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ORIGINAL RESEARCH

Selection in Early Generations to Shift Allele Frequency for Seminal Root Angle in Wheat

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ABSTRACT

A current challenge for plant breeders is the limited ability to phenotype and select for root characteristics to enhance crop productivity. The development of a high-throughput phenotyping method has recently offered new opportunities for the selection of root characteristics in breeding programs. Here, we investigated prospects for phenotypic and molecular selection for seminal root angle (SRA), a key trait associated with mature root system architecture in wheat (*Triticum aestivum* L.). We first investigated genetic diversity for this trait in a panel of 22 wheat lines adapted to Australian environments. The angle between the first pair of seminal roots ranged from 72 to 106°. We then evaluated selection gain via direct phenotypic selection in early generations by comparing the resulting shift in population distribution in tail populations selected for "narrow" and "wide" root angle. Overall, two rounds of selection significantly shifted the mean root angle as much as 10°. Furthermore, comparison of allele frequencies in the tail populations revealed genomic regions under selection, for which marker-assisted selection appeared to be successful. By combining efficient phenotyping and rapid generation advance, lines enriched with alleles for either narrow or wide SRA were developed within only 18 mo. These results suggest that there is a valuable source of allelic variation for SRA that can be harnessed and rapidly introgressed into elite wheat lines.

Core Ideas

- This is the first study to manipulate root system architecture through direct selection.
- Rapid and pronounced bidirectional selection for seminal root angle was achieved.
- The frequency of alleles for desirable root traits in wheat populations was shifted.

MAJOR RESOURCES are unevenly distributed in the soil profile in terms of depth and time. The spatial and temporal configuration of the root system in the soil, referred to as root system architecture (RSA), determines the ability of a plant to exploit those resources and is therefore an important aspect for plant productivity and yield stability (Ludlow and Muchow, 1990; Lynch, 1995). Roots are dynamic; they respond to changing moisture, nutrient status, temperature, and pH, and they interact with organisms present in the rhizosphere (Bao et al., 2014; Robbins and Dinneny, 2015). Through complex signaling pathways, roots are also able to communicate with the aboveground part of the plants, impacting their

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Abbreviations: DArT, diversity array technology; DArT-seq, sequencing-based diversity array technology; MAS, marker-assisted selection; Pop1–Ma/Dr, population derived from Mace/Drysdale//Mace cross; Pop2–Su/Dh, population derived from Suntop/Dharwar Dry//Suntop cross; Pop3–Sc/SB, population derived from Scout/SB062//Scout; QTL, quantitative trait loci; RSA, root system architecture; SRA, seminal root angle; SRN, seminal root number

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growth and development (DoVale and Fritsche-Neto, 2015). The plasticity of roots in response to the environment provides opportunities for exploring natural variation and to identify beneficial root traits to enhance plant productivity (Kano et al., 2011; Grossman and Rice, 2012).

Genotypic variation for root architectural traits and their functional implications for acquisition of water and nutrients have been reported for many crop species (Wasson et al., 2012; Comas et al., 2013; Lynch, 2013; Brown et al., 2013; Canè et al., 2014; Lynch et al., 2014; Paez-Garcia et al., 2015; Lynch and Wojciechowski, 2015). For example, a number of studies across species have reported that the angle between the first pair of seminal roots, the SRA, was associated with the three-dimensional growth and functioning of the root system later in the season (Nakamoto et al., 1991; Oyanagi et al., 1993; Nakamoto and Oyanagi, 1994; Bengough et al., 2004; Kato et al., 2006; Maccaferri et al., 2016; Voss-Fels et al., 2018). Seminal root angle is highly heritable, expressed at an early stage, and can be rapidly and cost-effectively screened in seedlings (Bengough et al., 2004; Richard et al., 2015). Hence, incorporation of this proxy trait into breeding programs would accelerate the deployment of desirable RSA genes in elite lines.

In this study, we examine genotypic variability for SRA in a panel of 22 cultivars and elite breeding lines of wheat adapted to Australian cropping regions and investigate the effectiveness of direct phenotypic selection and marker-assisted selection (MAS) for SRA. We used a recently developed phenotyping method based on transparent pots, the "clear-pot" method (Richard et al., 2015), to rapidly apply two consecutive rounds of bidirectional selection for SRA in early generations (BC_1F_2 and BC_1F_3) of three backcross populations. We examine the shift in population distributions resulting from the two selection rounds, and characterized fixed lines ($BC_1F_{4:5}$) generated in one of the backcross populations. We investigate shifts in allelic frequency to identify genomic regions under selection, which likely harbor genes controlling SRA. We also test the effectiveness of MAS for these regions in an independent $F_{4:5}$ reference population. We discuss the opportunities to integrate effective selection for SRA into breeding programs.

Materials and Methods

Plant Material

A panel of 22 wheat lines was assembled, comprising cultivars and elite breeding lines adapted or with potential adaptation to the Australian cropping conditions. This panel was used to identify six parental lines for developing three backcross populations of interest to breeders. Notably, in this panel, some lines share a common genetic background. For example, 'Mace', 'UQ01687', and 'Wallup' are derived from 'Wyalkatchem'; 'RIL114' and 'UQ01648' from 'H45'; and 'Spitfire' from 'Drysdale' (Supplemental Table S1).

High-Throughput Phenotyping (Clear-Pot Method)

Root angle in wheat seedlings was measured using the clear-pot method described by Richard et al. (2015).

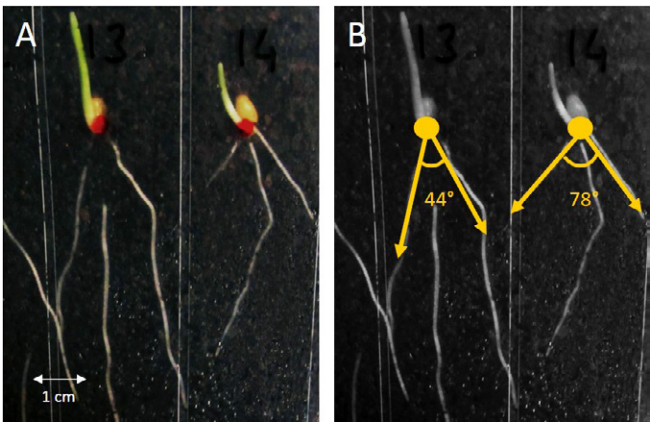

Fig. 1. Wheat seedlings phenotyped for seminal root traits via the clear-pot method. (A) Images recorded at 5 d after sowing. The camera parameters are set to enhance the contrast between the roots and the soil (red dot markings on the exterior of the transparent pots were used to guide the placement of seeds). (B) For each plant, the angle between the first pair of seminal roots was measured at approximately 3 cm distance from the seed using image analysis software (ImageJ, (http://rsb.info.nih.gov/ij/, accessed 9 Feb. 2018). The image was converted to black and white to enhance the visibility of the roots.

