.

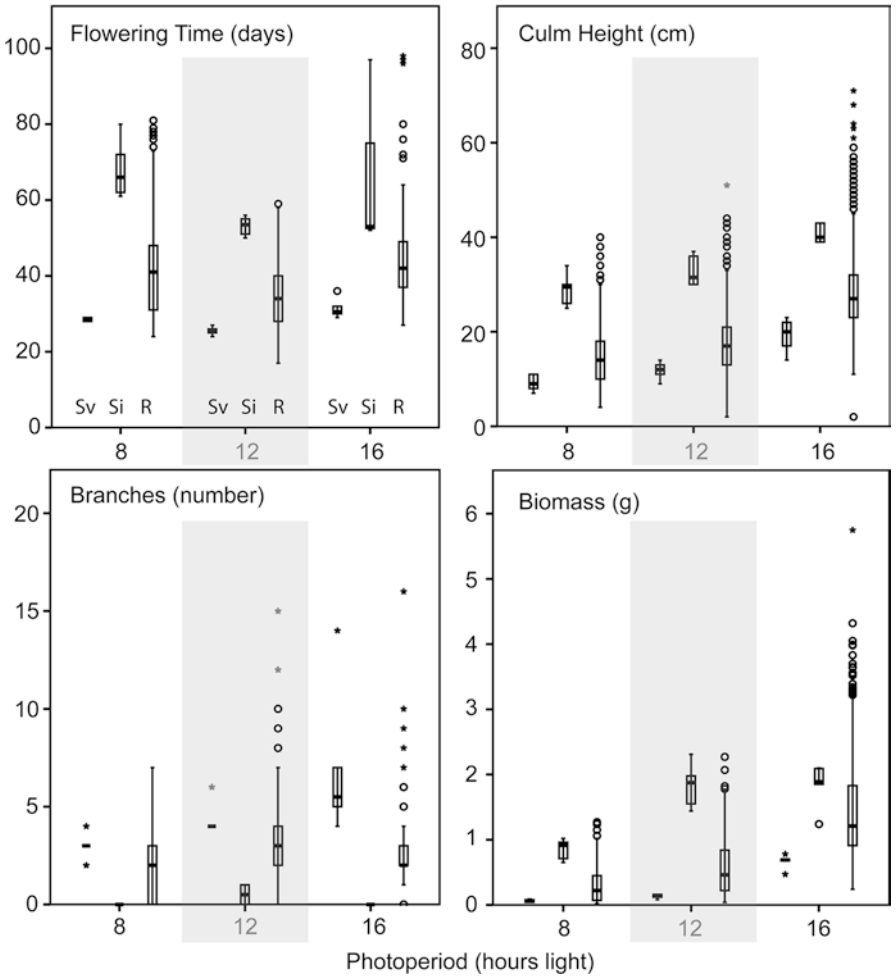

Fig. 12.1 Boxplots of trait values for each trait in each of the three photoperiod regimes. All boxplots show the distribution for each parent and for the RIL population (Sv = *S. viridis*, Si = *S. italica*, R = RIL population)

Height, branching, and biomass showed a generally positive response to increasing length of photoperiod, for both parents and RILs. The same was not true for flowering time, where the 12 h trial exhibited the shortest flowering times, followed by the 8 h and then the 16 h.

The phenotypic traits in each individual trial showed high positive correlations between flowering time, height, and biomass (Table 12.1); the correlations were also found when the effect of RIL (genotype) was removed. The relationship between branching and the other three variables was less consistent although in all but one comparison the relationship between height and branching was significantly negative. The correlation between branching and flowering time varied from trial to

Table 12.1 Correlations between traits in each of the photoperiod trials

		Trait values			Residuals[a]		
Photoperiod	Trait	Culm height	Total branches[b]	Biomass[b]	Culm height	Total branches[b]	Biomass[b]
8 h	Flowering time	++	ns	++	++	+	++
8 h	Culm height		−−	++		ns	++
8 h	Total branches[b]			ns			++
12 h	Flowering time	++	−−	++	++	ns	++
12 h	Culm height		−−	++		−−	++
12 h	Total branches[b]			−			ns
16 h	Flowering time	++	ns	++	++	++	++
16 h	Culm height		−−	++		−−	++
16 h	Total branches[b]			ns			++

[a]Residuals have the effect of genotype (RIL) removed
[b]The values for these traits have been square root transformed
Positive significant correlations: + = $P<0.05$, ++ = $P<0.01$
Negative significant correlations: − = $P<0.05$, −− = $P<0.01$
Nonsignificant correlations = ns

trial, both with and without the effect of genotype (RIL) (Table 12.1). Correlations of total amount of light received with each of the traits were significant and positive and explained 56 % of the variation in flowering time, 48 % in height, 78 % in biomass, but only 2 % in branching.

