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Optimizing tiller production and survival for grain yield improvement in a bread wheat × spelt mapping population

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• **Background and Aims** Tiller production and survival determine final spike number, and play key roles in grain yield formation in wheat (*Triticum aestivum*). This study aimed to understand the genetic and physiological basis of the tillering process, and its trade-offs with other yield components, by introducing genetic variation in tillering patterns via a mapping population of wheat × spelt (*Triticum spelta*).
• **Methods** The dynamics of tillering and red/far-red ratio (R:FR) at the base of a canopy arising from neighbouring plants in a bread wheat (*Triticum aestivum* 'Forno') × spelt (*Triticum spelta* 'Oberkulmer') mapping population were measured in the field in two growing seasons. Additional thinning and shading experiments were conducted in the field and glasshouse, respectively. Yield components were analysed for all experiments, followed by identification of quantitative trait loci (QTL) associated with each trait.
• **Key Results** Large genetic variation in tillering was observed, and more fertile shoots per plant were associated with more total shoots initiated, faster tillering rate, delayed tillering onset and cessation, and higher shoot survival. A total of 34 QTL for tillering traits were identified, and analysis of allelic effects confirmed the above associations. Low R:FR was associated with early tillering cessation, few total shoots, high infertile shoot number and shoot abortion, and these results concurred with the thinning and shading experiments. These effects probably resulted from an assimilate shortage for tiller buds or developing tillers, due to early stem elongation and enhanced stem growth induced by low R:FR. More fertile tillers normally contributed to plant yield and grain number without reducing yield and grain set of individual shoots. However, there was a decrease in grain weight, partly because of smaller carpels and fewer stem water-soluble carbohydrates at anthesis caused by pleiotropy or tight gene linkages.
• **Conclusions** Tillering is under the control of both genetic factors and R:FR. Genetic variation in tillering and tolerance to low R:FR can be used to optimize tillering patterns for yield improvement in wheat.

Key words: Carpel, grain number, grain weight, quantitative trait locus, QTL, red/far-red ratio, spelt, stem water-soluble carbohydrates, tillering, *Triticum aestivum*, *Triticum spelta*, wheat, yield.

INTRODUCTION

Tillering in wheat (*Triticum aestivum*) determines plant canopy size, photosynthetic area and, more importantly, the number of spikes bearing grains at maturity (fertile shoots), which is a key component of yield. Wheat plants undergo several events to form final fertile shoots: axillary bud initiation, first bud outgrowth, tillering cessation, tiller abortion and the development of surviving tillers. Tiller buds are initiated from the axillary meristems in the axils of developing leaves on the main shoots, and bud number is associated with total number of leaves (Baker and Gallagher, 1983; Longnecker *et al*., 1993). Early tillers can also be parent shoots producing secondary buds and tillers (Evers and Vos, 2013).

Outgrowth of the first tiller buds represents the onset of apparent tillering. In the field, this can occur from autumn to spring, depending on sowing date and temperature thereafter (Sylvester-Bradley *et al*., 2008). Tillering normally ceases just before stem elongation (Baker and Gallagher, 1983; Gomez-Macpherson *et al*., 1998; Sylvester-Bradley *et al*., 2008), and the remaining axillary buds become dormant. However, dormancy is not definitive, and can be released in cases such as early lodging and damage to the apices of parent shoots (Rameau *et al*., 2015). The timing of tillering cessation and number of total tillers initiated are regulated by many genetic, physiological and environmental factors. A tiller inhibition gene (*tin1*), which has been mapped on chromosome 1AS (Richards, 1988; Spielmeyer and Richards, 2004), has been found to reduce tillering through the early cessation of axillary bud outgrowth (Duggan *et al*., 2002; Kebrom *et al*., 2012). This inhibition may result from the sugar deficit for lateral tiller buds due to precocious internode elongation (Kebrom *et al*., 2012), concurring with the report of Langer *et al*. (1973). Environmental factors such as plant density and shade also affect tillering cessation. A larger plant population has been shown to be associated with earlier tillering cessation and fewer maximum tillers per plant (Evers *et al*., 2006; Sparkes *et al*., 2006). In dense communities, the red and blue wavelengths of light are absorbed by surrounding plants, and most far red is reflected and transmitted, resulting in a reduction in light intensity and quality (red/far-red ratio, R:FR), or shade. There is

evidence that tiller bud outgrowth responds to light quality and, to a lesser extent, light intensity (Sparkes *et al*., 2006). Cessation of axillary bud outgrowth coincides with a relatively conservative R:FR of 0·20–0·40 (Evers *et al*., 2006; Sparkes *et al*., 2006; Dreccer *et al*., 2013). High R:FR delays tillering cessation, and increases total tiller number (Toyota *et al*., 2014). Treatment with far-red light has the opposite effects, which can be reversed by adding red light, suggesting phytochrome perception (Kasperbauer and Karlen, 1986; Casal, 1988; Ugarte *et al*., 2010). Tillering (branching) response to low R:FR or shade has also been observed in ryegrass (*Lolium multiflorum*) (Casal *et al*., 1990), barley (*Hordeum vulgare*) (Davis and Simmons, 1994), sorghum (*Sorghum bicolor*) (Kebrom *et al*., 2006), soybean (*Glycine max*) (Kasperbauer, 1987) and arabidopsis (*Arabidopsis thaliana*) (Reddy *et al*., 2013). Shade acts as a warning signal of impending competition from neighbouring plants, and the consequent reduction of shoot branching is able to enhance apical growth for more incident light, known as a part of the shade avoidance syndrome (Gommers *et al*., 2013; Rameau *et al*., 2015).

Tiller abortion ensues immediately after the arrest of tiller bud outgrowth. Of the total tillers initiated, 10–80 % are destined to die, as affected by genotype, season, growing location, seeding rate and nutrient supply (Ishag and Taha, 1974; Hucl and Baker, 1989; Sharma, 1995; Berry *et al*., 2003). Tiller abortion usually takes place between the onset of stem elongation and anthesis, and those appearing last die first (Sylvester-Bradley *et al*., 2008). As there is a net loss of dry matter from non-surviving tillers, they have been thought to be detrimental for yield potential, especially when a further increase in harvest index is required (Sharma, 1995; Berry *et al*., 2003; Foulkes *et al*., 2011). Therefore, tiller survival needs to be improved in future breeding, and a first step would be to clarify its genetic and physiological basis, which remains unknown. In contrast, fertile shoot or spike number at maturity has been widely investigated. Three genes, *tin1* on chromosome 1AS (Richards, 1988; Duggan *et al*., 2005), *tin2* on 2A (Peng *et al*., 1998) and *tin3* on $3A^{m}L$ (Kuraparthy *et al*., 2007), have been identified to reduce final tiller number. This trait is often expressed quantitatively, and many quantitative trait loci (QTL) have been detected on at least 12 chromosomes (Kato *et al*., 2000; Deng *et al*., 2011; Naruoka *et al*., 2011; Jia *et al*., 2013; Zhang *et al*., 2013).

Despite the importance of tillering dynamics in terms of yield formation in wheat, knowledge of the genetic and environmental factors regulating this process remains scarce. The questions that need to be addressed include: (1) what are the genes or QTL controlling the timing and rate of tillering, tillering capacity, and the degree of tiller abortion and survival; (2) whether and how the shade from neighbouring plants affects tillering dynamics, particularly tiller abortion, and if so, what is the genetic basis of the shade kinetics arising from a genotype grown in the field; and (3) whether more fertile tillers contribute to plant productivity, given the possible negative effects on other yield components. In this study, we aimed to address these questions in a recombinant inbred line mapping population of bread wheat (*Triticum aestivum*) × spelt (*T. spelta*). Dynamics of the tillering and R:FR were measured consecutively in the field in two seasons, and this was also done in the thinning study. In the third season, a shading experiment was carried out in the glasshouse to determine its effect on fertile tiller number. Yield components of each genotype in all seasons were then analysed. Subsequently, the QTL underlying these traits were identified.

MATERIALS AND METHODS

Plant materials

A mapping population of Swiss winter bread wheat (*Triticum aestivum*) 'Forno' and Swiss winter spelt (*T. spelta*) 'Oberkulmer' was used to introduce genetic variation in tillering patterns. This population consists of 226 F_5 recombinant inbred lines (RILs) (Messmer *et al*., 1999), and showed large variation in tiller number at different developmental stages in the preliminary field trials. Based on these observations, a subset including 72 RILs was selected in the 2011–2012 season (hereafter 2012), with considerable difference in tillering but similar flowering time (± 4 d in 2009–2010 and ± 1 d in 2010–2011) to minimize the confounding effect of different phasic development. This subset was enlarged to 110 RILs in the 2012–2013 and 2013–2014 seasons (hereafter 2013 and 2014, respectively).

Growth conditions for field experiments

Field experiments were carried out at University of Nottingham Farm, Leicestershire, UK (52°50′N, 1°15′W, 50 m above sea level) in 2012 and 2013. The soil was a sandy loam (soil indices: N = 0, P = 4, K = 4, Mg = 4, pH = 7·6 in 2012; N = 0, P = 5, K = 4, Mg = 4, pH = 7·3 in 2013). An additional 140 and 160 kg N ha^{-1} were applied in three splits between March and May in 2012 and 2013, respectively. The whole population, including 'Forno' and 'Oberkulmer', was arranged in a randomized complete block design with three replicates. The seeds of each RIL were sown in 6 × 1·6-m plots on 19 October 2011 and in 12 × 1·6-m plots on 31 October 2012, with 250 seeds m^{-2}. Herbicides, fungicides and insecticides were applied when necessary to maintain undisturbed plant growth.