Seeds were sown in clear (transparent) ANOVApot pots (Anovapot Pty Ltd, Brisbane, QLD, Australia) (200 mm diameter, 190 mm height, 4 L) filled with pine bark potting media (70% composted pine bark 0–5 mm, 30% coco peat). Seeds were placed vertically, embryo pointing downward, at 2 cm depth with a 2.5-cm space between seeds (as shown in Fig. 1), providing a density of 24 seeds per pot. After sowing, the clear pots were placed in black pots (ANOVApot, 200 mm diameter, 190 mm height, 4 L) to exclude light from the developing roots. Seedlings were watered once after sowing. No other nutrients were supplied. Seedlings were grown in a climate-controlled growth facility. A constant temperature (17 ± 2°C) was maintained over 24 h with a 12-h photoperiod (artificial light) for the first 5 d. Roots were imaged with a camera (Canon PowerShot SX600 HS 16MP Ultra-Zoom Digital camera, Canon, Sydney, NSW, Australia) 5 d after sowing. Image analysis was performed with ImageJ software (http://rsb.info.nih.gov/ij/, accessed 9 Feb. 2018). For each plant, SRA, defined as the angle between the first pair of seminal roots, was measured at approximately 3 cm distance from the embryo of the grain (Fig. 1).

Selection of Parental Lines

The panel of 22 candidate parental wheat lines was characterized for SRA using the clear-pot method described above in two repeated experiments: candidate parent Experiments 1 and 2. The two experiments used a randomized complete block design, where 10 seeds of each of the 22 lines were randomized across 10 pots. Data from repeated characterization of the panel were analyzed using a mixed model, containing "Line" (i.e., cultivars or breeding lines) and "Replicate" as random components. Best linear unbiased predictions were obtained for each line in

each experiment (1 and 2) with ASReml-R (Gilmour et al., 2009) and R software version 3.2.0 (R Core Team, 2013).

Six parental lines displaying different phenotypes for SRA were selected from the panel and used to develop three backcross populations segregating for the trait of interest. These three backcross populations used three Australian spring wheat cultivars as recurrent parents and three diverse donor parents.

Two populations with parents that had contrasting phenotypes for SRA (i.e., narrow SRA for donor parents versus wide SRA for recurrent parents) were developed, namely Pop1–Ma/Dr (Mace/Drysdale//Mace) and Pop2–Su/Dh ('Suntop'/'Dharwar Dry'//Suntop). One population with parents that had intermediate SRA phenotypes was also developed, namely Pop3–Sc/SB ('Scout'/'SB062'// Scout). Pop1–Ma/Dr and Pop2–Su/Dh were designed to recombine alleles for narrow and wide SRA, whereas Pop3–Sc/SB provided an opportunity to explore the ability to manipulate SRA in crosses derived from parents exhibiting similar SRA phenotypes. Despite having a similar SRA phenotype, Scout and SB062 are genetically distant. These parental lines have a coefficient of parentage of 0.19, based on a calculation from Kempthorne (1969) under the assumptions that each parent contributes equally and that ancestors without known pedigrees are unrelated. As SRA is under complex genetic control and determined by a combination of alleles with both positive and negative effects (Christopher et al., 2013), lines displaying similar phenotypes could have different combinations of alleles.

Growing Conditions (Speed Breeding)

All generations (except F_2 for Pop1–Ma/Dr) were grown in the "speed breeding" rapid generation advance system at The University of Queensland, using controlled temperature (22 ± 3°C) and constant (24 h) light to accelerate plant development (Watson et al., 2018). At 10 d after sowing, slow release Osmocote NPK fertilizer (Scotts Australia, Bella Vista, NSW, Australia) (N–P–K: 21.2–1.9–5.7, with trace elements) was supplied (5 g L^{-1}). Using this system, BC1F4:5 or F4:5 lines were developed within 18 mo.

For generations that were subject to phenotyping, seedlings were grown for 5 d from sowing in a climate-controlled growth facility with environmental conditions maintained with a 12 h photoperiod and at a constant temperature of 17°C, as required for SRA assessment.

Development of Tail Backcross Populations via Phenotypic Selection

Three F_1 crosses were made (Mace × Drysdale, Suntop × Dharwar Dry, and Scout × SB062) and backcrossed to Mace, Suntop, and Scout, respectively. The resulting BC_1F_1 seeds were bulked for each backcross population and grown in the glasshouse to produce BC_1F_2 seeds for the three backcross populations Pop1–Ma/Dr, Pop2–Su/Dh, and Pop3–Sc/SB, respectively. The three backcross populations were progressed to the BC_1F_4 generation by combining generations of single-seed descent with phenotypic selection for SRA (Fig. 2). As Pop1–Ma/Dr was observed clearly to be segregating for the trait of interest (Fig. 3), this population was progressed an additional generation to obtain $BC_1F_{4:5}$ plants (Fig. 2).

Two consecutive rounds of bidirectional selection for SRA were applied to segregating generations (BC_1F_2 and BC_1F_3) of the three populations. Seminal root angle was assessed via the clear-pot method, as described above. The number of plants assessed for SRA varied between populations and across generations of screening (Supplemental Table S2). This was because some roots were hidden by the soil, others were too short (<3 cm) at the time of imaging or the seeds did not germinate. Thus, to maintain the population size throughout the development of the backcross populations, different selection intensities were applied for each population and generation (Supplemental Table S2).

For screening the BC_1F_2 generation, 552 seeds per population were sown in clear pots. The experiment was blocked according to each of the three populations and 12 replicates for each of the respective parents were included in each block. Each block contained 24 pots, where each pot contained 23 progeny plus one parent line randomly allocated to a position. At 5 d after sowing, SRA was determined for each individual plant via image analysis. Each BC_1F_2 plant screened for SRA was considered to be an individual genotype; thus no replication was possible and raw values were used to generate population distributions. Within each population, individuals displaying extreme phenotypes, thus representing both the lower ("narrow" angle) and upper ("wide" angle) tails of the population distribution were selected, respectively (represented by the pink and blue shaded areas in Fig. 3). Selection intensity ranged from 21 to 25%, resulting in tail populations comprising 57 to 60 BC_1F_2 plants (Supplemental Table S2). The selected plants were grown-on to produce self-pollinated seeds (BC_1F_3), but the nonselected plants were discarded. The BC_1F_3 seeds were harvested on a per-plant basis (i.e., per BC_1F_2 family). In total, six separate tail populations were created: 'narrow' and 'wide' SRA for each of the three main populations. Four to five BC_1F_3 seeds from the selected BC_1F_2 plants were sampled, bulked, and grown to be screened again and provided the next generation.

For screening the BC_1F_3 generation, 276 seeds for each of the six tail populations were sown in clear pots and assessed for SRA. Again, the experiment was blocked according to population, and replicates for each of the respective parents were included, as described above. Similarly, each BC_1F_3 plant screened for SRA was considered to be an individual genotype and raw values were used to generate population distributions. Within narrow tail populations, individuals exhibiting extremely narrow phenotypes were selected again, whereas within the wide tail populations, plants displaying extremely wide phenotypes were selected again (Fig. 3). Within each tail, selection intensity ranged from 27 to 31%, resulting in tail populations of 32 to 41 BC_1F_3 plants. Sometimes, no individuals from a BC_1F_2 family were retained and thus not all families selected in the BC_1F_2 generation were necessarily carried forward in subsequent generations. Here, selected

