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Identifying seedling root architectural traits associated with yield and yield components in wheat

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• **Background and Aims** Plant roots growing underground are critical for soil resource acquisition, anchorage and plant–environment interactions. In wheat (*Triticum aestivum*), however, the target root traits to improve yield potential still remain largely unknown. This study aimed to identify traits of seedling root system architecture (RSA) associated with yield and yield components in 226 recombinant inbred lines (RILs) derived from a cross between the bread wheat *Triticum aestivum* 'Forno' (small, wide root system) and spelt *Triticum spelta* 'Oberkulmer' (large, narrow root system).
• **Methods** A 'pouch and wick' high-throughput phenotyping pipeline was used to determine the RSA traits of 13-day-old RIL seedlings. Two field experiments and one glasshouse experiment were carried out to investigate the yield, yield components and phenology, followed by identification of quantitative trait loci (QTLs).
• **Key Results** There was substantial variation in RSA traits between genotypes. Seminal root number and total root length were both positively associated with grains m^{-2}, grains per spike, above-ground biomass m^{-2} and grain yield. More seminal roots and longer total root length were also associated with delayed maturity and extended grain filling, likely to be a consequence of more grains being defined before anthesis. Additionally, the maximum width of the root system displayed positive relationships with spikes m^{-2}, grains m^{-2} and grain yield. Ten RILs selected for the longest total roots exhibited the same effects on yield and phenology as described above, compared with the ten lines with the shortest total roots. Genetic analysis revealed 38 QTLs for the RSA, and QTL coincidence between the root and yield traits was frequently observed, indicating tightly linked genes or pleiotropy, which concurs with the results of phenotypic correlation analysis.
• **Conclusions** Based on the results from the Forno × Oberkulmer population, it is proposed that vigorous early root growth, particularly more seminal roots and longer total root length, is important to improve yield potential, and should be incorporated into wheat ideotypes in breeding.

Key words: Phenology, QTL, root, spelt, *Triticum aestivum*, *Triticum spelta*, wheat, yield.

INTRODUCTION

The roots, known as 'the hidden half' growing underground, are of fundamental importance to plant growth. For example, the roots acquire water and nutrients from soil, and can function as a storage site for nutrients (Kutman *et al*., 2012; Smith and De Smet, 2012; Gaju *et al*., 2014). They provide the anchorage for plants against lodging, and serve as a major interface sensing, and interacting with, biotic and abiotic factors in the rhizosphere (Smith and De Smet, 2012). An efficient root system is even more crucial for crops grown in poor soils, where water and nutrients are in short supply (Lynch, 2007). In addition, improving rooting efficiency could decrease the input of chemical fertilizers into the agroecosystem, leading to a reduction of both greenhouse gas emissions incurred during their production and the eutrophication of the natural environments from their application (White *et al*., 2013).

In wheat (*Triticum aestivum*), there are two types of roots: the seminal roots and the nodal roots (Kirby, 2002). While germinating, the radicle bursts through the coleorhiza of the seed, and then 3–6 other seminal roots emerge. Each seminal root has a root cap, followed sequentially by the zones of division, elongation and maturation. Numerous root hairs develop in the zone of maturation, and further back from the root hair zone the lateral roots grow from the stele. The seminal root system can reach 1·5–2·0 m in depth and support the plant throughout the crop's life (Sylvester-Bradley *et al*., 2008). Unlike the seminal roots growing directly from the seed, the nodal (crown or adventitious) roots arise from the stem basal nodes and begin to extend when tillering starts. These roots are usually thicker than the seminal roots, and appear relatively horizontally. At anthesis, the whole root system consisting of seminal and nodal roots in the field reaches a maximum: approx. 17 km m^{-2} in root density (sample depth: 0–100 cm) and 0·8 t ha^{-1} in root dry weight, and this varies with genotype, sowing date, temperature, soil structure and environmental stress (Sylvester-Bradley *et al*., 2008; White *et al*., 2015). Roots then senesce during grain filling, though some in the deeper soil layers may continue growing.

The root system of wheat is complex, and a series of quantitative traits have been used to describe the root spatial configuration in soil, namely root system architecture (RSA). The architectural traits include root number and length, tip and emergence angles, rooting width and depth, convex hull area and root mass centre (Pound *et al.*, 2013; Atkinson *et al.*, 2015). However, quantifying these traits in the field is difficult since extraction of all intact roots from the soil is extremely laborious, time-consuming and expensive. Alternatively, a number of indoor approaches have been applied in practice, for instance using hydroponic culture (Ayalew *et al.*, 2015; Kabir *et al.*, 2015), gel-filled chambers (Manschadi *et al.*, 2008; Christopher *et al.*, 2013), germination bags (Robertson *et al.*, 1979), sand-filled pots (Waines and Ehdaie, 2007; Hamada *et al.*, 2012), soil-filled pots (Cao *et al.*, 2014), clear pots (Richard *et al.*, 2015), a germination paper-based 'pouch and wick' system (Atkinson *et al.*, 2015), a paper-based 'cigar roll' system (Li *et al.*, 2011; Bai *et al.*, 2013) and soil-filled columns scanned by X-rays (Gregory *et al.*, 2009). Coupled with digital image analysis software, some of these systems permit the fast measurement of RSA over a large number of genotypes, which have been used to investigate the diversity and genetics of RSA in wheat.

Root system architecture shows large genetic variation among different ploidy levels, between domesticated and wild forms, and within each of the diploids, tetraploids and hexaploids (Robertson *et al.*, 1979; Manschadi *et al.*, 2008; Ayalew *et al.*, 2015). Hexaploid wheat has longer roots than the other ploidy levels (Ayalew *et al.*, 2015); however, compared with the landraces, modern bread wheat cultivars have smaller root mass (Waines and Ehdaie, 2007), suggesting that the RSA needs to be optimized for further yield improvement, particularly under infertile soil conditions (Lynch, 2007). In addition, high-throughput phenotyping techniques also allow identification of the quantitative trait loci (QTLs) associated with the RSA. To date, about 339 QTLs have been detected for different root architectural traits, and distributed on all wheat chromosomes except 1D (Ren *et al.*, 2012; Bai *et al.*, 2013; Christopher *et al.*, 2013; Liu *et al.*, 2013; Atkinson *et al.*, 2015; Kabir *et al.*, 2015; Maccaferri *et al.*, 2016).

Although many studies have initiated the first step to understand wheat RSA *per se*, the root architectural traits that are associated with increased yield remain largely unknown. The aim of the current study is to identify desirable root architectural traits at the seedling stage which could be associated with yield and yield components in the field. A mapping population, including 226 recombinant inbred lines (RILs) derived from a cross between the bread wheat *Triticum aestivum* 'Forno' and spelt *Triticum spelta* 'Oberkulmer', was quantified for the seedling RSA traits by using a 'pouch and wick' high-throughput phenotyping pipeline. The yield and yield components as well as phasic development of the population were determined in two field experiments and one glasshouse experiment. The physiological and genetic (i.e. QTL co-location) relationships between the RSA and yield traits were then analysed.

MATERIALS AND METHODS

Plant materials

Spelt is a relative of bread wheat. A preliminary experiment showed that spelt has a larger, deeper root system and resultant higher nitrogen uptake efficiency compared with bread wheat. To introduce the genetic variation in root traits from spelt, a cross between the bread wheat cultivar 'Forno' and spelt landrace 'Oberkulmer' was made, and the subsequent F_5 RIL mapping population consisting of 226 lines was used in the present study (Messmer *et al.*, 1999). Forno and Oberkulmer are both winter types and were widely grown in Switzerland.

Root phenotyping of the RIL seedlings in growth chamber

A germination paper-based 'pouch and wick' phenotyping system, coupled with digital image analysis, was adopted to measure the root architectural traits in 2014 (Bonser *et al.*, 1996; Atkinson *et al.*, 2015). All 226 RILs, together with Forno and Oberkulmer, were arranged in a randomized complete block (RCB) design with 20 plants per line. Due to limited space within the growth chamber, the 20 plants of each RIL were grown in eight runs: three plants per RIL in each of Run 1, 2, 3, 5, 6 and 7, as well as one plant per RIL in each of Run 4 and 8. In each run, intact, uniform and representative seeds of each RIL were selected from the combined grain samples in 2013, and grown at the top of the growth pouches (see below), with the embryos pointing downwards.

The growth system consisted of the pouches and tanks filled with deionized water. Each growth pouch included two black polythene sheets (24 × 28 cm; Cransford Polythene Ltd, Suffolk, UK), two blue germination papers (24 × 30 cm; Anchor Paper Company, St Paul, MN, USA), a plastic rod (Acrylic Online, Hull, UK) and two 19 mm fold-back clips (Fig. 1A). One polythene sheet and one germination paper were attached to one side of the rod, and the other polythene sheet and germination paper to the other side, fixed with two fold-back clips. Two seeds were grown on a growth pouch (one each side), and there were four tanks with a total of 360 pouches, allowing a maximum of 720 plants to be screened per run. Each tank was filled with 3 L of deionized water at the beginning of the experiment, and the water level was topped up at 2 d intervals to ensure that the germination papers remained saturated throughout the experiment. The tanks were placed in an environmentally controlled growth chamber, with 12 h light [photosynthetically active radiation (PAR) = 240 μmol m^{-2} s^{-1} at the tank level, temperature = 17 °C] and 12 h dark (temperature = 13 °C).

