



Genetic Architecture of Nitrogen-Deficiency Tolerance in Wheat Seedlings Based on a Nested Association Mapping (NAM) Population

Deqiang Ren^{1†}, Xiaoqian Fang^{1†}, Peng Jiang¹, Guangxu Zhang², Junmei Hu¹, Xiaoqian Wang¹, Qing Meng¹, Weian Cui¹, Shengjie Lan¹, Xin Ma¹, Hongwei Wang^{1*} and Lingrang Kong^{1*}

¹ State Key Laboratory of Crop Biology, Shandong Key Laboratory of Crop Biology, College of Agronomy, Shandong Agricultural University, Tai'an, China, ² Lianyungang Academy of Agricultural Sciences, Lianyungang, China

OPEN ACCESS

Edited by:

Yiwei Jiang,
Purdue University, United States

Reviewed by:

Shuanghe Cao,
Institute of Crop Sciences (CAAS),
China
Xingwang Yu,
North Carolina State University,
United States

*Correspondence:

Hongwei Wang
wanghongwei@sdau.edu.cn
Lingrang Kong
lkong@sdau.edu.cn

[†] These authors have contributed
equally to this work.

Specialty section:

This article was submitted to
Evolutionary and Population Genetics,
a section of the journal
Frontiers in Plant Science

Received: 05 March 2018

Accepted: 31 May 2018

Published: 26 June 2018

Citation:

Ren D, Fang X, Jiang P, Zhang G,
Hu J, Wang X, Meng Q, Cui W, Lan S,
Ma X, Wang H and Kong L (2018)
Genetic Architecture
of Nitrogen-Deficiency Tolerance
in Wheat Seedlings Based on
a Nested Association Mapping (NAM)
Population. *Front. Plant Sci.* 9:845.
doi: 10.3389/fpls.2018.00845

Genetic divergence for nitrogen utilization in germplasms is important in wheat breeding programs, especially for low nitrogen input management. In this study, a nested association mapping (NAM) population, derived from “Yanzhan 1” (a Chinese domesticated cultivar) crossed with “Hussar” (a British domesticated cultivar) and another three semi-wild wheat varieties, namely, “Cayazheda 29” (*Triticum aestivum* ssp. *tibetanum* Shao), “Yunnan” (*T. aestivum* ssp. *yunnanense* King), and “Yutian” (*T. aestivum* *petropavloski* Udats et Migusch), was used to detect quantitative trait loci (QTLs) for nitrogen utilization at the seedling stage. An integrated genetic map was constructed using 2,059 single nucleotide polymorphism (SNP) markers from a 90 K SNP chip, with a total coverage of 2,355.75 cM and an average marker spacing of 1.13 cM. A total of 67 QTLs for RDW (root dry weight), SDW (shoot dry weight), TDW (total dry weight), and RSDW (root to shoot ratio) were identified under normal nitrogen conditions (N⁺) and nitrogen deficient conditions (N⁻). Twenty-three of these QTLs were only detected under N⁻ conditions. Moreover, 23 favorable QTLs were identified in the domesticated cultivar Yanzhan 1, 15 of which were detected under N⁺ conditions, while only four were detected under N⁻ conditions. In contrast, the semi-wild cultivars contributed more favorable N⁻-specific QTLs (eight from Cayazheda 29; nine from Yunnan), which could be further explored for breeding cultivars adapted to nitrogen-deficient conditions. In particular, *QRSDW-5A.1* from YN should be further evaluated using high-resolution mapping.

Keywords: nitrogen utilization, nested association mapping (NAM), semi-wild wheat, quantitative trait loci (QTLs), wheat breeding

INTRODUCTION

Nitrogen (N), an essential plant nutrient, is vital for various aspects of crop growth and development, including seed germination, root architecture regulation, shoot development, flowering, and grain production (Lea and Morot-Gaudry, 2001; Alboresi et al., 2005; Kiba and Krapp, 2016; Yuan et al., 2016). Wheat production mainly depends on fertilizer input, particularly

N fertilizer (Peng et al., 2009). From 2008 to 2015, the total global N consumption increased annually by 3.5%. In 2015, the total global N consumption was 223 million tons, and the average N application to wheat was 71–370 kg/hm² (FAOSTAT, 2015), which is far higher than the safety threshold of 260 kg/hm² (Liu, 2017) in many areas. This excessive N input not only raises the cost of production, but it also causes various soil and environmental issues (Peng et al., 2009). Thus, it is necessary that N use in agriculture is reduced without decreasing grain yields. Wheat varieties are typically developed for maximum production with high N fertilizer input, which results in a decrease in N use efficiency (Dong et al., 2014). Therefore, in order to increase production without further damage to the environment, high-yield crop varieties tolerant of N deficient conditions, or those that can efficiently utilize limited N, are desirable.