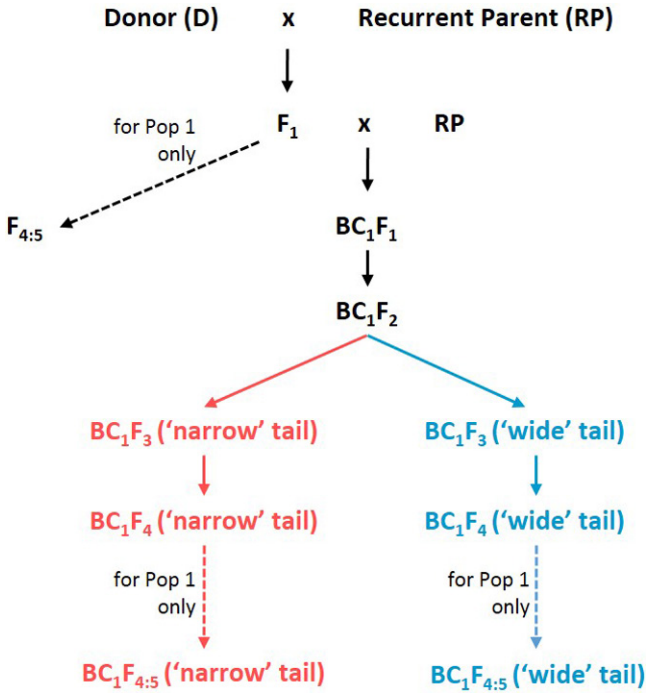

Fig. 2. Scheme for developing tail backcross wheat populations selected for seminal root angle and an independent unselected population. This scheme was applied to develop the narrow and wide tail populations up to the BC_1F_4 generation (represented in pink and blue respectively) for three populations: Pop1–Ma/Dr (Mace/Drysdale//Mace), Pop2–Su/Dh (Suntop/Dharwar Dry//Suntop), and Pop3–Sc/SB (Scout/SB062//Scout). Pop1–Ma/Dr was progressed an additional generation to the $BC_1F_{4:5}$ generation (represented by the pink dashed line). The F_1 generation from Pop1–Ma/Dr was independently progressed to the $F_{4:5}$ generation through selfing (represented by the black dashed line). No selection was applied in this independent reference population. RP: recurrent parent (i.e., Drysdale, Dharwar Dry, and SB062 for Pop1–Ma/Dr, Pop2–Su/Dh, and Pop3–Sc/SB, respectively); D: donor (i.e., Mace, Suntop, and Scout for Pop1–Ma/Dr, Pop2–Su/Dh, and Pop3–Sc/SB, respectively).

BC_1F_3 plants were sampled from 24 to 28 BC_1F_2 families (Supplemental Table S2). The selected BC_1F_3 plants were retained and grown-on to produce BC_1F_4 seeds but the nonselected plants were discarded. The BC_1F_4 seeds were harvested on a per-plant basis. Six to nine BC_1F_4 seeds were sampled per selected BC_1F_3 plant, bulked, and grown on to be phenotyped.

For screening the BC_1F_4 generation, a total of 276 seeds for each of the six tail populations were sown in clear pots and assessed for SRA. The experiment was conducted as described above for screening the BC_1F_3 generation. To compare shifts in population distributions over the course of bi-directional selection, a Welch two-sample *t*-test was used to compare SRA means attained by the narrow and wide tail populations for each backcross population in the BC_1F_3 and BC_1F_4 generations.

The SRAs of the six parental lines displayed across these first three generations of phenotypic screening were analyzed via a two-way ANOVA, with the two factors "genotype" and "generation of phenotypic screening". Tukey's

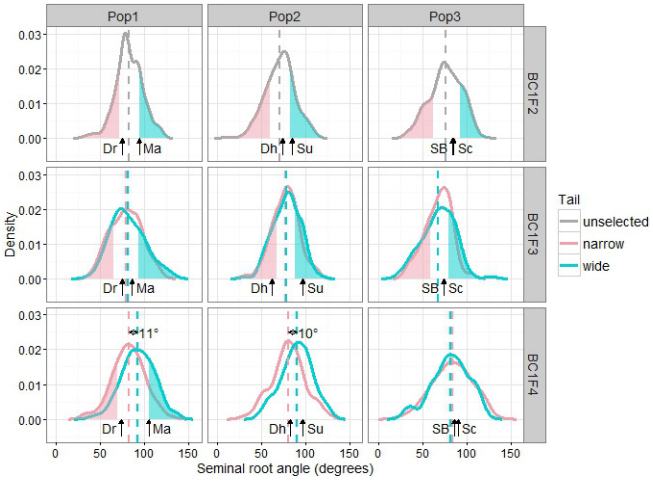

Fig. 3. Distribution of seminal root angle (SRA) for each generation and each wheat population. The frequency distribution of SRA is presented for individuals from the BC_1F_2, BC_1F_3 and BC_1F_4 generations for each of the three populations: Pop1–Ma/Dr (Mace/Drysdale//Mace), Pop2–Su/Dh (Suntop/Dharwar Dry//Suntop), and Pop3–Sc/SB (Scout/SB062//Scout). The shaded portion of the distribution indicates the selected individuals retained following bidirectional selection in each generation, where pink shading indicates the narrow tail and blue shading indicates the wide tail. The gray dashed line represents the mean SRA attained by the BC_1F_2 population; the pink and blue dashed lines display the mean SRA for the BC_1F_3 and BC_1F_4 plants from the narrow and wide tail populations, respectively. Arrows display the average SRA for the donor lines Drysdale (Dr), Dharwar Dry (Dh), and SB062 (SB) and the respective recurrent parents Mace (Ma), Suntop (Su), and Scout (Sc) for each population for each generation of phenotypic screening.

multiple comparisons of means test was conducted to detect differences between genotypes and between generations of phenotypic screening, with a 95% familywise confidence interval with the function HSD.test from the package agricolae (de Mendiburu and de Mendiburu, 2016).

Development and Characterization of $BC_1F_{4:5}$ Lines

Pop1–Ma/Dr, which was visibly segregating for SRA, was progressed an additional generation to obtain $BC_1F_{4:5}$ plants (Fig. 2 and Fig. 3). A selection intensity of 24% was applied to select the lower tail of the SRA distribution, resulting in the selection of 46 BC_1F_4 plants, derived from 20 BC_1F_2 families (Supplemental Table S2). A selection intensity of 24% was applied to select the upper tail of the SRA distribution, resulting in the selection of 44 BC_1F_4 plants, derived from 18 BC_1F_2 families (Supplemental Table S2). The selected 90 BC_1F_4 plants from Pop1–Ma/Dr were grown-on in the glasshouse to produce $BC_1F_{4:5}$ seeds, which were considered fixed lines.

The 46 $BC_1F_{4:5}$ lines from the narrow tail and the 44 $BC_1F_{4:5}$ lines from the wide tail of Pop1–Ma/Dr were characterized for SRA, along with the parental lines Mace and Drysdale via the clear-pot method described above. As the $BC_1F_{4:5}$ lines were considered to be fixed, a randomized complete block design was used, where 10 replicate seeds of each of the 90 lines along with the two

parental lines (and four checks) were randomized across 40 pots. Seminal root angle was analyzed via ANOVA, and Tukey's multiple comparisons of means test was conducted to detect differences among pairs of means as described above. A Welch two-sample *t*-test was also used to compare SRA means attained by the $BC_1F_{4:5}$ lines from the narrow and wide tails.