The images of the vertically grown root systems (Fig. 1B, C) were taken 13 d after transferring them onto the germination papers, using a Nikon D5100 DSLR camera and modified copy stand. The images were then analysed using the semi-automated software RootNav V1.7.5 (Pound *et al.*, 2013). The parameters used in this study included: (1) seminal root number, excluding the lateral roots; (2) average seminal root length; (3) total root length, the total length of seminal and lateral roots for a given plant; (4) maximum width, the horizontal distance between the two widest points achieved by the root system for all depths; (5) maximum depth, the vertical distance from the base to the tip of the deepest seminal root; (6) width to depth ratio, the ratio of maximum width to maximum depth; (7) tip angle of seminal roots, the average angle of all seminal root tips relative to the vertical axis; and (8) emergence angle of seminal roots, the average angle of two outermost seminal root
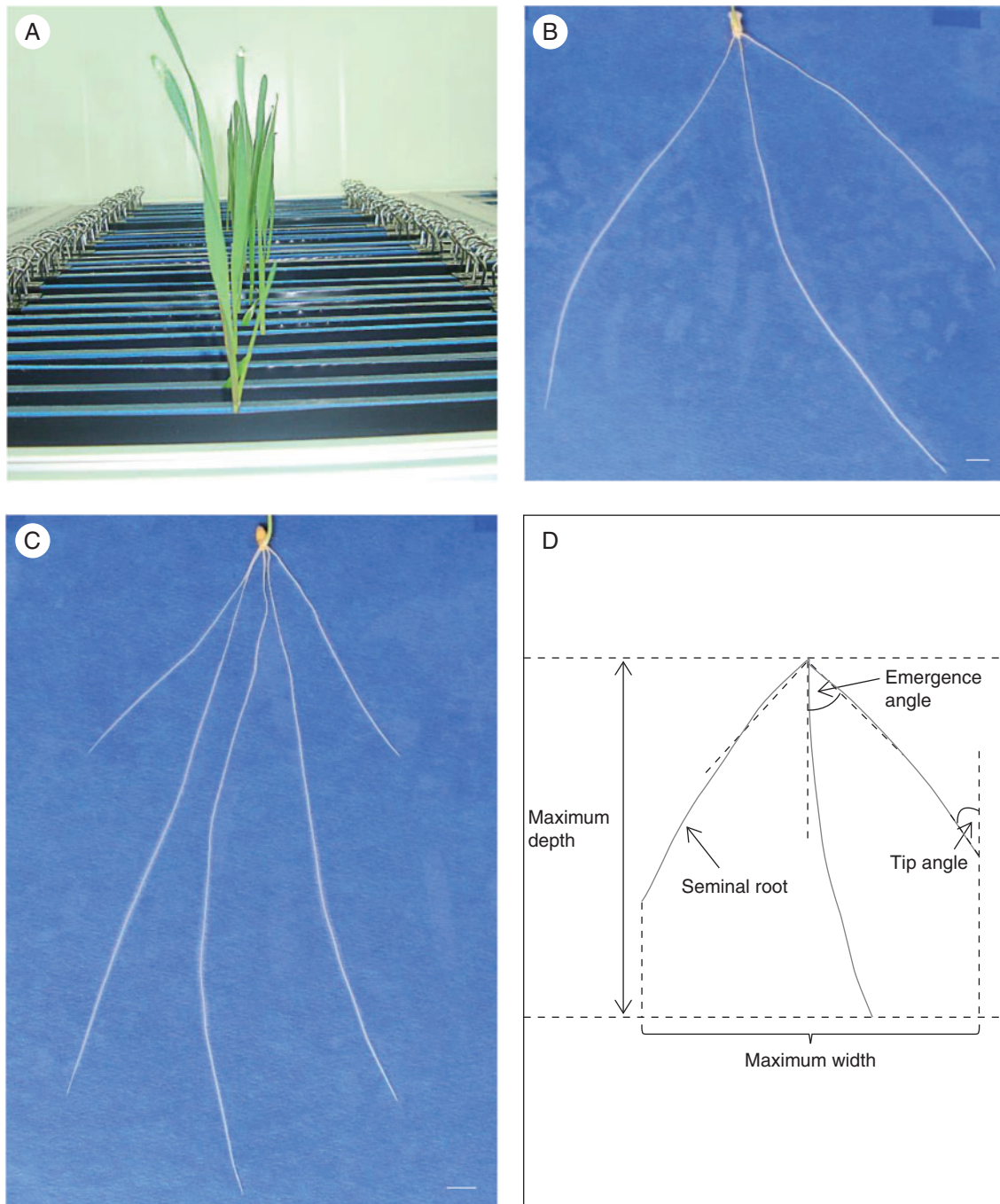
FIG. 1. Root phenotyping pipeline for the Forno × Oberkulmer mapping population grown in a growth chamber during 2014. (A) Germination paper-based 'pouch and wick' system. (B and C) Example root images of Forno and Oberkulmer, respectively (scale bar = 1 cm). (D) Measurement of root architectural traits on a recombinant inbred line.

bases (by 50 % of root length) relative to the vertical axis (Fig. 1D). Among these parameters, total root length was considered to represent the size of the root system, with seminal root number and average seminal root length as components, while width to depth ratio was used to represent the shape of the root system, with maximum width and depth as components.

Field experiments

Yield and yield components. The yield and yield components of the Forno × Oberkulmer mapping population were investigated in two seasons: 2011–2012 (referred to as 2012) and 2012–2013 (referred to as 2013). Field experiments were performed at the University of Nottingham Farm, Leicestershire, UK

(52°50′N, 1°15′W, 50 m a.s.l.). Data on the air temperature, rainfall and solar radiation at the growing sites during the two seasons were collected from the nearby meteorological station and are summarized in Supplementary Data Fig. S1, together with the historic weather data from the Met Office (http://www.metoffice.gov.uk/). Both field sites were planted with winter oats in the previous cropping seasons. The soil was a sandy loam, with the nutrient indices similar over two sites: N = 0, P = 5, K = 4 and Mg = 4 (pH 7·5). The 226 RILs and two parents were grown in an RCB design with three replicates. The seeds were sown in 6 × 1·6 m plots on 19 October 2011 and in 12 × 1·6 m plots on 31 October 2012, with a sowing density of 250 seeds m^{-2} and row width 0·125 m in both seasons. Nitrogen fertilizer was applied as ammonium nitrate (NH$_4$NO$_3$, 34·5 % N) in three splits: early tillering, onset of stem elongation and flag leaf emergence at 40, 40 and 60 kg N ha^{-1} in 2012, and 40, 60 and 60 kg N ha^{-1} in 2013, respectively. Plant growth regulators were applied to reduce the risk of lodging, and prophylactic crop protection chemicals were applied to minimize diseases, weeds and pests.

Two sub-sets comprising 72 RILs in 2012 and 110 RILs in 2013 (including the former 72 lines) were selected in the light of their considerable differences in yield traits but similar flowering time (± 4 d in 2010 and ± 1 d in 2011). The sub-sets were used to calculate spikes m^{-2}, grains per spike and biomass m^{-2} (in g), while machine-harvested grain yield (in t ha^{-1}), grains m^{-2} and thousand grain weight (TGW, in g) were determined for the whole population (226 RILs).

At maturity, the plants in an area of 0·5 × 0·5 m (four central rows) were collected from each plot in the sub-sets. After cutting at ground level, the infertile shoots (bearing no spikes) were discarded as they were negligible in terms of dry mass in all RILs. The spike number of fertile shoots was then counted, which was multiplied by four to generate the spikes m^{-2}. Subsequently, a sub-sample of approx. 70 g, with the fertile shoots counted, in 2012, and 20 fertile shoots in 2013, were obtained. For each sub-sample, the spikes were cut from these shoots, and the resultant spikes and stems were dried separately in an oven at 85 °C for 48 h. After weighing, above-ground biomass per shoot was calculated by dividing the sum of spike and stem dry weight by the fertile shoot number. The above-ground biomass m^{-2} was the biomass per shoot multiplied by the spikes m^{-2}. The dried spikes were threshed completely, and the total grain dry weight was recorded. The average grain weight of an individual spike was calculated by dividing the total grain weight by shoot number in the sub-sample. Next, individual grain weight was calculated by counting 250 grains and weighing, and grains per spike were the result of grain weight per spike divided by individual grain weight.

After sampling, the actual machine-harvested area of each of the 226 RILs was measured (namely A_c, in m^2). All the plots were then harvested with a combine harvester (2010, Sampo Rosenlew, Finland), and the combined grain samples were recorded for fresh weight (W_c, in g). Because of the non-free-threshing habit of spelt, the grain samples of many RILs could not be threshed completely. The machine-harvested grain yield, therefore, was derived from the threshability analysis. A sub-sample of 10 g was isolated randomly from each of the combined grain samples. Both threshed and unthreshed grains were counted by hand, recorded as N_{10}. Total grain number in that combined grain sample (N_c) was estimated as: $N_c = N_{10} \times (W_c/10)$, and grain number m^{-2} (N_{psm}) as: $N_{psm} = N_c/A_c$. In addition, another grain sub-sample of approx. 1 kg from each plot was partially threshed, and 200 grains were counted, oven-dried and weighed for TGW (W_{tg}). Then, machine-harvested yield (Y_m) was calculated as: $Y_m = N_c \times (W_{tg} \times 10^{-3}) \times 10^{-6}/(A_c \times 10^{-4})$.