Tillering, R:FR and yield components in the field experiments

Ten central plants per plot were selected and labelled after emergence in 2012 and 2013. To create relatively uniform populations among plots, plant density was adjusted by removing extra surrounding plants. When the tiller buds grew out at the leaf–stem junctions and became new tillers, the shoot number of each plant was counted approximately every 100 degree days (°Cd, base temperature 0 °C) until tillering cessation. Dying tillers, whose newest leaves started yellowing, were tagged using wires so that all shoots produced during tillering were taken into account. At the late stage of grain filling, the fertile shoots bearing spikes were counted. Immediately after each shoot count, R:FR at the base of each plant was measured using a two-channel radiometer (SKR 116, Skye Instruments, Llandrindod Wells, UK), following the method of Sparkes *et al*. (2006). Measurements were made under sunny days, with the sensor facing north against the stem bases, which allowed the light reflected and transmitted from the neighbouring plants to reach the sensor.

Data of shoots per plant and R:FR from each plot were then fitted over the accumulated thermal time from sowing using a logistic function (Fig. 1) (Sparkes *et al*., 2006).

$$S \text{ or } R = A + \frac{C}{1 + e^{-B(t-M)}}$$

where S is the numbers of shoots per plant, R is R:FR, A is the lower asymptote, $(A + C)$ is the upper asymptote, B is the doubled relative rate of tillering or R:FR reduction at time M, M is the accumulated thermal time when tillering rate or R:FR decline rate is at maximum and when shoot number or R:FR reaches $(A + 0{\cdot}5C)$, and t is the accumulated thermal time after sowing. The parameters used to describe the kinetics of tillering and R:FR are: total shoots per plant $(A + C)$, fertile shoots per plant (counted at late grain filling), shoot survival (fertile shoots divided by total shoots, %), infertile shoots per plant (the difference between total and fertile shoots), shoot abortion (infertile shoots divided by total shoots, %), tillering onset (t_{to}, when $A + 0{\cdot}1C$ is reached, $t_{to} = M - 2{\cdot}1972/B$), tillering cessation ($t_{tc}$, when $A + 0{\cdot}9C$ is reached, $t_{tc} = M + 2{\cdot}1972/B$), tillering duration ($t_{td}$, $t_{td} = t_{tc} - t_{to}$), tillering rate ($0{\cdot}8C/t_{td}$), the onset of R:FR reduction ($t_{R:FRor}$, when $A + 0{\cdot}9C$ is reached $t_{R:FRor} = M + 2{\cdot}1972/B$), the end of R:FR reduction ($t_{R:FRer}$, when $A + 0{\cdot}1C$ is reached $t_{R:FRer} = M - 2{\cdot}1972/B$) and stabilized R:FR (the lower asymptote A). In addition, R:FR at tillering onset and cessation were calculated.

Another key event during tillering is the onset of stem elongation (Growth Stage 31, GS31) (Zadoks *et al*., 1974). Five plants in central rows from each plot were split to observe the first internodes every 4 d. A line was judged to enter this stage when three or more main shoots had the first internodes longer than 1 cm. R:FR at GS31 was then calculated. In 2013, 15 RILs were selected randomly; five plants from each plot were measured for R:FR, counted for shoot number, and split for initial stem length (removing leaf sheaths and spikes) on 9 May (around GS31).

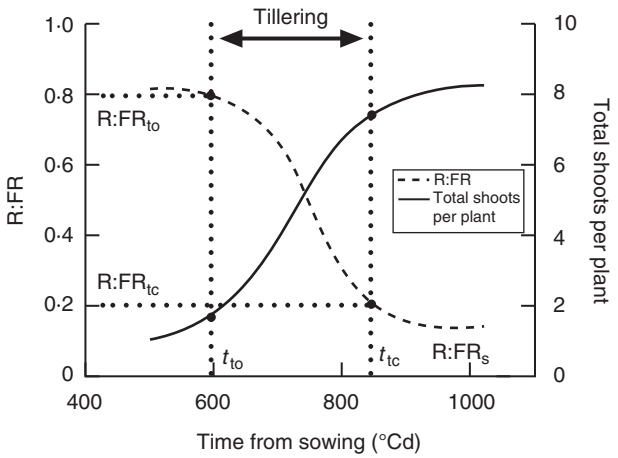

Fig. 1. Dynamics of tillering and red/far-red ratio (R:FR) at the base of the canopy in the mapping population of 'Forno' and 'Oberkulmer'. Data of shoot number per plant and R:FR from each plot were fitted over the accumulated thermal time from sowing using a logistic function. Definitions of the parameters: t_{to}, the time at tillering onset; t_{tc}, the time at tillering cessation; R:FR$_{to}$, R:FR at tillering onset; R:FR$_{tc}$, R:FR at tillering cessation; R:FR$_s$, stabilized R:FR.

Thinning was carried out in five RILs selected randomly in 2013. Plant density in these lines was reduced to 50 % by removing every other plant after emergence. Ten central plants in the thinned area in each plot were selected, and another ten plants without thinning taken as control. The dynamics of shoot number and R:FR of these plants were recorded for curve fitting, as described above.

Plant height, carpel size and stem water-soluble carbohydrate (WSC) content at anthesis were analysed in both seasons. For each plot of the subsets, five (in 2012) and ten (in 2013) shoots with the first anthers on spikes just visible were collected. Plant height was measured from the shoot bases to spike tips, excluding awns. Five spikes of each sample were used for carpel analysis. Two middle spikelets of each spike in 2012 and three spikelets (the third spikelets counted from the bases and tips, and the middle one between them on one side of a spike) in 2013 were dissected carefully. The carpels in the first three florets of a spikelet counting from the rachis were removed, oven-dried at 85 °C for 48 h, and weighed using an electronic balance (0·0001 g) (125A, Precisa, Dietikon, Switzerland). Average dry weight of individual carpels was then calculated. After removing leaves, all the stems (plus leaf sheaths) from the same shoots were collected, oven-dried immediately, weighed and finely ground. Stem carbohydrates were extracted (80 % ethanol and water), and WSCs were measured using the anthrone method, following the protocols of van Herwaarden *et al*. (1998), and Yemm and Willis (1954). Average dry weight of stem WSC per shoot was then calculated.

At maturity, five and 20 spikes from each plot in 2012 and 2013, respectively, were collected and threshed by a thresher and then by hand. The grains were oven-dried at 85 °C for 48 h and weighed, and yield per shoot was calculated. Then, approx. 200 grains were counted to calculate thousand grain weight (TGW) and grains per shoot. Yield and grains per plant were obtained by multiplying yield and grains per shoot by fertile shoot number, respectively.

Shading experiment in the glasshouse

A glasshouse experiment was conducted to test the effects of shade on tiller number and yield components in 2014. Green shade was achieved by using a green plastic filter (122 Fern Green; LEE Filters, Andover, UK) (Kegge *et al*., 2013). This green filter reduced photosynthetically active radiation (PAR, measured with a ceptometer: AccuPAR, Decagon Devices, Pullman, WA, USA) to 220 μmol m^{-2} s^{-1} and R:FR (SKR 116, Skye Instruments, Llandrindod Wells, UK) to 0·2, compared with the control using clear filters (PAR = 680 μmol m^{-2} s^{-1} and R:FR = 1·0). The filters were fixed on four sides and top of a wooden frame, but a 15-cm gap was left at the top of each side for ventilation. Daily temperature inside the frames during treatment was recorded using a data logger (Tinytag Ultra 2, Gemini Data Loggers, Chichester, UK), and the average temperature between shading and control was the same (15·3 °C). The seeds of the subset (110 RILs) were sown on 17 December 2013. The seedlings were vernalized at 6 °C for 9 weeks, and then transplanted into 1-litre pots (one plant per pot) filled with a loam-based compost (No. 3, John Innes, Norwich, UK). The RILs were arranged in a randomized complete block design with three

replicates for both the shading and the control. Frames were put on the plants from 27 March (onset of tillering) to 2 May 2014. The plants were watered frequently, and individually fed with 40 kg N ha^{-1} at the beginning of stem elongation. At maturity, fertile shoots of each plant were counted, and all spikes were threshed. Total grains were oven-dried at 85 °C for 48 h, weighed and counted. Yield per plant, yield per shoot, grains per plant, grains per shoot and TGW were then calculated.

Statistical analysis of phenotypic data

Analysis of variance (ANOVA) was used to test the differences between RILs and between treated (thinned and shaded) and control plants. Pearson correlations and regression analysis were carried out to determine the phenotypic relationships between different traits. Data were transformed to improve their normality, if necessary. Statistical analyses, including curve fitting, were performed with Genstat v17 and GraphPad Prism v6.05.

QTL analysis

A genetic map of 'Forno' × 'Oberkulmer' is available in the GrainGenes database (http://wheat.pw.usda.gov/GG2/index.shtml). Linkage analysis was repeated with 182 polymorphic markers [restriction fragment length polymorphism (RFLP) and simple sequence repeat (SSR)] using the JoinMap v4 (Van Ooijen, 2006), resulting in the same genetic map, with slightly different total coverage. This map included 230 segregating loci and 23 linkage groups, covering 2469 cM (approx. two-thirds of the whole genome of bread wheat and spelt) with an average marker density of 13·6 cM (Messmer *et al*., 1999). QTL analysis was performed with the MapQTL v6 (Van Ooijen, 2009), using the mean values of quantitative traits over replicates in each year. Interval mapping was used to estimate the QTL locations, logarithm of the odds (LOD) scores, additive effects and phenotypic variation explained by individual QTL (R^2). A genome-wide significance threshold ($P < 0{\cdot}05$) was computed by the permutation test with 1000 iterations. Co-factors, which were the markers nearest to QTL peaks, were selected, tested for significance ($P < 0{\cdot}02$), and used for the multiple-QTL model (MQM) mapping. QTL symbols were designed according to the Catalogue of Gene Symbols for Wheat (http://wheat.pw.usda.gov/GG2/Triticum/wgc/2008/). Locations of the significant QTL were presented using the 1-LOD support intervals (fall-off from the QTL maximum LOD peaks), and drawn using MapChart v2.2 (Voorrips, 2002). For each QTL, the allele increasing the quantitative trait value was defined as increasing allele, and the other one as decreasing allele. Those parents conferring increasing or decreasing alleles were analysed.

