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

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1 ABSTRACT

2 • **Background and Aims** Plant roots growing underground are critical for soil resource
3 acquisition, anchorage and plant-environment interactions. In wheat (*Triticum aestivum*),
4 however, the target root traits to improve yield potential still remain largely unknown. This
5 study aimed to identify traits of seedling root system architecture (RSA) associated with yield
6 and yield components in 226 recombinant inbred lines (RILs) derived from a cross between
7 the bread wheat *Triticum aestivum* ‘Forno’ (small, wide root system) and spelt *Triticum spelta*
8 ‘Oberkulmer’ (large, narrow root system).

9 • **Methods** A ‘pouch and wick’ high-throughput phenotyping pipeline was used to determine
10 the RSA traits of 13-d-old RIL seedlings. Two field and one glasshouse experiments were
11 carried out to investigate the yield, yield components and phenology, followed by
12 identification of quantitative trait loci (QTL).

13 • **Key Results** There was substantial variation in RSA traits between genotypes. Seminal root
14 number and total root length were both positively associated with grains m⁻², grains per spike,
15 above-ground biomass m⁻², and grain yield. More seminal roots and longer total root length
16 were also associated with delayed maturity and extended grain filling, likely to be a
17 consequence of more grains being defined before anthesis. Additionally, the maximum width
18 of the root system displayed positive relationships with spikes m⁻², grains m⁻², and grain yield.
19 Ten RILs selected for longest total roots exhibited the same effects on yield and phenology as
20 described above, compared to the ten lines with shortest total roots. Genetic analysis revealed
21 38 QTL for the RSA, and QTL coincidence between the root and yield traits were frequently
22 observed, indicating tightly linked genes or pleiotropy, which concurs with the results of
23 phenotypic correlation analysis.

24 • **Conclusions** Based on the results from the Forno × Oberkulmer population, it is proposed

1 that vigorous early root growth, particularly more seminal roots and longer total root length, is
2 important to improve yield potential, and should be incorporated into wheat ideotypes in
3 breeding.

4 **Key words:** Phenology, quantitative trait locus, root system architecture, spelt, *Triticum*
5 *aestivum*, *Triticum spelta*, wheat, yield.

1 INTRODUCTION

2 The roots, known as ‘the hidden half’ growing underground, are of fundamental importance
3 to plant growth. For example, the roots acquire water and nutrients from soil, and can function
4 as a storage site for nutrients (Kutman *et al.*, 2012; Smith and De Smet, 2012; Gaju *et al.*,
5 2014). They provide the anchorage for plants against lodging, and serve as a major interface
6 sensing, and interacting with, biotic and abiotic factors in the rhizosphere (Smith and De Smet,
7 2012). An efficient root system is even more crucial for crops grown in poor soils, where
8 water and nutrients are in short supply (Lynch, 2007). In addition, improving rooting
9 efficiency could decrease the input of chemical fertilisers into the agroecosystem, leading to a
10 reduction of both greenhouse gas emissions incurred during their production and the
11 eutrophication of the natural environments from their application (White *et al.*, 2013).

12 In wheat (*Triticum aestivum*), there are two types of roots: the seminal roots and the nodal
13 roots (Kirby, 2002). While germinating, the radicle bursts through the coleorhiza of the seed,
14 and then three to six other seminal roots emerge. Each seminal root has a root cap, followed
15 sequentially by the zones of division, elongation and maturation. Numerous root hairs develop
16 in the zone of maturation, and further back from the root hair zone the lateral roots grow from
17 the stele. The seminal root system can reach 1.5–2.0 m in depth and support the plant
18 throughout the crop’s life (Sylvester-Bradley *et al.*, 2008). Unlike the seminal roots growing
19 directly from the seed, the nodal (crown or adventitious) roots arise from the stem basal nodes
20 and begin to extend when tillering starts. These roots are usually thicker than the seminal ones,
21 and appear relatively horizontally. At anthesis, the whole root system consisting of seminal
22 and nodal roots in the field reaches a maximum: approximately 17 km m⁻² in root density
23 (sample depth: 0–100 cm) and 0.8 t ha⁻¹ in root dry weight, and this varies with genotype,
24 sowing date, temperature, soil structure and environmental stress (Sylvester-Bradley *et al.*,
25 2008; White *et al.*, 2015). Roots then senesce during grain filling though some in the deeper

1 soil layers may continue growing.