Genotyping and Comparative Marker Allele Frequency Analysis of BC_1F_4 Lines

Genomic DNA was extracted from young leaf tissue with the hexadecyltrimethylammonium bromide-based extraction protocol recommended by Diversity Arrays Technology Pty Ltd (DArT; www.diversityarrays.com, accessed 9 Feb. 2018). The samples submitted to DArT for genotyping consisted of selected lines from the narrow and wide tail populations of Pop1–Ma/Dr and Pop3–Sc/SB, as well as the respective parental lines. Individuals exhibiting extremely narrow phenotypes within the narrow tail populations and individuals displaying extremely wide phenotypes within the wide tail populations of Pop1–Ma/Dr and Pop3–Sc/SB were selected, with the selection intensity ranging from 24 to 26% (Supplemental Table S2). In total, 49 and 46 BC_1F_4 lines from the narrow and wide tail populations of Pop1–Ma/Dr, 34 and 35 BC_1F_4 lines from the narrow and wide tail populations of Pop3–Sc/SB, and one sample of each of the parental lines (Drysdale, Mace, SB062, and Scout) were genotyped with the wheat DArT genotyping-by-sequencing (DArT-seq) platform. Genotyping returned scores for dominant markers extracted in silico from sequences obtained from genomic representations referred to as SilicoDArT markers. Here, 4827 and 2640 polymorphic SilicoDArT presence–absence markers were returned for Pop1–Ma/Dr and Pop3–Sc/SB, respectively. SilicoDArT markers were positioned on the wheat DArT-seq consensus map provided by Dr. Andrzej Killian from DArT.

Marker data were processed with a quantitative allele frequency analysis method, referred to as comparative marker frequency analysis (Ziems et al., 2017). For both Pop1–Ma/Dr and Pop3–Sc/SB, the frequencies of the recurrent parent allele in the narrow and wide tail populations were compared in the BC_1F_4 progeny. For each marker, a discriminant value reflecting the difference in allele frequency between the two groups was calculated (Wenzl et al., 2006, 2007). This method identifies genetic loci conditioning phenotypic characteristics with at least 5-cM accuracy without the requirement of a linkage map (Wenzl et al., 2007). A χ^2 test was performed at each marker to detect significant discrimination between the expected and observed allele frequencies. A differential threshold of >0.4 discriminant value and a false discovery rate adjusted p-value < 0.01 were used to consider a marker significantly associated with a trait. Regions showing segregation distortion for SRA, referred to here as "hotspots", were identified when more than five significant marker–trait associations were found within 5 cM. For each hotspot, the parental allele most represented in the "narrow" tail population was considered the donor for narrow SRA, whereas the parental allele most represented in the wide tail population was considered the donor for wide SRA.

Previously reported quantitative trait loci (QTL) for traits related to RSA in wheat were collated from three published studies (Hamada et al., 2012; Christopher et al., 2013; Maccaferri et al., 2016). Seventy-seven QTL were reported, including 34 QTL for SRA (Christopher et al., 2013; Maccaferri et al., 2016), 39 QTL for seminal root number (SRN) (Hamada et al., 2012; Christopher et al., 2013; Maccaferri et al., 2016), and four QTL related to gravitropic responses of wheat roots (Hamada et al., 2012). Quantitative trait loci identified by Christopher et al. (2013) were reassigned from a previous map that used an older DArT marker system to the latest wheat DArT-seq consensus map (http://www.diversityarrays.com/, accessed 9 Feb. 2018).

The genomic locations of other QTL were projected onto the DArT-seq consensus map. A projection strategy using the single nucleotide polymorphism-based consensus map of tetraploid wheat as a bridge (Maccaferri et al., 2015) was followed (Mace and Jordan, 2011). A confidence interval of 5 cM (i.e., 2.5 cM above and below the peak marker location) was implemented for display purposes. The DArT consensus marker data and QTL positions were visually displayed using Map-Chart version 2.3 (Voorrips, 2002).

Marker-Assisted Selection in an Independent $F_{4:5}$ Reference Population

As segregation for SRA was clearly observed in Pop1–Ma/Dr (Fig. 3), seeds from the F_1 generation were progressed to the $F_{4:5}$ generation in parallel to the backcross populations to develop an independent reference population for MAS tests (Fig. 2). No selection was applied for SRA and all generations were grown in the speed breeding system described above, except the F_2 generation, which was sown in the field as 4-row 6-m long plots at The University of Queensland, Gatton, Queensland, Australia (27.54°S 152.34°E, 89 m asl). Single spikes from 52 F_2 plants were harvested green and dried with an air-forced dehydrator (Axyos, Brendale, QLD, Australia) at ambient temperature. The following generations were all produced in the speed breeding system via single-seed descent, resulting in 52 $F_{4:5}$ lines.

Genomic DNA was extracted from the 52 F_4 lines following the same protocol, as described above. Marker-assisted selection using markers associated with hotspots identified in Pop1–Ma/Dr was applied to the $F_{4:5}$ lines in silico. Five lines with the greatest total number of alleles for narrow SRA and five lines with the greatest total number of alleles for wide SRA were selected. Phenotyping for SRA was conducted as described above using a randomized complete block design with six seeds of each of the 52 lines. Seminal root angle was analyzed using a mixed model, containing "Line" and "Replicate" as random components and best linear unbiased predictions were obtained as described above. A Welch two-sample

t-test was used to compare SRA means attained by the the $F_{4:5}$ lines in the narrow and wide groups.

Results

Genotypic Variability for SRA

Phenotyping the panel of candidate parent wheat lines revealed a wide range in SRA in both experiments. In the first experiment, SRA ranged from 70 ± 6° for the narrowest candidate, Spitfire, to 101 ± 5° for the widest candidate, Suntop (Fig. 4). In the second experiment, SRA ranged from 73 ± 5° for '36:ZWW11' to 110 ± 5° for Suntop (Fig. 4). Despite some variation for SRA between the two experiments, the rank order of genotypes was quite similar, with 36:ZWW11, Drysdale, Dharwar Dry, Spitfire, and 'ZWW10–50' consistently exhibiting narrow SRA and 'Hartog', Mace, Suntop, and Wallup exhibiting wide SRA.

The six selected parental lines displayed contrasting SRA phenotypes, ranging from the narrowest to the widest: Dharwar Dry (75 ± 6°), Drysdale (76 ± 5°), SB062 (81 ± 6°), Scout (92 ± 6°), Mace (94 ± 6°), and Suntop (101 ± 5°) in the first experiment, and Drysdale (77 ± 5°), Dharwar Dry (80 ± 6°), Scout (88 ± 5°), SB062 (95 ± 5°), Mace (100 ± 5°), and Suntop (110 ± 5°) in the second experiment (Fig. 4).

