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Research Paper

SNP-based association analysis for seedling traits in durum wheat (*Triticum turgidum* L. *durum* (Desf.))

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In the present study, 150 accessions of worldwide originated durum wheat germplasm (*Triticum turgidum* spp. *durum*) were observed for major seedling traits and their growth. The accessions were evaluated for major seedling traits under controlled conditions of hydroponics at the 13th, 20th, 27th and 34th day-after germination. Biomass traits were measured at the 34th day-after germination. Correlation analysis was conducted among the seedling traits and three field traits at maturity, plant height, grain weight and 1000-grain weight observed in four consecutive years. Associations of the measured seedling traits and SNP markers were analyzed based on the mixed linear model (MLM). The results indicated that highly significant genetic variation and robust heritability were found for the seedling and field mature traits. In total, 259 significant associations were detected for all the traits and four growth stages. The phenotypic variation explained (R2) by a single SNP marker is higher than 10% for most (84%) of the significant SNP markers. Forty-six SNP markers associated with multiple traits, indicating non-neglectable pleiotropy in seedling stage. The associated SNP markers could be helpful for genetic analysis of seedling traits, and marker-assisted breeding of new wheat varieties with strong seedling vigor.

Key Words: durum wheat, seedling traits, growth and development, single nucleotide polymorphism, association mapping.

Introduction

Wheat (*Triticum* spp.) is one of the major cereal crops in the world. It mainly consists of two species, the hexaploid bread wheat (*Triticum aestivum*) and the tetraploid durum wheat (*Triticum durum*) (Peng *et al.* 2011). It is grown on more upland area worldwide than other crops, including the drought-prone environments (Philippe *et al.* 2012). The hexaploid modern bread wheat (AABBDD) is derived from the spontaneous hybridization of the diploid *Aegilops tauschii* (genome DD) with the tetraploid *Triticum turgidum* (genome AABB) (Peng *et al.* 2011).

Durum wheat is traditionally grown around the Mediterranean Sea and is one of the most important traditional food crops in West Asia. Nowadays, over 60% of the durum wheat is still grown in the Mediterranean basin, mainly in Italy, Spain, France, Greece, and West Asian and North African countries (Maccaferri *et al.* 2003). The cultivation of durum generates greater yield than other wheats in areas with low precipitation (3–5 dm), especially in the West Asian and North African countries. Good yields can be obtained by irrigation, but this is rarely done due to water limitation. In the Middle East and North Africa, local bread-making accounts for half the consumption of durum. Furthermore, many countries in Europe produce durum in commercially significant quantities (https://en.wikipedia.org/wiki/Durum#CITEREFMatz1992).

Association analysis is an effective approach to understanding the relationship between phenotypic variation and genetic polymorphisms. Single nucleotide polymorphisms (SNPs) are the most abundant type of molecular markers that can be used for genetic mapping and diverse applications in both animal and plant (Bhattramakki *et al.* 2002, Deschamps *et al.* 2010, Gupta *et al.* 2001, Ren *et al.* 2013, Trebbi *et al.* 2011). Many SNPs have been explored as a high-resolution marker for speeding up gene mapping of

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disease resistance or other traits (Trebbi *et al.* 2011, Wasson *et al.* 2012). Diploid crops such as rice and barley have benefited from an extensive genetic analysis and molecular breeding programs assisted by molecular markers (Kota *et al.* 2008, Nasu *et al.* 2002). SNP identification remains a challenge for large and polyploid genomes due to the size and complexity. In wheat, genome complexity has hindered this type of studies (Allen *et al.* 2011, Edwards *et al.* 2009, Kozlova *et al.* 2009). SNPs representing polymorphisms between wheat cultivars could provide an unprecedented resource for wheat diversity analysis and hence are very valuable for wheat breeding and genetics programs (Chao *et al.* 2009, Chono *et al.* 2015, Lorenc *et al.* 2012, Peng *et al.* 2009).

Seedling traits and the best crop establishment are essential for wheat production. It plays a major role in plant development in the adaptive response, especially under drought-stress conditions. Seedling vigor has been correlated to the better selection criteria for wheat crop establishment in the fields (Erayman *et al.* 2006). Seedling traits such as weights of fresh root and shoot, weights of dry root and shoot, and the height were correlated and considered to be inheritable (Khan *et al.* 2002). Several studies showed correlations of seedling traits with the other plant traits (Butt *et al.* 2001, Cisse and Ejeta 2003). Improvement in seedling traits and the crop establishment would likely result in the increased plant growth and yield (Nagel *et al.* 2014, Peleg *et al.* 2009, Rebetzke *et al.* 2007). Nevertheless, phenotypic selection for seedling traits is complicated and labor-intensive. The progress of genetic improvement based on direct selection of secondary seedling traits is quite limited so far. These restrictions for selection of the seedling traits could be overcome in breeding strategy by using molecular marker technology, i.e., marker-assisted selection (MAS) (Peng *et al.* 2000, Soleimani *et al.* 2002). However, very little is known about the molecular genetics of seedling traits and their growth in durum wheat (Nagel *et al.* 2014, Peleg *et al.* 2009). Therefore, identification of SNP markers associated with the seedling traits and their growth will be helpful in breeding for durum wheat varieties with quick and excellent crop establishment (Blum 2005, Nagel *et al.* 2014, Peleg *et al.* 2009).

Growth rate, growth gain, water content, respiratory rate, and dry biomass weight are very important physiological traits of plants (Monasterio 2001, Nagel *et al.* 2014, Peleg *et al.* 2009). The root system of plants also should be worthy of close attention because roots play a vital role in plant growth, development, and fitness (Bai *et al.* 2013, Kumar *et al.* 2014). Hydroponics is an excellent method of conducting studies on plant root system under controlled environmental conditions (Bai *et al.* 2013, Canè *et al.* 2014, Liu *et al.* 2013).

Association analysis based on linkage disequilibrium is helpful for gene discovery, and is an alternative approach and complementary tool for QTL mapping in crops. Identification of the chromosomal regions controlling seedling traits and their growth could be helpful for understanding the trait genetics in early development stage of wheat plant. Discovery of SNP markers associated with seedling traits would accelerate breeding durum wheat varieties with a strong root system and high productivity. Therefore, the major objective of this study is to figure out the candidate genome regions and the anchoring SNP markers associated with seedling traits in durum wheat.

Materials and Methods

Plant materials and growth conditions

A total of 150 accessions of durum wheat (*Triticum turgidum* L. ssp. *durum* Desf., 2n = 4x = 28, AABB) with a widespread origin covering various countries worldwide (Hu *et al.* 2015, Ren *et al.* 2013) were investigated in this study. The experiment was conducted in greenhouse during the year 2014-2015 at the College of Plant Science and Technology in Huazhong Agricultural University, Wuhan, China. The conditions of growth were set up at 15/20°C of temperature, 16-h day/8-h night light photoperiods, and relative humidity of 65%. After germination, five-day-old seedlings were transplanted to holes made in foam board (polystyrene) floating over the Hoagland's nutrient solution (Hoagland and Arnon 1950) in 30 plastic tanks with dimensions of 31.5 cm in length, 24 cm in width and 11.5 cm in depth. Each tank containing eight liters of nutrient solution. An experimental unit included 30 seedlings. The solution was renewed every seventh day during the plant growth period to prevent nutrient depletion. The accessions were grown in completely randomized design with six replicates. In each replication, one seedling was used for data collection.

