



Research Article

Genetic Bases of Root System Architecture in Durum Wheat Seedlings

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Article Information ABSTRACT

Key words:
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Root system architecture (RSA) traits are characterized by constitutive genetic inheritance components which may enable to predict the root phenotypes based on genetic information. The research presented in this study aimed at the identification of traits and genes that underlie root system architecture (RSA) in a population of 176 recombinant inbred lines (RILs) derived from the cross between two durum wheat cvs. Meridiana and Claudio, in order to eventually contribute to the genetic improvement of this species. The following seedling-stage RSA traits were: primary root length, seminal root length, total root length, diameter of primary and seminal roots. Results of ANOVA showed a significant difference among durum wheat cultivars for all traits and the largest heritability was observed for total root length (30.7%). In total, 14 novel QTLs for RSA traits were identified, and both parents contributed favorable alleles to the population.

INTRODUCTION

Durum wheat (*Triticum turgidum* L. var. durum, $2n = 4x = 28$) is the second most important wheat species and the only tetraploid species of wheat of commercial importance that is widely cultivated today.

Traditionally, efforts in plant breeding have focused on shoot characteristics, due to the challenges of in situ examination of roots and the complex influence of the environment on their growth (Tuberosa et al. 2002). Roots play several essential roles in the plant life cycle, including anchoring to the soil, mechanical support to stems, uptake of nutrients and water (therefore playing a role in environmental stress tolerance) and others (Osmont et al. 2007; Smith and De Smet 2012). While root system architecture has recently received increased attention in terms of genetic dissection of development and physiology, including the implementation of high-throughput phenotyping (Trachsel et al. 2011).

Analyses of genetic factors contributing to RSA are however still very limited, partly because of the difficulty

of observing the distribution of roots in field conditions, and partly because of the complexity of the effects of environmental conditions on root system architecture. However, the genetic mechanisms controlling root system architecture and function are still not well understood, especially in durum wheat, which possesses a complex and unsequenced tetraploid genome. Sanguineti et al. (2007) have reported the first results about genetic dissection of seminal root architecture in 57 elite durum wheat germplasm.

Quantitative trait loci (QTL) detection based on high-density molecular markers genetic maps and association mapping has improved the understanding of the complex genetic control of cereal root traits. Many of these studies have observed an overlap between QTL for root traits and those for nutrient uptake and productivity in maize (Tuberosa et al. 2002), wheat (An et al. 2006) and rice (Steele et al. 2007). However, for reported wheat QTLs, genome or gene-content sequence data is not currently available to allow confirmation of any relationship with root QTL in other crop species.

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MATERIALS AND METHODS

In order to study the genetic bases of RSA, a population of 176 recombinant inbred lines (RILs) from the cross between the two Italian elite durum wheat cvs. Meridiano and Claudio (Maccaferri *et al.* 2011) were grown in a randomized complete block design with two replications. The paper culture system at RSA traits was evaluated according to a protocol first described by Bengough *et al.* (2004) then modified by Sanguineti *et al.* (2007) and improved in the present work. In particular, for each genotype (RILs), 15 seeds were prepared and weighted exactly. Then, selected seeds were moved in the pre-germination step, in Petri dishes with a wet filter paper at 28 °C for 24 h. Then, seven germinated seeds for each genotype were placed on flat plastic rhizotrons covered with imbibed filter paper where seedlings were let to grow per one week, in the dark, at constant temperature of 25 °C. Five well developed and homogeneous seedlings were utilized for phenotypic scoring.

Five root system architecture traits were collected and traits, abbreviations and explanations are shown in Table 1. All traits were manually measured and/or recorded by plantlets' images using the software SmartRoot® (Lobet *et al.* 2011), after recording digital images of each seedling. Frequency distributions of the phenotypic data were inspected to assess the consistency of data and to investigate the complexity of the genetic control of the traits. The analysis of variance (ANOVA) for each trait was performed using the software Genstat V.15 and Heritability (h^2) was calculated on a mean basis across two replications.

Table 1: List of root system architecture traits with explanations and abbreviations

Trait	Abbreviation
Primary root length	PRL
Seminal root length	SRL
Total root length	TRL
diameter primary root	diamPR
diameter Seminal root	diamSR

In total, 899 markers (487 SNPs, 261 DARTs, 142 SSRs and 9 STSs) were utilized for QTL analysis. Single-marker analysis using linear regression, composite interval mapping (CIM) and multiple interval mapping (MIM) were carried out in Windows QTL Cartographer version 2.5 (WINQTL) (Wang *et al.* 2007). The reported QTLs were identified as having a threshold LOD ≥ 2.0 through composite, and multiple interval mapping. The markers were ordered according to a consensus map developed at the University of Bologna in the framework of an international cooperation for that purpose (Maccaferri *et*

al. 2014). The mapping population, Meridiano \times Claudio (MC RIL, Maccaferri *et al.* 2011) was developed by the University of Bologna in collaboration with Produttori Sementi Bologna SpA (Argelato, BO, Italy).