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

17 Root system architecture shows large genetic variation among different ploidy levels, between
18 domesticated and wild forms, and within each of the diploids, tetraploids and hexaploids
19 (Robertson *et al.*, 1979; Manschadi *et al.*, 2008; Ayalew *et al.*, 2015). Hexaploid wheat has
20 longer roots than the other ploidy levels (Ayalew *et al.*, 2015); however, compared to the
21 landraces, modern bread wheat cultivars have smaller root mass (Waines and Ehdaie, 2007),
22 suggesting that the RSA needs to be optimized for further yield improvement, particularly
23 under infertile soil conditions (Lynch, 2007). In addition, high-throughput phenotyping
24 techniques also allow identification of the quantitative trait loci (QTL) associated with the
25 RSA. To date, about 339 QTL have been detected for different root architectural traits, and

1 distributed on all wheat chromosomes except 1D (Ren *et al.*, 2012; Bai *et al.*, 2013;
2 Christopher *et al.*, 2013; Liu *et al.*, 2013; Atkinson *et al.*, 2015; Kabir *et al.*, 2015; Maccaferri
3 *et al.*, 2016).

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

1 MATERIALS AND METHODS

2 *Plant materials*

3 Spelt is a relative of bread wheat. A preliminary experiment showed that spelt has a larger,
4 deeper root system and resultant higher nitrogen uptake efficiency compared with bread
5 wheat. To introduce the genetic variation in root traits from spelt, a cross between the bread
6 wheat cultivar 'Forno' and spelt landrace 'Oberkulmer' was made, and the subsequent F₅ RIL
7 mapping population consisting of 226 lines was used in the present study (Messmer *et al.*,
8 1999). Forno and Oberkulmer are both winter-types and were widely grown in Switzerland.

9 *Root phenotyping of the RIL seedlings in growth chamber*

10 A germination paper-based 'pouch and wick' phenotyping system, coupled with digital image
11 analysis, was adopted to measure the root architectural traits in 2014 (Bonser *et al.*, 1996;
12 Atkinson *et al.*, 2015). All 226 RILs, together with Forno and Oberkulmer, were arranged in a
13 randomised complete block (RCB) design with 20 plants per line. Due to limited space within
14 the growth chamber, the 20 plants of each RIL were grown in eight runs: three plants per RIL
15 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
16 run, intact, uniform and representative seeds of each RIL were selected from the combined
17 grain samples in 2013, and grown at the top of the growth pouches (see below), with the
18 embryos pointing downwards.

19 The growth system consisted of the pouches and tanks filled with deionised water. Each
20 growth pouch included two black polythene sheets (24 × 28 cm; Cransford Polythene Ltd,
21 Suffolk, UK), two blue germination papers (24 × 30 cm; Anchor Paper Company, St Paul,
22 MN, USA), a plastic rod (Acrylic Online, Hull, UK) and two 19-mm fold-back clips (Fig. 1A).
23 One polythene sheet and one germination paper were attached to one side of the rod, and the
24 other polythene sheet and germination paper to the other side, fixed with two fold-back clips.

1 Two seeds were grown on a growth pouch (one each side), and there were four tanks with a
2 total of 360 pouches, allowing a maximum of 720 plants to be screened per run. Each tank
3 was filled with 3-L deionised water at the beginning of the experiment, and the water level
4 was topped up at 2-d intervals to ensure that the germination papers remained saturated
5 throughout the experiment. The tanks were placed in an environmentally controlled growth
6 chamber, with 12 h light (photosynthetically active radiation (PAR) = $240 \mu\text{mol m}^{-2} \text{s}^{-1}$ at the
7 tank level, temperature = $17 \text{ }^\circ\text{C}$) and 12 h dark (temperature = $13 \text{ }^\circ\text{C}$).

