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Dosage effect of the short arm of chromosome 1 of rye on root morphology and anatomy in bread wheat

Sundrish Sharma, Darleen A. DeMason, Bahman Ehdaie, Adam J. Lukaszewski and J. Giles Waines*

Department of Botany and Plant Sciences, University of California, Riverside, CA 92521-0124, USA

* To whom correspondence should be addressed: E-mail: giles.waines@ucr.edu

Abstract

The spontaneous translocation of the short arm of chromosome 1 of rye (1RS) in bread wheat is associated with higher root biomass and grain yield. Recent studies have confirmed the presence of QTL for different root morphological traits on the 1RS arm in bread wheat. This study was conducted to address two questions in wheat root genetics. First, does the presence of the 1RS arm in bread wheat affect its root anatomy? Second, how does root morphology and anatomy of bread wheat respond to different dosages of 1RS? Near-isogenic plants with a different number (0 to 4 dosages) of 1RS translocations were studied for root morphology and anatomy. The F_1 hybrid, with single doses of the 1RS and 1AS arms, showed heterosis for root and shoot biomass. In other genotypes, with 0, 2, or 4 doses of 1RS, root biomass was incremental with the increase in the dosage of 1RS in bread wheat. This study also provided evidence of the presence of gene(s) influencing root xylem vessel number, size, and distribution in bread wheat. It was found that root vasculature follows a specific developmental pattern along the length of the tap root and 1RS dosage tends to affect the transitions differentially in different positions. This study indicated that the inherent differences in root morphology and anatomy of different 1RS lines may be advantageous compared to normal bread wheat to survive under stress conditions.

Key words: 1RS translocation, dosage effect, heterosis, root anatomy, root morphology, stress adaptation.

Introduction

All higher plants have roots and the root fraction of the plant's total mass varies widely, even within the same species. Although, roots encounter many fluctuations in their external environments that affect their growth, it is their tendency to accommodate and survive these as a whole system that makes them strongly homoeostatic (Barlow, 1986). Knowledge of these modifications in the root system at a morphological and anatomical level, whether due to environmental response or genetic control, is of importance (Weaver, 1926).

Root morphological traits and root growth response to the external environment have been studied widely in a number of crop species. In a greenhouse study, drought-stressed *Lolium perenne* L. plants increased the number and growth of lateral roots (Jupp and Newman, 1987). In barley (*Hordeum vulgare* L.), potassium deficiency was associated with the reduced length of laterals and larger root diameter compared with phosphorus deficiency under greenhouse conditions (Hackett, 1968). In wheat (*Triticum aestivum* L.), temperature had a profound effect on dry weight and root length under greenhouse environments (Bowen and Rovira, 1971; Huang, 1991). Besides root morphology, anatomical traits are also greatly influenced by the surrounding environment and have been widely studied in different crop species such as maize (*Zea mays* L.) (Hose *et al.*, 2001), rice (*Oryza sativa* L.) (Uga *et al.*, 2008), and other cereals (Aloni and Griffith, 1991; Liljeroth, 1995). In controlled environmental growth chamber-grown winter and spring wheat, chilling and high temperature decreases the diameter of the central metaxylem vessel (Huang *et al.*, 1991; Terzioglu and Ekmekci, 1995).

There are reports which suggest that specific morphological and anatomical root traits help stress-tolerant plants survive a particular stress condition. In rice, traits such as

deep root-to-shoot ratio and deep specific root length were found to contribute to drought avoidance in the field (Yoshida and Hasegawa, 1982; Fukai and Cooper, 1995). Drought-tolerant wheat cultivars have smaller xylem vessel diameters (Yang *et al.*, 2007). This anatomical adaptation of the tolerant wheat genotypes proved to be an advantage for the survival and higher grain yield under water stress (Richards and Passioura, 1989; Huang *et al.*, 1991; Huang and Taylor, 1993; Ou *et al.*, 2005).

The genetic control of root characteristics is poorly understood especially in bread wheat. Robertson *et al.* (1979) characterized genetic variability for seedling root number within the genus *Triticum* to examine its value in a breeding programme and found this trait to be positively correlated with seed weight. In a field study in winter wheat, post-anthesis root growth was investigated and cultivars were found to be significantly different in root length and mass especially below 30 cm (Ford *et al.*, 2006). O'Brien (1979) examined different wheat genotypes for root development in glass-fronted growth containers in the greenhouse and reported that the variability in seminal roots was sufficient for the root morphology to be altered by hybridization and selection. In bread wheat, xylem vessel diameter was found to have more useful genetic variation and higher heritability than the number of seminal axes in the roots and they showed a significant response to selection (Richards and Passioura, 1989). In a wheat backcross breeding programme, lines selected for reduced xylem vessel diameter yielded 3–11% more grain in the driest environments than unselected controls, depending on the genetic background (Richards and Passioura, 1989). Besides these findings, there is still no report in bread wheat of the chromosomal localization of genes that affect root anatomy.

Weaver (1926) compared the root systems of rye and bread wheat under natural field conditions and reported that rye had deeper seminal roots. The spontaneous translocation of the short arm of chromosome 1 of rye (1RS) (*Secale cereale* L.) to the long arm of chromosome 1B (1BL) in bread wheat (*Triticum aestivum* L.) was first identified in the late 1930s (Kattermann, 1938; Mettin *et al.*, 1973). Over the past few decades, there have been several reports of higher grain yield of 1RS translocated wheats over other commonly grown wheat genotypes (Rajaram *et al.*, 1983; Villareal *et al.*, 1991; Owuoche *et al.*, 2003; Kim *et al.*, 2004). In other studies, an increase in grain yield among 1RS wheats was found to be positively correlated with higher root biomass (Ehdaie *et al.*, 2003) while there were no significant differences found for shoot traits (Kim *et al.*, 2004). Roots of 1RS.1BL translocation wheats were thinner and there was a higher root length density when grown in acid soils and this enhanced the root surface area (Manske and Vlek, 2002).