RESULTS

Phenotypic variation in tillering dynamics in the 'Forno' × 'Oberkulmer' mapping population

Tillering traits, including total shoots per plant, fertile and infertile shoots per plant, shoot survival and abortion, and tillering rate, were similar between the bread wheat 'Forno' and spelt 'Oberkulmer' in the field in 2012 (Fig. 2). However, 'Oberkulmer' had many more total, fertile and infertile shoots per plant, higher shoot survival and tillering rate but lower shoot abortion than 'Forno' in 2013. In the glasshouse experiment, fertile shoots per plant of 'Oberkulmer' (5.3 shoots) was similar to that of 'Forno' (5.0 shoots) under control conditions, but 'Oberkulmer' had 4·7 fertile shoots per plant under shading treatment, compared with 3·3 for 'Forno'. These indicate that the spelt can produce equal numbers of or more shoots than the bread wheat, depending on growth environments. Large genetic variation in all tillering traits was found in the RILs in each year (Fig. 2). In addition to genotype, year also affected tillering patterns: total shoots per plant (+38 %), fertile shoots per plant (+60 %), shoot survival (+9 %), infertile shoots per plant (+16 %) and tillering rate (+316 %) were higher in 2013 than in 2012 ($P < 0{\cdot}01$). The differences in tillering traits for the parents and RILs between years could result from the colder weather from sowing to March in 2013 (–2·6 °C for mean daily temperature) than in 2012, leading to delayed tillering and other consequent effects on the remaining traits, as demonstrated below. 'Oberkulmer' was more responsive to the growing years than 'Forno' in terms of tillering. Averaged across years, shoot survival was only 55 % over all the genotypes in the field.

Phenotypic correlations between tillering traits

Total shoots per plant was largely dependent on tillering rate rather than its duration (Table 1). There was no (in 2012) or weak (in 2013) negative relationship between fertile and infertile shoot number, indicating large independence. Both traits were positively associated with tillering rate. In addition, delayed onset and cessation of tillering appeared to be associated with more fertile shoots and higher shoot survival, and with fewer infertile shoots and lower shoot abortion. Tillering onset showed a positive relationship with tillering rate, but a negative one with tillering duration, suggesting that the later tillering onset, the faster tillering rate and the shorter tillering duration.

Identification of the QTL associated with tillering traits

A total of 34 QTL were identified for the tillering traits in the 'Forno' × 'Oberkulmer' mapping population, including one QTL for total shoots per plant, six for fertile shoots per plant, two for infertile shoots per plant, five for each of shoot survival and abortion, one for tillering rate, ten for tillering onset (containing nine for initial shoots per plant, which were recorded from the second tiller count at the beginning of tillering and used to measure tillering progress) and four for tillering cessation (Fig. 3 and Table 2). These QTL were scattered on ten chromosomes (1A, 2D, 3A, 3B, 4A, 4B, 5A, 5B, 7AL and 7B), and most of them (76 %) were located in the A genome. Phenotypic variation explained by individual QTL varied, ranging from 6·3 to 22·6 %.

The QTL coincidences between tillering traits were mainly found on chromosomes 3A, 4A and 5A (Fig. 3). For the QTL cluster on 3A, the alleles from the bread wheat 'Forno' delayed tillering onset and cessation, and increased shoot survival.

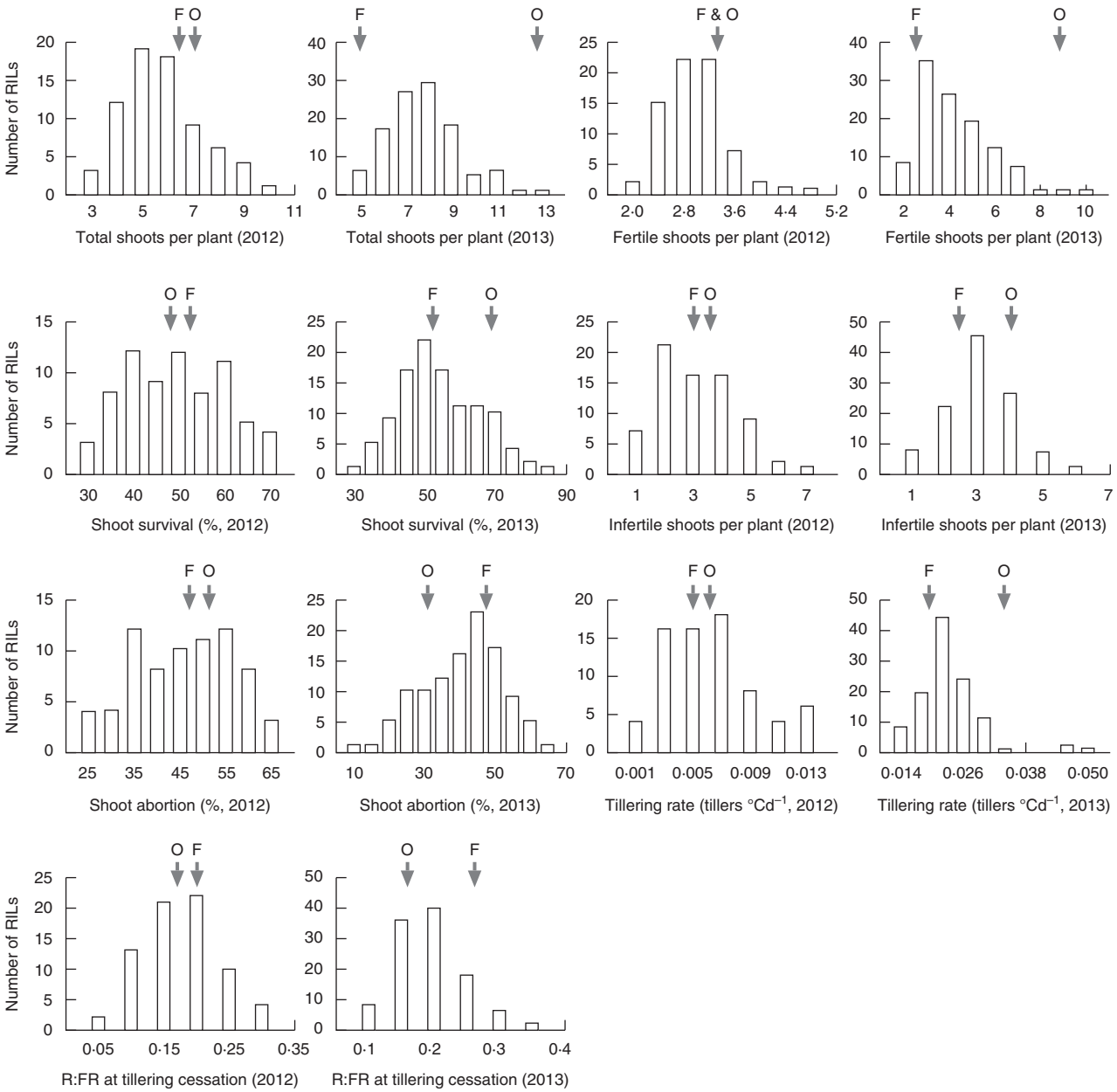

FIG. 2. Distributions of the recombinant inbred line (RIL) values for tillering and red/far-red ratio (R:FR) at tillering cessation. Parental values are indicated by arrows: F, 'Forno'; O, 'Oberkulmer'. A significant difference in each trait among the RILs was found ($P < 0{\cdot}01$).

There were two regions of QTL coincidences on 4A: one was located on 4AS, where 'Forno' conferred the alleles increasing fertile shoot number and shoot survival; the other was located on the distal region of 4AL, where 'Oberkulmer' conferred the alleles increasing initial and fertile shoot number. Likewise, there were also two regions of QTL coincidences on 5A: one was located on 5AS, where the alleles from 'Forno' delayed tillering onset and cessation, increased shoot survival, and decreased infertile shoots; the other was located on 5AL, where the alleles from 'Oberkulmer' delayed tillering onset and cessation, accelerated tillering rate, and increased total, fertile and infertile shoot number. However, one increasing and one decreasing allele for shoot survival were also identified from 'Oberkulmer' in this region; in other words, there were two closely linked alleles with the opposite effects on shoot survival, and their expressions depended on year.

Tillering dynamics as related to low R:FR

R:FR at the base of the canopy in the field showed relationships with tillering dynamics (Table 3). R:FR at tillering onset was positively associated with total and fertile shoot number, and shoot survival in 2013. Higher R:FR at that time was also associated with delayed tillering cessation across years. R:FR at tillering cessation and GS31, and stabilized R:FR, showed

TABLE 1. *Correlations between tillering traits in the mapping population of 'Forno' and 'Oberkulmer'*

Tillering trait†	Total shoots per plant	Fertile shoots per plant	Shoot survival	Infertile shoots per plant	Shoot abortion	Tillering rate	Tillering onset	Tillering cessation	Tillering duration
Total shoots per plant	1	0·46**	−0·76**	0·94**	0·76**	0·90**	0·23*	−0·02	−0·21
Fertile shoots per plant	0·80**	1	0·19	0·14	−0·19	0·33**	0·36**	0·24*	−0·12
Shoot survival	0·31**	0·80**	1	−0·92**	−1·00**	−0·76**	0·00	0·21	0·16
Infertile shoots per plant	0·35**	−0·28**	−0·77**	1	0·92**	0·88**	0·12	−0·12	−0·19
Shoot abortion	−0·31**	−0·80**	−1·00**	0·77**	1	0·76**	0·00	−0·21	−0·16
Tillering rate	0·64**	0·50**	0·15	0·25**	−0·15	1	0·29*	−0·23*	−0·43**
Tillering onset	0·22*	0·40**	0·43**	−0·27**	−0·43**	0·43**	1	0·26*	−0·64**
Tillering cessation	0·48**	0·66**	0·61**	−0·26**	−0·61**	0·01	0·25**	1	0·57**
Tillering duration	0·26**	0·28**	0·21*	−0·02	−0·21*	−0·31**	−0·54**	0·69**	1

* Significant at $P < 0·05$, ** significant at $P < 0·01$.
† Top right matrix: 2012 season; bottom left matrix: 2013 season.

negative relationships with infertile shoots per plant and shoot abortion, indicating that low R:FR established after tiller initiation promotes tiller death. R:FR at tillering cessation differed between the RILs, indicating the different responses of genotypes to low R:FR (Fig. 2). R:FR at tillering cessation was slightly higher in 2013 (0·21) than in 2012 (0·19) ($P < 0·05$).