8 The images of the vertically grown root systems (Fig. 1B, C) were taken 13 days after
9 transferring onto the germination papers, using a Nikon D5100 DSLR camera and modified
10 copy stand. The images were then analysed using the semi-automated software RootNav
11 V1.7.5 (Pound *et al.*, 2013). The parameters used in this study included: (1) seminal root
12 number, excluding the lateral roots; (2) average seminal root length; (3) total root length, the
13 total length of seminal and lateral roots for a given plant; (4) maximum width, the horizontal
14 distance between two widest points achieved by the root system for all depths; (5) maximum
15 depth, the vertical distance from the base to tip of deepest seminal root; (6) width to depth
16 ratio, the ratio of maximum width to maximum depth; (7) tip angle of seminal roots, the
17 average angle of all seminal root tips relative to the vertical axis; (8) emergence angle of
18 seminal roots, the average angle of two outermost seminal root bases (by 50% of root length)
19 relative to the vertical axis (Fig. 1D). Among these parameters, total root length was
20 considered to represent the size of the root system, with seminal root number and average
21 seminal root length as components, while width to depth ratio was used to represent the shape
22 of the root system, with maximum width and depth as components.

1 *Field experiments*

2 *Yield and yield components*

3 The yield and yield components of Forno × Oberkulmer mapping population were
4 investigated in two seasons: 2011–2012 (referred as 2012) and 2012–2013 (referred as 2013).
5 Field experiments were performed at the University of Nottingham Farm, Leicestershire, UK
6 (52°50' N, 1°15' W, 50 m above sea level).. Data on the air temperature, rainfall and solar
7 radiation at the growing sites during the two seasons were collected from the nearby
8 meteorological station and summarised in [Supplementary Data Fig. S1](#) [**Supplementary**
9 **Information**], together with the historic weather data from the Met Office
10 (<http://www.metoffice.gov.uk/>). Both field sites were planted with winter oats in the previous
11 cropping seasons. The soil was a sandy loam, with the nutrient indices similar over two sites:
12 N = 0, P = 5, K = 4 and Mg = 4 (pH = 7.5). The 226 RILs and two parents were grown in a
13 RCB design with three replicates. The seeds were sown in 6 m × 1.6 m plots on 19 October
14 2011 and in 12 m × 1.6 m plots on 31 October 2012, with a sowing density 250 seeds m⁻² and
15 row width 0.125 m in both seasons. Nitrogen fertiliser was applied as ammonium nitrate
16 (NH₄NO₃, 34.5% N) in three splits: early tillering, onset of stem elongation and flag leaf
17 emergence at 40, 40 and 60 kg N ha⁻¹ in 2012, and 40, 60 and 60 kg N ha⁻¹ in 2013,
18 respectively. Plant growth regulators were applied to reduce the risk of lodging, and
19 prophylactic crop protection chemicals were applied to minimise diseases, weeds and pests.

20 Two subsets comprising 72 RILs in 2012 and 110 RILs in 2013 (including the former 72
21 lines), were selected in the light of their considerable differences in yield traits but similar
22 flowering time (± 4 d in 2010 and ± 1 d in 2011). The subsets were used to calculate spikes
23 m⁻², grains per spike and biomass m⁻² (in grams), while machine-harvested grain yield (in
24 tonnes ha⁻¹), grains m⁻² and thousand grain weight (TGW, in grams) were determined for the

1 whole population (226 RILs).