Phenotyping of seedling traits

Phenotypes of seedling traits were evaluated under the above-described controlled environment, hydroponic culture. The traits were measured at four growth stages (13, 20, 27 and 34 days after germination) on the same six seedlings as the following: root length (RL, cm), number of main roots (NR), seedling height (SH, cm), number of leaf (NL), fresh weight (FW, g), and leaf area (LA, cm^2). Growth rate was calculated for root length (GRRL), seedling height (GRSH), number of leaf (GRNL), number of roots (GRNR), fresh weight (GRFW), and leaf area (GRLA), respectively. Growth gain was also calculated for fresh weight (GFW, g), number of leaf (GNL), number of roots (GNR), seedling height (GSH, cm), root length (GRL, cm), and leaf area (GLA), respectively. The final dry weight of root (RDW, g), shoot (SDW, g), and total biomass (DW, g) were measured at the 34^{th} day-after germination.

Field trials

The field experiments were conducted on the experimental farm of the Huazhong Agricultural University in four growing seasons of 2010, 2011, 2012 and 2013. The experimental details and results were reported in Hu *et al.* (2015).

Means of plant height (PH, cm), grain weight per plant (GWP, g) and 1000-grain weight (KGW, g) over the four years were used for correlation analysis with the seedling traits.

Statistical analysis

The mean phenotypic values of the seedling traits were subjected to statistical analysis using software SAS (2000). Analysis of variance (ANOVA), the broad-sense heritability (H^2) and correlation coefficients among seedling traits and the three field traits were calculated. Frequency distribution of the traits was analyzed using GraphPad Prism version 5.01 (www.graphpad.com).

Association analysis

The 21 seedling traits described above were subjected to association analysis with the SNP markers (Ren *et al.* 2013). The associations were estimated under the mixed linear model (MLM) using software TASSEL 3.0.124 (http//www.Misogynistic.net/tassel). A probability level of 0.001 that is equivalent to LOD = 3 is used as the threshold for a significant trait-marker association. Both Q-Matrix of the population structure and K matrixes used in the analysis were described in the previous studies (Hu *et al.* 2015, Ren *et al.* 2013).

Results

Genetic variation of the seedling traits

Distribution histograms of the 22 seedling traits are shown in **Figs. 1**, **2**. The trait distribution patterns were similar among the four seedling stages, i.e., basically fitted the normal distribution and thus are quantitatively inherited. Results of the ANOVA analyses for the traits were summarized in the **Table 1**. The genotypic variation was highly significant ($P < 0.001$) for all the 21 seedling traits.

Ten of the 21 seedling traits, RL, NR, FW, LA, GRRL, GRFW, GRLA, GNL, GLA and SDW, were very variable among genotypes with CV > 10%. Among these traits, GNL and GRFW were the most genetically variable with CV > 20%. Other 11 traits, SH, NL, GRSH, GRNL, GRNR, GSH, GRL, GNR, GFW, RDW, and DW, were relatively low variable among genotypes with CV < 10%. DW was the most genetically stable trait with CV = 2.36%.

Broad-sense heritability (H^2) was estimated for each of the 21 traits (**Table 1**). H^2 was over 50% for all the traits observed. Most (17) of the traits have high heritability ($H^2 > 75\%$). Therefore, it is meaningful and necessary to further perform association analyses for the seedling traits with the SNP markers.

Correlation among the observed traits

Correlation analyses were performed among the 21 seedling traits and three field traits, and results were shown in **Table 2**. Out of the 276 possible correlation pairs, about 60% (163) were significant or highly significant. GWP, the most important economic trait of wheat production, showed significant and positive correlation with three seedling traits, LA, GRLA, GLA and the field trait KGW. Interestingly, KGW, one of the most important yield components of wheat, was significantly or highly significantly and positively correlated with most (14) of the 21 seedling traits, NL, SH, RL, FW, SDW, RDW, DW, LA, GRLA, GRSH, GRFW, GRNL, GFW and GLA. The final plant height was significantly or highly significantly correlated positively with four seedling traits, SH, RL, GRSH and GRRL, and negatively with other five seedling traits, NL, RDW, GRNL, and GNL. Therefore, it is of great significance to conduct genetic analyses of seedling traits in durum wheat.

Marker-trait associations

In the present study, a marker-trait association is significant when $p \leq 0.001$, which is equivalent to $LOD \geq 3$. **Tables 3**, **4** and **Supplemental Tables 1**, **2** presented an overview and details of trait-marker associations under MLM model in four consecutive seedling stages, respectively. The analyses showed 259 significant associations in total for 21 seedling traits, including 18 measured at 4 seedling stages and 3 observed at the final stage, the 34th day-after germination. Most (84%) of these significant SNP markers can explain individually over 10–21% of the phenotypic variation. R^2 of a single SNP was higher than 15% for 36 trait-SNP association pairs (**Supplemental Tables 1**, **2**). There are 46 SNPs associated with multiple seedling traits (**Table 4**). The associations between SNP markers and seedling traits were varying with the growth stages. A total of 196 unduplicated significant associations were identified in the four seedling stages for 18 seeding traits (**Table 3**, **Supplemental Table 1**). Over 25 SNP markers were detected to be significantly associated with FW, GRFW and GRNR, whereas only a few (<5) SNP markers significantly associated with NR, LA, GRSH, GRLA and GRRL, and no significant SNPs were found for RL (**Table 3**).

For the three final seedling traits measured at the last stage (34 days after germination), RDW, SDW and DW, 63 significant associations with $R^2 = 7.99–18.06\%$ in total were identified (**Supplemental Table 2**). Large numbers of associated SNP markers, 34 and 28, were found for SDW and DW, respectively. Only one of SNP marker with $R^2 = 13.27\%$ was significantly associated with the RDW.

SNP markers associated with six seedling traits

Association analysis was first performed for six seedling traits in four growth stages: fresh weight (FW), number of leaf (NL), seedling height (SH), root length (RL), number of main roots (NR) and leaf area (LA). The number of associated SNP markers was quite variable among the traits and also among the growth stages for a same trait (**Table 3**, **Supplemental Table 1**).