Lukaszewski (1993) reconstructed the complete chromosomes of 1B and 1R from a 1RS.1BL translocation and, later, produced three new centric translocations namely, 1RS.1AL, 1RS.1BL, and 1RS.1DL in 'Pavon 76' (Lukaszewski, 1997). Each of three translocations had the same 1RS arm but in different locations in the genome and each was mitotically stable. All three translocations had greater root biomass and

higher grain yield under irrigated and water-stressed greenhouse conditions and under irrigated field conditions (Ehdaie *et al.*, 2003; Kumlay *et al.*, 2003). These translocation lines were ranked for root biomass as Pavon 1RS.1AL>Pavon 1RS.1DL>Pavon 1RS.1BL>Pavon 76 (Ehdaie *et al.*, 2003). Recently, a genetic map was generated using 1RS-1BS recombinant breakpoints in Pavon 76 wheat and their genetic analysis indicated the distal 15% of the physical length of chromosome 1RS may carry the gene(s) for better rooting ability and root morphological traits (Sharma *et al.*, 2009).

The present study was conducted to address the dosage effect of the 1RS translocation in bread wheat. Wheat genotypes were used that differ in their number of the 1RS translocations in a spring bread wheat 'Pavon 76' genetic background. For generating F_1 seeds, Pavon 1RS.1AL was the preferred parent of choice due to its better performance for root biomass than other 1RS lines (Ehdaie *et al.*, 2003). The dosage effect of a 1RS chromosome arm on different root traits including root biomass, root diameter, stele diameter, central metaxylem (CMX) vessel diameter, CMX vessel area, CMX vessel number, and number of xylem poles of wheat roots is reported here. The results from this study may validate previous results of the presence of genes for rooting ability on the 1RS chromosome arm. This study also provides evidence for the presence of genes affecting root anatomy on the 1RS arm.

Materials and methods

Plant material

Five genotypes, Pavon 76, F_1 (Pavon 1RS.1AL×Pavon 76), Pavon 1RS.1AL, Pavon 1RS.1DL, and Pavon 1RS.1AL-Pavon 1RS.1DL were used. They were coded as R_0, RA_1, RA_2, RD_2, and RAD_4, respectively. Here, 'R' denoted the dosage of chromosome arm 1RS, the second letter A and D denoted the chromosome 1 of the respective genome of wheat to which 1RS is translocated and numbers in the subscripts are dosage number of 1RS chromosome arms present in the genotype. Pavon 76 is a spring bread wheat from the breeding programme of Centro Internacional de Mejoramiento de Maíz y Trigo (CIMMYT), Mexico. For a single dose of 1RS, crosses were made between Pavon 1RS.1AL and Pavon 76 and F_1 seed was used as RA_1. Seeds of RA_2, RD_2, and RAD_4 genotypes were provided by Dr AJ Lukaszewski, University of California, Riverside.

Experimental set-up

To study the effect of different dosages of the 1RS chromosome arm of rye in a wheat background and their effect on root morphology, an experiment was set up in a randomized complete block design with six replicates per genotype during January 2007, April 2007, January 2008, and January 2009. The genotypes were grown under a natural photoperiod in a temperature controlled greenhouse (20–30 °C and 50–90% relative humidity). Anatomy of the primary seminal roots or specifically the primary axile roots (Watt *et al.*, 2008) was studied for two of these seasons. Seminal roots and primary-axile roots are mentioned from here onwards as tap root in accordance with root nomenclature guidelines (http://rootrap.boku.ac.at/index.php?id=28). Seeds from the above-mentioned five genotypes were surface-sterilized with 5% commercial bleach for 5 min, washed for 10 min in distilled water, soaked in water for 24 h, and then germinated on wet filter paper in Petri dishes. 3–5-d-old seedlings were transplanted to 7.5 l cylindrical pots (21×21.5 cm

diameter), lined with a plastic bag containing 6.5 kg of washed silica sand no. 30 with bulk density of 1.42 g ml^{-1} and 24% field capacity (w/w). Small holes were made at the bottom of the plastic bags to allow drainage of excess water. Plants were watered, when necessary, with half-strength Hoagland's solution to ensure that nutrients and water were not limiting.

Phenotypic data

Plants were harvested 45 d after germination at the mid-to-late tillering stage or Decimal code of 28–29 for growth stages (Zadoks et al., 1974) and pots containing roots in polythene bags were stored at 4 °C until processed. Each polythene bag was laid out on a screen frame in a tub half-filled with water and a cut was made on the side of the bag. The frame was moved gently in the tub to separate sand from the roots. Roots were recovered without damage using the floatation technique (Böhm, 1979) and attached sand was removed by hand. Different plant characters were measured including plant height (PH), number of tillers (NT), longest leaf length (LLL), maximum width of the longest leaf (LLW), leaf area of the longest leaf (LA), longest root length (LRL), root biomass (RB), shoot biomass (SB), and root biomass to shoot biomass ratio (R/S). Root and shoot biomass was determined from dry matter after drying to constant weight in a forced-air drier for 96 h at 65 °C. Leaf area of the longest leaf was calculated by LLL×LLW×0.78 for each genotype (Quarrie and Jones, 1977).

Heterosis

Mid-parent heterosis (MPH) and best-parent heterosis (BPH) of the F_1 hybrid (RA_1) over its parents (R_0 and RA_2) for root biomass and shoot biomass were calculated as:

$$MPH = (F_1 - \text{mean P})/\text{mean P in per cent}$$

$$BPH = (F_1 - \text{best P})/\text{best P in per cent}$$

Root anatomy

After washing, root samples were collected in two different seasons from the tap roots of six plants of each genotype at three different locations, namely root tip, middle of the root, and 1 cm from the base of the root adjacent to the seed. Root samples were immersed in half-strength Karnovosky's fixative (2.5% gluteraldehyde and 4% formaldehyde in 50 mM phosphate buffer, pH 7.2). The roots were left in the fixative at 4 °C for 24 h. The roots were dehydrated by passing through a graded ethanol series of 10%, 35%, 50%, and 70% and then left overnight in 70% ethanol followed by another 2 h treatment in 85%, 90%, and 100% and then subsequently overnight in 100% with 1% Erythrosine B (C.I. 11A). Then the samples were transferred through an ethanol/xylene series of 3/1, 1/1, and 1/3 for 30 min to 1 h each. Tissues were then placed in pure xylene for 2 h followed by overnight treatment. Infiltration and embedding was done in paraffin.