2 At maturity, the plants in an area of 0.5×0.5 m (four central rows) were collected from each
3 plot in the subsets. After cutting at ground level, the infertile shoots (bearing no spikes) were
4 discarded as they were negligible in terms of dry mass in all RILs. The spike number of fertile
5 shoots was then counted, which was multiplied by four to generate the spikes m^{-2} .
6 Subsequently, a subsample of *c.* 70 g, with the fertile shoots counted, in 2012, and 20 fertile
7 shoots in 2013, were obtained. For each subsample, the spikes were cut from these shoots,
8 and the resultant spikes and stems were dried separately in an oven at 85°C for 48 h. After
9 weighing, above-ground biomass per shoot was calculated by dividing the sum of spike and
10 stem dry weight by the fertile shoot number. The above-ground biomass m^{-2} was the biomass
11 per shoot multiplied by the spikes m^{-2} . The dried spikes were threshed completely, and the
12 total grain dry weight was recorded. The average grain weight of an individual spike was
13 calculated by dividing the total grain weight by shoot number in the subsample. Next,
14 individual grain weight was calculated by counting 250 grains and weighing, and grains per
15 spike were the result of grain weight per spike divided by individual grain weight.

16 After sampling, the actual machine-harvested area of each of the 226 RILs was measured
17 (namely A_c , in square meters). All the plots were then harvested with a combine harvester
18 (2010, Sampo Rosenlew, Finland), and the combined grain samples were recorded for fresh
19 weight (W_c , in grams). Because of the non-free-threshing habit of spelt, the grain samples of
20 many RILs could not be threshed completely. The machine-harvested grain yield, therefore,
21 was derived from the threshability analysis. A subsample of 10 g was isolated randomly from
22 each of the combined grain samples. Both threshed and unthreshed grains were counted by
23 hand, recorded as N_{10} . Total grain number in that combined grain sample (N_c) was estimated
24 as: $N_c = N_{10} * (W_c/10)$, and grain number m^{-2} (N_{psm}) as: $N_{\text{psm}} = N_c/A_c$. In addition, another
25 grain subsample of *c.* 1 kg from each plot was partially threshed, and 200 grains were counted,

1 oven-dried and weighed for TGW (W_{tg}). Then, machine-harvested yield (Y_{m}) was calculated
2 as: $Y_{\text{m}} = N_{\text{c}} * (W_{\text{tg}} * 10^{-3}) * 10^{-6} / (A_{\text{c}} * 10^{-4})$.

3 ***Phasic development***

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

15 ***Glasshouse experiment***

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

1 dried at 85°C for 48 h, weighed and counted to calculate grains per spike and TGW.

2 ***Statistical analysis of phenotypic data***

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

9 ***Identification of quantitative trait loci***

10 The genetic linkage map of Forno × Oberkulmer was downloaded from the
11 GrainGenes database (<http://wheat.pw.usda.gov/GG2/index.shtml>), and consisted of 230
12 segregating loci (restriction fragment length polymorphisms and microsatellites) and 23
13 linkage groups covering 2,469 cM. Analysis of QTL was performed using MapQTL v6 (Van
14 Ooijen, 2009), with the average values of phenotypic data over replicates. An initial interval
15 mapping was carried out to identify the putative QTL. A locus was considered significant
16 when its peak of logarithm of the odds (LOD) was higher than the genome-wide threshold for
17 the trait, which was computed from a permutation test with 1,000 iterations. Subsequently,
18 multiple-QTL model (MQM) mapping was performed by using the markers closest to the
19 QTL peaks as the co-factors ($P < 0.02$). The nomenclature of QTL followed the Catalogue of
20 Gene Symbols for Wheat (<http://wheat.pw.usda.gov/GG2/Triticum/wgc/2008/>). The chart of
21 linkage groups and 1-LOD (the LOD peak values minus one) support intervals representing
22 the QTL positions were drawn using MapChart v2.2 (Voorrips, 2002).