As expected, thinning across the five RILs selected randomly raised R:FR at tillering onset (+17 %), leading to more total (+31 %) and fertile (+47 %) shoots per plant, higher shoot survival (+12 %) and lower shoot abortion (–8 %) (Fig. 4 and Table 4). A detailed analysis showed that thinning did not change the onset and rate of tillering, but delayed tillering cessation. These results are consistent with the above observations. There was no difference between thinned and control lines in R:FR at either tillering cessation or GS31, as well as stabilized R:FR.

R:FR around GS31 was measured in the 15 RILs on a given day in 2013, and showed a positive relationship with fertile shoots per plant (Fig. 5). In addition, shading in the glasshouse also reduced fertile shoots per plant by 12 % (Table 5).

Genetic analysis revealed a total of seven QTL for R:FR, including one QTL for each of R:FR at tillering onset and GS31 on chromosome 5A, two for stabilized R:FR on 4A and 5A, and three for the timing of R:FR reduction on 2A and 5A (Fig. 3 and Table 2). A QTL for stabilized R:FR was coincident with those for tillering traits on 4A; the increasing alleles from 'Forno' raised stabilized R:FR, fertile shoot number and shoot survival. In addition, the QTL coincidences between R:FR and tillering occurred on chromosome 5A. 'Forno' provided the alleles on 5AS increasing R:FR at GS31, delaying tillering onset and cessation, increasing shoot survival, and decreasing infertile shoot number and shoot abortion. In contrast, 'Oberkulmer' provided the alleles on 5AL increasing R:FR at tillering onset, delaying tillering onset and cessation, and increasing total and fertile shoots per plant, as well as shoot survival. A QTL for stabilized R:FR was also coincident with the other QTL for shoot survival in this region, with the increasing alleles from 'Forno'. These results support the above phenotypic relationships between R:FR and tillering.

Responses of the onset of stem elongation and plant height to low R:FR

There were positive relationships between the R:FR just before GS31 and the accumulated thermal time for GS31 ($r = 0·40$, $P < 0·01$ in 2012; $r = 0·33$, $P < 0·01$ in 2013), indicating that the lower R:FR, the earlier onset of stem elongation. Consistent with this, R:FR around GS31 was negatively associated with the initial stem length at the same time (Fig. 5). In addition, R:FR was increased by thinning, resulting in a delay of the onset of stem elongation (Table 4).

Plant height at anthesis was negatively associated with R:FR at tillering cessation ($r = –0·28$, $P < 0·05$ in 2012; $r = –0·20$, $P < 0·05$ in 2013), and with stabilized R:FR in 2012 ($r = –0·31$, $P < 0·01$).

Synchrony among tillering cessation, R:FR stabilization and the onset of stem elongation

Tillering ceased at 1196 and 844 °Cd after sowing over all RILs in 2012 and 2013, respectively, coincident with R:FR stabilization (1273 °Cd in 2012 and 862 °Cd in 2013) and GS31 (1214 °Cd in 2012 and 905 °Cd in 2013). This was also found in the thinning experiment, including both control and treatment (Table 4). The onset of stem elongation was slightly later than tillering cessation and R:FR stabilization. However, taking account of the measurement of GS31 (first internodes > 1 cm), the exact beginning of stem elongation might coincide with the other two events.

Relationships between tillering and yield components

Total shoots per plant contributed to yield and grain number per plant, but did not affect yield or grain number per shoot, or TGW (Table 6). Similarly, fertile shoots per plant and shoot survival in the field in 2012 and 2013, and fertile shoots per plant in the glasshouse in 2014 (both control and shading), were closely and positively associated with yield and grains per plant. More fertile shoots and higher shoot survival did not reduce yield per shoot, and even showed associations with slightly increased grains per shoot, despite an accompanying slight decline in TGW. One exception was the fertile shoots per plant in the shading treatment, where more fertile shoots were associated with lower yield per shoot, which resulted mainly from reduced grains per shoot (Tables 5 and 6).

To understand how more fertile shoots reduced TGW, carpel size and stem WSC content at anthesis were analysed (Table 7). Both carpel size and stem WSC content were

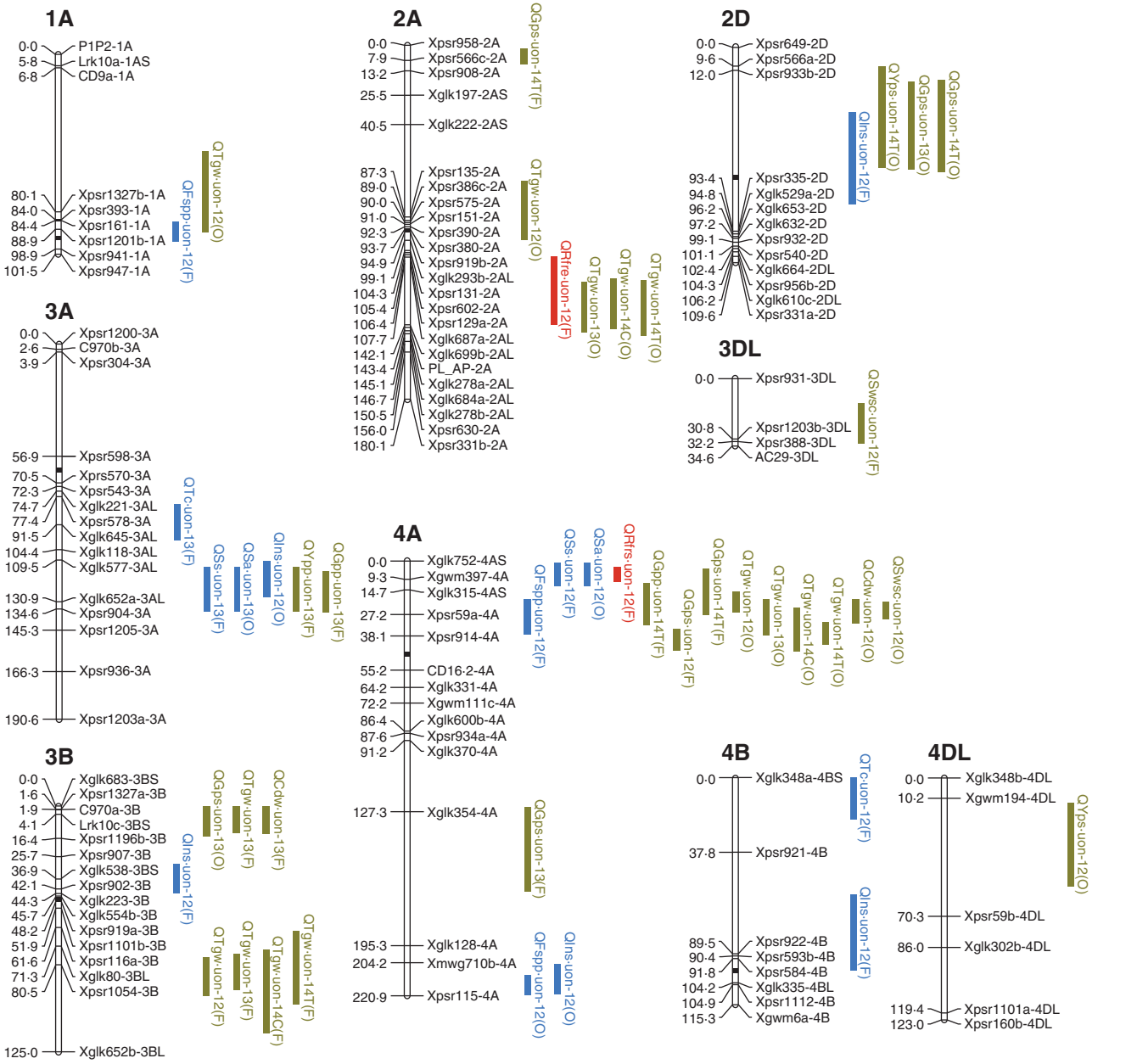

Fig. 3. Quantitative trait loci (QTL) for tillering, red/far-red ratio (R:FR) and yield components in the mapping population of 'Forno' and 'Oberkulmer'. The 1-LOD support intervals of significant QTL are indicated by blue (tillering), red (R:FR) and green (yield components) vertical bars. For QTL symbols, the 'Q' is followed by the abbreviated names of quantitative traits and laboratory (*uon*). Abbreviations for traits: Tspp, total shoots per plant; Fspp, fertile shoots per plant; Ss, shoot survival (%); Ispp, infertile shoots per plant; Sa, shoot abortion (%); Tr, tillering rate; Ins, initial shoots per plant; To, the time at tillering onset; Tc, the time at tillering cessation; Rfrto, R:FR at tillering onset; Rfr31, R:FR at GS31 (onset of stem elongation); Rfrs, stabilized R:FR; Rfre, the time at the end of R:FR reduction; Ypp, yield per plant; Yps, yield per shoot; Gpp, grains per plant; Gps, grains per shoot; Tgw, thousand grain weight; Cdw, carpel dry weight at anthesis; Swsc, stem water-soluble carbohydrate dry weight at anthesis. The QTL found in 2012 (field), 2013 (field), 2014 (glasshouse, control) and 2014 (glasshouse, shading treatment) are indicated by the suffixes 12, 13, 14C and 14T, respectively. The parental lines providing the alleles increasing trait values are given in parentheses: F, 'Forno'; O, 'Oberkulmer'.

positively associated with TGW, confirming their roles in determining grain weight. Furthermore, they showed negative relationships with fertile shoots per plant, so more fertile shoots tended to produce smaller carpels and less stem WSC per shoot, and in turn smaller grains.