Paraffin blocks containing tissues were sectioned at 10 μm thick using an A/O rotary Microtome and stainless steel knives. Root tips were serially sectioned in the transverse plane starting at the tip of the root cap. Sections were mounted on slides and stained with Safranin O (C.I. 50240) and Fast Green FCF (C.I.# 19) using Sass's procedure (Ruzin, 1999). Samples were observed on a Zeiss standard compound brightfield microscope. Digital images were taken using an Infinity 1 digital camera (Luminere Inc, Canada). Data were recorded for the different root anatomical features such as root diameter, stele diameter, central metaxylem (CMX) vessel diameter, CMX vessel area, CMX vessel number, and number of xylem poles from different regions of the roots of all the above-mentioned five genotypes. Data on CMX vessel diameter and area were made on transverse sections of root tips at a distance of 1370 μm from the tip for all genotypes. At this position the CMX vessel

was clearly evident and there was no significant difference in the position of its appearance from the root tip between the genotypes. Calibrations and measurements were carried out by camera compliant software 'Analyse Infinity' (Luminere Inc, Canada).

Statistical analysis

The morphological data were subjected to analysis of variance (ANOVA) for each year (Steel et al., 1997). The combined ANOVA across seasons was performed for each measured and calculated trait using a split plot design in time. Season was treated as the main plot and genotype as a sub-plot. In combined ANOVA, all the factors were considered fixed. The anatomical data were also subjected to ANOVA to determine the genetic differences among genotypes. Simple correlation coefficients were calculated to determine the relationship among different anatomical traits. Linear regression analysis was carried out for different anatomical root traits to assess the relationship between dosage of the 1RS chromosome arm and the root anatomical traits in bread wheat. Statistix 8 program (Analytical Software, Tallahassee FL) was used to carry out correlation and regression analysis.

Results

Phenotypic study

There was lesser total root biomass in Pavon 76 (R_0) than any of its 1RS sister lines. Among the 1RS lines, F_1 (RA_1) and RAD_4 had the highest root biomass (Fig. 1). There were significant differences among genotypes for plant height, number of tillers, length of longest leaf, maximum width of longest leaf, root biomass, shoot biomass, and root to shoot ratio ($P < 0.05$) (Table 1). For leaf area, differences among genotypes were not significant. All the genotypes showed significant difference for all the phenotypic traits across the seasons ($P < 0.05$). Genotype × season interactions ($P < 0.01$) were not significant for leaf area and

Fig. 1. Roots of different wheat genotypes with different number of 1RS translocations in spring bread wheat 'Pavon 76' background harvested 45 d after germination (grown in pots). Pavon 76 (R0)=0 dose of 1RS, Pavon 1RS.1AL×Pavon 76 (RA1)=1 dose of 1RS, Pavon 1RS.1AL (RA2)=2 doses of 1RS on 1AL, Pavon 1RS.1DL (RD2)=2 doses of 1RS on 1DL, and Pavon 1RS.1AL- Pavon 1RS.1DL (RAD4) = 4 doses of 1RS on 1AL and 1DL.

Table 1. Summary of combined ANOVA and mean values of plant height (PH), number of tillers (NT), longest leaf length (LLL), maximum width of the longest leaf (LLW), leaf area of the longest leaf (LA), root biomass per plant (RB), shoot biomass per plant (SB), and root to shoot biomass ratio (R/S) for bread wheat Pavon 76 (R_0), F_1, Pavon 1RS.1AL×Pavon 76 (RA_1), Pavon 1RS.1AL (RA_2), Pavon 1RS.1DL (RD_2), and Pavon 1RS.1AL-Pavon 1RS.1DL (RAD_4) grown in sand pots for 45 d (mid to late tillering stage) averaged across four seasons

Genotype	PH[a] (cm)	NT[a] (no.)	LLL[a] (cm)	LLW[a] (cm)	LA[a] (cm^2)	RB[a] (mg)	SB[a] (mg)	R/S[a]
R_0	54 b	11 b	35 c	1.38 ab	38.1 ab	1073 bc	3692 b	0.33 bc
RA_1	55 b	11 b	37 b	1.31 b	37.7 ab	1549 a	4319 a	0.36 ab
RA_2	58 a	11 b	37 b	1.42 a	40.9 a	1232 b	3802 b	0.36 ab
RD_2	58 a	9 c	36 bc	1.35 ab	38.3 ab	1087 b	3637 b	0.31 c
RAD_4	52 c	13 a	39 a	1.20 c	37.1 b	1461 a	3743 b	0.38 a
Season	**	**	**	**	**	**	**	**
Genotype	**	**	**	**	NS	**	**	**
Genotype×Season	**	**	**	**	NS	**	**	NS
CV (%)[b]	7	15	7	10	14	24	17	16

[a] Means followed by the same small letter within a column are not significantly different at $P <0.05$ according to LSD test; **=significant ($P <0.01$); NS=not significant ($P >0.05$).
[b] CV=Coefficient of variation.

root-to-shoot ratio. The RAD_4 genotype had the highest number of tillers, longest leaf length, and maximum root-to-shoot ratio but the shortest plant height, minimum width of longest leaf measured at its widest point, and minimum leaf area of the longest leaf. The RA_1 hybrid performed significantly differently from other genotypes for root biomass and shoot biomass ($P <0.05$). Mean values of RA_2 were higher for maximum width of the longest leaf and leaf area. For root biomass, RA_1 and RAD_4 were significantly different from RA_2, RD_2, and check R_0 ($P <0.05$) (Fig. 1; Table 1). RA_2 was significantly different from RD_2, and check R_0 only at P <0.01 data not shown). All the genotypes were significantly similar for shoot biomass except RA_1 which was significantly different from them ($P <0.05$) (Table 1).