Phasic development. In the sub-sets, the key growth stages (GSs) of each RIL were determined in both seasons according to the decimal code compiled by Tottman and Broad (1987). For GS31 (onset of stem elongation), five main shoots selected randomly in the central rows of a plot were opened to measure the length of the first internodes every 4 d. A RIL was considered to enter GS31 when ≥3 shoots had their first internodes ≥1 cm. GS39 (flag leaf emergence), GS61 (beginning of anthesis) and GS92 (physiological maturity) were the dates when 50 % of the main shoots in a plot showed fully expanded flag leaves, visible anthers and yellow peduncles, respectively, as observed on a daily basis. The durations between different growth stages were also derived, including GS31−GS39, GS39−GS61, GS31−GS61 and GS61−GS92. The calendar dates for phenology were then converted into accumulated thermal time (degree-days, °Cd), with a base temperature 0 °C.

Glasshouse experiment

The 110 RILs in the sub-set, together with two parents, were grown in the glasshouse in 2013 − 2014 (referred as 2014) to investigate two key yield components: grains per spike and TGW. The seeds were sown in modular trays on 17 December 2013 for germination, followed by vernalization at 6 °C for 9 weeks. The plants were then individually transferred into 1 L pots, filled with a loam compost (No. 3, John Innes, Norwich, UK). The experiment was conducted in an RCB design with three replicates, and the blocks were arranged to account for the variation in PAR along the glasshouse. The plants were grown under natural daylight, watered every other day, and fed with 40 kg N ha^{-1} at early stem elongation. At maturity, the spikes of each plant were harvested, counted and threshed by hand. The grains were then dried at 85 °C for 48 h, weighed and counted to calculate grains per spike and TGW.

Statistical analysis of phenotypic data

Statistical analysis was performed using Genstat v17 and GraphPad Prism v6. Analysis of variance, multiple comparisons [Fisher's unprotected least significant difference (LSD)] and Student's *t*-test were applied to determine the significant differences between the parents and between the RILs. The average values over replicates were used for Pearson correlation analysis between different traits. All the phenotypic data were subjected to normality test before statistical analysis, and transformed if necessary.

Identification of quantitative trait loci

The genetic linkage map of Forno × Oberkulmer was downloaded from the GrainGenes database (http://wheat.pw.usda.gov/GG2/index.shtml), and consisted of 230 segregating loci

(restriction fragment length polymorphisms and microsatellites) and 23 linkage groups covering 2469 cM. Analysis of QTLs was performed using MapQTL v6 (Van Ooijen, 2009), with the average values of phenotypic data over replicates. An initial interval mapping was carried out to identify the putative QTLs. A locus was considered significant when its peak of logarithm of the odds (LOD) was higher than the genome-wide threshold for the trait, which was computed from a permutation test with 1000 iterations. Subsequently, multiple-QTL model (MQM) mapping was performed by using the markers closest to the QTL peaks as the co-factors ($P < 0.02$). The nomenclature of QTLs followed the Catalogue of Gene Symbols for Wheat (http://wheat.pw.usda.gov/GG2/Triticum/wgc/2008/). The chart of linkage groups and 1 – LOD (the LOD peak values minus one) support intervals representing the QTL positions were drawn using MapChart v2.2 (Voorrips, 2002).

RESULTS

Phenotypic variation in seedling root traits between genotypes

The bread wheat Forno and spelt Oberkulmer differed significantly in RSA examined in 'the pouch and wick' system in the growth chamber in 2014 (Figs 1 and 2; Table 1). Compared with Forno, Oberkulmer had more (17 %) and longer (12 %) seminal roots, and its total root length was also greater (20 %). No significant difference in root maximum depth was found, but the maximum width, width to depth ratio, and tip and emergence angles of seminal roots were smaller in Oberkulmer, indicating a narrow root system. As expected, there was a large variation in RSA among different RILs derived from Forno × Oberkulmer (Fig. 2; Table 1). Transgressive segregation (values beyond the parental phenotypic range) was seen for each trait. For example, the seminal root number of RIL-230 (4·7),

seminal root length of RIL-164 (186 mm) and total root length of RIL-248 (766 mm) were 12, 30 and 27 % greater than those of the higher parent Oberkulmer.

Phenotypic correlations between the seedling root traits

Significant correlations between different root traits were found (Table 2). Root system size represented by the total root length was positively correlated with both the number and length of seminal roots, and a closer relationship was found with the former. Root system shape represented by the ratio of width to depth was closely associated with the maximum width ($r = 0.85$, $P < 0.01$), rather than the depth. Total root length was positively associated with both the maximum width and depth, and had only a slight effect on root system shape. In addition, larger tip and emergence angles of seminal roots were associated with a wider root system, and a larger tip angle was also associated with a shallower root system.

Phenotypic correlations of the root traits with yield and yield components

Field experiments showed that Oberkulmer had lower machine-harvested yield than Forno (Table 1). Also, grain number components, including grains m^{-2}, spikes m^{-2} and grains per spike, tended to be lower in Oberkulmer. Thousand grain weight was higher in Oberkulmer in 2012, but not in 2013. In terms of the above-ground biomass m^{-2}, there was no significant difference between the two parental lines. In the RIL population, a large variation was found for each yield trait investigated (Table 1).

Correlation analysis exhibited significant relationships between the seedling root traits, yield and yield components
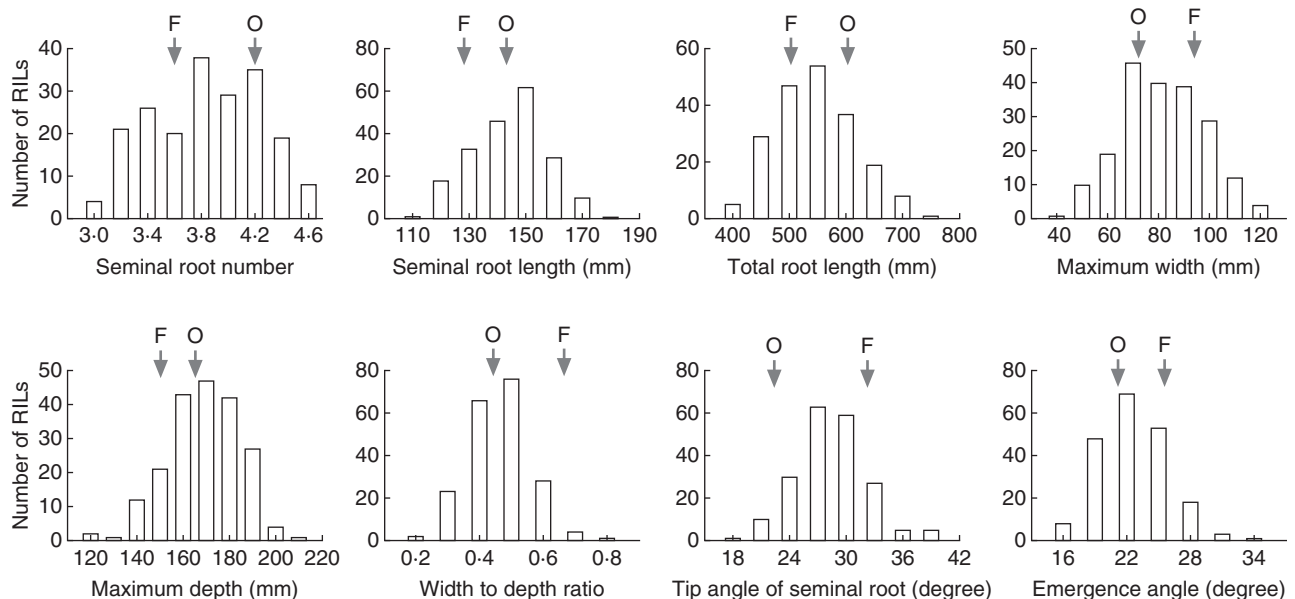

FIG. 2. Frequency distributions of the root architectural traits measured in the recombinant inbred line (RIL) mapping population of Forno (F) × Oberkulmer (O) in the 'pouch and wick' system in a growth chamber during 2014.