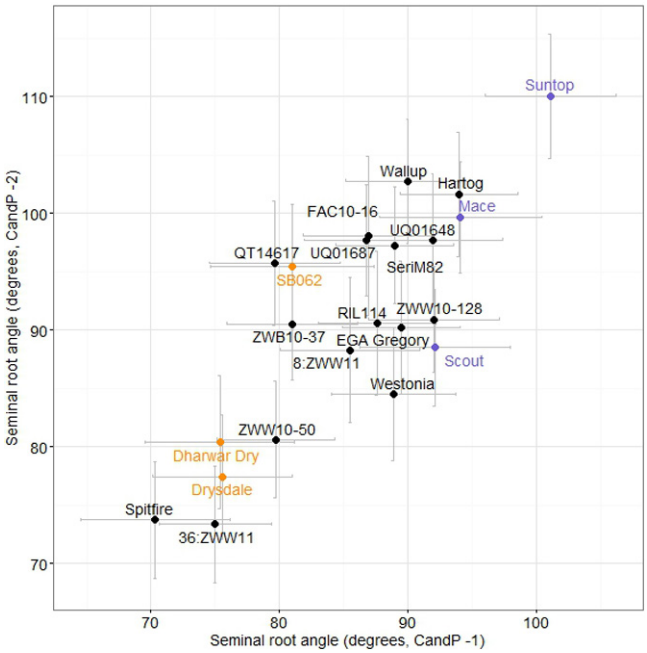

Fig. 4. Mean seminal root angle (best linear unbiased predictions) for the panel of 22 Australian-adapted wheat lines tested as candidate parents for phenotypic selection experiments. The panel was evaluated in two experiments, namely CandP–1 and CandP–2. The panel includes the donor lines (tan) and the recurrent parents (purple) selected to develop the backcross populations in this study. Error bars in gray represent the SE of the mean.

Comparison of Population Distribution in Tail Populations

Following selection for SRA in the BC_1F_2 generation (with a 21–25% selection intensity; Supplemental Table S2), assessment of the BC_1F_3 progeny representing the narrow and wide tails within each backcross population revealed an extensive overlap in SRA phenotypes and little shift in distribution (Fig. 3). Further, no significant differences were found between the SRA means (Table 1). The SRA averaged 79 ± 17°, 77 ± 15°, and 68 ± 15° in the narrow tail populations of Pop1–Ma/Dr, Pop2–Su/Dh, and Pop3–Sc/SB, respectively (Table 1, represented by the pink dashed lines in Fig. 3). There was little change in the wide tail populations, where the SRA averaged 81 ± 19°, 78 ± 15°, and 68 ± 19° for Pop1–Ma/Dr, Pop2–Su/Dh, and Pop3–Sc/SB, respectively (Table 1, represented by the blue dashed lines in Fig. 3).

Following a second round of selection in the BC_1F_3 generation (27–31% selection intensity; Supplemental Table S2), assessment of the BC_1F_4 progeny representing the narrow and wide tails within each backcross population revealed a significant difference in SRA for Pop1–Ma/Dr and Pop2–Su/Dh, but not for Pop3–Sc/SB (Table 1 and Fig. 3). For Pop1–Ma/Dr, the mean SRA was 82 ± 18° for the narrow tail and 92 ± 18° for the wide tail. This represented a significant change of 10° as a result of bidirectional selection performed in the BC_1F_2 and BC_1F_3 generations (Table 1). Similarly for Pop2–Su/Dh, the mean SRA was 80 ± 19° for the narrow tail and 90 ± 18° for the wide tail, also providing a significant difference of 10° (Table 1). For Pop3–Sc/SB, generated using parents with intermediate SRA phenotypes, no significant difference was found between the SRA means of the two tail populations (Table 1). The phenotypic distribution of raw SRA values revealed similar patterns (Fig. 3).

For Pop1–Ma/Dr and Pop2–Su/Dh, the difference between the narrow and wide tail populations at the BC_1F_4 generation in comparison to the BC1F3 generation was due to a wider mean SRA in the wide tail populations (11° and 12° wider, respectively), whereas SRA remained almost constant in the narrow tail populations (both 3° wider; Table 1). Interestingly for Pop3–Sc/SB where little differentiation between the narrow and wide tails was observed, the mean SRA for the narrow and wide tails was 16° and 14° wider, respectively, when comparing the BC_1F_4 to BC_1F_3 generations.

Phenotyping of the six parental lines in each generation of phenotypic screening also revealed some variation between experiments performed (Fig. 3, represented by arrows). Parental lines were significantly wider in the BC1F4 experiment compared to the BC1F3 experiment (data not shown). However, the rank between parental lines was maintained across experiments, with Drysdale and Dharwar Dry exhibiting significantly narrower SRA than Mace and Suntop during each generation of phenotypic screening. This rank consistency was expected, as a common seed source of each parental line was used as a benchmark and not subjected to selection for SRA.

Table 1. Comparison of mean seminal root angle (SRA) attained by tail populations. Means are presented for tail populations selected for narrow or wide SRA from three backcross populations, Pop1–Ma/Dr (Mace/Drysdale//Mace), Pop2–Su/Dh (Suntop/Dharwar Dry//Suntop), and Pop3–Sc/SB (Scout/SB062//Scout).

		Mean root angle for tail populations			
Generation	Population	Narrow	Wide	Shift†	P-value‡
BC_1F_3	Pop1–Ma/Dr	79°	81°	2°	0.3 (ns§)
	Pop2–Su/Dh	77°	78°	1°	0.4 (ns)
	Pop3–Sc/SB	68°	68°	0°	0.8 (ns)
BC_1F_4	Pop1–Ma/Dr	82°	92°	10°	$4.5.10^{-8}$ (***)
	Pop2–Su/Dh	80°	90°	10°	$1.1.10^{-6}$ (***)
	Pop3–Sc/SB	84°	82°	-2°	0.4 (ns)

*** Significant at the 0.001 probability level.

† Difference between the mean SRA for narrow tail population and mean SRA for wide tail population.

‡ The p-values from a Welch two-sample t-test is displayed for each comparison between tail populations selected for narrow and wide SRA.

§ ns, nonsignificant at P = 0.05.

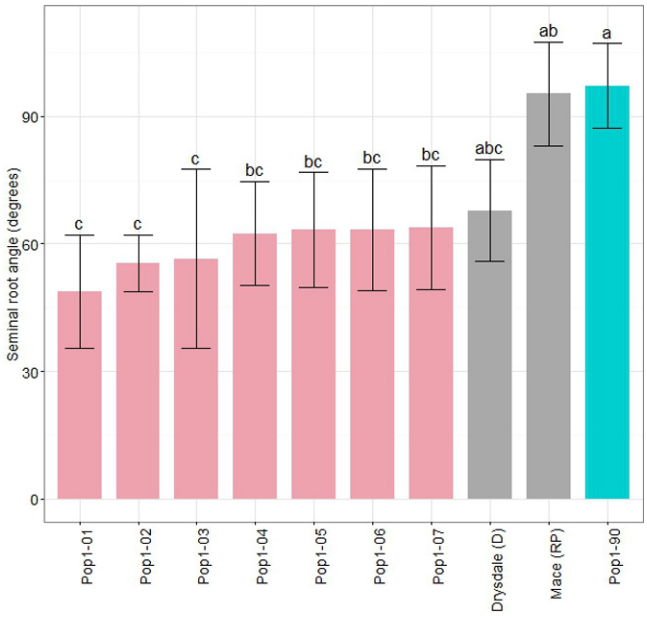

Fig. 5. Seminal root angle (SRA) for selected $BC_1F_{4:5}$ wheat lines and their respective parents. Seminal root angle was measured for seven $BC_1F_{4:5}$ lines displaying the narrowest SRA from Pop1–Ma/Dr (pink), one $BC_1F_{4:5}$ lines displaying the widest SRA from Pop1–Ma/Dr (blue), and the associated parents (gray) [i.e., the recurrent parent (RP) Mace and the donor line (D) Drysdale]. Mean SRA for lines labeled with the same letter are not significantly different according to Tukey's multiple comparisons of means test ($p > 0.05$). Error bars represent the SE of the means in this experiment.