Mid-parent and best-parent heterosis

Mean values of the F_1 hybrid (RA_1) were significantly different from both of its parents (R_0 and RA_2) for root biomass and shoot biomass ($P <0.05$) (Table 1). MPH and BPH for root biomass were calculated to be 34.4% and 25.7%, respectively (Table 2). For shoot biomass, heterotic gain of F_1 over mid-parent and best-parent was 15.3% and 13.6%, respectively (Table 2).

Root anatomy along the root

To study root anatomy, the two seasons selected were representative of the four seasons used for the study. For most of the phenotypic traits, including root and shoot biomass, genetic differences observed across four years were also statistically significant when the data across the two years (in which the microscopy was done) were analysed separately. The size, number, and arrangement of metaxylem vessels varied along the length of the tap root from the top to the tip region for each genotype. In the top region, there were 3–6 large metaxylem vessels that were not necessarily central in position. More of these were present in

Table 2. Mid-parent heterosis (MPH) and best-parent heterosis (BPH) for root and shoot biomass (per plant) of bread wheat hybrid F_1 - Pavon 1RS.1AL×Pavon 76 (RA_1) over parents Pavon 1RS.1AL (RA_2) and Pavon 76 (R_0) grown in sand pots for 45 d (mid-tillering stage).

Mean values are averaged across four seasons.

Trait	Mean			MPH (%)	BPH (%)
	Hybrid (RA_1) (mg)	Mid-parent (mg)	Best-parent (RA_2) (mg)		
Root biomass	1549	1153	1232	34.4	25.7
Shoot biomass	4319	3747	3802	15.3	13.6

the transverse sections of R_0, RA_1, and RA_2 than in RD_2 and RAD_4 (Fig. 2A–E). In the latter genotypes, especially RAD_4, there was variation for number and arrangement of CMX vessels in the basal portion transverse sections of the tap root (Fig. 3A, B, C). A partial ring of smaller metaxylem vessels surrounding the bigger CMX vessel was observed in some of the tap roots where there was only a single CMX vessel. Similar variation was also observed in RD_2 (data not shown). The diameter of the stele was larger in R_0 and RA_1 than RA_2, RD_2, and RAD_4 (Fig. 2A–E).

In the middle region of the root, there were two centrally located metaxylem vessels in the stele of R_0 and RA_1 and a single CMX vessel in RD_2 and RAD_4 (Fig. 2F–J). The diameter of the stele was smaller in the middle than in the top region of the root for most of the genotypes (data not shown). In the root tip region, there was a single differentiating CMX vessel in all the genotypes which was narrower in RA_2, RD_2, and RAD_4 genotypes (Fig. 2M, N, O). There was not much variation for other anatomical traits in the root tip region among the genotypes (Fig. 2K–O).
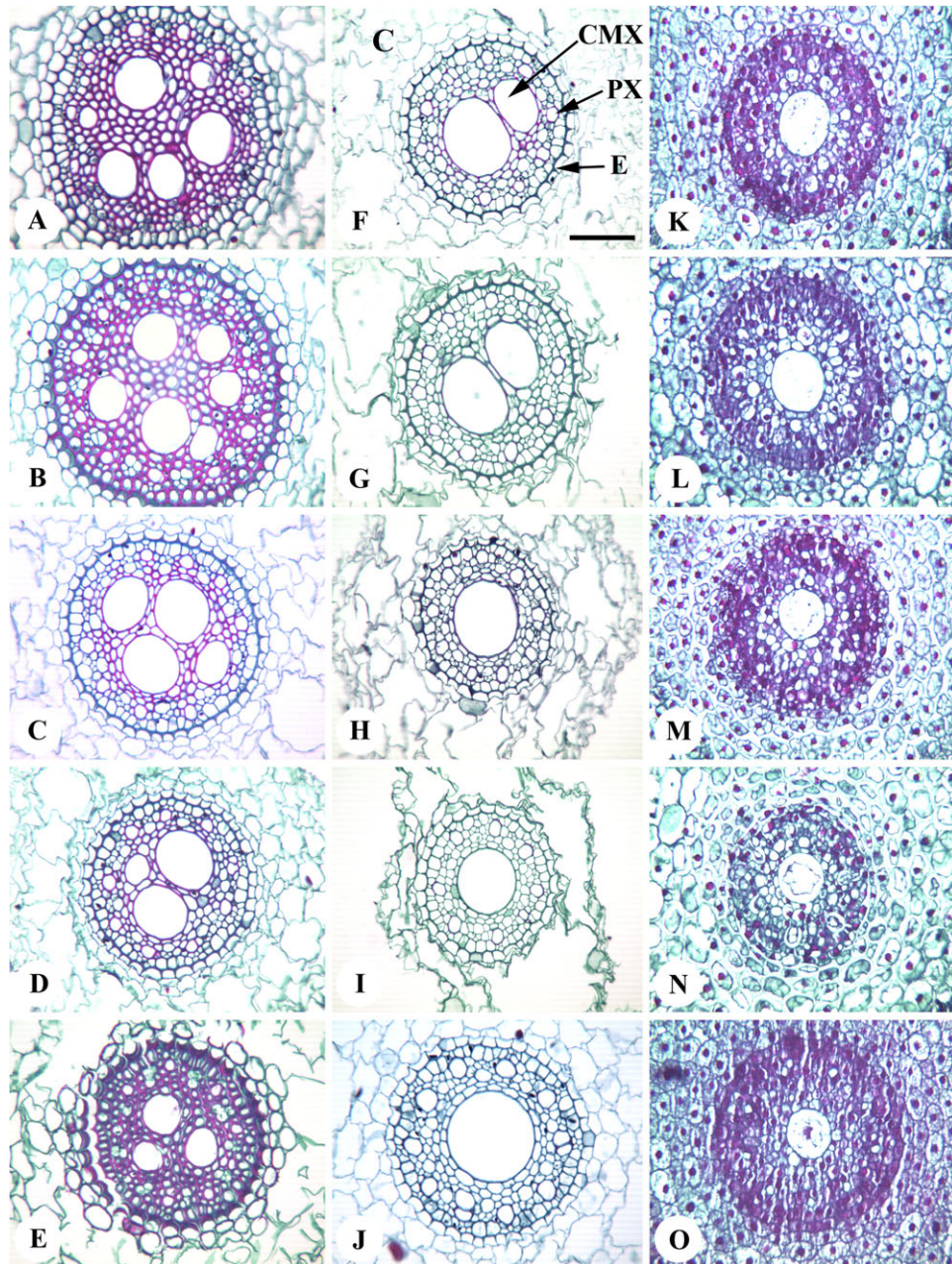
Fig. 2. Stained transverse sections of different regions (A–E top, F–J middle, K–0 tip) of roots of wheat genotypes. (A, F, K) Pavon 76 (R_0); (B, G, L) F_1 of Pavon 1RS.1AL×Pavon 76 (RA_1); (C, H, M) Pavon 1RS.1AL (RA_2); (D, I, N) Pavon 1RS.1DL (RD_2); (E, J, O) Pavon 1RS.1AL-Pavon 1RS.1DL (RAD_4); (F) is labelled with C, cortex; CMX, central metaxylem vessel; E, endodermis; PX, protoxylem; Scale bar, 100 µm.
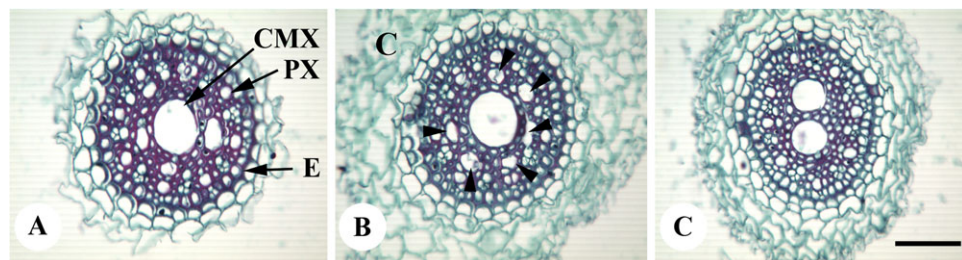