The effect of photoperiod on each of the four traits was analyzed in the parents of the population by ANOVAs with and without the effect of total amount of illumination received. *Setaria viridis* was more sensitive to photoperiod changes than *S. italica* for both flowering time (*S. viridis* $p<0.001$, *S. italica* not significant) and branching (*S. viridis* $p<0.001$, *S. italica* $p<0.05$). Both *S. viridis* and *S. italica* had highly significant differences in height and biomass across photoperiods.

The effect of photoperiod and RIL genotype on each of the four traits was analyzed with ANOVAs using the residuals from a regression of the trait values against the total amount of illumination received. There were highly significant differences amongst both Photoperiod and RIL, and for the interaction between them. However, the amount of variation explained by each factor (partial eta squared values) and their interaction varied between traits (Table 12.2). RIL and Photoperiod * RIL explained large proportions of the variance for all traits, but Photoperiod by itself only explained large proportions of the variance for flowering time and for

Table 12.2 Partial eta squared values for the ANOVA using the residuals of the four traits (after removing the effect of total amount of illumination received), showing the degree to which each factor explains variation in the traits

Source factor	Flowering time	Height	Biomass (sqrt)	Branching (sqrt)
Photoperiod	0.68	0.07	0.13	0.38
RIL	0.61	0.69	0.6	0.73
Photoperiod * RIL	0.85	0.56	0.53	0.43

All factors were significant for all traits
Note: Because RIL is a random sampling of all possible genotypes it is treated as a random factor. Therefore, the mean square used as an error term for the Photoperiod and RIL comparisons is the mean square for Photoperiod * RIL, and that for Photoperiod * RIL is the error mean square

branching, and very little of the variation in height and biomass. This suggests that the main driver for height and biomass is carbon gain driven by the number of illumination hours rather than photoperiod length.

12.3.2 QTL Analyses

Across all individual trials ten QTL regions for flowering were identified, with five QTL in both the 8 and 12 h trials, and three in the 16 h trial. Three of the genomic regions contained QTL from multiple trials, these being on chromosomes IV and VII (8 and 12 h), and on chromosome VIII (12 and 16 h) (Fig. 12.2). There were 11 joint main effect QTL and six GxE effect QTL, indicating that the control of flowering has a significant environmental component. Eight QTL regions identified in individual trials overlapped with either a main or GxE QTL of the joint analysis. However, four of the joint QTL did not align with any of the individual QTL.

Across all individual trials nine QTL regions for height were identified, with three in the 8 h, five in the 12 h, and five in the 16 h trial, with only three genomic regions where QTL maximum LOD positions overlapped. These were on chromosomes IV (8 and 12 h), V (12 and 16 h), and IX (all three trials). Six main effect and four GxE effect QTL were identified in the joint analysis, of which one on chromosome IV overlaps with the 8 and 12 h trials, one on chromosome V overlaps with the 12 h trial, and one on chromosome IX that overlaps with all three trials.

Across all individual trials 11 QTL regions for biomass were identified, with five in the 8 h trial, five in the 12 h, and six in the 16 h trial. Four genomic regions contained overlapping QTL from the individual trials, these were on chromosomes IV (8, 12, and 16 h), V (8 and 12 h), VIII (12 and 16 h), and IX (8 and 12 h). There were eight joint main effect and six GxE effect QTL, of which four overlapped with QTL from the individual trials, on chromosomes II, III, IV, and V.