Characterization of Fixed Lines Selected via Direct Phenotypic Selection

Fixed backcross lines ($BC_1F_{4:5}$) with extremely narrow and wide phenotypes for SRA from Pop1–Ma/Dr only were compared with their associated parental lines Drysdale and Mace to validate the shift in SRA observed after three rounds of selection. Seminal root angle for the selected 46 $BC_1F_{4:5}$ lines from the narrow tail of Pop1–Ma/Dr ranged from 49 ± 13° to 87 ± 12°, and averaged 72 ± 8° (Supplemental Table S3). Seminal root angle for the selected 44 $BC_1F_{4:5}$ lines from the wide tail of Pop1–Ma/Dr ranged from 63 ± 12° to 97 ± 10° and averaged 79 ± 9° (Supplemental Table S3). This corresponded to a significant difference of 7° ($p = 2.4.10^{-5}$) between the $BC_1F_{4:5}$ lines from the narrow and wide tails. As in previous experiments, SRAs for parental lines were contrasting, with Drysdale being the narrowest (68 ± 12°) and Mace the widest (95 ± 12°; Supplemental Table S3 and Fig. 5).

Interestingly, the three narrowest $BC_1F_{4:5}$ lines from the narrow tail population were not significantly different from the donor parent Drysdale but were significantly narrower than Mace, the recurrent parent (Fig. 5). The narrowest line (49 ± 13°) was 46° narrower than Mace, representing a 48% change in SRA. The widest line from the wide tail (97 ± 10°) was not significantly different from the parental lines but was significantly wider than seven $BC_1F_{4:5}$ lines from the narrow tail (Fig. 5).

Comparison of Allele Frequency in Tail Populations

Marker alleles contributed by the recurrent parent varied between 0 and 100% along the genome in both the narrow and wide tail populations of Pop1–Ma/Dr and Pop3–Sc/SB (data not shown). Hotspots, representing genomic regions under selection for SRA, were identified, along with the parent contributing narrow and wide SRA alleles in each case. The genomic interval and number of markers varied for each hotspot (Table 2). Eight hotspots were identified in Pop1–Ma/Dr (*hp1.Sra–hp8.Sra*) on chromosomes 1A, 2B, 3A, 3B, 3D, 5A, 7A, and 7D and five hotspots in Pop3–Sc/SB (*hp9.Sra–hp13.Sra*, Table 2) on chromosomes 1D, 2B, 4A, 6A, and 6B. Among these 13 hotspots, *hp2.Sra* discovered in Pop1–Ma/Dr and *hp10.Sra* discovered in Pop3–Sc/SB overlapped on chromosome 2B. The other 11 hotspots had locations on the genome that were unique to each population (Table 2).

Out of the 13 hotspots identified for SRA, seven hotspots collocated with previously reported genomic regions related to the RSA (Supplemental Fig. S1). On chromosome 2B, *hp10.Sra* collocated with three QTL for SRA and two QTL for SRN identified in Maccaferri et al. (2016), as well as a QTL for SRA identified in Christopher et al. (2013). On chromosomes 2B, 3B, 4A, 6A, 6B, and 7A, *hp2.Sra, hp4.Sra, hp11.Sra, hp12.Sra, hp13.Sra*, and *hp7.Sra*, respectively, collocated with three QTL for SRA and three QTL for SRN identified in Maccaferri et al. (2016), as well as two QTL for SRA and a QTL for SRN identified in Christopher et al. (2013).

Interestingly, in Pop1–Ma/Dr, 50% of the narrow SRA alleles were contributed by recurrent parent Mace, whereas 80% were contributed by Scout in Pop2–Su/Dh (Table 2). Backcross lines displayed different combinations of alleles for both narrow and wide SRA alleles at each hotspot. For example, in Pop1–Ma/Dr, some backcross lines from the narrow tail population carried some alleles associated with wide SRA, whereas some

Table 2. Genomic hotspots identified through comparative frequency analysis. Comparison of marker frequency between the narrow and wide tail populations of Pop1–Ma/Dr (Mace/Drysdale//Mace) and Pop3–Sc/SB (Scout/SB062//Scout) at the BC_1F_4 generations revealed regions under selection for seminal root angle (SRA).

Population	Hotspot	Chromosome	Start position†	Stop position	Number of significant markers	Origin of the allele for narrow SRA
			cM			
Pop1–Ma/Dr	*hp1.Sra*	1A	6.2	13.9	24	Mace
	hp2.Sra	2B	73.7	80.8	48	Drysdale
	hp3.Sra	3A	12.0	19.0	20	Drysdale
	hp4.Sra	3B	13.5	32.5	23	Drysdale
	hp5.Sra	3D	137.6	151.1	40	Mace
	hp6.Sra	5A	59.5	78.2	25	Mace
	hp7.Sra	7A	74.4	97.6	33	Drysdale
	hp8.Sra	7D	78.5	97.3	16	Mace
Pop3–Sc/SB	*hp9.Sra*	1D	25.3	57.5	30	Scout
	hp10.Sra	2B	62.5	82.6	44	Scout
	hp11.Sra	4A	19.8	30.9	107	SB062
	hp12.Sra	6A	97.6	100.8	37	Scout
	hp13.Sra	6B	2.4	9.3	18	Scout

† The distances in cM refer to the latest wheat diversity array technology (DArT) consensus map (http://www.diversityarrays.com/, accessed 12 Feb. 2018).

backcross lines from the wide tail population carried some alleles associated with narrow SRA (Fig. 6).

Fig. 6. Heatmap of the alleles for narrow and wide seminal root angle (SRA) in wheat lines developed from the recurrent parent Mace and the donor line Drysdale. Representation of the alleles for narrow and wide SRA at the eight hotspots, comprising between 16 and 48 marker loci, detected in the 49 lines from the wide tail population and the 46 lines from the narrow tail population of Pop1–Ma/Dr (Mace/Drysdale//Mace). The parent contributing the allele for narrow SRA at each hotspot is indicated in brackets. White indicates that the source for an allele in a particular line is unassigned. The genomic interval for each hotspot is provided in Table 2.

Validation of MAS for SRA

Characterization for SRA in the independent $F_{4:5}$ reference population derived from the same parental lines as Pop1–Ma/Dr (i.e., Mace and Drysdale) revealed phenotypic variation ranging from 67 ± 5° to 90 ± 5° (Fig. 7). For the group of five lines selected as having the greatest number of desirable alleles for narrow SRA (i.e., five or six alleles out of eight), the SRA ranged from 71 ± 5° to 81 ± 5° and averaged 76 ± 5° (Fig. 7). For the group of five lines selected as having the lowest number of desirable alleles for narrow SRA (i.e., zero or one allele out of eight), the SRA ranged from 76 ± 5° to 90 ± 5° and averaged 84 ± 5° (Fig. 7). A Welch two-sample *t*-test between the two groups indicated a significant difference of 8° (*p*-value = 0.04). Interestingly, in this population, the narrowest line (67 ± 5°) and the widest line (90 ± 5°) were found among the unselected lines (Fig. 7). Despite this, the narrowest line still carried a greater number of narrow alleles (i.e., four alleles) than the widest line (i.e., three alleles).