A thorough understanding of the mechanisms of N-deficiency tolerance and N-use efficiency in crop plants is required for the development of wheat varieties that are less dependent on N fertilizer. Plants have developed complex adaptive response pathways to cope with N fluctuations (Sandrine et al., 2014). N, as a metabolite, has been well studied. For instance, the nitrate transporter NRT1.1 not only transports NO₃⁻, but also enhances the movement of basipetal auxin out of the roots, leading to the repression of lateral root development (Krouk et al., 2006; Wang et al., 2017). Many other proteins, such as the transcription factors ANR1 (nitrite regulator 1, Gan et al., 2005), SPL9 (squamosa promoter binding protein-like 9, Krouk et al., 2010), and NLP7 (NIN-like protein 7, Marchive et al., 2013), as well as the RING-type ubiquitin ligase NLA (Peng et al., 2007), respond to N metabolites. Transcriptome studies have shown that a wide range of physiological and developmental processes are controlled by N signals (Sandrine et al., 2014). Most N-responsive genes are also regulated by hormone and carbon signaling, indicating that N signaling mechanisms are highly integrated with other regulatory pathways (Sandrine et al., 2014). Despite this thorough understanding of N metabolites, the genetic mechanisms by which wheat tolerates or efficiently uses limited N are largely unclear.

Quantitative trait loci (QTL) mapping is a powerful tool for dissecting and understanding the genetic regulation of complex quantitative traits (Cui et al., 2014). Previous QTL studies have focused on morphological traits and crop yields in plants with low N tolerance or with efficient N uptake in hydroponic culture experiments (An et al., 2006; Laperche et al., 2007) and in field experiments (Quarrie et al., 2005; An et al., 2006; Laperche et al., 2007, 2008; Fontaine et al., 2009; Cui et al., 2014), leading to the identification of important QTLs on chromosomes 2A, 2B, 4A, 5A, 7A, and 7B. For instance, Quarrie et al. (2005) reported that major QTLs for grain yield components (ears per plant, grains per ear, and 1000s grain weight) under nitrogen deficiency condition were mapped on chromosomes 4AS, 7AL, 7BL, and around centromeres of chromosomes 4B and 6A using a spring wheat doubled haploid (DH) population derived from the cross Chinese Spring × SQ1. Laperche et al. (2007) detected 233 QTL for traits measured in each combination of environment and clustered into 82 genome regions, the dwarfing gene (*Rht-B1*), the photoperiod sensitivity gene (*Ppd-D1*) and the awns inhibitor gene (*B1*)

coincided with regions that contained the highest numbers of QTL. Cui et al. (2016) reported that the *Rht-B1* affected not only plant height but also grain quality and its adaptability to N-deficient environments. Several other co-localizations between QTLs related to yield, physiological traits and enzyme activities involved in the control of N assimilation and recycling were detected for nitrate reductase (NR) and glutamate dehydrogenase (GDH) in maize (Hirel et al., 2001; Gallais and Hirel, 2004), glutamine synthetase (GS) in wheat (Habash et al., 2007; Fontaine et al., 2009). It is important to understand the identified specific QTLs associated with the adaptation of the plant to different N supply conditions. QTLs controlling high levels of N uptake and utilization can be detected specifically under high N conditions, and QTLs specifically detected under N limited conditions are involved in N-deficiency tolerance and adaption processes (Gaju et al., 2011). Direct selection for QTLs specifically detected under low N supply would be effective for the genetic improvement of N-deficiency tolerance traits (Dong et al., 2014).

Most studies were conducted on single, bi-parental population, thus the genetic polymorphisms are limited between two parents. Joint-multiple family analyses, such as “NAM,” potentially detect more QTLs, more accurately estimate QTL effects, better resolve QTL positions, and directly assess the distribution of functional allelic variation across multiple families, as compared to QTL analysis by bi-parental population (Yu et al., 2008; McMullen et al., 2009; Li et al., 2013; Ogut et al., 2015; Vatter et al., 2017). NAM population is a joint-multiple family comprising multiple bi-parental mapping families all sharing one common reference parent (Yu et al., 2008). For example, Sophie et al. (2015) developed a sorghum NAM population comprised of 2,400 recombinant inbred lines (RILs) from 10 families with the sorghum hybrid RTx430 as the common parent. The recombination rate of the NAM population was 4 cM/Mb, estimated based on 96,000 SNPs generated with a genotyping-by-sequencing approach, and 57,500 recombination events were observed (Sophie et al., 2015). Using this NAM population, Sophie et al. (2015) detected 41 QTLs and reduced the QTL mapping region to between 63 Kb and 1.9 Mb.

Here, we studied the genetic architecture of N-deficiency tolerance in wheat seedlings using a NAM dataset comprised of four related RIL populations. We constructed an integrative genetic map using high-density SNP markers genotyped with a 90K SNP chip. We aimed to detect QTLs involved in N-deficiency tolerance and to identify favorable alleles.