1 RESULTS

2 *Phenotypic variation in seedling root traits between genotypes*

3 The bread wheat Forno and spelt Oberkulmer differed significantly in RSA examined in 'the
4 pouch and wick' system in the growth chamber in 2014 (Figs 1 and 2, Table 1). Compared
5 with Forno, Oberkulmer had more (17%) and longer (12%) seminal roots, and its total root
6 length was also greater (20%). No significant difference in root maximum depth was found,
7 but the maximum width, width to depth ratio, and tip and emergence angles of seminal roots,
8 were smaller in Oberkulmer, indicating a narrow root system. As expected, there was a large
9 variation in RSA among different RILs derived from Forno × Oberkulmer (Fig. 2 and Table
10 1). Transgressive segregation (values beyond the parental phenotypic range) was seen for
11 each trait. For example, the seminal root number of RIL-230 (4.7), seminal root length of
12 RIL-164 (186 mm), and total root length of RIL-248 (766 mm) were 12%, 30% and 27%
13 greater than those of the higher parent Oberkulmer.

14 *Phenotypic correlations between seedling root traits*

15 Significant correlations between different root traits were found (Table 2). Root system size
16 represented by the total root length was positively correlated with both the number and length
17 of seminal roots, and a closer relationship found with former. Root system shape represented
18 by the ratio of width to depth was closely associated with the maximum width ($r = 0.85$, $P <$
19 0.01), rather than the depth. Total root length was positively associated with both the
20 maximum width and depth, and had only a slight effect on root system shape. In addition,
21 larger tip and emergence angles of seminal roots were associated with a wider root system,
22 and larger tip angle was also associated with a shallower root system.

1 ***Phenotypic correlations of the root traits with yield and yield components***

2 Field experiments showed that Oberkulmer had lower machine-harvested yield than Forno
3 (Table 1). Also, grain number components, including grains m^{-2} , spikes m^{-2} and grains per
4 spike, tended to be lower in Oberkulmer. Thousand grain weight was higher in Oberkulmer in
5 2012, but not in 2013. In terms of the above-ground biomass m^{-2} , there was no significant
6 difference between two parental lines. In the RIL population, a large variation was found for
7 each yield trait investigated (Table 1).

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

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

21 ***Phenotypic correlations of the root traits with phenology***

22 Similarly, there were significant phenotypic differences in growth stages between genotypes
23 in the RIL population (Table 1), and significant relationships of the growth stages with RSA
24 (Table 3). Seminal root number and total root length were positively associated with GS92

1 and GS61–GS92, indicating that a larger root system can be associated with delayed maturity
2 and a longer grain filling period. Additionally, longer seminal roots were associated with
3 earlier GS61 and shorter GS39–GS61. One of the root system shape parameters, maximum
4 depth, showed a consistent negative relationship with GS61.

5 ***Performance of the top and bottom RILs categorised by total root length***

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

14 ***Detection of quantitative trait loci***

15 ***Quantitative trait loci for the root architectural traits***

16 A total of 38 QTL associated with the RSA were discovered, including 12 for seminal root
17 number, two for seminal root length, 13 for total root length, five for each of root maximum
18 width and depth, and one for the emergence angle of seminal roots (Fig. 3 and Table 5). These
19 QTL were located on 12 chromosomes, i.e. 2A (five QTL), 2B (one QTL), 3A (three QTL),
20 3B (three QTL), 3DL (two QTL), 4A (six QTL), 4DL (one QTL), 5A (six QTL), 5B (three
21 QTL), 7AL (two QTL), 7B (three QTL) and 7D (three QTL), individually explaining
22 6.3–22.3% of the phenotypic variation observed. Forno provided 17 (45%) increasing alleles
23 (increasing the values of the traits) while Oberkulmer provided the remaining ones. Most of
24 the QTL identified (71%) were associated with the parameters of root system size, and

1 distributed on all 12 chromosomes where QTL were detected.

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

9 *Quantitative trait locus coincidence between the root and yield traits*

10 For yield and yield components, 41 QTL were detected on 11 chromosomes, including seven
11 for machine-harvested yield, 11 for grains m^{-2} , four for spikes m^{-2} , nine for grains per spike
12 and ten for TGW (Fig. 3 and Table 5). These QTL explained phenotypic variation between
13 6.3% and 35.1%.