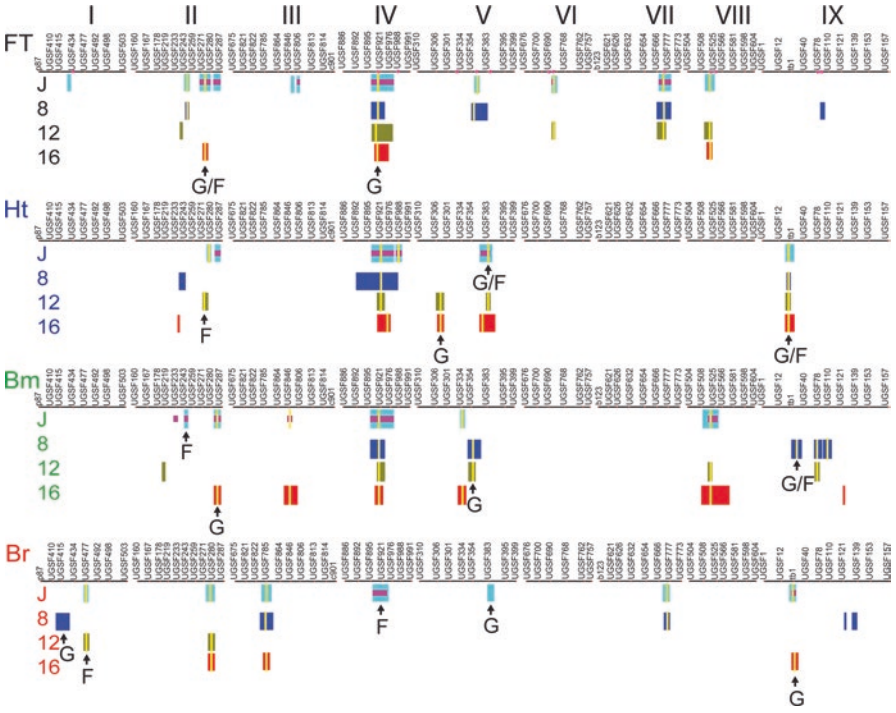

Fig. 12.2 QTL map showing the distribution of QTL for each of the traits in each of the photoperiod environments (8 h—*dark blue*, 12 h—*ochre*, 16 h—*red*, as well as joint main QTL calculated for each trait across the three environments (*light blue*) and QTL by environment effects (*mauve* bars, often nested within *light blue* joint main QTL). G (greenhouse) and F (field) refer to regions where QTL from greenhouse and field trials (Mauro-Herrera and Doust 2016; Mauro-Herrera et al. 2013) overlap with QTL from this study

Across all individual trials eight QTL regions for branching were identified, with five QTL in the 8 h trial, two in the 12 h, and three in the 16 h trial. QTL overlapped in two genomic regions, the 12 and 16 h on chromosome II and the 8 and 16 h on chromosome III. There were seven joint main effect and two GxE effect QTL identified, of which five overlapped with individual trials. These were on chromosomes I (with 12 h), II (with 12 and 16 h), III (with 8 and 16 h), VII (with 8 h), and IX (with 16 h).

QTL for flowering time, height, and biomass show a striking overlap, especially on chromosome IV. Generally speaking, approximately one third of the QTL positions identified across the three trials were found in more than one trial (Table 12.3). In all but three of these regions, a joint QTL was also found, the exceptions being height on chromosome V (12 and 16 h), biomass on chromosome V (8 and 12 h), and biomass on chromosome IX (8 and 12 h). There was a greater percentage of overlap between individual trial QTL and joint QTL, as would be expected considering that the data from each individual trial contributes to the joint analysis (Table 12.3).

Table 12.3 Average percentages of shared QTL between the different trials

Trait	Amongst growth chamber individual trials[a]	QTL from individual trials that overlap with joint QTL[b]	QTL from greenhouse that overlap with growth chamber QTL[c]	QTL from field that overlap with growth chamber QTL[d]	Greenhouse versus field[e]
Flowering time	3/10	7/12	2/8	1/5	4/9
Height	3/9	3/6	3/10	3/10	5/15
Biomass	4/11	3/7	3/12	2/10	5/17
Branching	2/8	5/7	3/10	2/5	1/14
Mean ± S.D.	0.31 ± 0.05	0.45 ± 0.12	0.28 ± 0.03	0.28 ± 0.10	0.28 ± 0.16