TABLE 1. *Descriptive statistics on seedling root traits in the 'pouch and wick' system in a growth chamber in 2014 as well as yield, yield components and phenology in field trials during 2012 and 2013 for the recombinant inbred line (RIL) mapping population of Forno (F) × Oberkulmer (O)*

Trait	Year	Parental lines			RILs		
		F	O	*P*-value	Mean (min; max)	*P*-value	s.e.d.*
Seedling root traits ($n = 20$)							
Seminal root number	2014	3·6	4·2	<0·01	3·9 (3·0; 4·7)	<0·001	0·3
Seminal root length (mm)	2014	128	143	<0·01	148 (118; 186)	<0·001	12
Total root length (mm)	2014	502	604	<0·01	577 (404; 766)	<0·001	55
Maximum width (mm)	2014	94	72	<0·001	86 (49; 127)	<0·001	16
Maximum depth (mm)	2014	150	165	>0·05	174 (125; 215)	<0·001	13
Width to depth ratio	2014	0·66	0·44	<0·001	0·51 (0·28; 0·80)	<0·001	0·10
Tip angle of seminal root (°)	2014	32·3	22·3	<0·001	29·9 (18·1; 41·1)	<0·001	3·6
Emergence angle of seminal root (°)	2014	25·6	21·2	<0·01	23·9 (16·7; 35·4)	<0·001	3·1
Yield traits ($n = 3$ in each year)							
Machine-harvested yield (t ha^{-1})	2012	7·37	5·28	<0·01	5·01 (2·62; 7·75)	<0·001	0·58
	2013	9·56	5·60	<0·01	6·42 (3·74; 10·39)	<0·001	0·97
Grains m^{-2}	2012	20 296	12 393	<0·01	12 478 (7244; 18 937)	<0·001	1309
	2013	19 367	12 519	<0·01	14 420 (8720; 24 864)	<0·001	2086
Spikes m^{-2}	2012	726	472	<0·01	545 (392; 848)	<0·001	77
	2013	480	468	>0·05	449 (300; 628)	<0·001	54
Grains per spike	2012	40	33	>0·05	37 (24; 51)	<0·001	4·6
	2013	38	36	>0·05	40 (26; 54)	<0·001	2·8
	2014†	32	30	>0·05	31 (17; 48)	<0·001	6
Thousand grain weight (g)	2012	34·9	43·1	<0·01	39·9 (31·9; 48·8)	<0·001	1·7
	2013	48·5	43·9	<0·01	45·0 (34·9; 53·6)	<0·001	1·3
	2014†	52·5	47·5	>0·05	47·3 (33·5; 58·8)	<0·001	4·4
Biomass m^{-2} (above-ground, g)	2012	1765	1443	>0·05	1570 (1018; 1914)	<0·05	182
	2013	1719	1914	>0·05	1829 (1391; 2428)	<0·001	249
Phenology ($n = 3$ in each year)							
GS31 (onset of stem elongation, °Cd)	2012	1184	1239	<0·01	1214 (1175; 1267)	<0·001	20
	2013	872	904	<0·05	906 (843; 953)	<0·001	15
GS39 (full flag leaf emergence, °Cd)	2012	1606	1672	<0·01	1648 (1606; 1672)	<0·001	10
	2013	1174	1228	<0·01	1204 (1163; 1253)	<0·001	9
GS61 (anthesis, °Cd)	2012	1876	1916	<0·01	1900 (1840; 1944)	<0·001	9
	2013	1442	1509	<0·01	1485 (1434; 1547)	<0·001	9
GS92 (maturity, °Cd)	2012	2658	2661	>0·05	2680 (2553; 2811)	<0·001	27
	2013	2066	2212	<0·01	2106 (1975; 2268)	<0·001	30
GS31−GS39 (°Cd)	2012	422	433	>0·05	434 (389; 497)	<0·001	21
	2013	302	324	>0·05	298 (246; 358)	<0·001	18
GS39−GS61 (°Cd)	2012	270	244	<0·05	252 (209; 293)	<0·001	12
	2013	268	281	>0·05	281 (248; 320)	<0·001	12
GS31−GS61 (°Cd)	2012	692	677	>0·05	686 (628; 753)	<0·001	23
	2013	570	605	<0·05	579 (530; 652)	<0·001	17
GS61−GS92 (°Cd)	2012	782	745	>0·05	780 (658; 902)	<0·001	24
	2013	624	703	<0·01	622 (515; 753)	<0·001	28

*s.e.d., standard error of the difference; †Glasshouse experiment in 2014 ($n = 3$).

TABLE 2. *Phenotypic correlations between the seedling root traits measured in the 'pouch and wick' system in a growth chamber during 2014 for the Forno × Oberkulmer mapping population*

	SRN	SRL	TRL	MW	MD	W/D	TA
SRL	−0·19**						
TRL	0·71**	0·54**					
MW	0·44**	0·18*	0·52**				
MD	0·10	0·85**	0·69**	0·29**			
W/D	0·38**	−0·23**	0·19**	0·85**	−0·20**		
TA	0·14*	−0·24**	−0·05	0·35**	−0·34**	0·52**	
EA	0·21**	−0·11	0·14*	0·49**	−0·08	0·54**	0·36**

Trait abbreviations: SRN, seminal root number; SRL, seminal root length; TRL, total root length; MW, maximum width; MD, maximum depth; W/D, width to depth ratio; TA, tip angle of seminal root; EA, emergence angle of seminal root.
*Significant at $P < 0.05$, **significant at $P < 0.01$.

TABLE 3. *Phenotypic correlations of the seedling root traits in the 'pouch and wick' system with yield, yield components and phenology in (1) field trials during 2012 and 2013, and (2) in pot trials in a glasshouse in 2014 for the Forno × Oberkulmer mapping population*

	SRN		SRL		TRL		MW		MD		W/D		TA		EA	
(1)Field	2012	2013	2012	2013	2012	2013	2012	2013	2012	2013	2012	2013	2012	2013	2012	2013
MHY	−0·01	0·22**	0·09	0·17*	0·05	0·31**	0·09	0·20**	0·10	0·31**	0·07	0·05	−0·06	−0·14*	0·05	−0·03
GPSM	0·22	0·29**	0·01	0·18	0·22	0·40**	0·25*	0·27**	0·09	0·33**	0·24*	0·09	0·02	−0·12	0·07	−0·14
SPSM	0·21	0·18	−0·03	0·02	0·19	0·17	0·25*	0·21*	−0·02	0·02	0·25*	0·16	0·01	0·02	−0·05	−0·01
GPS	0·32**	0·31**	0·06	−0·05	0·35**	0·22*	0·22	0·04	0·18	0·06	0·18	0·03	0·09	0·05	−0·03	0·01
TGW	−0·47**	−0·37**	0·12	0·13	−0·36**	−0·23*	−0·25*	−0·07	−0·07	0·03	−0·21	−0·09	−0·08	0·03	0·14	0·08
BPSM	0·22	0·25**	0·11	−0·01	0·30**	0·18*	0·29*	0·16	0·08	0·03	0·25*	0·13	0·05	0·16	0·16	0·14
GS31	0·14	0·22**	−0·05	−0·14	0·11	0·08	0·16	−0·04	−0·03	−0·10	0·15	0·01	0·03	0·02	0·09	−0·03
GS39	0·00	0·06	−0·13	−0·09	−0·11	−0·03	−0·04	−0·09	−0·14	−0·11	−0·01	−0·04	0·09	0·01	0·09	−0·06
GS61	0·17	0·14	−0·30**	−0·20*	−0·07	−0·04	0·08	−0·06	−0·24*	−0·19*	0·16	0·04	0·21	0·13	0·01	−0·03
GS92	0·35**	0·30**	−0·10	−0·12	0·23*	0·16	0·24*	0·04	0·03	−0·09	0·25*	0·10	0·19	0·09	0·01	−0·05
GS31−GS39	−0·14	−0·21*	−0·05	0·09	−0·19	−0·12	−0·18	−0·04	−0·08	0·02	−0·16	−0·06	0·04	−0·01	−0·02	−0·02
GS39−GS61	0·22	0·16	−0·25*	−0·23*	0·03	−0·02	0·14	0·02	−0·16	−0·18	0·22	0·14	0·18	0·22*	−0·08	0·04
GS31−GS61	0·03	−0·11	−0·22	−0·05	−0·15	−0·13	−0·06	−0·03	−0·17	−0·09	0·02	0·03	0·15	0·12	−0·07	0·00
GS61−GS92	0·34**	0·34**	0·01	−0·07	0·30*	0·23*	0·24*	0·08	0·14	−0·03	0·23	0·12	0·14	0·06	0·01	−0·05
(2) Glasshouse in 2014																
GPS	0·27**		−0·01		0·23*		0·06		0·12		0·00		−0·02		−0·09	
TGW	−0·22*		0·01		−0·20*		−0·08		−0·06		−0·06		0·10		−0·10	

Trait abbreviations: SRN, seminal root number; SRL, seminal root length; TRL, total root length; MW, maximum width; MD, maximum depth; W/D, width to depth ratio; TA, the tip angle of seminal root; EA, the emergence angle of seminal root; MHY, machine-harvested yield; GPSM, grains m^{-2}; SPSM, spikes m^{-2}; GPS, grains per spike; TGW, thousand grain weight; BPSM, biomass m^{-2}; GS31, GS39, GS61 and GS92, the accumulated thermal time at the onset of stem elongation, full flag leaf emergence, anthesis and maturity, respectively.
*Significant at $P < 0.05$, **significant at $P < 0.01$.

(Table 3). For the size parameters of the root system, total root length and its key component, seminal root number, were positively correlated with machine-harvested yield as well as grains m^{-2} in 2013. The two root parameters also showed positive relationships with grains per spike and biomass m^{-2}, and negative relationships with TGW, in both years. For the shape parameters of the root system, both root maximum width and depth were positively correlated with machine-harvested yield in 2013. Consistent positive correlations of the maximum width with grains m^{-2} and spikes m^{-2} were found over both years. In general, there was no significant correlation between the tip and emergence angles of seminal roots, and yield traits, except for tip angle in 2013.