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

1 also co-located with two QTL for grains m⁻² and one for spikes m⁻².

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

7 ***Quantitative trait locus coincidence between the root traits and phasic development***

8 For the growth stages, a total of 49 QTL were identified, individually explaining 11.6–29.6%
9 of the phenotypic variation (Fig. 3 and Table 5). The spelt Oberkulmer provided 34 (69%)
10 alleles delaying phenology.

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

1 DISCUSSION

2 ***Significant links of the seedling RSA with yield and yield components***

3 Using a germination paper-based ‘pouch and wick’ phenotyping pipeline, the seedling root
4 architectural traits of Forno and Oberkulmer were measured in a fast, cost-effective way.
5 Although the seedling roots growing in the pouches may not develop exactly as those of
6 plants growing in the field, this technique allowed us to characterise the differences in RSA
7 between genotypes. Comparing the indoor seedling RSA traits with field agronomical
8 performance has been reported in a number of studies, for instance, in bread wheat (Liu *et al.*,
9 2013; Atkinson *et al.*, 2015), durum wheat (*Triticum durum*) (Cane *et al.*, 2014), maize (*Zea*
10 *mays*) (Li *et al.*, 2015), and oilseed rape (*Brassica napus*) (Thomas *et al.*, 2016). The present
11 study identifies significant links between the seedling RSA, and field yield and yield-related
12 traits, suggesting that the RSA of 13-d-old seedlings grown in the growth pouches could be
13 predictive of field performance of these agronomic traits.

14 The root system size of wheat seedlings, as represented by seminal root number and total root
15 length, was positively associated with grain yield, in line with a previous report by Liu *et al.*
16 (2013). The positive correlations between the two root traits and yield were not found in 2012,
17 likely due to the heavy rainfall and consequent high disease levels (e.g. glume blotch that
18 affects grain development) during grain filling in June and July of this year ([Supplementary](#)
19 [Data Fig. S1](#)) [**Supplementary Information**]. The bread wheat parent Forno is highly
20 susceptible to glume blotch whereas the spelt parent Oberkulmer has a high level of resistance
21 (Messmer *et al.*, 1999), which can at least partly explain the low TGW of Forno in 2012.
22 Furthermore, the high disease pressure, rather than resource capture by roots, might limit the
23 yields of many RILs in this year (sink limited), so the correlations between the root system
24 size and yield could not be seen. Increase in yield as a result of larger root system was

1 mainly due to increased grain number rather than individual grain weight in the present study.
2 Grains per spike, the major component of grain number, is substantially determined during
3 stem elongation before anthesis (González *et al.*, 2011). During this period, the spikes and
4 stems are both growing rapidly, resulting in an intensive intra-plant competition for available
5 assimilates (source limited). The growth of spikes, which is a key determinant of spike
6 fertility and, in turn, grains per spike, may be limited in such a case (Fischer, 1985, 2011;
7 González *et al.*, 2011). There is evidence that vigorous seedling roots are beneficial to take up
8 more soil resources (e.g. N and P) for early plant growth (An *et al.*, 2006; Zhu *et al.*, 2006;
9 Cao *et al.*, 2014), leading to a release of the intra-plant competition to a great extent. The
10 resultant availability of more resources for young spike growth would increase grains per
11 spike and yield. However, total root length and seminal root number had negative effects on
12 TGW, likely because increased grain number is often associated with decreased individual
13 grain weight (Slafer and Andrade, 1993; Miralles and Slafer, 1995; Griffiths *et al.*, 2015).
14 Grain size contributes to end-use quality of wheat (Gegas *et al.*, 2010), so care must be taken
15 that a larger root system should be selected for the purpose of improving grain yield, whereas
16 a smaller one may improve grain quality but be accompanied with a penalty for yield.