Fresh weight (FW): 31 significant SNP markers were detected across the three seedling stages, i.e., Stage 2–4 (**Table 3**). Only one associated SNP marker, *BG314205_*

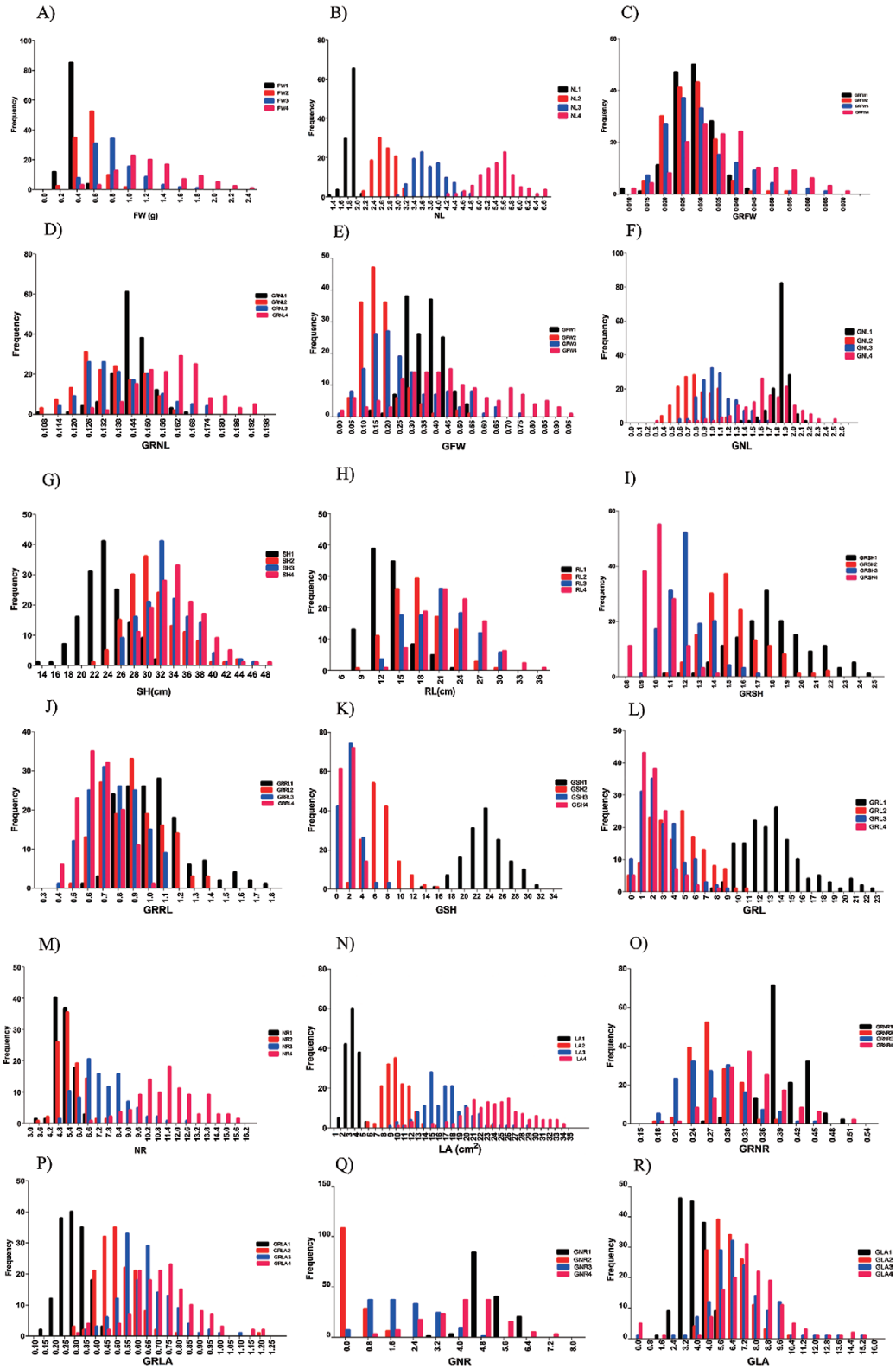

Fig. 1. Frequency distribution of the 18 examined seedling traits of durum wheat in four consecutive seedling stages. (a) Fresh weight (FW), (b) number of leaves (NL), (c) growth rate of fresh weight (GRFW), (d) growth rate for number of leaves (GRNL), (e) growth gain of fresh weight (GFW), (f) growth gain in number of leaves (GNL), (g) seedling height (SH), (h) root length (RL), (i) growth rate of seedling height (GRSH), (j) growth rate of root length (GRRL), (k) growth gain of seedling height (GSH), (l) growth gain of root length (GRL), (m) number of main roots (NR), (n) leaf area (LA), (o) growth rate for number of roots (GRNR), (p) growth rate of leaf area (GRLA), (q) growth gain in number of roots (GNR) and (r) growth gain of leaf area (GLA).

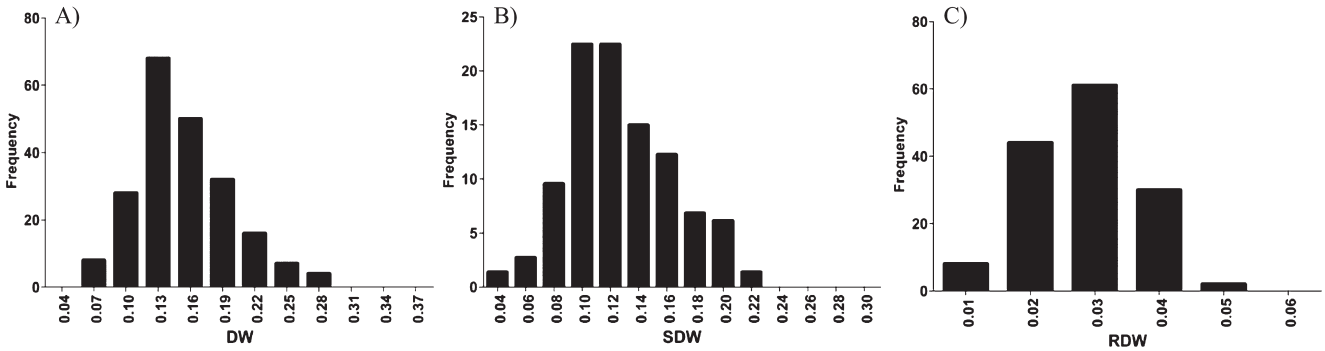

Fig. 2. Frequency distribution of the chlorophyll content and biomass traits for the 34-day old seedlings in durum wheat. (a) total dry weight (DW), (b) shoot dry weight (SDW) and (c) root dry weight (RDW).

Table 1. Analysis of variance (ANOVA) and heritability (H^2) for the 21 seedling traits in durum wheat

Trait[a]	Mean	Range	MS[b]	F-value	CV (%)[c]	H^2 (%)
RL	20.65	12.36–34.8	69.128	6.49***	16.2	87
SH	33.13	25.43–45.5	73.617	11.00***	7.8	92
NL	5.44	4.23–6.57	1.194	8.00***	7.1	89
NR	11.46	6.00–17.4	20.949	6.56***	15.5	87
FW	0.95	0.41–3.12	0.537	18.26***	18.2	95
LA	21.78	10.10–41.12	190.245	4.59***	19.5	82
GRRL	0.58	0.36–1.02	0.058	5.13***	18.2	84
GRSH	0.97	0.80–1.38	0.065	9.07***	8.6	90
GRNL	0.16	0.13–0.19	0.001	8.95***	7.14	90
GRNR	0.33	0.18–0.51	0.024	8.59***	5.8	90
GRFW	0.04	0.01–0.09	0.001	15.62***	21.5	94
GRLA	0.64	0.31–1.21	0.165	4.59***	19.5	82
GNL	1.685	0.25–2.55	0.54	3.59***	22.5	78.2
GSH	2.06	0.10–7.70	5.82	1.88***	8.31	65.3
GRL	2.06	0.10–5.70	5.79	1.44**	9.61	58.9
GNR	4.17	1.20–7.33	7.88	2.80***	4.24	73.7
GFW	0.47	0.01–2.01	0.23	8.97***	3.76	90.0
GLA	6.14	2.52–9.98	7.30	3.39***	13.9	77.2
SDW	0.13	0.05–0.30	0.008	8.57***	13.2	90
RDW	0.04	0.01–0.14	0.041	1.87***	8.84	65
DW	0.16	0.06–0.37	0.013	7.71***	2.36	89