Discussion

We believe this to be the first report of direct phenotypic selection for RSA in early generations of a crop species. We applied bidirectional selection in the BC_1F_2 and BC_1F_3 generations, which successfully shifted the mean SRA by 10° in two wheat populations segregating for the trait. By combining efficient phenotyping and rapid generation advance, backcross-derived lines ($BC_1F_{4:5}$) enriched with alleles for narrow and wide SRA were developed within 18 mo. Furthermore, application of MAS in an independent reference population successfully identified lines with narrow and wide SRA. We propose that a similar root trait-based approach could be implemented in breeding programs to directly target RSA (Maccaferri et al., 2016).

Useful Genotypic Diversity for SRA was Identified

The panel of 22 candidate parental wheat lines evaluated in this study revealed a high degree of phenotypic variation for SRA, suggesting there are valuable sources of genetic diversity that can be exploited to improve RSA in breeding programs. In this panel, wheat genotypes displayed variation of 34° for SRA, with mean phenotypes for two experiments ranging from 72° to 106°. In a previous study by Richard et al. (2015), a panel of 24 Australian spring wheat lines was characterized for SRA via the clear-pot method; however, narrower phenotypes and a smaller range were observed (60–84°, i.e., a range of 24°). Manschadi et al. (2008) used the gel-filled chamber method to characterize a collection of 30 wheat genotypes for SRA, including some in common with Richard et al. (2015), and reported a range from 72° to 113° (i.e., a range of 41°). Notably, of the lines that were common across studies performed by Manschadi et al. (2008) and Richard et al. (2015), the genotypes displaying extreme phenotypes (i.e., the narrowest and the widest) were largely in agreement,

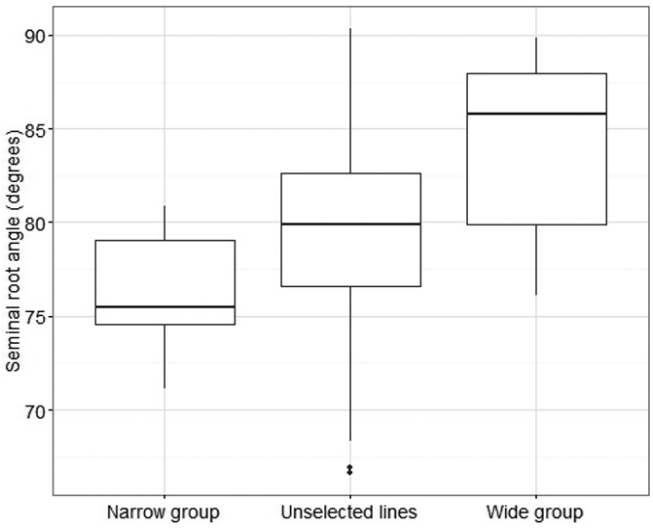

Fig. 7. Phenotypic variation for seminal root angle (SRA) of an independent population of wheat. Box and whisker plots of SRA of 52 $F_{4:5}$ lines from an unselected reference population derived for Mace × Drysdale independently of the selected tail populations described above. Five lines carrying the greatest number of alleles for narrow SRA represent the narrow group (left). Five lines carrying the greatest number of alleles for wide SRA represent the wide group (right). The remaining 42 $F_{4:5}$ lines represent the unselected lines (center). The bottom and the top of the boxes display the first and third quartile values. The band inside the box displays the median; the ends of the whiskers display the minimum and maximum values.

despite the differences in screening methods. It seems likely that the range in SRA reported in these studies may not represent the full extent of genetic variation in wheat germplasm, as the panels mostly comprised spring wheats from CIMMYT and Australia, some of which share similar genetic backgrounds. In comparison, barley (*Hordeum vulgare* L.) appears to display a broader range of SRA phenotypes: Robinson et al. (2016) reported a range from 13° to 82° (i.e., a range of 69°) via the clear-pot method for a panel of 30 Australian cultivars and breeding lines.

Segregating populations Adapted to Australian Environments were Developed

The three populations examined in this study were developed for their relevance to Australian wheat breeders. The three recurrent parents Mace, Scout, and Suntop are high-performing cultivars widely grown throughout the western, southern, and eastern production regions of the Australian wheatbelt, respectively. The three donor lines Drysdale, SB062, and Dharwar Dry combine drought and/or heat adaptation traits, which are considered desirable for improving and expanding wheat production in Australia.

The six parental lines displayed contrasting SRA phenotypes, with a narrow to intermediate SRA for the three donor lines and an intermediate to wide SRA for the three recurrent lines. Crosses for Pop1–Ma/Dr and Pop2–Su/Dh were selected with the intention of recombining alleles from donors with narrow SRA (Drysdale and Dharwar Dry) with alleles from locally adapted cultivars with wide SRA (Mace and Suntop). For Pop3–Sc/SB, the cross was selected to test for transgressive segregation for SRA using donors that contrasted genetically but that have similar intermediate root angles.

Seminal Root Angle was Modified by Selection

We examined the phenotypic distribution of SRA over the course of selection for either narrow or wide SRA in segregating generations. After one round of selection, there was no significant difference between the distributions of SRA for the narrow and wide tails within each of the three backcross populations. However, following two rounds of selection (i.e., BC_1F_2 and BC_1F_3 screens), a significant shift of approximately 10° was observed between SRA distributions of the narrow and wide tails for two of the three backcross populations (i.e., Pop1–Ma/Dr and Pop2–Su/Dh). Although contrasting SRA phenotypes were displayed by the parents for Pop1–Ma/Dr and Pop2–Su/Dh, the donor parent and the recurrent parent for Pop3–Sc/SB both displayed intermediate SRA. Thus, in this study, phenotypic selection in early generations for SRA was only effective when applied to populations derived from parents that were phenotypically distinct.

The significant shifts observed in the two backcross populations were caused by the wide tail population getting wider. These results could suggest that it is easier to select for wide SRA than further reducing SRA to produce narrower phenotypes. However, wider phenotypes were observed in the BC_1F_4 experiments compared to previous generations. For example, both the narrow and wide tail populations of Pop3–Sc/SB, as well as parental lines, were significantly wider when assessed in the BC_1F_4 screening experiment compared to the BC_1F_3 screening experiment. Though effort was made to minimize variation in environmental factors between experiments, some variation in results across experiments could be attributable to subtle differences in temperature, water, and nutrient content, all of which are known to influence root growth (Al-Khafaf et al., 1989; Vincent and Gregory, 1989; Adalsteinsson, 1994). Hence, the results are best compared within an experiment, and in relative terms rather than comparing absolute values across experiments. Thus, although a shift was clearly observed in the latter generation of population development, we cannot, on the basis of the current evidence, determine whether this shift was attributed to the wide tail populations getting wider, the narrow tail populations getting narrower, or both.

We compared allele frequencies between tail populations selected for narrow and wide SRA in two of the three backcross populations: first, in Pop1–Ma/Dr, where a significant shift in phenotype was observed for the selected tail populations; second, for Pop3–Sc/SB, where this did not occur. In both populations, the bidirectional phenotypic selection resulted in changes to allele frequencies at several genomic locations that probably harbor genes influencing SRA. Where a significant shift for SRA was observed in Pop1–Ma/Dr, eight regions under selection for SRA were identified on chromosomes 1A,

2B, 3A, 3B, 3D, 5A, 7A, and 7D. Interestingly, in Pop3–Sc/SB, where no shift was observed for SRA, five regions under selection for SRA were still identified on chromosomes 1D, 2B, 4A, 6A, and 6B, including one in common with Pop1–Ma/Dr on chromosome 2B. In recent years, QTL have been reported in wheat for SRA, SRN, and related root architectural traits (Hamada et al., 2012; Liu et al., 2013; Bai et al., 2013; Christopher et al., 2013; Maccaferri et al., 2016). In this study, seven hotspots identified for SRA colocated with previously reported QTL; six hotspots appeared to be novel.