17 Seedling root architectural traits also influence the phasic development. Notably, a larger root
18 system is associated with delayed maturity and, in turn, extended grain filling, likely because
19 of improved water and nutrient uptake for post-anthesis photosynthesis (Pinto and Reynolds,
20 2015). A longer period of grain filling was thought to enlarge final grain size, which contrasts
21 the result that the TGW is actually reduced. A further analysis showed that the GS61–GS92
22 duration was positively correlated with grains per spike ($r = 0.43$, $P < 0.001$ in 2012; $r = 0.60$,
23 $P < 0.001$ in 2013), indicating that more grains defined before anthesis as a consequence of a
24 larger early root system require a longer period to fill thereafter. At the same time, more
25 grains per spike are associated with smaller grains, as discussed above, which leads to an

1 apparent negative correlation between grain size and grain filling period.

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

3 A total of 25 QTL for seminal root number and total root length were identified in this study,
4 clustering on seven chromosomes (2A, 3DL, 4A, 5A, 5B, 7AL and 7B). Two clusters for the
5 two traits were found on each of the short and long arms of 4A, similar to the findings of
6 Christopher *et al.* (2013), and the clusters on 2AS and 5AS could correspond to those of Kabir
7 *et al.* (2015) and Hamada *et al.* (2012). There was also a QTL identified in the distal region of
8 7DS for seminal root number, where the QTL for total length of all roots and of seminal roots
9 as well as grain yield have been detected previously (Atkinson *et al.*, 2015). In addition, a
10 QTL for total root length was mapped around the centromere of 3B in the present study,
11 concurring with the reports by Bai *et al.* (2013) and Liu *et al.* (2013). The favourable QTL
12 identified here can be transferred into elite wheat cultivars for RSA improvement after fine
13 mapping. Furthermore, the top ten RILs selected for their highest total root length, each of
14 which carries mostly all the positive alleles underlying the above QTL, will be utilised as the
15 intermediate materials for introgression.

16 The phenotypic correlations of seminal root number and total root length with yield traits
17 were confirmed by genetic analysis, showing tightly linked genes or pleiotropy. Recently, the
18 QTL coincidence between the two root traits, yield and TGW was also found in tetraploid
19 wheat (Maccaferri *et al.*, 2016). *qTaLRO-B1*, a major 2BS QTL controlling the root length of
20 wheat seedlings, has been used to develop near-isogenic lines with long roots and efficient
21 phosphate uptake and biomass accumulation (Cao *et al.*, 2014). In rice (*Oryza sativa*), a QTL
22 increasing root length by 9.6 cm has been introgressed into an upland cultivar, giving rise to
23 an increase of 0.2 t ha⁻¹ in grain yield (Steele *et al.*, 2013). These examples suggest the
24 potential of introgressed coincident QTL to improve both RSA and agronomical traits.

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

2 The significant variation in RSA in the present study is largely attributed to the use of spelt.
3 Spelt is an ancient crop and has been cultivated since 5,000 BC. As an old-world grain, spelt
4 is adapted to high precipitation, heavy and infertile soils, and cool temperatures, and is still a
5 minor crop grown on a considerable acreage in marginal areas at present (Campbell, 1997).
6 Additionally, previous studies found that spelt has a faster early crop growth rate, and
7 accumulates more biomass and N than bread wheat does (Koutroubas *et al.*, 2012). These
8 aspects of the performance of spelt may depend largely on its vigorous root system. In this
9 study, the spelt Oberkulmer showed a large but narrow seminal root system, represented by
10 more seminal roots, longer seminal and total roots, less root maximum width, and smaller tip
11 and emergence angles. A narrow but strong root system could help to penetrate the heavy soil
12 for foraging and anchoring, while a large root system would enhance its ability of nutrient
13 acquisition to support plant growth. In another experiment, spelt (three cultivars:
14 ‘Oberkulmer’, ‘Tauro’ and ‘SB’) had much more root biomass at all soil layers than bread
15 wheat (two cultivars: ‘Xi-19’ and ‘Forno’), and this is also true for root length density (root
16 length per unit soil volume) (unpubl. res.), which, at the depth of 80 cm, is still above the
17 threshold of *c.* 1 cm cm⁻³ for potential water and nitrate capture (Foulkes *et al.*, 2011).
18 Genetically, the current study presents 14 and 7 favourable alleles originating from the spelt
19 Oberkulmer for the size and shape of root system, respectively. Taken together, spelt is a
20 critical genetic resource for the root system improvement in bread wheat, and its nature as a
21 hexaploid will allow efficient hybridisation with bread wheat to transfer these traits.