*** Significant at 0.001 probability level.

[a] RL, root length (cm); SH, seedling height (cm); NL, number of leaf; NR, number of main roots; FW, fresh weight (g); LA, leaf area (cm^2); GRRL, growth rate of root length; GRSH, growth rate of seedling height; GRNL, growth rate for number of leaf; GRNR, growth rate for number of roots; GRFW, growth rate of fresh weight; GRLA, growth rate of leaf area; SDW, shoot dry weight (g); RDW, root dry weight (g); DW, total dry weight (g); GNL, growth gain for number of leaf; GSH, growth gain of seedling height; GRL, growth gain of root length; GNR, growth gain for number of main roots; GFW, growth gain of fresh weight (g); GLA, growth gain of leaf area.

[b] MS, mean square.

[c] CV, coefficient of variation.

1_B_33 was detected in the two stages. All other 30 SNP markers were detected only in one seedling stage, maubly in the stage 4. These FW-associated SNP markers were distributed across all the durum chromosomes except for 4B (**Supplemental Table 1**).

Number of leaf (NL): nine SNP markers were detected to be significantly associated with NL two seedling stages, i.e., Stages 1 and 3 (**Table 3**). All the 9 SNP markers were found to be significant only in one seedling stage (**Supplemental Table 1**). The SNP markers were located on six chromosomes, 2A, 5A, 7A, 1B, 5B and 6B.

Seedling height (SH): seven SNP markers were significantly associated with SH in three of the four seedling stages (**Table 3**). Three of the 7 SNP markers, *BG314205_1_B_33*, *BE443538_5_A_1436* and *BE590521_6_B_N_331*, were found to be significantly associated with SH in two of the four stages. The other four SNP markers were significant in one of the four seedling stages. These SNP markers were located on five chromosomes, 5A, 7A, 1B, 2B and 6B (**Supplemental Table 1**).

Number of main roots (NR): only one SNP marker, *BG313722_3_A_281*, was significantly associated with NR in two of the four seedling stages, i.e., stage 2 and 3 (**Table 3**). This SNP marker was located on chromosome 3A. (**Supplemental Table 1**).

Leaf area (LA): two SNP markers were found to be significantly associated with LA in one stages, i.e., stage 4 (**Table 3**). These two SNP markers, *BG274687_1_B_Y_287* and *BQ169448_6_B_252*, were located on the chromosomes 1B and 6B (**Supplemental Table 1**).

SNP markers associated with growth rate of the seedling traits

Association analysis was then performed for growth rate of the six seedling traits described above in four growth stages: growth rate for leaf area (GRLA), fresh weight (GRFW), number of leaf (GRNL), number of main roots (GRNR), seedling height (GRSH) and root length (GRRL). The number of associated SNP markers was also quite variable among the traits and also among the growth stages for a same trait (**Table 3**, **Supplemental Table 1**).

Growth rate of fresh weight (GRFW): 31 SNPs in total were found to be significantly associated with GRFW in three of the four seedling stages (**Table 3**). Only one SNP marker, *BG314205_1_B_33* was detected in the two stages, i.e., stages 3 and 4. the other 30 SNPs were significant in one seeding stage, mainly stage 4. These SNP markers were

Table 2. Correlation coefficients among the 21 seedling and three field mature traits in durum wheat

Trait[a]	NL	SH	RL	NR	FW	SDW	RDW	DW	LA	GRLA	GRNL	GRSH
NL	1											
SH	0.15	1										
RL	0.21*	0.23*	1									
NR	0.51***	0.14*	0.07	1								
FW	0.58***	0.53***	0.22*	0.57***	1							
SDW	0.59***	0.54***	0.23*	0.59***	0.96***	1						
RDW	0.46***	0.21*	0.33***	0.48***	0.69***	0.68***	1					
DW	0.60***	0.49***	0.26**	0.60***	0.95***	0.98***	0.82***	1				
LA	0.18*	0.59***	0.16	0.28**	0.69***	0.67***	0.38***	0.64***	1			
GRLA	0.18*	0.59***	0.16	0.28**	0.69***	0.67***	0.38***	0.64***	0.94***	1		
GRNL	0.94***	0.15	0.21*	0.51***	0.58***	0.59***	0.46***	0.60***	0.18*	0.18*	1	
GRSH	0.02	0.93***	0.27**	0.13	0.52***	0.53***	0.23**	0.48***	0.58***	0.58***	0.02	1
GRRL	0.15	0.07	0.96***	0.11	0.19*	0.15	0.05	0.13	0.12	0.12	0.15	0.08
GRNR	0.46***	0.15	0.11	0.98***	0.54***	0.56***	0.44***	0.56***	0.27**	0.27**	0.46***	0.13
GRFW	0.58***	0.53***	0.22*	0.57***	0.98***	0.96***	0.69***	0.95***	0.69***	0.69***	0.58***	0.52***
PH	**–0.18***	**0.40*****	**0.20***	**–0.09**	**–0.01**	**–0.06**	**–0.19***	**–0.10**	**0.09**	**0.09**	**–0.18***	**0.36*****
GWP	**0.06**	**–0.01**	**0.13**	**0.07**	**0.13**	**0.12**	**0.08**	**0.11**	**0.18***	**0.18***	**0.06**	**0.05**
KGW	**0.16***	**0.28****	**0.18***	**0.14**	**0.40*****	**0.35*****	**0.24****	**0.34*****	**0.46*****	**0.46*****	**0.16***	**0.31*****
GNL	0.60***	–0.20*	–0.10	0.25**	0.16*	0.16*	0.12	0.16	0.02	0.02	0.60***	–0.28**
GSH	–0.16*	0.11	0.14	0.02	0.16*	0.17*	0.09	0.16*	0.20*	0.20*	–0.16*	0.61***
GRL	–0.02	0.01	0.03	–0.12	–0.10	–0.11	–0.12	–0.12	–0.03	–0.03	–0.02	–0.30**
GNR	0.01	0.04	0.10	0.68***	0.06	0.06	0.03	0.06	0.04	0.04	0.00	0.03
GFW	0.52***	0.26**	0.08	0.51***	0.87***	0.83***	0.60***	0.82***	0.48***	0.48***	0.52***	0.29***
GLA	0.25**	0.59***	0.21*	0.29**	0.70***	0.71***	0.42***	0.67***	0.88***	0.88***	0.25**	0.58***