A total of 44 QTL for yield components were identified in the field and glasshouse experiments, including three QTL for yield per plant, four for yield per shoot, five for grains per plant, 12 for grains per shoot and 20 for TGW (Fig. 3 and Table 2). These QTL were scattered on 11 chromosomes (1A, 2A, 2D, 3A, 3B, 4A, 4DL, 5A, 5B, 5DL and 7B), individually explaining 11·5–37·6 % of the phenotypic variation. The QTL for grains per shoot on 2D, 4A and 7B were stable across 2–3 environments, while those for TGW on 2A, 3B, 4A, 5DL and 7B

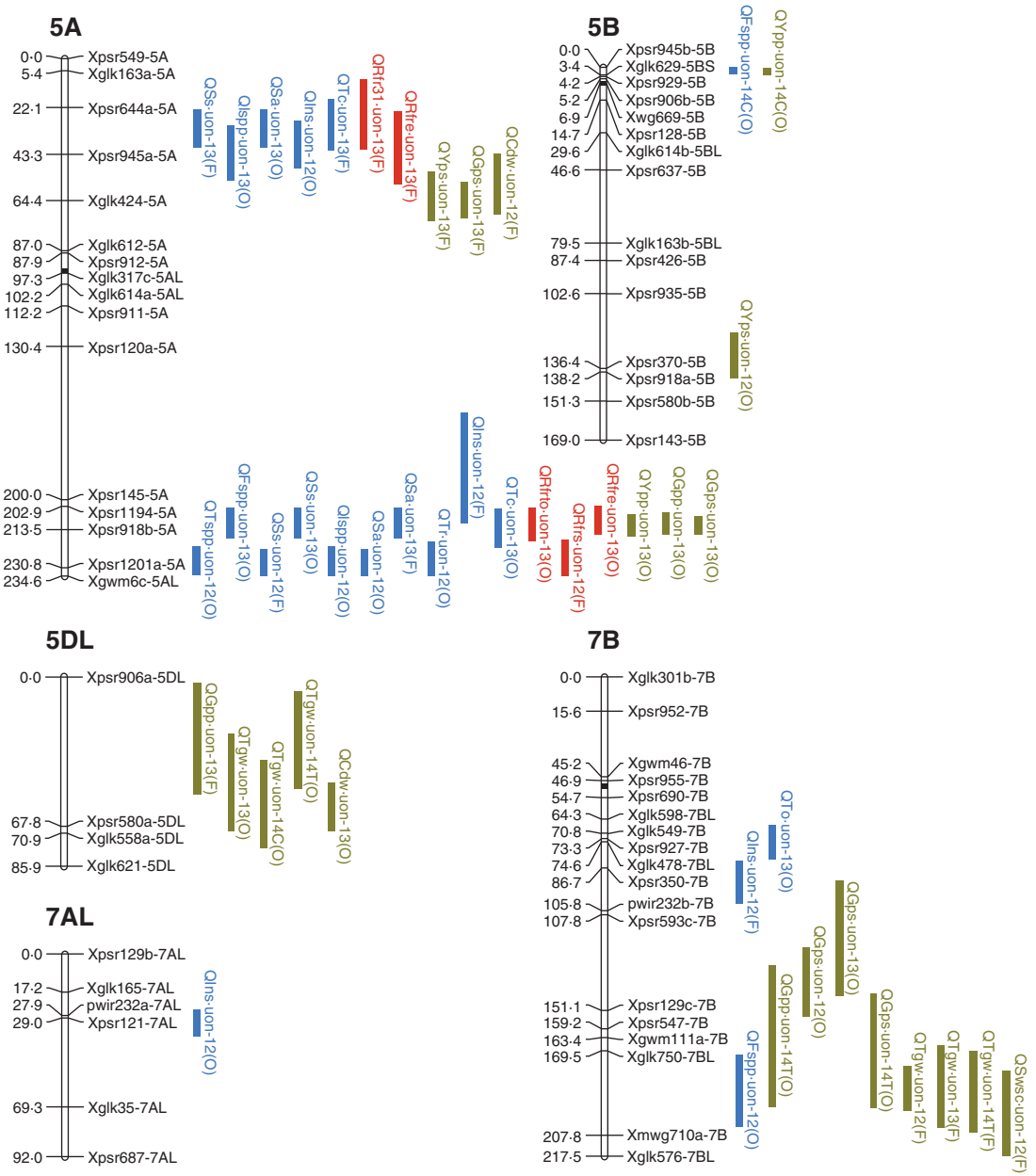

Fig. 3. Continued.

were stable across 3–4 environments. In the glasshouse experiment, one QTL for yield per shoot, two for grains per plant, four for grains per shoot and one for TGW were identified only under the shading treatment, indicating that they may be involved in shade responses. In terms of carpel size and stem WSC content at anthesis, four and three QTL were detected, respectively, individually explaining 16·4–27·5 % of the phenotypic variation (Fig. 3 and Table 2).

The QTL coincidences between tillering traits and yield components were found on seven chromosomes (1A, 2D, 3A, 4A, 5A, 5B and 7B) (Fig. 3). One QTL for total shoots per plant was coincident with one for each of yield and grains per plant as well as grains per shoot on 5A, with their increasing alleles conferred by 'Oberkulmer'. Likewise, eight QTL for fertile shoots per plant and shoot survival were coincident with those for yield and grains per plant, and grains per shoot on 3A, 4A, 5A, 5B and 7B; their increasing alleles were provided by the same parents. In contrast, four QTL for fertile shoots per plant and shoot survival were also coincident with eight QTL for TGW on 1A, 4A and 7B, but their increasing alleles were provided by the opposite parents, confirming the negative relationships between them. A further analysis showed that three QTL for carpel size and two for stem WSC content at anthesis were coincident with 11 QTL for TGW on 3B, 4A, 5DL and 7B, with the increasing alleles provided by the same parents; additionally, one QTL for carpel size and two for stem WSC content were coincident with two QTL for fertile shoots per plant on 4A and 7B, with the increasing alleles conferred by the

TABLE 2. *Quantitative trait loci (QTL) for tillering, red/far-red ratio (R:FR) and yield components in the 'Forno' × 'Oberkulmer' mapping population*

Trait/chromosome	Year*	Position (cM)	LOD	R^2†	Additive effect‡	Closest marker
Tillering						
Total shoots per plant						
5A	2012	229·5	3·68	21·5	−0·7	*Xpsr1201a-5A*
Fertile shoots per plant						
1A	2012	88·9	3·28	19·4	0·2	*Xpsr1201b-1A*
4A	2012	30·2	3·13	18·6	0·2	*Xpsr59a-4A*
	2012	219·2	3·56	20·9	−0·2	*Xpsr115-4A*
5A	2013	209·9	5·85	21·7	−0·8	*Xpsr918b-5A*
5B	2014C	0·1	3·42	13·3	−0·4	*Xpsr945b-5B*
7B	2012	188·5	3·45	20·3	−0·2	*Xmwg710a-7B*
Shoot survival (%)						
3A	2013	124·5	3·28	12·8	5·1	*Xglk652a-3AL*
4A	2012	8·0	3·33	19·7	5·2	*Xgwm397-4A*
5A	2012	230·8	2·99	17·9	4·6	*Xpsr1201a-5A*
	2013	32·1	5·29	19·9	6·3	*Xpsr644a-5A*
	2013	209·9	6·12	22·6	−6·4	*Xpsr918b-5A*
Infertile shoots per plant						
5A	2012	231·8	3·62	21·2	−0·6	*Xpsr1201a-5A*
	2013	37·1	5·49	20·5	−0·5	*Xpsr945a-5A*
Shoot abortion (%)						
3A	2013	124·5	3·28	12·8	−5·1	*Xglk652a-3AL*
4A	2012	8·0	3·33	19·7	−5·2	*Xgwm397-4A*
5A	2012	230·8	2·99	17·9	−4·6	*Xpsr1201a-5A*
	2013	32·1	5·29	19·9	−6·3	*Xpsr644a-5A*
	2013	209·9	6·12	22·6	6·4	*Xpsr918b-5A*
Tillering rate (tillers °Cd^{-1})						
5A	2012	228·5	3·28	19·4	−0·0016	*Xpsr1201a-5A*
Initial shoots per plant						
2D	2012	55·9	3·53	7·8	0·3	*Xpsr335-2D*
3A	2012	119·5	4·78	10·4	−0·2	*Xglk577-3AL*
3B	2012	38·9	3·51	7·8	0·1	*Xglk538-3BS*
4A	2012	213·2	4·24	9·3	−0·2	*Xpsr115-4A*
4B	2012	91·8	3·54	7·8	0·1	*Xpsr584-4B*
5A	2012	35·1	5·88	12·7	−0·2	*Xpsr945a-5A*
	2012	205·9	4·59	10·0	0·2	*Xpsr1194-5A*
7AL	2012	27·9	3·18	7·1	−0·1	*pwir232a-7AL*
7B	2012	91·7	2·84	6·3	0·1	*Xpsr350-7B*
Time at tillering onset (°Cd)						
7B	2013	75·6	3·24	12·7	−12	*Xglk478-7BL*
Time at tillering cessation (°Cd)						
3A	2013	89·4	3·29	12·9	14	*Xglk645-3AL*
4B	2012	8·0	3·10	18·4	72	*Xglk348a-4BS*
5A	2013	30·1	3·35	13·1	15	*Xpsr644a-5A*
	2013	210·9	3·67	14·2	−15	*Xpsr918b-5A*
R:FR						
R:FR at tillering onset						
5A	2013	211·9	3·37	13·2	−0·02	*Xpsr918b-5A*
R:FR at GS31 (onset of stem elongation)						
5A	2013	31·1	3·59	13·9	0·02	*Xpsr644a-5A*
Stabilized R:FR						
4A	2012	9·3	3·13	18·6	0·01	*Xgwm397-4A*
5A	2012	225·5	4·46	25·5	0·02	*Xpsr1201a-5A*
Time at the end of R:FR reduction (°Cd)						
2A	2012	125·7	2·59	15·7	91	*Xglk699b-2AL*
5A	2013	35·1	3·45	13·5	15	*Xpsr945a-5A*
	2013	208·9	4·73	17·9	−16	*Xpsr918b-5A*
Yield components						
Yield per plant (g)						
3A	2013	124·5	3·26	12·8	1·37	*Xglk652a-3AL*
5A	2013	211·9	6·94	25·2	−1·76	*Xpsr918b-5A*
5B	2014C	1·0	3·15	12·3	−0·80	*Xpsr945b-5B*
Yield per shoot (g)						
2D	2014T	40·9	3·60	14·0	−0·22	*Xpsr933b-2D*
4DL	2012	37·2	3·15	18·7	−0·15	*Xgwm194-4DL*
5A	2013	63·3	3·38	13·2	0·10	*Xglk424-5A*
5B	2012	136·4	3·30	19·5	−0·07	*Xpsr370-5B*