Fig. 3. (A, B, C) Examples of patterns seen in the number of CMX vessels in the top region of tap root of the RAD_4 genotype (four doses of 1RS chromosome arm). (B) is labelled with arrowheads, inner xylem vessels; C, cortex; CMX, central metaxylem vessel; E, endodermis; PX, protoxylem; Scale bar, 100 µm.

Top region of the root (TOP)

There were significant differences among genotypes for stele diameter, total metaxylem vessel area, number of CMX vessels, and number of xylem poles in the top region of the tap root. Differences among genotypes were not significant for xylem diameter, CMX number, and root diameter but all genotypes showed the same trend in their performance for all the root anatomical traits measured (Fig. 4A–F). Hybrid RA1 was the largest among genotypes, except for root diameter, followed by R_0, for all the traits in the top region of the tap root while RAD_4 was the smallest.

The calculation of correlation coefficients (r) revealed a strong degree of association among traits of root anatomy in the top region of the root (Table 2). Stele diameter showed a positive correlation with CMX vessel area (0.82), number of xylem poles (0.81), and root diameter (0.84). CMX vessel area was further associated highly with CMX vessel diameter (0.77), number of xylem poles (0.71)), and root diameter (0.86). There was also a high correlation between root diameter and number of xylem poles (0.79) (Table 3).

Linear regression analysis showed significant R^2 values for all anatomical root traits measured in this study. The number of CMX vessels in the top of the root was the best explained trait (51%) due to 1RS dosage. Forty-seven per cent of the variation of two root traits, CMX area and number of xylem poles, was explained by the linear regression on the number of 1RS dosages in a genotype (Table 4). The number of 1RS dosages explained 45% and 38% of the variation in stele diameter and root diameter, respectively. The negative slope of the regression equation showed a negative relationship between root anatomical traits in the top region and number of 1RS dosages (Table 4). This is also evident from the negative trends in Fig. 4.

Mid-region of the root (MID)

ANOVA showed no significant differences among genotypes for any of the anatomical root traits in the mid-region of the tap root except number of xylem poles (data not shown). However, all the traits showed stronger association among themselves except CMX vessel number (Table 3).
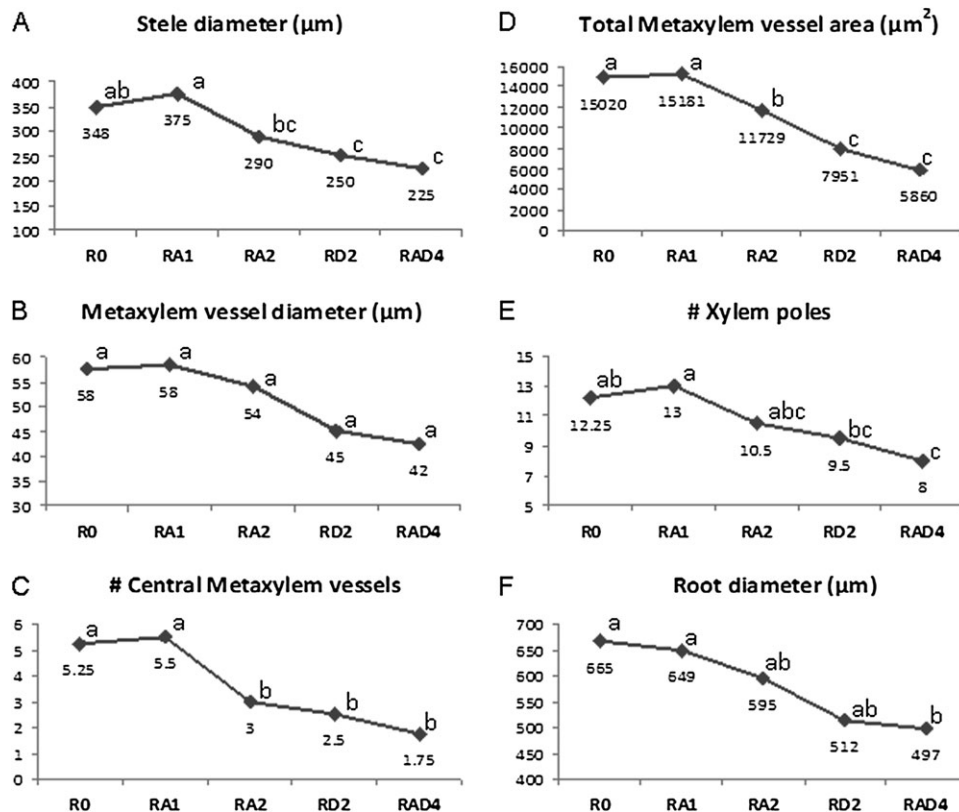