[a]Numerator is number of overlapping QTL between individual trials, denominator is total number of regions identified. Overlapping QTL can be in all three trials or in just two of the trials
[b]Numerator is number of overlapping QTL between individual trials and the joint analysis, denominator is total number of regions identified in the joint analysis
[c]Numerator is number of overlapping QTL between greenhouse QTL and individual + joint growth chamber analyses, denominator is total number of individual + joint growth chamber QTL identified
[d]Numerator is number of overlapping QTL between field QTL and individual + joint growth chamber analyses, denominator is total number of individual + joint growth chamber QTL identified
[e]Numerator is number of overlapping QTL between greenhouse and field, denominator is total number of regions identified in greenhouse and field

12.3.3 Comparisons of Growth Chamber Trials with Previous Greenhouse and the Field Trials

QTL identified in the growth chamber trials were compared with those discovered in previous greenhouse and field trials (Mauro-Herrera et al. 2013, Mauro-Herrera and Doust 2016). The percentage of overlap of QTL between these different environments was similar to that between the individual growth chamber trials, and between greenhouse and field trials (Table 12.3). The region on chromosome IV that was significantly correlated with flowering time, height, and biomass in all three growth chamber photoperiod trials was also found for flowering time in the greenhouse and for branching in the field trial. Other QTL that were found in more than one growth chamber trial and in either or both of the greenhouse and field trials include those on chromosomes VII and VIII for flowering time. However, QTL from multiple trials for biomass on chromosome VIII and for branch number on chromosomes II and III in the growth chamber trials were not found in the greenhouse or field trials.

12.4 Discussion

Two main trends are seen in these trials. One is related to total amount of illumination received (although confounded with variation in average temperature) while the other is related to the duration of light and dark intervals. The correlations and

boxplots show that architectural and biomass traits have a positive response to increasing length of photoperiod, whereas the 12 h photoperiod regime gave the shortest flowering time, followed by the 8 h and then the 16 h regime. This may have been because the 12 h regime was the shortest viable photoperiod in terms of light quanta received for this C4 plant, and that 8 h light per day was simply not enough to allow flowering quickly. This is being tested in further experiments underway in our lab. The much longer time to flowering under the 16 h regime suggests that Setaria should be considered a facultative short day plant.

The positive response of height, branching and biomass to increasing photoperiod most probably reflects both the increase in light (and temperature) received each day as well as the increase in number of days to flowering that allows plants to continue to grow for a longer time period. This relationship is supported by the finding that flowering time is significantly correlated with height and biomass in all three trials, with or without the effect of genotype. When the effect of genotype is not considered, there is a significant positive correlation between flowering time and branch number in two of the three photoperiod regimes, but, when the effect of genotype is included, the relationship between branch number and flowering time is not significant, suggesting that different genotypes perform differently in different photoperiod regimes.

In the ANOVA analyses, we chose to concentrate on photoperiod and genotype (RIL), by eliminating confounding variation due to different levels of light intensity and/or temperature due to the different photoperiod lengths. ANOVA analyses of the four traits showed that Photoperiod, RIL, and Photoperiod * RIL explained significant proportions of the variance of all four traits. Given the very different appearance and time to maturity of the parents, it is not surprising that RIL was significant, but the analyses also show a significant interaction between Photoperiod and RIL. This suggests that the different RILs react differently to the different regimes, pointing to differences in sensitivity to photoperiod in the parents of the cross. Significant differences between trials for Photoperiod for all four traits indicates that the length of the day:night cycle affects flowering time and morphology irrespective of the amount of light received. However, this effect, while significant for all traits, explained most variation for flowering time, but only some for branching, and relatively little for biomass or height. Thus, biomass and height appear most affected by genotype and by amount of light received rather than by the day:night duration, pointing to the rate of carbon gain through photosynthesis as their main controlling factor.

The QTL analyses suggest a number of shared QTL regions along with multiple regions found only in individual trials that control flowering time, architecture, and biomass. Not surprisingly, QTL for flowering time, height, and biomass overlap in several regions, most notably on chromosome IV. There is less overlap with branching, reinforcing the conclusions of the ANOVA and correlation analyses that branching is controlled by a set of factors that are partially distinct from those for the other traits.