(Continued)

TABLE 2. *Continued*

Trait/chromosome	Year*	Position (cM)	LOD	R^2†	Additive effect‡	Closest marker
Grains per plant						
3A	2013	125·5	3·08	12·1	32	*Xglk652a-3AL*
4A	2014T	21·7	4·21	16·1	13	*Xpsr59a-4A*
5A	2013	209·9	7·74	27·7	−46	*Xpsr918b-5A*
5DL	2013	31·0	3·68	14·3	60	*Xpsr906a-5DL*
7B	2014T	156·1	4·24	16·3	−12	*Xpsr547-7B*
Grains per shoot						
2A	2014T	7·0	3·08	12·1	2	*Xpsr566c-2A*
2D	2013	45·9	4·69	17·8	−6	*Xpsr933b-2D*
	2014T	44·9	4·23	16·2	−5	*Xpsr933b-2D*
3B	2013	8·1	3·72	14·4	−3	*Lrk10c-3BS*
4A	2012	38·1	3·49	20·5	2	*Xpsr914-4A*
	2013	152·3	3·61	14·0	6	*Xglk354-4A*
	2014T	10·3	2·93	11·5	2	*Xgwm397-4A*
5A	2013	64·4	4·53	17·3	3	*Xglk424–5A*
	2013	213·5	5·10	19·2	−3	*Xpsr918b-5A*
7B	2012	138·8	3·15	18·7	−3	*Xpsr129c-7B*
	2013	128·8	3·09	12·1	−3	*Xpsr593c-7B*
	2014T	165·4	3·85	14·9	−2	*Xgwm111a-7B*
Thousand grain weight (g)						
1A	2012	80·1	3·57	20·9	−2·55	*Xpsr1327b-1A*
2A	2012	94·9	3·47	20·4	−2·50	*Xpsr919b-2A*
	2013	133·7	3·54	13·8	−2·00	*Xglk699b-2AL*
	2014C	133·7	2·98	11·7	−2·01	*Xglk699b-2AL*
	2014T	143·4	3·52	13·7	−1·60	*PL_AP-2A*
3B	2012	80·5	4·73	26·8	2·97	*Xpsr1054-3B*
	2013	2·9	3·44	13·4	1·60	*C970a-3B*
	2013	80·5	5·11	19·3	1·91	*Xpsr1054-3B*
	2014C	100·5	3·39	13·2	2·90	*Xpsr1054-3B*
	2014T	78·3	3·02	11·9	1·62	*Xpsr1054-3B*
4A	2012	20·7	7·17	37·6	−3·95	*Xglk315-4AS*
	2013	31·2	4·76	18·1	−2·09	*Xpsr59a-4A*
	2014C	32·2	4·10	15·8	−2·13	*Xpsr59a-4A*
	2014T	34·2	7·93	28·2	−2·61	*Xpsr914-4A*
5DL	2013	45·0	3·88	15·0	−3·17	*Xpsr580a-5DL*
	2014C	58·0	5·54	20·7	−3·05	*Xpsr580a-5DL*
	2014T	32·0	4·17	16·0	−3·62	*Xpsr906a-5DL*
7B	2012	187·5	5·97	32·5	4·64	*Xglk750-7BL*
	2013	187·5	3·24	12·7	2·24	*Xglk750-7BL*
	2014T	189·5	3·15	12·4	2·27	*Xmwg710a-7B*
Carpel dry weight at anthesis (mg)						
3B	2013	0·1	4·37	16·7	0·03	*Xglk683-3BS*
4A	2012	27·2	3·46	20·4	−0·06	*Xpsr59a-4A*
5A	2012	56·3	3·78	22·0	0·07	*Xglk424-5A*
5DL	2013	67·8	4·29	16·4	−0·03	*Xpsr580a-5DL*
Stem water soluble carbohydrate dry weight at anthesis (g)						
3DL	2012	23·0	3·49	20·5	0·069	*Xpsr1203b-3DL*
4A	2012	27·2	4·88	27·5	−0·069	*Xpsr59a-4A*
7B	2012	192·5	3·51	20·6	0·080	*Xmwg710a-7B*

* 2012 and 2013: field experiments; 2014: glasshouse experiment (C, control; T, shading treatment).

† The proportion of phenotypic variation explained by individual QTL.

‡ Positive additive effects indicate that the alleles from 'Forno' increase the values of the traits, whereas negative additive effects indicate that the alleles from 'Oberkulmer' increase the values of the traits.

opposite parents. There was no QTL coincidence between total and fertile shoot number, nor yield per shoot; only one QTL for shoot survival was coincident with one for yield per shoot on 5AS, with the increasing alleles conferred by 'Forno'. These results agree with the above physiological relationships between tillering and yield components: more total and fertile shoots, and higher shoot survival, were associated with higher yield and grain number per plant without reducing those of individual shoots; however, more fertile shoots and higher shoot survival were associated with reduced TGW because of smaller carpels and less stem WSC per shoot.

DISCUSSION

Large variation in tillering dynamics and its genetic control

Significant variation in tillering traits between genotypes has been observed in the present and previous studies (Ishag and Taha, 1974; Hucl and Baker, 1989; Sharma, 1995; Berry *et al.*, 2003; Dreccer *et al.*, 2013). Thus, it is possible to optimize wheat tillering patterns by genetic selection. A major target of tillering optimization is to increase fertile shoot number per plant, an important component of grain number to enlarge sink size. Fertile shoots per plant was positively associated with total

TABLE 3. *Correlations between tillering traits and red/far-red ratio (R:FR) in the mapping population of 'Forno' and 'Oberkulmer'*

Tillering trait	$R:FR_{to}$		$R:FR_{tc}$		$R:FR_{31}$		$R:FR_{s}$		$R:FR_{or}$		$R:FR_{er}$	
	2012	2013	2012	2013	2012	2013	2012	2013	2012	2013	2012	2013
Total shoots per plant	0·12	0·33**	−0·55**	−0·34**	−0·59**	0·11	−0·59**	0·08	−0·22	0·03	0·06	0·31**
Fertile shoots per plant	−0·04	0·43**	−0·30**	−0·22*	−0·11	0·45**	−0·24*	0·30**	−0·12	−0·01	0·15	0·60**
Shoot survival	0·08	0·38**	0·40**	−0·05	0·59**	0·62**	0·50**	0·41**	0·16	−0·04	0·04	0·66**
Infertile shoots per plant	−0·10	−0·14	−0·50**	−0·21*	−0·62**	−0·52**	−0·56**	−0·33**	−0·19	0·06	0·01	−0·45**
Shoot abortion	−0·08	−0·38**	−0·40**	0·05	−0·59**	−0·62**	−0·50**	−0·41**	−0·16	0·04	−0·04	−0·66**
Tillering rate	−0·13	−0·18	−0·47**	0·12	−0·57**	0·03	−0·51**	0·06	−0·17	−0·03	0·03	0·18
Tillering onset	0·33**	0·10	−0·33**	0·02	0·18	0·25**	0·01	0·29**	0·09	0·04	0·21	0·37**
Tillering cessation	0·34**	0·44**	−0·30**	−0·61**	0·32**	0·49**	−0·04	0·30**	0·20	0·10	0·18	0·58**
Tillering duration	0·15	0·31**	−0·49**	−0·55**	0·10	0·24*	−0·04	0·04	0·08	0·06	−0·03	0·23*

$R:FR_{to}$, R:FR at tillering onset; $R:FR_{tc}$, R:FR at tillering cessation; $R:FR_{31}$, R:FR at GS31 (onset of stem elongation); $R:FR_{s}$, stabilized R:FR; $R:FR_{or}$, the time at the onset of R:FR reduction; $R:FR_{er}$, the time at the end of R:FR reduction.