Fig. 4. Graphical representation of mean performances of anatomical traits: (A) stele diameter; (B) metaxylem vessel diameter; (C) number of central metaxylem vessels; (D) total metaxylem vessel area; (E) number of xylem poles (protoxylem); and (F) root diameter measured from top region of the roots of each wheat genotype with different number of 1RS translocation arms in the genetic background of spring bread wheat 'Pavon 76'. The same small letters within a graph are not significantly different. *X*-axis, bread wheat genotypes namely Pavon 76 (R0)=0 dose of 1RS, Pavon 1RS.1AL×Pavon 76 (RA1)=1 dose of 1RS, Pavon 1RS.1AL (RA2)=2 doses of 1RS on 1AL, Pavon 1RS.1DL (RD2)=2 doses of 1RS on 1DL, and Pavon 1RS.1AL- Pavon 1RS.1DL (RAD4)=4 doses of 1RS on 1AL and 1DL; Y axis, scale for root trait.

Table 3. Simple correlation coefficients between different root anatomical traits in different root regions in the genetic background of spring bread wheat 'Pavon 76' with different number of 1RS translocation arms; $n=20$ for TOP and TIP regions, $n=25$ for MID regions

Root traits	Root regions	Root traits[a]				
		Stele diameter	CMX vessel area	CMX vessel diameter	CMX vessel number	Xylem poles
CMX vessel area	TOP	0.82**				
	MID	0.96**				
	TIP	0.68**				
CMX vessel diameter	TOP	0.46*	0.77**			
	MID	0.96**	0.96**			
	TIP	0.73**	0.99**			
CMX vessel number	TOP	0.86**	0.85**	0.68**		
	MID	0.29	0.19	0.21		
	TIP	–	–	–		
Xylem poles	TOP	0.81**	0.71**	0.53*	0.84**	
	MID	0.91**	0.88**	0.89**	0.27	
	TIP	0.14	–0.02	–0.01	–	
Root diameter	TOP	0.84**	0.86**	0.56*	0.84**	0.79**
	MID	0.83**	0.75**	0.78**	0.08	0.84**
	TIP	0.75**	0.59**	0.64**	–	–0.08

[a] ** Significant ($P \leq 0.01$); * significant ($P \leq 0.05 > 0.01$).

Table 4. Summary of regression analysis

The independent variable is number of 1RS translocation arms in the spring bread wheat 'Pavon 76' genetic background ($n=20$)

Dependent variable	Intercept	Slope	R^2	F	P
Root top					
Stele diameter (µm)	363.4	–36.5	0.45	14.66	0.00
Total CMX area (µm^2)	15720.0	–2540.0	0.47	16.01	0.00
CMX diameter (µm)	59.0	–4.2	0.21	4.65	0.04
CMX (number)	5.4	–1.0	0.51	18.63	0.00
Xylem pole (number)	12.9	–1.2	0.47	15.78	0.00
Root diameter (µm)	667.7	–44.5	0.38	6.60	0.03
Root tip					
Stele (µm)	250.8	–5.4	0.10	2.00	0.17
CMX area (µm^2)	6161.4	–773.5	0.54	21.30	0.00
CMX diameter (µm)	87.7	–6.0	0.52	19.69	0.00
Xylem pole (number)	8.8	0.1	0.01	0.21	0.65
Root diameter (µm)	655.8	–15.1	0.06	1.19	0.29

Regression analysis showed a negligible dependence of root traits on the number of 1RS dosages (data not shown).

Tip of the root (TIP)

In root tips of the tap roots, there were significant differences among genotypes for CMX vessel area and CMX vessel diameter. For both the traits, R_0 and RA_1 showed significantly higher mean values than 1RS double dosage genotypes (RA_2 and RD_2) and quadruple dosage of 1RS in RAD_4 genotype (Fig. 5A, B). Correlation coefficient values of both the above traits also showed a highly positive association with each other followed by stele and root diameter

(Table 3). Again in the regression analysis, both the traits, CMX vessel area and CMX vessel diameter, were significantly influenced by different 1RS dosages in the genotypes. Their regression equation showed negative slope and the variation explained for CMX vessel area and CMX vessel diameter was 54% and 52%, respectively (Table 4).

Discussion

From previous studies (Ehdaie *et al.*, 2003; Sharma *et al.*, 2009), it was clear that there was a gene (or genes) present on the 1RS chromosome arm which affects root traits in bread wheat. These authors did not attempt to determine if this chromosomal region affects any root anatomical traits in bread wheat. The purpose of this study was to look for variation in root morphology and anatomy among different wheat genotypes and then to determine how these differences are related to different dosages of 1RS in bread wheat. During this study, we came to some interesting conclusions: (i) F_1 hybrids showed a heterotic effect for root biomass and there was an additive effect of the 1RS arm number on root morphology of bread wheat; (ii) there was a specific development pattern in the root vasculature from top to tip in wheat roots and 1RS dosage tended to affect root anatomy differently in different regions of the tap root. Further, the differences in root biomass, and traits associated with CMX of the different genotypes may have specific bearing on their ability to tolerate water and heat stress.