RESULTS AND DISCUSSION

The Phenotypic results indicated a wide range of variation for RSA traits. In detail, TRL was the trait that showed the largest range of variation, from 95.8 to 142.39 cm, with a mean value of 122.5 cm and diamSR indicated the lowest range of variation, from 0.07 to 0.09 cm, with a mean value of 0.08 cm. Additionally, PRL ranged from 24.7 to 34.5 cm, with a mean value of 30.7 cm. The treatment effect and genetic variation within the 176 RIL population for RSA traits are reported in Table 2. Results of ANOVA showed significant differences among durum wheat RILs for all RSA traits and coefficient of variation (CV) ranged from 5.0% for diamPR to 7.1% for TRL (Table 2). In among of the considering traits, the diamPR with amount of 14.4 percent had the least of heritability and earliness, the TRL with 30.7 percent has shown the most of heritability and for the other considering traits were 26.3 to 29.7 percent in average. Frequency distributions for RSA traits are shown in Figure 1. All the RSA traits exhibited continuous variation in the RIL population with normal distributions, indicating a polygenic control underlying these traits and allowing us to find QTLs for RSA traits.

Table 2: Summary of traits heritability, coefficient of variation and genetic differences as obtained in the analysis of 176 Meridiano \times Claudio RILs at the seedling stage

Trait	PRL	SRL	TRL	diamPR	diamSR
Mean	30.7	22.9	122.5	0.08	0.08
Max	34.5	26.2	142.4	0.10	0.09
Min	24.7	17.7	95.8	0.07	0.07
F (P)	**	**	**	*	*
CV (%)	5.7	6.6	7.1	5.0	5.2
h^2 (%)	29.7	27.5	30.7	26.3	14.4

A total of 176 RIL population from the cross between the two Italian elite durum wheat cvs. Meridiano and Claudio were used to construct the genetic linkage map. The linkage groups were assigned to durum wheat chromosomes 1A through to 7B. For the five RSA traits examined, 14 QTLs were identified using QTL Cartographer with a LOD score significant at $P \geq 2$. The results of QTL detection are presented in Table 3. In total, we identified 3 QTLs for PRL, 3 for SRL, 3 for TRL, 2 for diamPR and 3 for diamSR. The QTLs were identified on all

chromosomes and both parents contributed favorable alleles to the population, with 9 from Meridiano and 5 from Claudio.

Out of the nine RSA-QTLs that were detected, three (QPRL1-6A, QPRL2-6B and QPRL3-7B) co-located for PRL, three (QSRL1-1B, QSRL1-2B and QSRL1-6B) for SRL and three (QTRL1-1B, QTRL2-3B and QTRL3-4B) for TRL. The largest effect of QTL for PRL was QPRL2-6B, related to a SSR marker wmc182-6B on chromosome 6B with R² value 7.05%. The SRL and TRL associated QTLs with the greatest effects were located on chromosome 1B (QSRL1-1B and QTRL1-1B) and flanked by a DART marker wPt-733882-1B with R² values of 10.25% and 11.85%, respectively. According to the results of additive effects, the favorable alleles in QPRL2-6B, QSRL1-1B and QTRL1-1B have been contributed by Claudio and the other QTLs contributed by Meridiano (Table 3).

Among the RSA-QTLs that were identified, two (QdiamPR1-1A and QdiamPR2-7A) co-located for diamPR, three (QdiamSR1-1A, QdiamSR2-2A and QdiamSR3-7A) for diamSR. The diamPR and diamSR associated QTLs with the highest effects were located on chromosome 1A (QdiamPR1-1A) and 7A (QdiamSR3-7A) with R² values of 9.02% and 8.79%, respectively. The favorable alleles of all QTLs associated with diamPR and diamSR were contributed by Meridiano (Table 3).

Durum wheat has a dense fibrous root system which is difficult to quantify and to select directly by breeders. Therefore, mapping QTL for root system architecture traits will help breeders to select root traits desirable for efficient acquisition of water and nutrients from soils. Analysis of genetic effect showed that both parents, Meridiano and Claudio, contributed favorable alleles. Detection of these QTLs further show that they are still segregating in cultivars adapted to the target production region and could potentially be responsive to the conventional or marker-assisted selection.