In the glasshouse experiment, seminal root number and total root length also showed positive relationships with grains per spike, but negative relationships with TGW, concurring with the results found in the field experiments.

Phenotypic correlations of the root traits with phenology

Similarly, there were significant phenotypic differences in growth stages between genotypes in the RIL population (Table 1), and significant relationships of the growth stages with RSA (Table 3). Seminal root number and total root length were positively associated with GS92 and GS61–GS92, indicating that a larger root system can be associated with delayed maturity and a longer grain filling period. Additionally, longer seminal roots were associated with earlier GS61 and shorter GS39–GS61. One of the root system shape parameters, maximum depth, showed a consistent negative relationship with GS61.

Performance of the top and bottom RILs categorized by total root length

To confirm the effects of total root length representing the root system size on yield and yield-related traits, the top ten (RIL-31, 63, 69, 79, 89, 101, 134, 202, 230 and 248) and bottom ten (RIL-4, 7, 21, 67, 124, 180, 185, 186, 210 and 236) lines with the highest and lowest total root length, respectively, were selected (Table 4). The top ten RILs had 44 % longer total root length than the bottom ten, showing significantly more seminal roots, and a wider and deeper root system. In addition, the top RILs exhibited significantly higher yield, more grains per m^{-2} and grains per spike, higher biomass m^{-2}, delayed maturity and a longer grain filling period, but lower TGW, consistent with the above observations.

Detection of quantitative trait loci

Quantitative trait loci for the root architectural traits. A total of 38 QTLs associated with the RSA were discovered, comprising 12 for seminal root number, two for seminal root length, 13 for total root length, five for each of root maximum width and depth, and one for the emergence angle of seminal roots (Fig. 3; Table 5). These QTLs were located on 12 chromosomes, i.e. 2A (five QTLs), 2B (one QTL), 3A (three QTLs), 3B (three QTLs), 3DL (two QTLs), 4A (six QTLs), 4DL (one QTL), 5A (six QTLs), 5B (three QTLs), 7AL (two QTLs), 7B (three QTLs) and 7D (three QTLs), individually explaining 6·3–22·3 % of the phenotypic variation observed. Forno provided 17 (45 %) increasing alleles (increasing the values of the traits) while Oberkulmer provided the remaining increasing alleles. Most of the QTLs identified (71 %) were associated with

TABLE 4. *Performance of the top and bottom ten recombinant inbred lines (RILs) with the highest and lowest total root length*

RIL	TRL	SRN	MW	MD	MHY	GPSM	GPS	TGW	BPSM	GS92	GS61–GS92
Top ten (mean)	690	4·5	105	186	6·3	14110	43	41·2	1808	2443	741
Bottom ten (mean)	480	3·3	80	165	5·7	12166	35	45·6	1639	2374	673
Difference (value)	210	1·2	25	21	0·6	1944	8	−4·4	169	69	68
Difference (%)	44	36	31	13	11	16	23	−10	10	3	10
P-value	<0·001	<0·001	<0·01	<0·01	<0·05	<0·01	<0·001	<0·01	<0·01	<0·01	<0·01

Trait abbreviations: TRL, total root length (mm); SRN, seminal root number; MW, maximum width (mm); MD, maximum depth (mm); MHY, machine-harvested yield (t ha^{-1}); GPSM, grains m^{-2}; GPS, grains per spike; TGW, thousand grain weight (g); BPSM, biomass m^{-2} (g); GS92, the accumulated thermal time at maturity (°Cd); GS61–GS92, the duration between anthesis and maturity (°Cd).

the parameters of root system size, and distributed on all 12 chromosomes where QTLs were detected.

Twelve QTLs for total root length co-located with 12 QTLs for seminal root number and length, with the positive effects coming from the same parents (either Forno or Oberkulmer) (Fig. 3), which is in line with their positive phenotypic relationships (Table 2). Likewise, eight QTLs for total root length co-located with three QTLs for root maximum width and five for maximum depth on seven chromosomes; one QTL for the emergence angle of seminal roots co-located with one QTL for root maximum width. The increasing alleles of these coincident QTLs were from the same parents.

Quantitative trait locus coincidence between the root and yield traits. For yield and yield components, 41 QTLs were detected on 11 chromosomes, comprising seven for machine-harvested yield, 11 for grains m^{-2}, four for spikes m^{-2}, nine for grains per spike and ten for TGW (Fig. 3; Table 5). These QTLs explained phenotypic variation between 6·3 and 35·1 %.

Quantitative trait locus coincidence between root and yield traits was frequently observed (Fig. 3). For the size parameters of the root system, 2–4 QTLs for seminal root number were co-incident with two QTLs for yield (on 4A and 5A), four for grains m^{-2} (on 4A, 5A and 7B), three for spikes m^{-2} (on 4A and 7B), two for grains per spike (on 4A and 5A) and six for TGW (on 2A, 4A, 5B and 7B). Two QTLs for seminal root length were coincident with three QTLs for yield, two for grains m^{-2} and one for spikes m^{-2} on 3A and 3B. A similar result was found for total root length: 2–5 QTLs were coincident with five QTLs for yield (on 3A, 3B, 4A and 5A), six for grains m^{-2} (on 3A, 4A, 5A and 7B), four for spikes m^{-2} (on 3A, 4A and 7B), two for grains per spike (on 4A and 5A) and six for TGW (on 2A, 4A, 5B and 7B). For the shape parameters of the root system, one and two QTLs for root maximum width and depth, respectively, co-located with those for yield. In addition, two QTLs for root maximum width also co-located with two QTLs for grains m^{-2} and one for spikes m^{-2}.

All coincident QTLs between the RSA and yield traits had the increasing alleles conferred by the same parents. The only exceptions were the coincident QTLs between the RSA and TGW, and between the RSA and grains per spike detected on 5AL, with the increasing alleles originating from the opposite parents. The results indicate tightly linked genes or pleiotropy, consistent with the correlation analysis between the RSA and yield traits.

Quantitative trait locus coincidence between the root traits and phasic development. For the growth stages, a total of 49 QTLs were identified, individually explaining 11·6–29·6 % of the phenotypic variation (Fig. 3; Table 5). The spelt Oberkulmer provided 34 (69 %) alleles delaying phenology.

Analysis of the QTL coincidence between the RSA and phasic development showed that six QTLs for each of seminal root number and total root length co-located with seven QTLs for GS92 and ten for GS61–GS92, with the increasing alleles from the same parents except for those on 5AL (Fig. 3). One QTL for seminal root length co-located with one QTL for GS39–GS61 on 3B, with the positive effects coming from Forno and Oberkulmer, respectively. The nature of tight gene linkages or pleiotropy is in line with the positive phenotypic correlations between root system size, delayed maturity and extended grain filling period, as well as the negative correlation between seminal root length and the duration of flag leaf emergence to anthesis.

DISCUSSION

Significant links of the seedling RSA with yield and yield components

Using a germination paper-based 'pouch and wick' phenotyping pipeline, the seedling root architectural traits of the RILs derived from Forno and Oberkulmer were measured in a fast, cost-effective way. Although the seedling roots growing in the pouches may not develop exactly as those of plants growing in the field, this technique allowed us to characterize the differences in RSA between genotypes. Comparing the indoor seedling RSA traits with field agronomical performance has been reported in a number of studies, for instance in bread wheat (Liu *et al*., 2013; Atkinson *et al*., 2015), durum wheat (*Triticum durum*) (Cane *et al*., 2014), maize (*Zea mays*) (Li *et al*., 2015) and oilseed rape (*Brassica napus*) (Thomas *et al*., 2016). The present study identifies significant links between the seedling RSA, and field yield and yield-related traits, suggesting that the RSA of 13-day-old seedlings grown in the growth pouches could be predictive of field performance of these agronomic traits.