Trait	GRRL	GRNR	GRFW	**PH**	**GWP**	**KGW**	GNL	GSH	GRL	GNR	GFW	GLA
GRRL	1											
GRNR	0.10	1										
GRFW	0.19*	0.54***	1									
PH	**0.27****	**–0.08**	**–0.01**	**1**								
GWP	**0.04**	**0.08**	**0.13**	**0.14**	**1**							
KGW	**0.12**	**0.12**	**0.40*****	**0.12**	**0.52*****	**1**						
GNL	0.10	0.22*	0.16*	**–0.18***	**0.06**	**0.01**	1					
GSH	0.04	0.01	0.16*	**0.08**	**0.10**	**0.13**	–0.21*	1				
GRL	0.03	–0.07	–0.10	**–0.06**	**–0.01**	**–0.10**	0.13	–0.48***	1			
GNR	0.02	0.45***	0.06	**0.02**	**0.03**	**–0.02**	0.01	–0.01	0.07	1		
GFW	0.20*	0.48***	0.87***	**–0.04**	**0.07**	**0.25****	0.33***	0.15	–0.06	0.05	1	
GLA	0.09	0.26**	0.70***	**0.08**	**0.16***	**0.38*****	–0.05	0.20*	–0.07	0.02	0.50***	1

*, **, *** significant at the probability level of 0.05, 0.01 and 0.001, respectively.

[a] NL, number of leaves; SH, seedling height (cm); RL, root length (cm); NR, number of main roots; FW, fresh weight (g); SDW, shoot dry weight (g); RDW, root dry weight (g); DW, total dry weight (g); LA, leaf area (cm^2); GRLA, growth rate of leaf area; GRFW, growth rate of fresh weight; GRNL, growth rate for number of leaf; GRSH, growth rate of seedling height; GRRL, growth rate of root length; GRNR, growth rate for number of roots; PH, plant height at the mature stage in the field; GWP, grain weight per plant; KGW, 1000-grain weight; GNL, growth gain for number of leaf; GSH, growth gain of seedling height (cm); GRL, growth gain of root length (cm); GNR, growth gain for number of main roots; GFW, growth gain of fresh weight (g); GLA, growth gain of leaf area (cm^2).

located in all but the 4B chromosomes of durum wheat (**Supplemental Table 1**).

Growth rate for the number of leaf (GRNL): 10 SNPs, in total, were detected to be significantly associated with GRNL in two of the four seedling stages (**Table 3**). All of these SNPs were found to be significant associated in only one seedling stage, i.e., stage 1 or 3. These SNP markers were located in six chromosomes, i.e., 2A, 3A, 5A, 1B, 3B, and 6B (**Supplemental Table 1**).

Growth rate of seedling height (GRSH): four SNPs were significantly associated with GRSH in the two seedling stages, i.e., stage 1 or 3 (**Table 3**). All four SNP markers were distributed in four chromosomes, i.e., 5A, 7A, 1B and 6B (**Supplemental Table 1**).

Growth rate of root length (GRRL): two different SNP markers, *BE444305_1_B_433* and *BF485380_7_B_Y_479*, were significantly associated with GRRL in two seedling stages 3 and 4, respectively (**Table 3**). These two SNP markers were located on chromosomes 1B and 7B , respectively (**Supplemental Table 1**).

Growth rate for the number of main roots (GRNR): 27 SNPs in total were observed to be significantly associated with GRNR in three seedling stages (**Table 3**). SNP markers, *BG313722_3_A_281* was detected to be significantly associated with GRNR in two seedling stages with $R^2 > 11\%$. The other SNPs were significantly in only one of the four stages, stage 3 or 4. These GRNR-associated SNP markers were located in 11 of the 14 chromosomes of

Table 3. Number of SNP markers associated with the observed seedling traits, their growth rate and growth gain in four consecutive seedling stages of durum wheat

Trait[a]	Number of the associated SNP markers				
	Stage 1[b]	Stage 2	Stage 3	Stage 4	Total[c]
FW	0	1	1	30	31
NL	4	0	5	0	9
SH	1	0	3	6	7
RL	0	0	0	0	0
NR	0	1	1	0	1
LA	0	0	0	2	2
GRLA	0	0	0	2	2
GRFW	0	1	1	30	31
GRNL	4	0	6	0	10
GRNR	0	1	11	16	27
GRSH	1	0	3	0	4
GRRL	0	0	1	1	2
GFW	0	1	2	12	15
GNL	4	3	4	0	8
GNR	0	9	0	0	9
GSH	1	0	9	0	10
GRL	0	7	2	5	14
GLA	1	2	1	10	14
Total[d]	16	26	50	114	**196**

[a] FW, fresh weight (g); NL, number of leaf; SH, seedling height (cm^2); RL, root length (cm); NR, number of main roots; LA, leaf area (cm^2); GRLA, growth rate of leaf area; GRFW, growth rate of fresh weight; GRNL, growth rate for number of leaf; GRNR, growth rate for number of roots; GRSH, growth rate of seedling height; GRRL, growth rate of root length; GFW, growth gain of fresh weight; GNL, growth gain for number of leaves; GNR, growth gain for number of roots; GSH, growth gain of seedling height; GRL, growth gain of root length; GLA, growth gain of leaf area.

[b] Stage 1, the period from the 0 to13th day; Stage 2, the period from the 13th to 20th day; Stage 3, the period from the 20th to 27th day; Stage 4, the period from the 27th to 34th day.

[c] Total of the non-duplicated SNP markers across the four growth stages.

[d] Total of the SNP markers over the 18 traits.

durum wheat, i.e., 1A, 3A, 4A, 5A, 6A, 7A, 1B, 2B, 5B, 6B and 7B. The SNP marker *BG607308_5_A_Y_101* has the highest phenotypic effect of $R^2 = 20.59\%$ (**Supplemental Table 1**).

Growth rate of root length (GRRL): two different SNP markers were detected to be significantly associated with GRRL in two seedling stages (**Table 3**). These SNP markers were located on chromosomes 1B and 7B (**Supplemental Table 1**).

Growth rate of leaf area (GRLA): two SNP markers, *BG274687_1_B_Y_287* and *BQ169448_6_B_252*, were significantly associated with GRLA in one of the four stages, i.e., stage 4 (**Table 3**). These SNP markers were located in the chromosomes 1B and 6B, respectively, and could explain over 10% of phenotypic variation (**Supplemental Table 1**).

SNP markers associated with growth gain of the seedling traits

Association analysis was further performed on growth gain of six seedling traits described above in four growth stages: growth gain for fresh weight (GFW), number of leaf (GNL), number of main roots (GNR), seedling height (GSH), root length (GRL) and leaf area (GLA). The number of associated SNP markers was also quite variable among the traits and across the growth stages for a same trait (**Table 3**, **Supplemental Table 1**).

Growth gain of fresh weight (GFW): 15 SNPs in total were revealed to be significantly associated with GFW in seeding stages 2–4 (**Table 3**). All the 15 SNPs were detected in only one seeding stages, one in stage 2, two in stage 3 and 12 in stage 4. These GFW-associated SNP markers were distributed on nine of the 14 chromosomes, 1A, 3A, 4A, 5A, 6A, 1B, 5B, 6B and 7B (**Supplemental Table 1**).