The major shared QTL region on chromosome IV has been shown to contain a number of genes involved in the photoperiod signaling pathway leading to flowering, including the Setaria orthologs of *HD1* (*CONSTANS*) and several copies of *FT*

(Mauro-Herrera et al. 2013). There is both a joint main QTL and a GxE QTL for branching in the same region, which also overlaps with a QTL for flowering time in a greenhouse trial and a QTL for branching in a field trial (Mauro-Herrera and Doust 2016). If one examines the QTL region of chromosome IV closely, it is apparent that the maximum LOD position for the QTL of the 8 and 12 h trials for flowering time and height are slightly offset from that for the 16 h trial, suggesting that the regulation of these traits in the long day 16 h trial is different from the short day 8 and 12 h trials. Evidence for this from the QTL analyses would be if it appeared more likely for QTL for the 8 and 12 h trials to group together than with QTL for the 16 h trial, but in fact it appears equally likely for 8 and 12 h trials to overlap as it is for 12 and 16 h trials to overlap. However, over all four traits there is only one example of QTL for the 8 h trial overlapping with the 16 h trial to the exclusion of the 12 h trial, which implies that the groupings of 8 and 12 h or 12 and 16 h QTL are nonrandom, suggesting a differentiation between shorter and longer photoperiods. Thus, the QTL analyses do give some support for separate short- and long-day responses in Setaria.

There is one other QTL region, apart from that on chromosome IV, where all three trials and the joint analysis have overlapping QTL. That is for height on chromosome IX, in the same region as the repressor of branching gene *teosinte branched1* (*tb1*) (Fig. 12.2). This was also found in both greenhouse and field trials. While it is possible that *tb1* is itself affecting height by repressing branch elongation, it is also possible that other genes in this region are involved.

There are a number of previously published QTL from greenhouse and field trials that overlap with QTLs found in the photoperiod growth chamber trials, but these do not appear to overlap any more frequently than QTL between the photoperiod trials. It is not surprising that QTL patterns differ between growth chamber, greenhouse, and field, as such patterns are well known in literature from other model systems, such as the difference in Arabidopsis mapping populations grown in greenhouse and field environments (Brachi et al. 2010; Malmberg et al. 2005). Those QTL regions that are constant between such varied environments, such as on chromosome IV for flowering time and V and IX for height and biomass should be investigated further for genes that differ between the parents and control these traits. There is less overlap of QTL for branching between environments indicating that this trait has strong and significant GxE interactions that govern the expression of the phenotype.

The QTL analyses are not sufficiently detailed to infer whether the different photoperiod regimes invoke different genetic pathways, in the manner of the differences between *HD3a* and *RFT1* expression under short and long days in rice. However, it is striking that the QTL intervals cover several of the major genes involved in the CONSTANS/*HD1* pathway but neither *EHD1* nor *GHD7*. It would be inappropriate to read too much into these analyses, but they suggest that further qRT-PCR analyses of plants at the floral transition should be undertaken to search for the participation of the *EHD1*/*GHD7* pathway in the regulation of photoperiod changes. The QTL analyses did not pick up significant differences between parental alleles at the *ZCN8* locus on chromosome III although our unpublished results do show that it is up-regulated at flowering. However, genome searches reveal that

several of the FT co-orthologs are present in QTL regions IV, VII, and VIII, making it possible that the parents differ in expression of the FT homologs but not the *ZCN8* homolog.

This study has uncovered interesting variation in the genetic regulation of flowering time and architectural traits and laid the stage for more intensive analyses. It has also shown that Setaria is variable in its architecture when grown under different environments (see also Chap. 10), suggesting that close attention needs to be paid to environmental conditions in order to understand phenotypic variation. The overlap between QTL identified in this study and in previous studies with genes in the CONSTANS photoperiod pathway suggests that variation in this pathway explains a significant proportion of the differences in flowering time seen between the two parents of the cross, as well as much of the variation in height and biomass. While not conclusive, the evidence presented here suggests that Setaria is a facultative short-day plant and that there may be differences in genetic regulation between short- and long-day photoperiod regimes. QTL for branching overlapped less frequently than those for height and biomass between trials and between this study and previous work, emphasizing the large environmental component to control of branching, and the weak relationship between branching and other architectural traits such as height and biomass gain. The insights gained in this study could not easily have been achieved in larger C_4 grasses such as maize or sorghum, and was only possible due to the small size, rapid life cycle and ease of growth of the Setaria system.

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