The results in this study were obtained at an early stage of growth in the greenhouse environment. Although the positive correlation of higher root biomass of 1RS wheats in the greenhouse and their yield performance in field
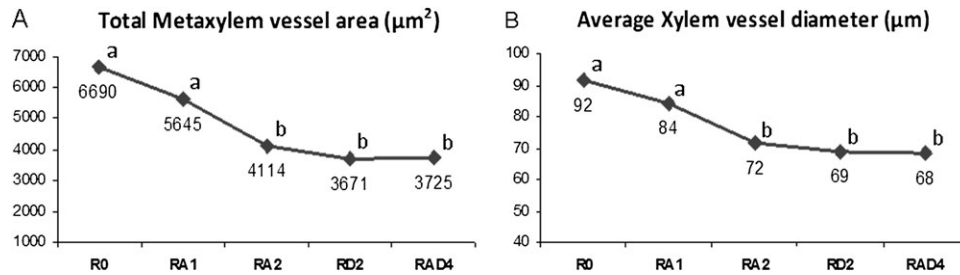
Fig. 5. Graphical representation of mean performances of (A) total metaxylem vessel diameter; and (B) average metaxylem diameter measured in root tips of the roots of different wheat genotypes with different number of 1RS translocation arms in the genetic background of spring bread wheat 'Pavon 76'. The same small letters within a graph are not significantly different. *X*- axis, bread wheat genotypes, namely Pavon 76 (R0)=0 dose of 1RS, Pavon 1RS.1AL×Pavon 76 (RA1)=1 dose of 1RS, Pavon 1RS.1AL (RA2)=2 doses of 1RS on 1AL, Pavon 1RS.1DL (RD2)=2 doses of 1RS on 1DL, and Pavon 1RS.1AL - Pavon 1RS.1DL (RAD4)=4 doses of 1RS on 1AL and 1DL; Y -axis, scale for root trait.

conditions was demonstrated (Ehdaie *et al.*, 2003), it may be difficult to translate the observed effects of this study to wheat root systems in the field at later stages, especially the anatomical traits on xylem vessel number, size etc which were observed on only one tap root per plant.

Effect of 1RS dosage on root morphology

The effect of number of 1RS translocation arms in bread wheat was clearly evident from their averaged mean values for root biomass. RA_1 and RAD_4 were ranked highest while R_0 ranked at the bottom (Fig. 1; Table 1). Genotype RD_2 performed only slightly better than R_0 for root biomass because of its poor performance in one season, otherwise it showed better rooting ability in the other three seasons. Otherwise, all the genotypes with 1RS translocations showed higher root biomass than R_0 which carried a normal 1BS chromosome arm. Data in this study suggested two types of effects of 1RS on wheat roots. First, an additive effect of 1RS, there was increase in root biomass with the increase in 1RS dosage from zero (R_0) to two (RA_2 and RD_2) and then to four (RAD_4).

A second major effect noted was a heterotic effect of 1RS and 1AS on root biomass and shoot biomass. MPH and BPH of the F_1 hybrid (RA_1) were higher for root biomass than for shoot biomass (Table 2). This further explained the more pronounced effect of 1RS on root biomass than shoot biomass. Significant positive heterosis was observed for root traits among wheat F_1 hybrids and 27% of the genes were differentially expressed between hybrids and their parents (Wang *et al.*, 2006). The possible role of differential gene expression was suggested to play a role in root heterosis of wheat and other cereal crops (Wang *et al.*, 2006). In a recent molecular study of heterosis, it was speculated that up-regulation of TaARF, an open reading frame (ORF) encoding a putative wheat ARF protein, might be contributing to heterosis observed in wheat root and leaf growth (Yao *et al.*, 2009).

Root anatomy in three dimensions in different doses of 1RS arms

There is a large void in root research involving the study of root anatomy in wheat as well as other cereal crops. Most of the anatomical literature is either limited to root

anatomy near the base of the root (Aloni and Griffith, 1991; Watt *et al.*, 2008) or near the root tip (Huang *et al.*, 1991) in young seedlings (Richards and Passioura, 1989). There is still a general lack of knowledge about the overall structure and pattern of whole root vasculature during the later stages of growth in cereals, especially in wheat. Recently, a study was conducted on root anatomy of various classes of winter wheat, barley, and triticale roots, including primary axile (tap) roots, scutellar, coleoptile, and leaf node axile roots, as well as branch roots of various orders (Watt *et al.*, 2008). These authors sampled axile (tap) roots at 3–5 cm from the base of young plants grown in the greenhouse and other branch roots from field-grown plants at flowering.

In the present study, root anatomical traits were studied in the tap roots of different spring wheat genotypes containing different dosages of 1RS translocation arms harvested during the late-tillering stage. Root sections were made from three regions along the length of the root, namely top of the root, middle of the root, and root tip, to get an overview of the complete structure and pattern of root anatomy relative to differences in 1RS dosage. A comparison of the different regions of the root of a genotype showed a transition for CMX vessel number and CMX area from higher in the top region of the root to a single CMX vessel in the root tip. In barley, a similar pattern was reported only in the nodal roots retaining the central pith (Luxová, 1989, 1991), but the author also observed a few instances of more than one CMX vessel in the base of the seminal (tap) root. Watt *et al.* (2008) reported one to three CMX vessels in the primary axile (tap) roots of hexaploid winter wheat and tritcale (*Triticale hexaploid*). The variation that was found along a single tap root of spring wheat spans the differences between different root types in winter wheat observed by Watt *et al.* (2008). In the root tip only CMX vessel diameter and area were traceable as other cell types were still differentiating. This developmental pattern was consistent across the different wheat genotypes used in this study. Interestingly, there was variation in timing for the transitions in root anatomy among genotypes and this variation was explained by dosage of the 1RS arm in bread wheat. RD_2 and RAD_4 transitioned earlier, from having multiple

metaxylem vessels and a larger stele to a single, CMX vessel and smaller stele, than did R_0 and RA_1.