Growth gain for number of leaf (GNL): eight SNPs were found to be significantly associated with GNL in the seeding stages 1–3 (**Table 3**). Three SNP markers, *BE443538_5_A_1436*, *BE590521_6_B_N_331*, and *BG314205_1_B_33* were detected in the two of the four growth stages. Other SNPs were significantly in only one of the four stages, four in stage 1 and one in stage 3. These GNL-associated SNP markers were distributed on five of the 14 chromosomes, 5A, 7A, 1B, 5B and 6B (**Supplemental Table 1**).

Growth gain of seedling height (GSH): 10 SNPs in total were detected to be significantly associated with GSH in the two growth stages, i.e., one in stage 1 and nine in stage 3 (**Table 3**). These SNP markers were distributed on seven chromosomes, i.e., 1A, 4A, 5A, 6A, 7A, 4B and 6B (**Supplemental Table 1**).

Growth grain of root length (GRL): 14 SNPs were identified to be significantly associated with GRL in three growth stages (**Table 3**). All the 14 associations were significant in only one growth stages, i.e., seven in stage 2, two in stage 3 and five in stage 4. These GRL-associated SNP markers were present in six chromosomes, 1B, 2A, 5A, 5B, 6B and 7A (**Supplemental Table 1**).

Growth gain in number of roots (GNR): a total of nine SNPs were found to be significantly associated with GNR in one growth stage, i.e., stage 2 (**Table 3**). These SNP markers were distributed across eight of the 14 chromosomes, 2A, 3A, 7A, 2B, 3B, 4B, 6B and 7B (**Supplemental Table 1**).

Growth gain for leaf area (GLA): 14 SNPs in total were found to be significantly associated with GLA in the four stages (**Table 3**). All of these GLA-associated SNPs were detected in only one growth stage, 1, 2, 1 and 10 in stages 1, 2, 3, and 4, respectively. These GLA-associated SNP markers were distributed on seven of the 14 chromosomes, 2A, 3A, 5A, 6A, 7A, 1B and 6B (**Supplemental Table 1**).

Associations of biomass traits with SNP markers

Association analysis was conducted for several traits measured at the 34th day of seedlings, dry weight of root (RDW), shoot (SDW), and total biomass (DW). The results are summarized in the **Supplemental Table 2**.

DW is the total biomass, and is consisted of dry weight of root (RDW) and shoot (SDW) of seedlings. DW is actually

Table 4. The SNP marker loci associated with multiple seedling traits in durum wheat

SNP loci	Trait[a]	Sequence resource/candidate gene
BE443538_5_A_1436	NL, SH, GRNL, GRSH, GNL, GRL, GLA	WHE1115_B01_D01ZS Wheat etiolated seedling root normalized cDNA library
BE590521_6_B_N_331	NL, SH, GRNL, GRSH, GNL, GRL, GLA	WHE0854_D04_H08ZS Wheat 20–45 DAP spike cDNA library
BG314205_1_B_33	FW, NL, SH, GRFW, GRNL, GRSH, GFW, GNL, GRL, SDW, DW, GLA	WHE2460_F01_K02ZS *Triticum monococcum* early reproductive apex cDNA library
BG313722_3_A_281	FW, NR, GRFW, GRNR, GFW, GNR, SDW, DW, GLA	WHE2057_F12_L23ZS Wheat salt-stressed sheath cDNA library
BE426214_6_A_N_191	FW, GRFW, GRNR, GFW, SDW, DW	WHE0329_D02_G03ZS Wheat unstressed seedling shoot cDNA library
BE438226_4_A_N_681	FW, GRFW, GRNR, GFW, SDW, DW	WHE0006.C12R000701 ITEC Wheat Endosperm Library
BE442905_6_B_N_1225	FW, GRFW, GRNR, GFW, SDW, DW	WHE1108_A02_B04ZS Wheat etiolated seedling root normalized cDNA library
BE490763_2_A_1462	FW, GRFW, GNR, SDW, DW, GLA	WHE0368_G02_M04ZS Wheat cold-stressed seedling cDNA library
BE494023_4_A_N_380	FW, GRFW, GRNR, GFW, SDW, DW	WHE1277_A01_B01ZS *Secale cereale* anther cDNA library
BE517711_5_B_49	FW, GRFW, GRNR, GFW, SDW, DW	WHE0802_G04_M08ZS Wheat vernalized crown cDNA library
BE591423_5_B_Y_580	FW, GRFW, GRNR, GFW, SDW, DW	WHE1659-1662_M09_M09ZS Wheat heat stressed flag leaf cDNA library
BF474569_1_A_Y_382	FW, GRFW, GRNR, GFW, SDW, DW	WHE2102_E11_I22ZS Wheat salt-stressed crown cDNA library
BF484496_1_B_N_150	FW, GRFW, GRNR, GFW, SDW, DW	WHE2324_B12_D24ZS Wheat pre-anthesis spike cDNA library
BF484606_1_A_390	FW, GRFW, GRNR, GFW, SDW, DW	WHE2317_E09_I17ZS Wheat pre-anthesis spike cDNA library
BM137384_5_A_444	FW, GRFW, GRNR, GFW, SDW, DW	WHE0463-0466_E07_E07ZS Wheat *Fusarium graminearum* infected spike cDNA library (PDR1 gene)
BQ169448_6_B_252	FW, GRLA, GRFW, LA, GNR, SDW, DW, GLA	WHE1793_G02_N03ZT Wheat pre-anthesis spike cDNA library
BE405834_1_B_Y_216	SH, GRNL, GRL	WHE0437_B05_C09ZS Wheat etiolated seedling root cDNA library
BE606541_6_B_Y_676	NL, SH, GRNL,GRL, SDW, GLA	WHE0903_G11_N21ZS Wheat 5–15 DAP spike cDNA library
BE637476_7_B_N_544	FW, GRFW, GFW, GNR	WHE0859_D12_G23ZS Wheat 20–45 DAP spike cDNA library (LOC542902 gene)
CD453593_6_A_N_238	FW, GRFW, SDW, DW, GLA	WHE0810_H09_O18ZT CS wheat vernalized crown cDNA library
BE443500_4_A_N_610	FW, GRFW, GFW, SDW, DW	WHE1115_E07_J13ZS Wheat etiolated seedling root normalized cDNA library
BE445587_7_A_N_347	GRNR, GNL	WHE1451_D08_G15ZS Wheat etiolated seedling root normalized cDNA library
BE494028_7_A_Y_108	FW, GRFW, SDW, DW	WHE1277_A07_B13ZS *S. cereale* anther cDNA library
BG274119_1_A_Y_221	FW, GRFW, GFW, SDW, DW	WHE2231_G05_N09ZS *Aegilops speltoides* anther cDNA library
BG274687_1_B_Y_287	GRLA, LA, GRL, GLA	WHE2229_G09_M17ZS *A. speltoides* anther cDNA library
BG314551_3_A_Y_33	FW, GRFW, SDW, DW, GLA	WHE2488_D03_G06ZS *T. monococcum* early reproductive apex cDNA library
BE405667_5_B_305	FW, GRFW, SDW, DW	WHE1209_H12_O23ZS Wheat etiolated seedling root cDNA library (LOC100037524 gene)
BE438495_6_B_Y_71	FW, GRFW, SDW, DW	WHE0007.H12R000701 ITEC WHE Wheat Endosperm Library (LOC543474 gene)
BF292596_3_A_439	FW, GRFW, SDW	WHE2215_F05_L09ZS *A. speltoides* anther cDNA library
BF292614_6_B_189	FW, GRFW, SDW, DW	WHE2215_H02_P03ZS *A. speltoides* anther cDNA library
BF485305_1_A_Y_29	FW, GRFW, SDW, DW	WHE1790_H04_P08ZS Wheat pre-anthesis spike cDNA library
BG604857_7_B_N_74	FW, GRFW, SDW, DW	WHE0944_B05_D10ZS Wheat 5–15 DAP spike cDNA library (LOC543429 gene)
BQ159615_6_B_N_189	FW, GRFW, SDW, DW	WHE2216_F01_L02ZT *A. speltoides* anther cDNA library
BE497740_3_B_120	FW, GRFW, SDW, DW	WHE0956_G06_M12ZS Wheat pre-anthesis spike cDNA library
BE500206_2_B_Y_148	FW, GRFW, SDW, DW	WHE0980_D07_G14ZS Wheat pre-anthesis spike cDNA library
BE500714_1_B_Y_237	FW, GRFW, SDW	WHE0991-0994_G23_G23ZS Wheat pre-anthesis spike cDNA library
BF473138_7_A_Y_206	FW, GRFW, SDW	WHE0922_B01_C02ZS Wheat 5–15 DAP spike cDNA library
BF145580_2_A_107	NL, GRNL, GRL	WHE1835_A04_B07ZS *S. cereale* anther cDNA library
BF293181_7_A_Y_332	NL, GNL	WHE2164_E07_I14ZS *T. turgidum* L. var. durum (durum wheat) whole plant cDNA library
BE517858_6_A_198	RDW, DW, GLA	WHE0803_A05_B09ZS Wheat vernalized crown cDNA library
BE591002_7_A_Y_158	SH, GRSH, GSH	WHE1655-1658_N02_N02ZS Wheat heat stressed flag leaf cDNA library
BE490200_6_B_Y_171	NL, GNL	WHE0366_E07_J14ZS Wheat cold-stressed seedling cDNA library
BE493868_7_A_Y_93	NL, GNL	WHE1276_F09_K18ZS *S. cereale* anther cDNA library
BE495277_5_B_336	NL, GNL	WHE1268_H03_O06ZS *S. cereale* anther cDNA library
BE444305_1_B_433	GRRL, GRL	WHE1117_F03_K05ZS Wheat etiolated seedling root normalized cDNA library
BG313767_1_B_107	GRNR, GRL	WHE2058_C03_F06ZS Wheat salt-stressed sheath cDNA library