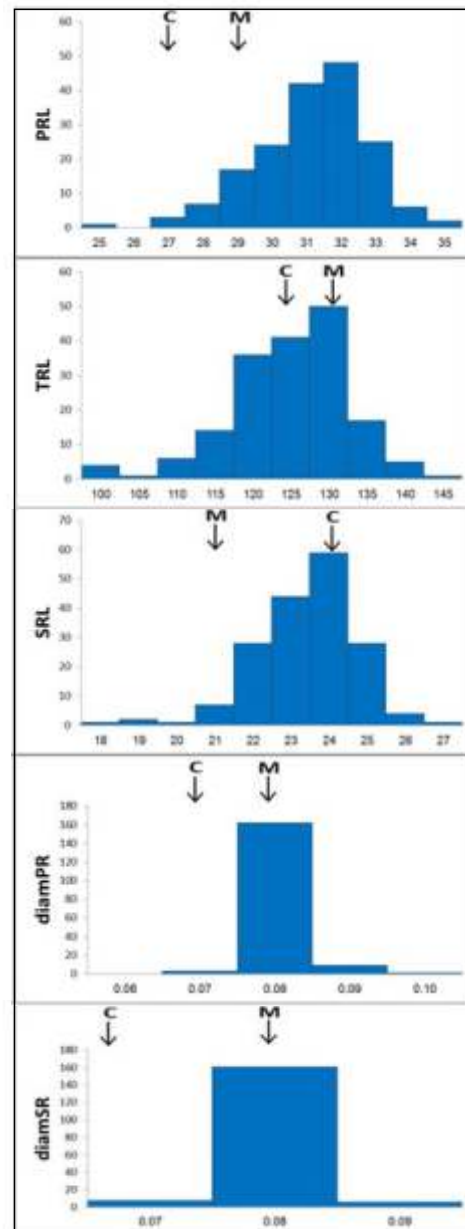


Figure 1: Frequency distribution for the RSA traits in 176 Meridiano x Claudio RILs collected at seedling stage. Arrows indicate mean values for Meridiano (M), and Claudio (C)

Table 3: QTL detected for RSA traits in 176 Meridiano x Claudio RILs of durum wheat collected at the seedling stage

Trait	QTL	Chro.	Marker	LOD	Position	R ² %	Additive effect	Parent
PRL	QPRL1-6A	6A	wPt-732328	3	83	6.78	0.42	Meridiano
	QPRL2-6B	6B	wmc182-6B	2.9	135	7.05	-0.44	Claudio
	QPRL3-7B	7B	wPt-4300b-7B	2.85	202	6.88	0.44	Meridiano
SRL	QSRL1-1B	1B	wPt-733882-1B	3.69	55	10.25	-0.42	Claudio
	QSRL1-2B	2B	wPt-2430-2B	2.98	139	6.46	-0.33	Claudio
	QSRL1-6B	6B	IWA8011	2.88	129	8.4	-0.38	Claudio
TRL	QTRL1-1B	1B	wPt-733882-1B	3.84	50	11.85	-2.72	Claudio
	QTRL2-3B	3B	IWB72454	2.42	108	5.17	1.79	Meridiano
	QTRL3-4B	4B	kbo-0236-4B	5.16	97	10.93	2.60	Meridiano
diamPR	QdiamPR1-1A	1A	wPt-4676-1A	3.88	0	9.02	0.01	Meridiano
	QdiamPR2-7A	7A	IWB73246	2.56	7	6.59	0.01	Meridiano
diamSR	QdiamSR1-1A	1A	wmc336-1A	3.05	18	6.77	0.01	Meridiano
	QdiamSR2-2A	2A	wmc658-2A	2.09	23	4.6	0.01	Meridiano
	QdiamSR3-7A	7A	IWB73246	3.22	8	8.79	0.01	Meridiano

CONCLUSION

In the research study, the paper culture system with the imaging technique employed together was able to distinguish among RSA traits in young seedlings of durum wheat population. We investigate the main RSA features might be useful for plant breeding activity, since RSA plays a key role in the response of the plants to different environments, soil type and rainfall (Tuberosa *et al.* 2002; Wasson *et al.* 2012). Significant differences and normal distributions were identified among RIL population of durum wheat for RSA traits, indicating a polygenic control underlying RSA traits. These results are noteworthy especially considering that the tested materials are from the cross between the two Italian elite durum wheat cvs. Meridiano and Claudio, both characterized by high yield potential and stability. Among the considered RSA traits, 14 novel QTLs were identified, and both parents contributed favorable alleles to the population.

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