The root system size of wheat seedlings, as represented by seminal root number and total root length, was positively associated with grain yield, in line with a previous report by Liu *et al*. (2013). The positive correlations between the two root traits and yield were not found in 2012, probably due to the relatively heavy rainfall and consequent high disease levels (e.g. glume blotch that affects grain development) during grain
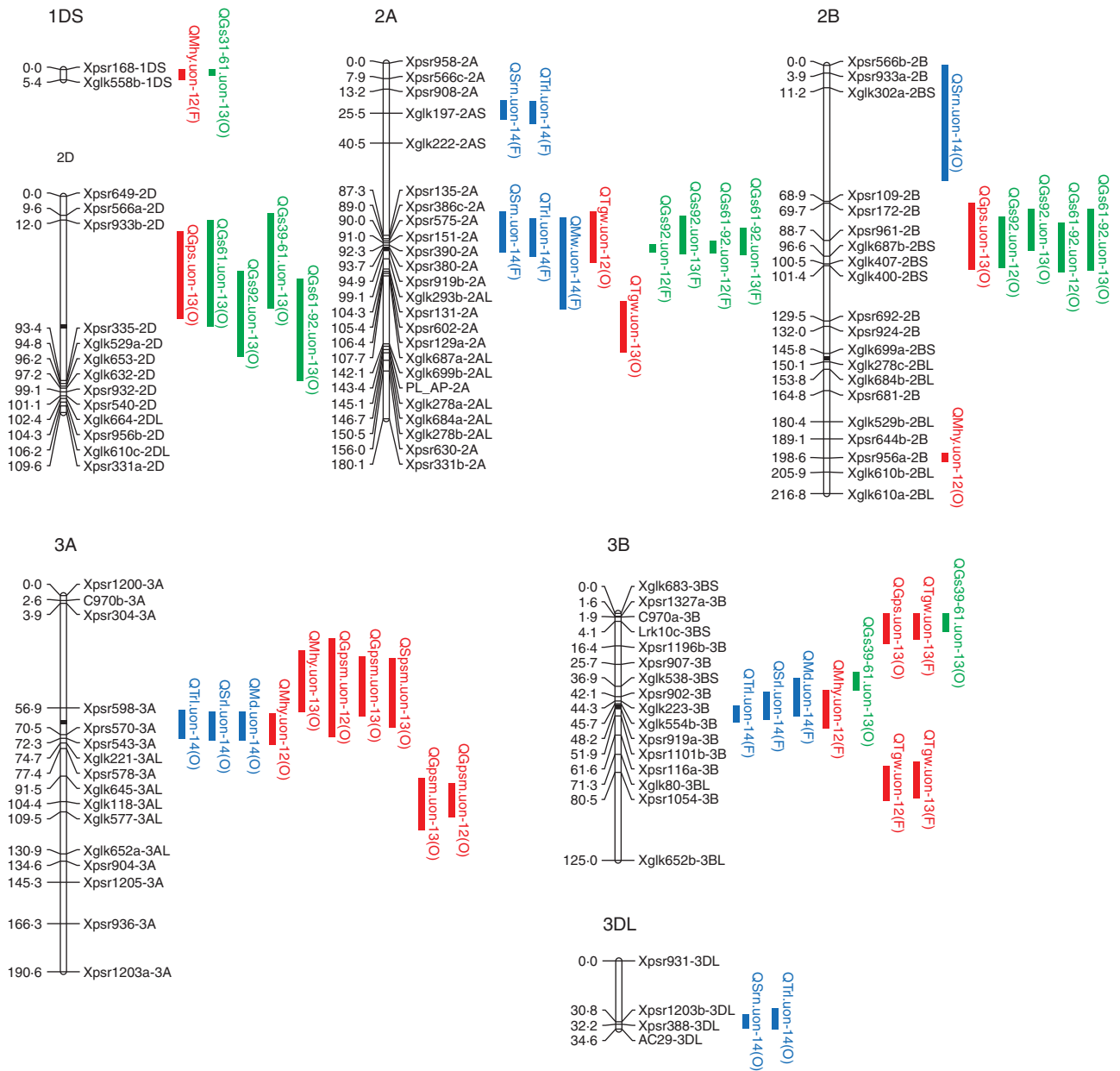
FIG. 3. Quantitative trait loci (QTLs) associated with seedling root traits, yield, yield components and phenology in the Forno × Oberkulmer mapping population. For each linkage group, the molecular marker identifiers are placed on the right side of the chromosome (hollow bar) and their locations are shown on the left side in centiMorgans (cM). The vertical bars on the right sides represent the 1 – LOD support intervals of significant QTLs: blue (root traits), red (yield and yield components) and green (phenology). Each QTL symbol consists of an upper case letter 'Q' and the abbreviations of quantitative trait, institution (uon), years when the QTL was detected ('12', 2012; '13', 2013; '14', 2014) and the parent conferring the allele with positive effect ('F', Forno; 'O', Oberkulmer). Trait abbreviations: Srn, seminal root number; Srl, seminal root length; Trl, total root length; Mw, maximum width of root system; Md, maximum depth of root system; Ea, emergence angle of seminal roots; Mhy, machine-harvested yield; Gpsm, grains m^{-2}; Spsm, spikes m^{-2}; Gps, grains per spike; Tgw, thousand grain weight; Gs39, the time at full flag leaf emergence; Gs61, the time at anthesis; Gs92, the time at maturity; Gs31–39, the duration from GS31 (the time at the onset of stem elongation) to GS39; Gs39–61, the duration from GS39 to GS61; Gs31–61, the duration from GS31 to GS61; Gs61–92, the duration from GS61 to GS92.

filling in June and July of this year (Fig. S1). The bread wheat parent Forno is highly susceptible to glume blotch, whereas the spelt parent Oberkulmer has a high level of resistance (Messmer *et al.*, 1999), which can at least partly explain the low TGW of Forno in 2012. Furthermore, the high disease pressure, rather than resource capture by roots, might limit the yields of many RILs in this year (sink limited), so the

correlations between the root system size and yield could not be seen. An increase in yield as a result of a larger root system was mainly due to increased grain number rather than individual grain weight in the present study. Grains per spike, the major component of grain number, is substantially determined during stem elongation before anthesis (González *et al.*, 2011). During this period, the spikes and stems are both growing
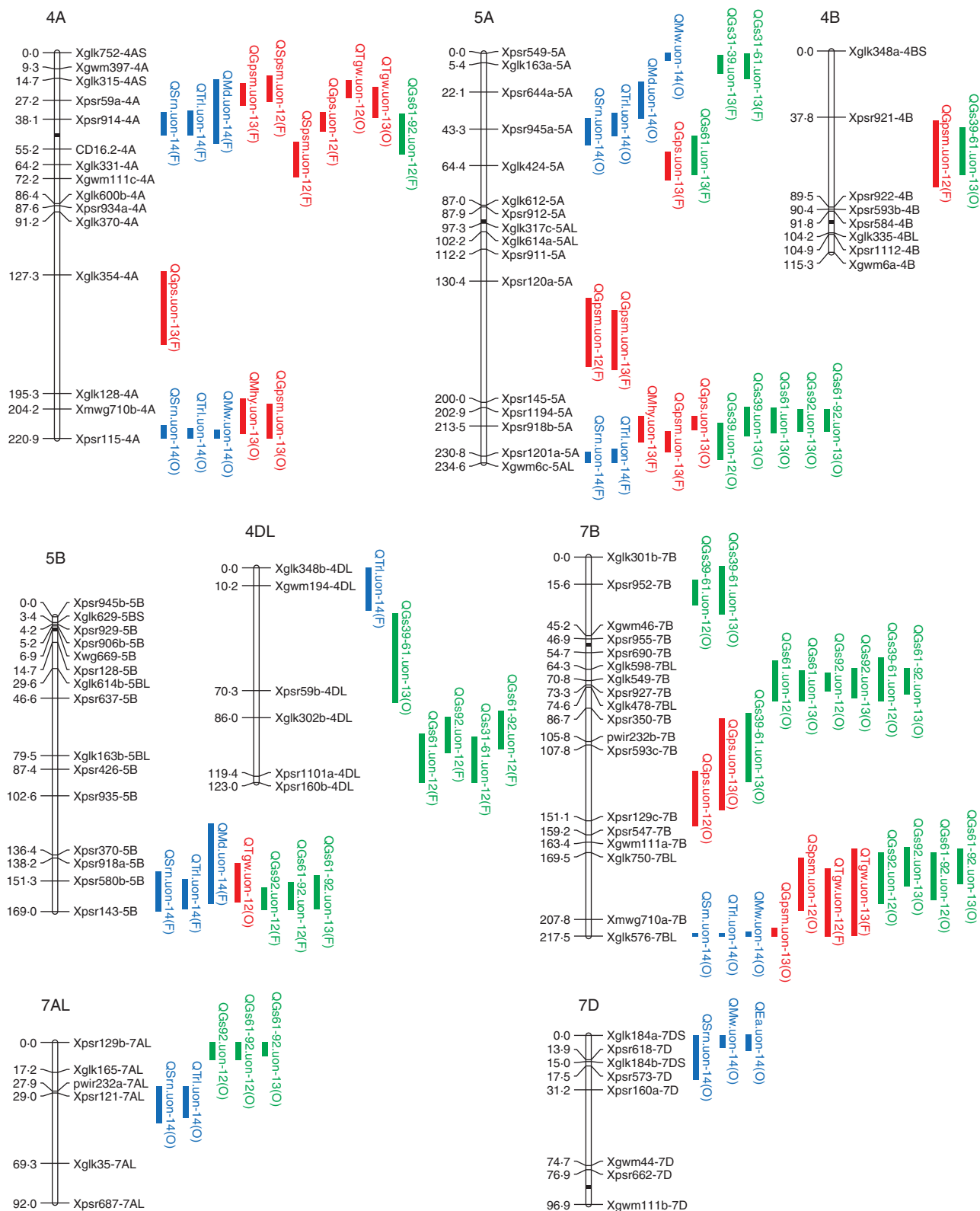
Fig. 3. Continued.