[a] FW, fresh weight; NL, number of leaf; SH, seedling height; NR, number of main roots; LA, leaf area; GRLA, growth rate of leaf area; GRFW, growth rate of fresh weight; GRNL, growth rate for number of leaf; GRNR, growth rate for number of roots; GRSH, growth rate of plant/seedling height; GRRL, growth rate of root length; GFW, growth gain of fresh weight; GNL, growth gain in number of leaf; GNR, growth gain in number of roots; GSH, growth gain of seedling height; GRL, growth gain of root length; GLA, growth gain of leaf area; RDW, root dry weight; SDW, shoot dry weight; DW, total biomass.

equal to summation of RDW and SDW. In total, 1, 34 and 28 SNP loci were revealed to be significantly associated with RDW SDW and DW, respectively. The only one SNP locus, *BE517858_6_A_198* associated with RDW (R^2 = 13.27%) was also associated with DW (R^2 = 7.99%). There were 27 SNPs common between SDW and DW. The 27 SNP loci were distributed on all of the 14 chromosomes except 4B, with preference to chromosome 1A (4) and 6B (5) (**Supplemental Table 2**).

Discussion

Seedling traits are of great importance for wheat production

In the present study (**Table 2**), we confirmed the significant and positive correlations of three seedling traits, leaf area (LA), growth rate of leaf area (GRLA) and growth gain of leaf area (GLA) with the final yield, grain weight per plant (GWP). The yield is closely related to the yield component trait, 1000-grain weight (KGW). This yield component trait is significantly correlated with most (14) of the 21 seedling traits measured in this study, including the three seedling biomass traits, shoot dry weight (SDW), root dry weight (RDW) and the total seedling dry weight (DW). The final plant height is very an important agronomic trait of wheat, and also closely correlated, positively or negatively, with nine seedling traits (**Table 2**). Truly, it is of great significance for wheat production to nurse healthy and vigorous seedlings and to conduct genetic improvement of seedling traits in durum wheat.

SNP-based associations verify genetic control of wheat seedling traits

ANOVA analysis showed highly significant variations among genotypes and high heritability for wheat seedling traits (**Table 1**). The SNP markers are mainly derived from the mapped wheat ESTs (Ren *et al.* 2013) and thus could represent the functional genes. A total 259 SNP marker loci were detected to be significantly associated with all the seedling traits measured in the four growth stages and on the 34th day after germination (**Table 3**, **Supplemental Tables 1**, **2**). The large number of significant associations between SNP markers and seedling traits verified the obvious genetic control of the traits at an early growth stage as previously reported (Canè *et al.* 2014). These associated SNP marker loci are non-randomly distributed across the whole genome of durum wheat. In general, slightly more associated SNP markers were detected in the genome A (131) than the B (128) genome (**Supplemental Tables 1**, **2**). This is agree, to some extent, from what reported previously by Chao *et al.* (2010), Chen *et al.* (2012) and Peng *et al.* (2011), genome A has more genes controlling important adaptive traits in wheat. Similarly, Akhunov *et al.* (2010) and Chao *et al.* (2010) reported that the chromosome 4B had the lowest haplotype diversity and lowest number of haplotypes per locus.

Seedling height

Seedling height (SH) is one of the most important seedling traits in cereal crops. Börner *et al.* (2002) and Griffiths *et al.* (2012) reported multiple QTLs for final height of wheat plant. Hu *et al.* (2015) found six SNP markers associated with plant height and located on chromosomes 1A, 2A, 4B, 6A and 6B in durum wheat. In this study, we detected 7, 4 and 10 SNP marker loci associated with seedling height (SH), growth rate of SH, and growth gain of SH, respectively, in various growth stages. One SNP locus *BE591002_7_A_Y_158* is common in the three seedling height traits with R^2 > 10%. The following three SNP loci *BG314205_1_B_33*, *BE443538_5_A_1436* and *BE590521_6_B_N_331* are common in two height-related traits, SH and GRSH, (**Supplemental Table 1**). Plant height is controlled by multiple genes (Ahmed *et al.* 2000) and mainly by semi-dwarf genes in wheat (Bai *et al.* 2013). Wheat plant height associated significantly with SSR marker *Xfbb250*-6B and two QTLs for this trait was found in the chromosome region 6BL5-0.40–1.00 (Cadalen *et al.* 1998). Therefore, in this 6B chromosome region, the association of seedling height with SNP loci *BE590521_6_B_N_331* should be reliable with R^2 = 8.11–10.69%.