In the top region, all the root traits were significantly different among genotypes except average CMX vessel diameter and CMX vessel number (Fig. 4A–F). The average CMX diameter was calculated from the average of diameters of all the CMX number of that subsequent genotype and, hence, the number of CMX vessels was different in each genotype and so was the total CMX vessel area. Interestingly, all the root traits in the top region showed negative slope in regression analysis and most of them were significant, especially stele diameter, total CMX vessel area, and peripheral xylem pole number. Variation in all the traits was explained by number of 1RS dosages in wheat genotypes and root anatomical traits were smaller with a higher number of 1RS dosages (Fig. 2A–E). Significant positive correlation among almost all the root traits from the top region and mid-region of the roots (except CMX vessel number) suggested their inter-dependences in growth and development. Root diameter could not be measured for all the replicates of each genotype because of the degeneration and mechanical damage to the cortex and epidermis. Earlier, a study on the rate of cortical death in seminal (tap) roots was investigated in different cereals. It was found that rate of cortical death was faster in hexaploid wheat and was positively associated with root age (Liljeroth, 1995). Our results suggest there are genes on the 1RS arm that affect xylem anatomy which is further affected by 1RS dosage.

Structural adaptation to stress

Root morphology and root architecture are responsible for both water and nutrient uptake while in root anatomy, xylem vessels are essential for their transportation to the shoots to allow continued photosynthesis and growth. Variations in xylem anatomy and hydraulic properties occur at interspecific, intraspecific, and intraplant levels (Zimmermann, 1983; Sperry and Saliendra, 1994; Jackson *et al.*, 2000). Variations in xylem vessel diameter can drastically affect the axial flow because of the fourth-power relationship between radius and flow rate through a capillary tube, as described by the Hagen–Poiseuille law (Zimmermann, 1983; Tyree and Ewers, 1991). Thus, even a small increase in mean vessel diameter would have exponential effects on specific hydraulic conductivity (K_s) for the same pressure difference across a segment (McElrone *et al.*, 2004). Xylem diameters tend to be narrower in drought-tolerant genotypes (Ou *et al.*, 2005; Yang *et al.*, 2007), and at higher temperature (Huang *et al.*, 1991). Smaller xylem diameters pose higher flow resistance and slower water flow which may help the wheat plant to survive water-stressed conditions. Richards and Passioura (1989) reported increased grain yield of two Australian wheat cultivars by selecting for narrow xylem vessels in seminal (tap) roots. Reduced water flow in seminal axile (tap) roots may be most important at the early stages of plant development since they are the first to emerge and seedling survival is often a critical stage, especially in rainfed environments, where seedling survival solely depends on the available sub-soil moisture.

A model for water and nitrogen capture in cereal root systems predicted greater economic returns from crops with greater partitioning of dry matter to the roots (King *et al.*, 2003). The results in this study generally showed more allocation of dry matter to roots in the 1RS genotypes. It was observed that partitioning of dry matter to roots was incremental with an increase in dosage of the 1RS arm excluding the F_1 hybrid genotype (data not shown). The non-significant difference in R/S ratio between RD_2 (=0.31) and R_0 (=0.33) may be due to the poor performance of RD_2 during the 2008 season. Ehdaie *et al.* (2003) observed a better R/S ratio in RD_2 genotype than Pavon 76 (R_0) in a greenhouse experiment grown to maturity. These authors also studied resource capture involving genotypes RA_2 and RD_2 which showed better water use efficiency (Ehdaie *et al.*, 2003) and more nutrient uptake with less leachate concentration than check R_0 (B Ehdaie and JG Waines, unpublished data). These authors reported a larger and well-branched root system in 1RS wheats which is further positively correlated with an increase in grain yield and its components (B Ehdaie and JG Waines, unpublished data). The model for resource capture (King *et al.*, 2003) also predicted maximum returns from crops with smaller specific root weight, i.e. finer roots. It was suggested to be advantageous for the plants to invest in more surface area available for resource capture at lower depths.

The results of this study also showed that the presence of 1RS arm in bread wheat increased root biomass (Fig. 1; Table 1) and reduced the dimensions of some root parameters, especially the CMX vessel area and diameter in the root tips (Fig. 2A–E, Fig. 5A, B) as well as in the top of the root (Figs 2K–O, 4 B, D). Future experiments warrant inclusion of traits such as root number, extensiveness of root branching, and root surface area along with resource capture studies to address the effect of 1RS wheats. The present study also suggests the possibility of different genes present on the 1RS arm affecting root biomass and anatomy in wheat as dosage of the 1RS arm affects these traits differently. Manske and Vlek (2002) reported that wheat genotypes with the 1RS translocated chromosome arm had thinner roots and higher root-length density compared with normal wheat with a 1BS chromosome arm under field conditions. Similar differences were also observed for root length density, diameters of stele and CMX vessel between winter wheat and triticale (genome formula=AABBRR) (Watt *et al.*, 2008). Among 1RS translocation wheats, significant association was observed between root biomass and grain yield under well-watered and droughted environments (Ehdaie *et al.*, 2003). Narrow metaxylem vessels and higher root biomass may provide 1RS translocation wheats with better adaptability to water stress and make them better performers for grain yield.

Conclusion

In a recent study on rice root anatomy, QTL for metaxylem vessels were identified on the distal end of the long arm of

chromosome 10 of rice (Uga *et al.*, 2008) which showed synteny to the 1RS chromosome arm of rye (Hackauf *et al.*, 2009). These reports also support the present findings and may validate the purpose of the current study on wheat root anatomy. This study suggests that the short arm of chromosome 1 of rye carries important genes affecting root morphology and root anatomy in bread wheat. The 1RS arm showed heterotic effect and significant dosage effect on root biomass and root anatomy in bread wheat. Thus, higher root biomass and narrow metaxylem vessels in 1RS wheat strongly advocate their inclusion as a selection criterion in wheat breeding programmes.

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