TABLE 5. *Quantitative trait loci (QTLs) associated with seedling root traits in the 'pouch and wick' system in a growth chamber as well as yield, yield components and phenology in field trials for the recombinant inbred line mapping population of Forno ×* *Oberkulmer*

Trait/chromosome	Year	Position (cM)	LOD	R^2*	Additive effect[†]	Closest marker
Seedling root traits						
Seminal root number						
2A	2014	24·2	8·7	18·0	0·18	*Xglk197-2AS*
	2014	93·7	11·1	22·3	0·20	*Xpsr380-2A*
2B	2014	38·2	2·9	6·3	–0·19	*Xglk302a-2BS*
3DL	2014	34·2	3·5	7·7	–0·11	*AC29-3DL*
4A	2014	41·1	6·6	14·0	0·17	*Xpsr914-4A*
	2014	220·8	5·5	11·8	–0·15	*Xpsr115-4A*
5A	2014	43·3	3·1	6·8	–0·11	*Xpsr945a-5A*
	2014	234·0	4·4	9·5	0·13	*Xgwm6c-5AL*
5B	2014	158·3	3·1	6·7	0·13	*Xpsr580b-5B*
7AL	2014	27·9	4·2	9·0	–0·14	*pwir232a-7AL*
7B	2014	217·4	8·1	16·8	–0·17	*Xglk576-7BL*
7D	2014	7·0	3·7	8·0	–0·13	*Xpsr618-7D*
Seminal root length (mm)						
3A	2014	66·9	3·5	7·6	–4	*Xprs570-3A*
3B	2014	49·2	4·8	10·4	5	*Xpsr919a-3B*
Total root length (mm)						
2A	2014	25·5	4·1	9·0	22	*Xglk197-2AS*
	2014	95·9	8·5	17·6	31	*Xpsr919b-2A*
3A	2014	63·9	5·6	11·9	–30	*Xprs570-3A*
3B	2014	51·9	3·3	7·3	21	*Xpsr1101b-3B*
3DL	2014	34·2	3·0	6·6	–19	*AC29-3DL*
4A	2014	42·1	8·5	17·6	35	*Xpsr914-4A*
	2014	220·8	6·4	13·6	–28	*Xpsr115-4A*
4DL	2014	3·0	3·3	7·2	23	*Xglk348b-4DL*
5A	2014	43·3	4·5	9·8	–23	*Xpsr945a-5A*
	2014	232·8	4·7	10·2	25	*Xpsr1201a-5A*
5B	2014	160·3	5·2	11·1	29	*Xpsr143-5B*
7AL	2014	28·9	4·1	8·8	–24	*Xpsr121-7AL*
7B	2014	217·4	10·1	20·6	–34	*Xglk576-7BL*
Maximum width (mm)						
2A	2014	97·9	3·3	7·3	5	*Xglk293b-2AL*
4A	2014	220·9	4·5	9·8	–5	*Xpsr115-4A*
5A	2014	0·1	3·6	7·9	–5	*Xpsr549-5A*
7B	2014	217·4	4·0	8·6	–5	*Xglk576-7BL*
7D	2014	0·1	3·1	6·9	–4	*Xglk184a-7DS*
Maximum depth (mm)						
3A	2014	65·9	4·1	9·0	–6	*Xprs570-3A*
3B	2014	40·9	4·1	9·0	5	*Xpsr902-3B*
4A	2014	32·2	3·0	6·6	5	*Xpsr59a-4A*
5A	2014	28·1	3·5	7·6	–5	*Xpsr644a-5A*
5B	2014	134·6	3·2	6·9	5	*Xpsr370-5B*
Emergence angle of seminal root (°)						
7D	2014	2·0	3·4	7·5	–0·9	*Xglk184b-7DS*
Yield traits						
Machine-harvested yield (t ha^{-1})						
1DS	2012	2·0	3·2	7·1	0·26	*Xpsr168-1DS*
2B	2012	198·6	3·4	20·1	–0·36	*Xpsr956a-2B*
3A	2012	67·9	3·5	20·6	–0·42	*Xprs570-3A*
	2013	39·9	6·1	13·1	–0·74	*Xpsr598-3A*
3B	2012	42·1	2·8	6·3	0·23	*Xpsr902-3B*
4A	2013	210·2	3·8	8·4	–0·42	*Xmwg710b-4A*
5A	2013	218·5	9·3	19·2	0·68	*Xpsr918b-5A*
Grains m^{-2}						
3A	2012	37·9	4·1	9·0	–1202	*Xpsr598-3A*
	2012	106·4	5·0	11·0	–847	*Xglk118-3AL*
	2013	43·9	6·0	12·9	–1520	*Xpsr598-3A*
	2013	107·4	3·5	7·7	–838	*Xglk577-3AL*
4A	2013	23·7	4·6	10·0	1018	*Xpsr59a-4A*
	2013	212·2	2·8	6·3	–826	*Xmwg710b-4A*
4B	2012	61·8	3·2	7·1	1186	*Xpsr921-4B*
5A	2012	163·4	4·1	9·1	1562	*Xpsr120a-5A*
	2013	165·4	4·6	10·1	1955	*Xpsr145-5A*
	2013	222·5	6·2	13·3	1284	*Xpsr1201a-5A*

(continued)

TABLE 5. *Continued*

Trait/chromosome	Year	Position (cM)	LOD	R^2*	Additive effect[†]	Closest marker
7B	2013	217·4	3·9	8·5	−811	*Xglk576-7BL*
Spikes m^{-2}						
3A	2013	50·9	3·2	12·7	−27	*Xpsr598-3A*
4A	2012	20·7	3·7	21·4	45	*Xglk315-4AS*
	2012	60·2	3·6	21·0	42	*Xglk331-4A*
7B	2012	188·5	3·7	21·5	−58	*Xglk750-7BL*
Grains per spike						
2B	2013	94·7	3·1	12·0	−2·2	*Xglk687b-2BS*
2D	2013	44·9	4·7	17·8	−6·0	*Xpsr933b-2D*
3B	2013	8·1	3·7	14·4	−2·7	*Lrk10c-3BS*
4A	2012	38·1	3·5	20·5	2·5	*Xpsr914-4A*
	2013	151·3	3·6	14·0	5·5	*Xglk354-4A*
5A	2013	64·4	4·5	17·3	2·9	*Xglk424-5A*
	2013	213·4	5·1	19·2	−2·9	*Xpsr918b-5A*
7B	2012	137·8	3·2	18·7	−3·6	*Xpsr129c-7B*
	2013	127·8	3·1	12·2	−3·4	*Xpsr593c-7B*
Thousand grain weight (g)						
2A	2012	94·9	3·5	20·6	−1·74	*Xpsr919b-2A*
	2013	133·7	3·5	13·8	−2·00	*Xglk699b-2AL*
3B	2012	80·5	4·2	23·9	1·95	*Xpsr1054-3B*
	2013	2·9	3·4	13·4	1·61	*C970a-3B*
	2013	80·5	5·1	19·3	1·91	*Xpsr1054-3B*
4A	2012	20·7	6·6	35·1	−2·65	*Xglk315-4AS*
	2013	31·2	4·8	18·1	−2·09	*Xpsr59a-4A*
5B	2012	154·3	3·0	17·7	−1·80	*Xpsr580b-5B*
7B	2012	190·5	5·2	28·9	3·04	*Xmwg710a-7B*
	2013	187·5	3·2	12·7	2·24	*Xglk750-7BL*
Phenology						
GS39 (full flag leaf emergence, °Cd)						
5A	2012	222·4	2·9	17·5	−10·3	*Xpsr1201a-5A*
	2013	209·9	4·0	15·2	−10·3	*Xpsr918b-5A*
GS61 (anthesis, °Cd)						
2D	2013	45·9	3·7	14·3	−24·3	*Xpsr933b-2D*
4DL	2012	107·0	3·1	18·4	15·1	*Xpsr1101a-4DL*
5A	2013	58·3	3·8	14·8	13·3	*Xglk424-5A*
	2013	209·9	3·9	15·1	−12·0	*Xpsr918b-5A*
7B	2012	77·6	5·0	27·9	−15·8	*Xglk478-7BL*
	2013	77·6	4·5	17·1	−12·9	*Xglk478-7BL*
GS92 (maturity, °Cd)						
2A	2012	94·9	5·1	28·6	39·9	*Xpsr919b-2A*
	2013	94·9	4·2	16·1	32·8	*Xpsr919b-2A*
2B	2012	91·7	3·8	22·0	−38·0	*Xpsr961-2B*
	2013	81·7	4·3	16·4	−40·5	*Xpsr961-2B*
2D	2013	56·9	4·4	17·0	−81·1	*Xpsr335-2D*
4DL	2012	97·0	4·4	25·2	50·5	*Xglk302b-4DL*
5A	2013	210·9	3·8	14·8	−36·2	*Xpsr918b-5A*
5B	2012	162·2	3·7	21·7	41·9	*Xpsr143-5B*
7AL	2012	0·1	4·1	23·7	−39·1	*Xpsr129b-7AL*
7B	2012	72·7	5·2	29·1	−42·3	*Xpsr927-7B*
	2012	185·5	3·8	22·2	−51·2	*Xglk750-7BL*
	2013	73·3	5·3	19·7	−38·6	*Xpsr927-7B*
	2013	173·5	3·7	14·3	−35·9	*Xglk750-7BL*
GS31 (onset of stem elongation)−GS39 (°Cd)						
5A	2013	5·4	3·4	13·2	8·6	*Xglk163a-5A*
GS39−GS61 (°Cd)						
2D	2013	33·9	3·1	12·2	−10·1	*Xpsr933b-2D*
3B	2013	0·1	3·3	12·9	−5·4	*Xglk683-3BS*
	2013	35·7	3·8	14·6	−6·1	*Xglk538-3BS*
4B	2013	59·8	5·4	20·3	−12·0	*Xpsr921-4B*
4DL	2013	45·2	3·3	13·0	−10·0	*Xpsr59b-4DL*
7B	2012	18·6	5·3	29·6	−13·7	*Xpsr952-7B*
	2012	61·7	4·7	26·4	−11·6	*Xglk598-7BL*
	2013	11·0	4·6	17·6	−7·6	*Xpsr952-7B*
	2013	99·7	3·5	13·6	−6·4	*pwir232b-7B*
GS31−GS61 (°Cd)						
1DS	2013	0·1	3·2	12·5	−8·8	*Xpsr168-1DS*
4DL	2012	108·0	3·7	21·4	18·9	*Xpsr1101a-4DL*
5A	2013	3·0	3·3	12·9	9·2	*Xpsr549-5A*