* Significant at $P < 0·05$, ** significant at $P < 0·01$.

shoots per plant, tillering rate, and the time for tillering onset and cessation, indicating that genetic selection for delayed but fast tillering, and high tillering capacity, can result in more fertile shoots. An additional strategy to increase fertile shoot number is to improve tiller survival. The present study showed that only 55 % of the total shoots initiated produced spikes, and there was large variation in shoot survival among the RILs (31–87 %). This variation has been demonstrated in several studies, for example 37–68 % by Berry *et al.* (2003) and 70–93 % by Sharma (1995), suggesting an opportunity to select genotypes with high shoot survival for more spikes. Likewise, only about half of the florets initiated within spikelets set grains, and the remaining ones (mainly those at distal positions) are aborted just before anthesis (Kirby, 1988; González-Navarro *et al.*, 2015). Floret fertility has been known to largely determine grains per shoot at maturity, the other key component of grain number per unit land area (González *et al.*, 2011). It has been found that shoot and floret fertility respond to the availability of environmental resources such as nutrients and radiation (Ishag and Taha, 1974; Fischer and Stockman, 1980; Thorne and Wood, 1987; Alzueta *et al.*, 2012), indicating plasticity. This attribute of wheat plants may play a crucial role in accommodating various environments and forming yield (Sadras and Rebetzke, 2013).

The QTL for tillering dynamics were reported here for the first time, except the trait of fertile shoots per plant, which has been widely studied (Kato *et al.*, 2000; Deng *et al.*, 2011; Naruoka *et al.*, 2011; Jia *et al.*, 2013; Zhang *et al.*, 2013). Most QTL for tillering dynamics were located on chromosomes 3A, 4A and 5A. The most important QTL cluster was detected on 5AL, where the alleles from the spelt 'Oberkulmer' were associated with increased total, fertile and infertile shoot number, accelerated tillering rate, and delayed tillering onset and cessation. In a single-chromosome (spelt 5A) recombinant line mapping population, Kato *et al.* (2000) also mapped a QTL for fertile tiller number per plant at this location. Another QTL coincidence for fertile shoots per plant and shoot survival was found in the distal region of 4AS, where the QTL for tillers per plant was identified in a previous study (Jia *et al.*, 2013). In addition, the present study revealed a QTL for initial shoots per plant on 2D, corresponding to the *Ppd-D1* gene, indicating that the photoperiod response gene probably regulates the progress of tillering (Borras-Gelonch *et al.*, 2012). Two QTL for fertile shoots per plant were coincident with those for total shoots per plant, shoot survival, tillering rate, and tillering onset and cessation, and their increasing alleles were conferred by the same parents. This is in line with the above conclusion that more fertile shoots per plant can be achieved by increasing tillering capacity and survival, accelerating tillering rate, and delaying tillering onset and cessation. Although many QTL for tillering were identified here, none of them was stable over years, indicating the genetic complexity of the tillering process and the important roles of environmental factors such as shade, as discussed below. Future work is needed to dissect genetic elements for tillering per se and those responding to environmental cues. The QTL presented in this study provide an initial step for this purpose.

Low R:FR inhibits tiller production, and increases tiller abortion

It seems that wheat plants can sense R:FR at an early stage of tillering. Low R:FR at the beginning of tillering was associated with fewer total shoots per plant, as confirmed in the thinning experiment, indicating an inhibition of tiller production. Detailed analysis showed that low R:FR did not reduce tillering rate, but led to early tiller cessation. The same results have been observed with the treatments of low R:FR, far-red light, shade or high plant density (Evers *et al.*, 2006; Sparkes *et al.*, 2006; Ugarte *et al.*, 2010; Toyota *et al.*, 2014). The threshold of R:FR for tillering cessation in the field was on average 0·20, similar to that of previous reports (0·20–0·40) (Evers *et al.*, 2006; Sparkes *et al.*, 2006; Dreccer *et al.*, 2013). However, significant variation in this trait among the RILs (0·07–0·37) was also determined, suggesting genetic difference in the tolerance of tiller bud outgrowth to low R:FR. This difference has previously been reported between the *tiller inhibition* (*tin1*) lines and free-tillering lines. The *tin1* lines become more sensitive to light quality (0·18–0·22), compared with the free-tillering lines (0·09–0·11) (Moeller *et al.*, 2014). The *tin1* gene appears to be involved in the perception of R:FR. This gene inhibits tiller bud outgrowth by limiting sugar supply due to precocious internode development (Kebrom *et al.*, 2012). Early stem elongation can be induced by low R:FR, as shown in the present study. Therefore, it can be hypothesized that a low R:FR promotes the onset of stem elongation, leading to assimilate deprivation from

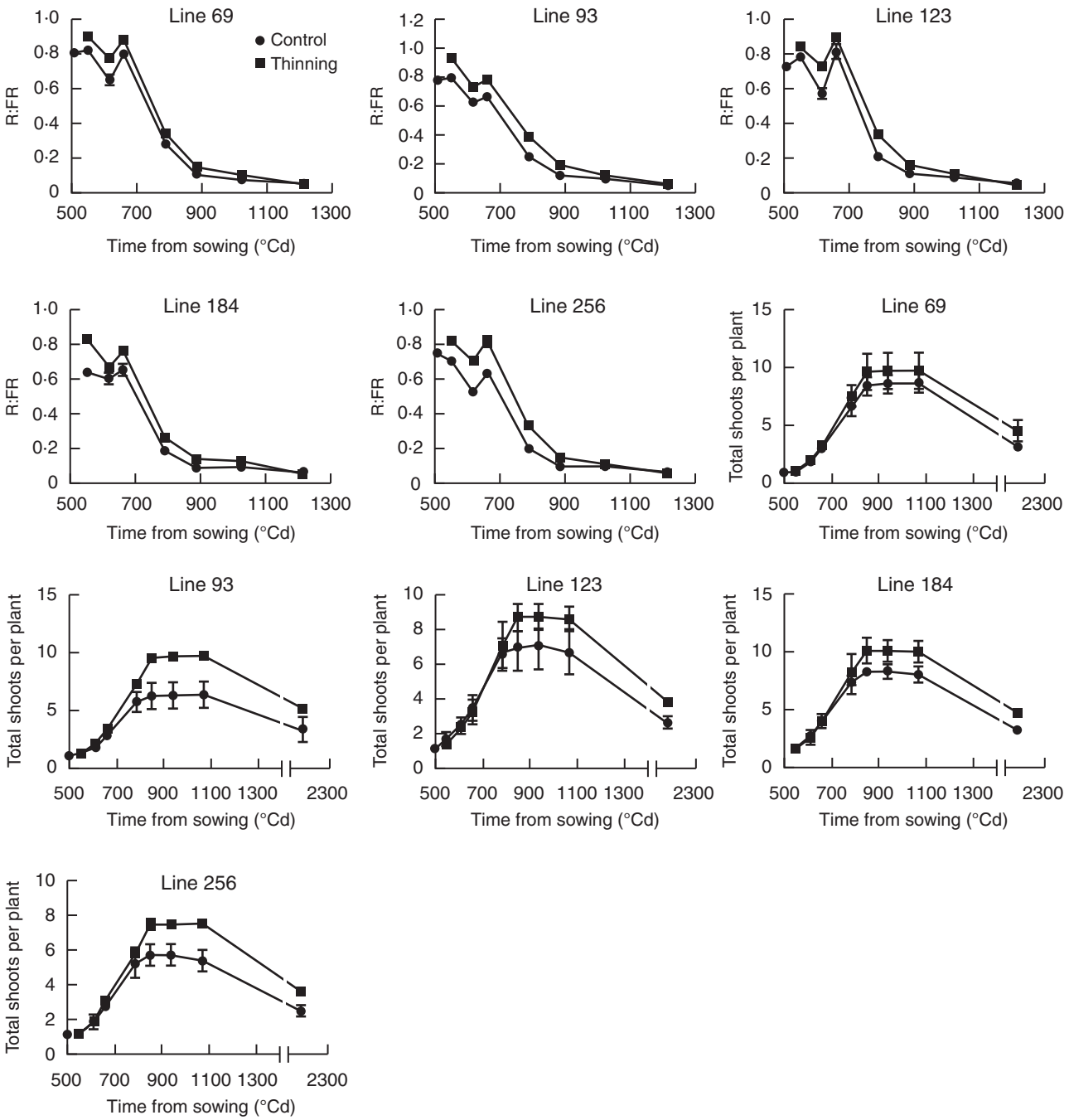

FIG. 4. Dynamics of tillering and red/far-red ratio (R:FR) in the control (circles) and thinned (squares) lines. Values of shoot number per plant and R:FR at each time point are shown as mean ± standard error of the mean (bars). The last count, representing the fertile shoot number per plant, was taken at late grain filling.

growing tiller buds and, in turn, bud dormancy. The *tin1* mutants respond to low R:FR earlier, and start stem elongation earlier, resulting in earlier cessation of axillary tiller bud outgrowth, fewer buds growing out and hence fewer total tillers. Thus, R:FR may function as a direct signal inhibiting tillering by inducing stem elongation in the *tin1* lines. This model can also be used to explain the coincidence between tillering cessation and the onset of stem elongation in the present and previous studies (Baker and Gallagher, 1983; Gomez-Macpherson *et al*., 1998; Sylvester-Bradley *et al*., 2008).

The results showed that low R:FR not only inhibits tiller bud outgrowth, but also promotes the abortion of young tillers initiated, which extends our understanding of the effect of low R:FR. The underlying mechanism is not yet clear. Tiller death normally starts from the onset of stem elongation, and ends around flowering (Sylvester-Bradley *et al*., 2008). During this period, stems and spikes are growing rapidly, suggesting source limitation (Gomez-Macpherson *et al*., 1998; González *et al*., 2011). More carbohydrates have to be diverted to these expanding sinks, leading to a shortage for developing young tillers

TABLE 4. *Thinning effects on tillering and red/far-red ratio (R:FR)*

Trait	Mean across five lines ($n = 3$)		*P*-value (n.s., not significant; $*P < 0{\cdot}05$; $**P < 0{\cdot}01$)			Thinning effect (%)
	Control	Thinning	Treatment	Line	Treatment × line	
Total shoots per plant	7·2	9·4	**	n.s.	n.s.	+ 31
Fertile shoots per plant	3·0	4·4	**	n.s.	n.s.	+ 47
Shoot survival (%)	42·2	47·1	*	*	n.s.	+ 12
Infertile shoots per plant	4·2	5·0	n.s.	*	n.s.	n.s.
Shoot abortion (%)	57·8	52·9	*	*	n.s.	–8
Tillering rate (tillers °Cd^{-1})	0·024	0·027	n.s.	n.s.	n.s.	n.s.
Tillering onset (°Cd)	580	590	n.s.	n.s.	n.s.	n.s.
Tillering cessation (°Cd)	789	838	**	n.s.	n.s.	+6
Tillering duration (°Cd)	210	248	n.s.	n.s.	n.s.	n.s.
R:FR at tillering onset	0·71	0·83	**	*	n.s.	+17
R:FR at tillering cessation	0·25	0·20	n.s.	n.s.	n.s.	n.s.
Onset of stem elongation (°Cd, GS31)	882	930	**	**	n.s.	+5
R:FR at GS31	0·10	0·12	n.s.	n.s.	n.s.	n.s.
End of R:FR reduction	832	854	**	**	*	+3
Stabilized R:FR	0·08	0·09	n.s.	n.s.	n.s.	n.s.