Previous studies in wheat suggest that genetic variation for SRA could be governed by multiple genes, each with a minor effect (Liu et al., 2013; Christopher et al., 2013). Thus the parental lines may have contributed different alleles for SRA at a number of loci. Regions under selection identified in Pop3–Sc/SB may have smaller effects than those identified in Pop1–Ma/Dr. This may help to explain why, despite a clear shift in allele frequencies in Pop3–Sc/SB, no phenotypic shift was observed in tail populations for narrow and wide SRA. However, further studies are required to estimate the allele effects to test this hypothesis.

The source of alleles contributing narrow SRA in the 'narrow' tail populations of Pop1–Ma/Dr and Pop3–Sc/SB were from both the donor and recurrent parental lines. Interestingly, Mace, which displayed wide SRA, contributed half of the alleles for narrow SRA in Pop1–Ma/Dr. This tends to confirm that SRA is under complex genetic control, possibly involving epistatic, additive, antagonist, and/or synergetic genetic effects. If this is the case, it could help to explain why particular combinations of alleles for narrow SRA may result in different phenotypes.

Lines Enriched with Narrow and Wide SRA Alleles were Developed

The 46 $BC_1F_{4:5}$ lines derived from the narrow tail of Pop1–Ma/Dr displayed a mean SRA that was significantly narrower than that of the 44 $BC_1F_{4:5}$ lines derived from the wide tail of Pop1–Ma/Dr (a difference of 7°). Among the 46 selected $BC_1F_{4:5}$ lines representing the narrow tail, three lines exhibited SRA phenotypes that were significantly narrower than their respective recurrent parent (Mace). These three narrow $BC_1F_{4:5}$ lines developed in this study combined favorable alleles for narrow SRA in an elite background and thus could be directly used in breeding programs for top-crossing or for further testing in the field. Among the 44 selected $BC_1F_{4:5}$ lines representing the wide tail, one line displayed a SRA phenotype that was significantly wider than seven $BC_1F_{4:5}$ lines from the narrow tail. These results demonstrate how repeated rounds of selection (three in this case) for narrow or wide SRA in early generations can shift trait values.

The five lines from the independent set of unselected $F_{4:5}$ lines that were subsequently selected for narrow SRA through MAS displayed a mean SRA that was significantly narrower than that of the five $F_{4:5}$ lines selected for wide SRA from this set (a difference of 8°). This result indicates the potential for molecular selection in early generations of wheat to combine favorable alleles for SRA in breeding lines. However, there were also lines in this set that displayed extreme phenotypes for narrow and wide SRA but were not selected via MAS. This may reflect the complexity of the trait and the possibility that specific gene combinations are critical for superior phenotypes. Some regions influencing SRA may not have been identified through comparative marker frequency analysis because of the small population size. Alternatively, the dominant marker system may have caused some regions that were still segregating to be indicated as fixed, influencing the marker analysis. Regions influencing SRA used for MAS are likely to be specific to this population. Hence, further genetic studies using large multiparent populations that incorporate high genetic diversity and recombination events are needed to detect QTL or combinations of QTLs with the highest breeding value to fully exploit the potential of MAS.

Opportunities to Breed for SRA

In this study, we revealed significant genetic variation for SRA that was amenable to rapid and cost-effective phenotypic and molecular selection. But will breeders adopt selection for such traits? Richards (1996) suggests that breeders will remain unconvinced until there is evidence suggesting they could make important yield gains by selecting for specific traits.

Previous studies of several cereal species indicate that the angle at which roots emerge from the seed can influence rooting depth. In rice (*Oryza sativa* L.), a QTL controlling root growth angle was identified (Uga et al., 2011) and recently introgressed into a shallow-rooting rice cultivar to enhance its yield under drought conditions by increasing deep rooting (Uga et al., 2013). In wheat, characterization of SRA for 27 cultivars revealed that those adapted to drought-prone environments relying on soil moisture stored at depth were more likely to have a narrow growth angle and a deeper root system, as opposed to the cultivars adapted to Mediterranean environments (Manschadi et al., 2008). Furthermore, modeling studies suggest that selection for narrow SRA results in deeper root systems and higher yields (Manschadi et al., 2010; Veyradier et al., 2013). In bean (*Phaseolus vulgaris* L.), selection for wide SRA has resulted in varieties with shallow root systems with enhanced access to P in the topsoil (Lynch and Brown, 2001; Liao et al., 2001; Lynch, 2011). Hence, there is evidence that root traits expressed at early plant developmental stages, such as SRA, are associated with improved yield in some environments. However, many environmental factors can influence the shape and the size of the root system in the field (Fang et al., 2009). For example, root growth and distribution at depth can be influenced by soil temperature (Onderdonk and Ketcheson, 1973), soil structure (White and Kirkegaard, 2010), soil compaction (Jin et al., 2015; Ramalingam et al., 2017), and soil nutrient content (Bonser et al., 1996). As a consequence, the RSA in seedlings may not always be representative of the RSA of the mature plant (Watt et al., 2013). Thus proof of concept in the field is required to determine the value of specific seminal root traits in diverse

environments that typically vary in soil type and rainfall patterns (Potgieter et al., 2002; Chenu et al., 2013). Genetic resources developed in this study offer a novel opportunity to further investigate this link. For example, the tail populations selected for divergent SRA could, in future, be compared in a variety of environments in the field.

Conclusion

Breeding directly for favorable RSA in wheat has been hampered by the lack of efficient high-throughput phenotyping methods and a relatively poor understanding of the genetic controls. In this study, we rapidly developed lines enriched with alleles for narrow and wide SRA. The clear-pot method, which was designed to provide heritable, precise, and reproducible phenotypic information on the seedling roots, was used here for rapid SRA screening at early growth stages, out of season, and in a more homogeneous environment than in the field. We also applied MAS for regions influencing SRA, successfully selecting lines with divergent SRA. The benefit of performing selection in early generations is that once a beneficial root ideotype has been identified, individuals with undesirable gene combinations can be eliminated early in the breeding cycle, thereby allowing breeders to advance a smaller set of plants enriched with the target trait. Thus expensive field-testing is targeted to a potentially superior set of inbred lines. As only two rounds of selection were required to shift the population distribution for SRA, this strategy allows breeders to enrich their germplasm with favorable alleles for RSA rapidly. This could be readily integrated into breeding programs aimed at modifying RSA.

Supplemental Information

Supplemental Table S1. Details of the candidate parental wheat lines examined in this study.

Supplemental Table S2. Characteristics of the selection phases for developing tail populations.

Supplemental Table S3. Root angle phenotypes for the selected 90 BC1F4:5 lines.

Supplemental Figure S1. Hotspots for seminal root angle in wheat positioned on the consensus map.

Conflict of Interest

There are no conflicts of interests to disclose.

Acknowledgments

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