22 ***Conclusions***

23 This study demonstrated that vigorous root growth at the early seedling stage affects yield
24 formation in wheat. Seminal root number and total root length have positive effects on grain

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

1 **SUPPLEMENTARY DATA**

2 [Supplementary data](#) are available online at www.aob.oxfordjournals.org and consist of the
3 following. [Figure S1](#): mean air temperature, rainfall and solar radiation at University of
4 Nottingham Farm in 2011–2012 and 2012–2013 seasons.

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8

1 **FIGURE LEGENDS**

2 **FIG. 1.** Root phenotyping pipeline for the Forno × Oberkulmer mapping population grown in
3 a growth chamber during 2014. (A) Germination paper-based ‘pouch and wick’ system; (B)
4 and (C) Example root images of Forno and Oberkulmer, respectively (*bar* = 1 cm); (D)
5 Measurement of root architectural traits on a recombinant inbred line.

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

9 **FIG. 3.** Quantitative trait loci (QTL) associated with seedling root traits, yield, yield
10 components and phenology in the Forno × Oberkulmer mapping population. For each linkage
11 group, the molecular marker identifiers are placed on the right side of the chromosome
12 (*hollow bar*) and their locations are shown on the left side in centiMorgan (cM). The vertical
13 bars on the right sides represent the 1-LOD support intervals of significant QTL: blue (root
14 traits), red (yield and yield components) and green (phenology). Each QTL symbol consists of
15 an upper-case letter ‘Q’ and the abbreviations of quantitative trait, institution (*uon*), years
16 when the QTL was detected (‘12’, 2012; ‘13’, 2013; ‘14’, 2014), and the parent conferring the
17 allele with positive effect (‘F’, Forno; ‘O’, Oberkulmer). Trait abbreviations: Sm, seminal
18 root number; Srl, seminal root length; Trl, total root length; Mw, maximum width of root
19 system; Md, maximum depth of root system; Ea, emergence angle of seminal roots; Mhy,
20 machine-harvested yield; Gpsm, grains m⁻²; Spsm, spikes m⁻²; Gps, grains per spike; Tgw,
21 thousand grain weight; Gs39, the time at full flag leaf emergence; Gs61, the time at anthesis;
22 Gs92, the time at maturity; Gs31-39, the duration from GS31 (the time at the onset of stem
23 elongation) to GS39; Gs39-61, the duration from GS39 to GS61; Gs31-61, the duration from
24 GS31 to GS61; Gs61-92, the duration from GS61 to GS92.

25

TABLES

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	<i>P</i> -value	Mean (min; max)	<i>P</i> -value	SED*
Seedling root traits (<i>n</i> = 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 (degree)	2014	32.3	22.3	<0.001	29.9 (18.1; 41.1)	<0.001	3.6
Emergence angle of seminal root (degree)	2014	25.6	21.2	<0.01	23.9 (16.7; 35.4)	<0.001	3.1
Yield traits (<i>n</i> = 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	20296	12393	<0.01	12478 (7244; 18937)	<0.001	1309
	2013	19367	12519	<0.01	14420 (8720; 24864)	<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

TABLE 1. (continued)

Trait	Year	Parental lines			RILs		
		F	O	<i>P</i> -value	Mean (min; max)	<i>P</i> -value	SED
Phenology (<i>n</i> = 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

* SED: standard error of the difference; † Glasshouse experiment in 2014 (*n* = 3); ‡ °Cd, degree day.

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 $P < 0.01$.