(continued)

TABLE 5. *Continued*

Trait/chromosome	Year	Position (cM)	LOD	R^2*	Additive effect[†]	Closest marker
GS61−GS92 (°Cd)						
2A	2012	94·9	5·2	28·8	34·6	*Xpsr919b-2A*
	2013	94·9	5·6	20·7	28·0	*Xpsr919b-2A*
2B	2012	92·7	4·1	23·6	−34·1	*Xglk687b-2BS*
	2013	81·7	4·2	16·2	−30·3	*Xpsr961-2B*
2D	2013	61·9	4·0	15·4	−54·8	*Xpsr335-2D*
4A	2012	47·1	3·4	19·9	33·4	*CD16·2-4A*
4DL	2012	92·0	3·3	19·3	33·7	*Xglk302b-4DL*
5A	2013	211·9	3·0	11·9	−24·0	*Xpsr918b-5A*
5B	2012	161·2	3·3	19·4	34·7	*Xpsr143-5B*
	2013	157·2	2·9	11·6	24·8	*Xpsr580b-5B*
7AL	2012	0·1	4·4	25·3	−34·9	*Xpsr129b-7AL*
	2013	0·1	3·4	13·2	−23·3	*Xpsr129b-7AL*
7B	2012	185·5	4·5	25·5	−47·4	*Xglk750-7BL*
	2013	73·3	4·7	17·9	−27·7	*Xpsr927-7B*
	2013	174·5	4·9	18·5	−31·7	*Xglk750-7BL*

*R^2, the proportion of phenotypic variation explained by individual QTLs.

[†]Positive and negative additive effects indicate that the alleles from Forno and Oberkulmer increase the values of the traits, respectively.

rapidly, resulting in an intensive intra-plant competition for available assimilates (source limited). The growth of spikes, which is a key determinant of spike fertility and, in turn, grains per spike, may be limited in such a case (Fischer, 1985, 2011; González *et al*., 2011). There is evidence that vigorous seedling roots are beneficial to take up more soil resources (e.g. N and P) for early plant growth (An *et al*., 2006; Zhu *et al*., 2006; Cao *et al*., 2014), leading to a release of the intra-plant competition to a great extent. The resultant availability of more resources for young spike growth would increase grains per spike and yield. However, total root length and seminal root number had negative effects on TGW, probably because increased grain number is often associated with decreased individual grain weight (Slafer and Andrade, 1993; Miralles and Slafer, 1995; Griffiths *et al*., 2015). Grain size contributes to end-use quality of wheat (Gegas *et al*., 2010), so care must be taken that a larger root system should be selected for the purpose of improving grain yield, whereas a smaller one may improve grain quality but be accompanied by a penalty for yield.

Seedling root architectural traits also influence the phasic development. Notably, a larger root system is associated with delayed maturity and, in turn, extended grain filling, probably because of improved water and nutrient uptake for post-anthesis photosynthesis (Pinto and Reynolds, 2015). A longer period of grain filling was thought to enlarge final grain size, which contrasts with the result that the TGW is actually reduced. A further analysis showed that GS61–GS92 was positively correlated with grains per spike ($r = 0.43$, $P < 0.001$ in 2012; $r = 0.60$, $P < 0.001$ in 2013), indicating that more grains defined before anthesis as a consequence of a larger early root system require a longer period to fill thereafter. At the same time, more grains per spike are associated with smaller grains, as discussed above, which leads to an apparent negative correlation between grain size and grain filling period.

Genetic basis of the seedling RSA and its links with yield traits

A total of 25 QTLs for seminal root number and total root length were identified in this study, clustering on seven chromosomes (2A, 3DL, 4A, 5A, 5B, 7AL and 7B). Two clusters for the two traits were found on each of the short and long arms of 4A, similar to the findings of Christopher *et al*. (2013), and the clusters on 2AS and 5AS could correspond to those of Kabir *et al*. (2015) and Hamada *et al*. (2012). There was also a QTL identified in the distal region of 7DS for seminal root number, where the QTLs for total length of all roots and of seminal roots as well as grain yield have been detected previously (Atkinson *et al*., 2015). In addition, a QTL for total root length was mapped around the centromere of 3B in the present study, concurring with the reports by Bai *et al*. (2013) and Liu *et al*. (2013). The favourable QTLs identified here can be transferred into elite wheat cultivars for RSA improvement after fine mapping. Furthermore, the top ten RILs selected for their highest total root length, each of which carries mostly all the positive alleles underlying the above QTLs, will be utilized as the intermediate materials for introgression.

The phenotypic correlations of seminal root number and total root length with yield traits were confirmed by genetic analysis, showing tightly linked genes or pleiotropy. Recently, the QTL coincidence between the two root traits, yield and TGW was also found in tetraploid wheat (Maccaferri *et al*., 2016). *qTaLRO-B1*, a major 2BS QTL controlling the root length of wheat seedlings, has been used to develop near-isogenic lines with long roots and efficient phosphate uptake and biomass accumulation (Cao *et al*., 2014). In rice (*Oryza sativa*), a QTL increasing root length by 9·6 cm has been introgressed into an upland cultivar, giving rise to an increase of 0·2 t ha^{-1} in grain yield (Steele *et al*., 2013). These examples suggest the potential of introgressed coincident QTLs to improve both RSA and agronomic traits.

Spelt as a useful genetic resource for the RSA improvement in bread wheat

The significant variation in RSA in the present study is largely attributed to the use of spelt. Spelt is an ancient crop and has been cultivated since 5000 BC. As an old-world grain, spelt is adapted to high precipitation, heavy and infertile soils,

and cool temperatures, and is still a minor crop grown on a considerable acreage in marginal areas at present (Campbell, 1997). Additionally, previous studies found that spelt has a faster early crop growth rate, and accumulates more biomass and N than bread wheat (Koutroubas *et al.*, 2012). These aspects of the performance of spelt may depend greatly on its vigorous root system. In this study, the spelt Oberkulmer showed a large but narrow seminal root system, represented by more seminal roots, longer seminal and total roots, less root maximum width and smaller tip and emergence angles. A narrow but strong root system could help to penetrate the heavy soil for foraging and anchoring, while a large root system would enhance its ability for nutrient acquisition to support plant growth. In another experiment, spelt (three cultivars: 'Oberkulmer', 'Tauro' and 'SB') had much more root biomass at all soil layers than bread wheat (two cultivars: 'Xi-19' and 'Forno'), and this is also true for root length density (root length per unit soil volume) (unpubl. res.), which, at the depth of 80 cm, is still above the threshold of approx. 1 cm cm^{-3} for potential water and nitrate capture (Foulkes *et al.*, 2011). Genetically, the current study presents 14 and seven favourable alleles originating from the spelt Oberkulmer for the size and shape of the root system, respectively. Taken together, spelt is a critical genetic resource for the root system improvement in bread wheat, and its nature as a hexaploid will allow efficient hybridization with bread wheat to transfer these traits.

CONCLUSIONS

This study demonstrated that vigorous root growth at the early seedling stage affects yield formation in wheat. Seminal root number and total root length have positive effects on grain number, above-ground biomass and grain yield in the field, accompanied by a reduction of individual grain weight. A wider root system is also associated with higher grain number and yield. Delayed physiological maturity and a longer period of grain filling, as a consequence of a larger root system, may result from more grains defined before anthesis. These relationships are consistent with analysis of the QTL coincidence, indicating tight gene linkages or pleiotropy. The results suggest that the root architectural traits, particularly seminal root number and total root length, are favourable to improve yield potential, and should be incorporated into wheat ideotypes. The alleles with positive effects would permit marker-assisted selection, and the top ten RILs, which carry these alleles and also perform well in terms of yield, could be utilized as intermediate materials in breeding. In addition, spelt has a large root system, and may be a useful genetic resource for the RSA enhancement in bread wheat.

SUPPLEMENTARY DATA

Supplementary data are available online at https://academic.oup.com/aob and consist of the following. Figure S1: mean air temperature, rainfall and solar radiation at University of Nottingham Farm in the 2011 − 2012 and 2012 − 2013 seasons.

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