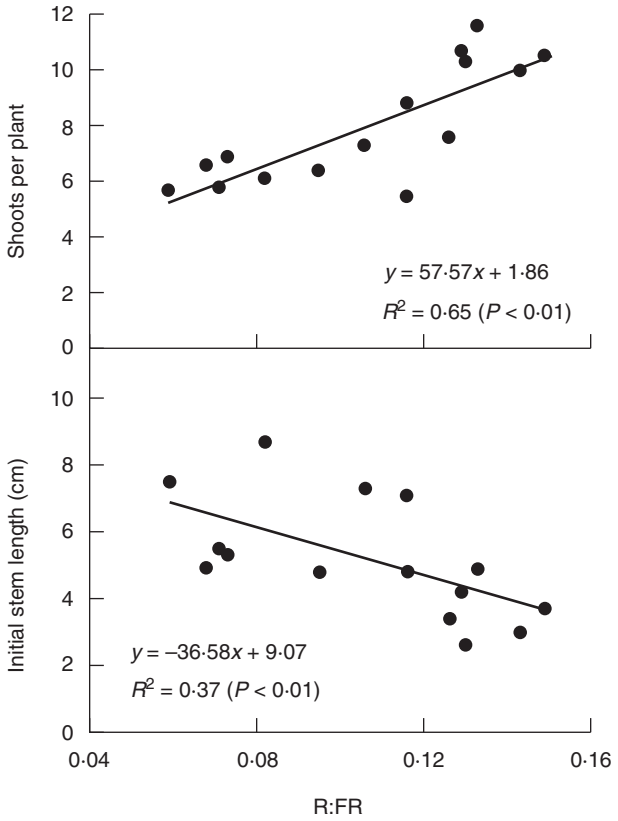

FIG. 5. Relationships between red/far-red ratio (R:FR), shoots per plant and initial stem length around the onset of stem elongation.

and, in turn, tiller death (Gomez-Macpherson *et al.*, 1998). On the other hand, a release in intraplant competition by increasing resource availability such as radiation improves tiller survival (Thorne and Wood, 1987). In this study, it was found that low R:FR was associated with early stem elongation and taller plants at anthesis. These responses have been well known as part of the shade avoidance syndrome in many other species, involved in phytochrome perception (mainly PHYB) and hormonal regulation (Gommers *et al.*, 2013; Rameau *et al.*, 2015). Therefore, low R:FR may increase stem sink, and intensify intraplant competition indirectly; as a result, tiller abortion is enhanced.

To improve tiller survival, the genotypes with either high tolerance to shade or well-established light environment can be selected. Genetic variation in shade tolerance has been determined in this study. For the latter, light quality under a canopy is a complex trait, depending on plant architecture, such as leaf characteristics (number, size, thickness, insertion angle, shape, stiffness and colour) and plant height. Redesigning these traits using a 3-D imaging and modelling method may improve the light environment at the bottom of the canopy.

Increasing fertile shoot number while maintaining other yield components

Fertile shoot number per plant largely contributed to plant productivity, confirming its role as a key yield determinant (Sharma, 1995; Kato *et al.*, 2000). This resulted from an increase in grain number per plant, rather than individual grain weight. A close look revealed that more fertile shoots did not significantly reduce yield and grains per shoot, as seen in previous studies (Kato *et al.*, 2000; Jia *et al.*, 2013); there was even a positive relationship between fertile shoots per plant and grains per shoot. In full sunlight, fertile shoots per plant were only negatively associated with individual grain weight, as supported by analyses of the QTL coincidences and allelic effects. Grains develop from the carpels growing mainly between booting and anthesis, and carpel size at anthesis has been considered as an upper limit to grain weight (Calderini *et al.*, 1999). Another pre-anthesis trait affecting grain weight is stem WSC remobilized into grains during grain filling (van Herwaarden *et al.*, 1998). Each of these two traits was positively associated with grain weight in this study, consistent with the results of QTL analysis, confirming their roles as grain weight determinants. Carpel growth and stem WSC accumulation concur with tiller death and final tiller formation before anthesis. More fertile shoots produced was associated with smaller carpels and less stem WSC. Genetic analysis showed the QTL coincidences

TABLE 5. *Shading effects on fertile shoot number and other yield components*

Trait	Mean across 112 lines ($n = 3$)		P-value (n.s., not significant; *$P < 0{\cdot}05$; **$P < 0{\cdot}01$)			Shading effect (%)
	Control	Shading	Treatment	Line	Treatment × line	
Fertile shoots per plant	5·1	4·5	**	**	n.s.	–12
Yield per plant (g)	7·60	5·23	**	**	n.s.	–31
Yield per shoot (g)	1·47	1·21	**	**	n.s.	–18
Grains per plant	162	111	**	**	n.s.	–31
Grains per shoot	31	25	**	**	n.s.	–19
Thousand grain weight (g)	47·4	47·8	n.s.	**	n.s.	n.s.

TABLE 6. *Correlations between tillering traits and yield components in the mapping population of 'Forno' and 'Oberkulmer'*

(A) Field

Tillering trait	Yield per plant		Yield per shoot		Grains per plant		Grains per shoot		Thousand grain weight	
	2012	2013	2012	2013	2012	2013	2012	2013	2012	2013
Total shoots per plant	0·31**	0·69**	−0·07	−0·12	0·26*	0·67**	−0·11	−0·01	0·01	−0·16
Fertile shoots per plant	0·70**	0·94**	−0·07	0·04	0·80**	0·93**	0·20	0·20*	−0·28*	−0·26**
Shoot survival	0·15	0·81**	0·04	0·17	0·27*	0·82**	0·26*	0·33**	−0·20	−0·27**
Infertile shoots per plant	0·09	−0·36**	−0·05	−0·25**	−0·01	−0·39**	−0·20	−0·33**	0·12	0·15
Shoot abortion	−0·15	−0·81**	−0·04	−0·17	−0·27*	−0·82**	−0·26*	−0·33**	0·20	0·27**
Tillering rate	0·25*	0·44**	−0·03	−0·06	0·20	0·41**	−0·07	−0·02	0·01	−0·05
Tillering onset	0·47**	0·37**	0·24*	−0·04	0·41**	0·40**	0·23*	0·12	0·05	−0·24**
Tillering cessation	0·31**	0·64**	0·19	0·08	0·21	0·64**	0·07	0·19*	0·16	−0·20*
Tillering duration	−0·15	0·28**	−0·05	0·10	−0·19	0·25**	−0·14	0·08	0·08	0·00

(B) Glasshouse (2014)

	Control	Shading	Control	Shading	Control	Shading	Control	Shading	Control	Shading
Fertile shoots per plant	0·71**	0·54**	0·11	−0·46**	0·76**	0·53**	0·22*	−0·40**	−0·16	−0·12

* Significant at $P < 0{\cdot}05$, ** significant at $P < 0{\cdot}01$.

TABLE 7. *Correlations between carpel size and stem water soluble carbohydrates (WSC) at anthesis, thousand grain weight and fertile shoots per plant at maturity in the mapping population of 'Forno' and 'Oberkulmer'*

Trait	Thousand grain weight		Fertile shoots per plant	
	2012	2013	2012	2013
Carpel dry weight	0·46**	0·34**	−0·31**	−0·19*
Stem WSC	0·55**	0·20*	−0·52**	−0·16

* Significant at $P < 0{\cdot}05$, ** significant at $P < 0{\cdot}01$.

between fertile shoots per plant, carpel size and stem WSC content on chromosomes 4A and 7B, indicating that the negative relationships between them at least partly result from the pleiotropic effects or tight gene linkages. To break the negative relationships, these genes may be excluded, and/or more independent ones have to be added; at the same time, leaf photosynthesis and soil nutrient supply during the pre-anthesis period should be improved to increase source availability.

CONCLUSIONS

This study describes the tillering dynamics of wheat in detail, and its genetic and environmental control. Large genetic variation in tillering traits was determined, and it is proposed that the genotypes with higher tillering capacity, faster tillering rate, delayed tillering onset and cessation, and higher tiller survival can be selected to increase fertile shoot number. Based on this variation, the QTL for tillering traits were identified, and QTL coincidence analysis agrees with the above proposition for fertile shoot improvement. R:FR has significant effects on tillering: low R:FR generated from neighbouring plants inhibits tiller production by accelerated tillering cessation, and promotes infertile tillers and tiller abortion, probably resulting from an assimilate shortage due to early stem elongation and enhanced stem growth induced by low R:FR. A few QTL for R:FR kinetics in the field were also detected. After these processes, final shoot number is defined. More fertile shoots at maturity contribute to plant yield and grain number, without reducing single-shoot productivity and grain set. However, this is accompanied by a slight decrease in individual grain weight, partly as an outcome of reduced carpel size and stem WSC content at anthesis. Therefore, this study improves our knowledge of the genetic and environmental determination of the tillering process and, in turn, grain yield formation in wheat.

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