Root system

Vigor of crop seedlings relies on the strong root system. QTL analysis revealed a relatively limited number of chromosomal regions related with the root traits in wheat. Most of the genome regions conferring root traits were in chromosome 1B, 2A, 5A, and 6A (Bai *et al.* 2013, Petrarulo *et al.* 2009). Canè *et al.* (2014) detected six QTLs for root length and agronomic performance. It is shown in a wheat association mapping that 1B, 2A and 6A are the most important chromosomes harboring QTLs for drought tolerance (Edae *et al.* 2014). In the present study on seedlings of durum wheat (**Table 3**), we detected two and 14 SNPs associated with growth rate of root length (GRRL) and growth gain of root length (GRL), respectively. The SNP marker *BE444305_1_B_433* (**Supplemental Table 1**) was associated with GRRL (R^2 = 10.16%), and also with GRL (R^2 = 8.86%). In contrast with root length traits, there are many more associated SNP loci for root number traits (**Table 3**), 1, 27 and 9 for number of main roots (NR), growth rate of number of main roots (GRNR), and growth gain of number of main roots (GNR), respectively. The SNP marker *BG313722_3_A_281* was closely associated with three root number traits, NR, GRNR and GNR with R^2 = 11.81–17.50%. This single SNP demonstrates great importance in genetic control of root system, and can be used together with other root number associated SNP markers (**Supplemental Table 1**) for the marker-assisted improvement (MAI) of wheat root system.

Leaves

Leaf is the most visible trait showing vigor of crop seedlings. The leaf-related traits, including leaf number and leaf

area were measured in the present study. We detected 9, 10 and 8 SNPs associated with number of leaves (NL), growth rate of leaf number (GRNL), and growth gain of leaf number (GNL), respectively. Among these SNPs, *BG314205_1_B_33*, *BE443538_5_A_1436* and *BE590521_6_B_N_331*, are common in the three leaf number traits with $R^2 > 8.4\%$. These three SNPs are located on chromosomes 5A, 1B and 6B, respectively (**Supplemental Table 1**), also associated with grain weight/plant (GWP), i.e., grain yield (Hu *et al.* 2015), and thus can be used for MAI of leaf number and the yield in wheat.

The significant and positive correlations (**Table 2**) of grain yield/plant (GWP) with leaf area (LA), growth rate of leaf area (GRLA) and growth gain of leaf area (GLA) verify the importance of early seedling growth in yield performance of wheat, as reported by Bai *et al.* (2013). Edae *et al.* (2013) found that the functional drought tolerance candidate genes for enhanced response to abscisic acid, ERA1-A and ERA1-B, were associated with flag leaf width, a parameter for leaf area. For LA, GRLA and GLA, 2, 2 and 14 associated SNPs were detected, respectively, in the present study. The two SNPs, *BQ169448_6_B_252* and *BG274687_1_B_Y_287*, are common in the three traits and have a high $R^2 = 10.61–13.70\%$ (**Supplemental Table 1**). These SNPs may be useful and reliable for MAI of leaf area and therefore the grain yield in wheat.

In a $F_{2–3}$ mapping population derived from wild emmer × durum wheat, Peng *et al.* (2003) discovered that multiple QTL effects of domestication traits clustered in the single chromosome regions. In the present study, 46 SNP marker loci were found to be associated with multiple seedling traits (>2) of durum wheat (**Table 4**). It means that a single SNP or gene locus can affect multiple seedling traits due to the pleiotropy of genes (Peng *et al.* 2003). This information is quite helpful for MAI in wheat.

SNP markers should be helpful for breeding wheat varieties with strong seedlings

Biomass traits of seedlings, i.e., the total biomass (DW), dry weight of root (RDW) and dry weight of shoot (SDW), are the good indication of healthy and vigorous seedlings as shown in **Table 2**. The large numbers and high R^2 (mostly over 10%) of SNP marker loci significantly associated with these two traits, SDW and DW (**Supplemental Table 2**) provides rich genomic resource for marker-assisted genetic improvement of wheat seedlings. Furthermore, the only one RDW-specific SNP locus, *BE517858_6_A_198* may be of great value for genetic analysis and further MAI of wheat root system.

Fresh weight (FW), a very important parameter of seedling biomass, and the related traits, growth rate of fresh weight (GRFW) and growth gain of fresh weight (GFW) can also reflect the seedling vigor to a great extent. We detected 31, 31 and 15 SNP marker loci associated with FW, GRFW and GFW, respectively (**Table 3**). Out of these 31 markers, 15, *BE637476_7_B_N_544*, *BG314205_1_B_33*, *BG313722_3_A_281*, *BE426214_6_A_N_191*, *BE438226_4_A_N_681*, *BE442905_6_B_N_1225*, *BE494023_4_A_N_380*, *BE517711_5_B_49*, *BE591423_5_B_Y_580*, *BF474569_1_A_Y_382*, *BF484496_1_B_N_150*, *BF484606_1_A_390*, *BM137384_5_A_444*, *BE443500_4_A_N_610*, and *BG274119_1_A_Y_221*, have high R^2 (mostly over 10%) and are common among the three traits (**Supplemental Table 1**). The large number and high significance of associated SNP markers provide more genomic resource for MAI of wheat seedlings.

Number of associated SNP markers are variable with the growth process of seedlings

Peng (1987), Peng and Gao (1988), and Peng and Li (1988, 1989) demonstrated that number of gene loci and the genetic effects varied with the growth and development of quantitative traits in maize. In the present study, we found that the number of SNP marker loci significantly associated with seedling traits is also quite variable among the four growth stages, and only small portion of the associated SNP loci could be consistently detected in multiple stages (**Table 3**, **Supplemental Tables 1**, **2**). Since these SNPs can represent the functional genes as stated above, we speculate that genes controlling the seedling traits are actually differentially expressed during the process of growth and development.

Conclusions

As demonstrated in the present study on durum wheat, seedling traits play a very important role in yield establishment, mainly through affecting 1000-grain weight (**Table 2**). Very large number (259) of trait-SNP associations are detected for the measured seedling traits (**Table 3**, **Supplemental Table 2**). Some (46) SNP markers associated with multiple traits, indicating non-neglectable pleiotropy in the seedling stage of durum wheat. These associations are not randomly distributed among the chromosomes and across the genome, with slightly larger number in A (131) than in B (128) genome (**Supplemental Tables 1**, **2**). The results further confirm the robust genetic control and the feasibility of genetic improvement of the seedling traits. The associations are quite variable with the growth process of seedlings (**Table 3**, **Supplemental Table 1**), and thus lay a foundation for developmental genetics analysis in durum wheat. The large number of associated SNP markers provides breeders with rich genomic resource for marker-assisted improvement of the seedling traits. Therefore, this study contributes to understanding the genetics, breeding for vigorous seedlings, and opening an opportunity for further improvement of wheat productivity.

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