MATERIALS AND METHODS

Plant Materials

The NAM population that we constructed was comprised of four RIL populations derived from crosses between a single female parent “Yanzhan 1” (YZ) and four different male parents. YZ is a good-quality, high-yield, disease-resistant variety of winter wheat cultivated in Henan Province of the Huanghuai region, China (in 2003). The male parents were “Hussar” (HR, a British dwarf cultivar), and three semi-wild wheat varieties from China: “Chayazheda” (CY, *Triticum aestivum* ssp. *tibetanum* Shao) from

Tibet, “Yunnanxiaomai” (YN, *T. aestivum* ssp. *yunnanense* King) from Yunnan, and Yutiandaomai (YT, *T. aestivum* *petropavloski* Udats et Migusch) from Xinjiang. We crossed YZ with HR, CY, YN, and YT to develop separate RILs using a single seed descent approach. The final population sizes for each cross were 97, 82, 98, and 93, respectively.

Experimental Design

All of the plants were grown in hydroponic culture (following Sun et al., 2013) in a greenhouse at Shandong Agricultural University, Shandong, China. We used Hoagland’s solution (Hoagland and Arnon, 1950) to optimize plant growth (**Supplementary Table S1**). To inhibit any potential nitrification of the nutrient solution, we added 2 mg/L dicyandiamide (a nitrification inhibitor). We tested two levels of N: normal (N^+ ; 5.0 mmol/L N) and low (N^- ; 0.5 mmol/L N). We used a randomized complete block design with three replicates for each treatment. Wheat seeds were sterilized for 10 min in 10% sodium hypochlorite, washed with distilled water, and then germinated in a germination tray. After 7 days, one healthy seedling from each line and each treatment was transferred to a 200-cell bottomless tray. The tray was placed in an opaque plastic tank containing 20 L nutrient solution. The tank was opaque in order to encourage healthy root growth and to restrict the growth of algae. The nutrient solution was renewed every 3 days, and the pH was adjusted to 6.0 every day. We repeated this entire procedure six times in 2017: February 26–March 31 (E1); March 2–April 7 (E2); March 9–April 14 (E3); March 16–April 21 (E4); March 23–April 28 (E5); and March 31–May 5 (E6). Our experiment thus comprised 12 environmental combinations: $E1N^+$, $E1N^-$, $E2N^+$, $E2N^-$, $E3N^+$, $E3N^-$, $E4N^+$, $E4N^-$, $E5N^+$, $E5N^-$, $E6N^+$, and $E6N^-$.

Trait Measurements

All of the plants were harvested after 30 days in the nutrient solution. The roots were cleaned with distilled water, and excess water was blotted with absorbent paper. Plants were then dried for 24 h at 56°C in a drying oven before measuring dry root weight (RDW, in mg) and dry shoot weight (SDW, in mg). The total dry weight (TDW, in mg) was calculated as $RDW + SDW$ and the ratio of dry root weight to dry shoot weight (RSDW) was calculated as RDW/SDW . To estimate the plant response to N deficiency, we calculated a “global” interaction variable ($G \times N$) as $(N_{y^-} - N_{y^+})/N_{y^+}$, where N_{y^-} and N_{y^+} represent the trait values in the N^- and N^+ treatments, respectively.

Genotyping and Genetic Map Construction

Genomic DNA was extracted from the seedling leaves of all five parents and all of the RILs (Doyle and Doyle, 1987). DNA samples were genotyped with an Illumina 90K assay (Wang et al., 2014). All of the SNP markers for each line were converted based on the alleles of the parents: ‘A’ for the common parent YZ, ‘B’ for the other parental lines, ‘H’ for the heterozygous genotype, and ‘-’ for missing information. Individual genetic maps for each RIL population were constructed using Kosambi mapping (Kosambi, 1944) and individual maps were combined with Joinmap v4.0

(Van Ooijen and Jansen, 2013)¹. The integrative map was drawn using MapChart v2.2 (Voorrips, 2002)².

Data Analysis and QTL Mapping

We tested the significance of the phenotypic differences between each pair of parents using Student’s *t*-tests. To estimate the variance across genotypes (G), environment (E), genotype/environment interactions (GEI), and replicates, we used analysis of variance (ANOVA) with generalized linear models (GLMs) in SPSS v20 (Bryman and Cramer, 2012)³. Heritability (h^2) was computed using the estimated variance components $V_G / (V_G + V_{GEI}/s + V_e/sr)$, where V_G , V_{GEI} , and V_e are the variances of G, GEI, and the residuals, respectively; *s* is the number of environments; and *r* is the number of replicates. The best linear unbiased estimates (BLUE) for each line with respect to each trait across all traits were used to analyze pairwise correlations. We mapped QTLs with the ICIM-ADD method (Li et al., 2007) using stepwise regression, and considered all of the marker information simultaneously in Ici-Mapping v4.1 (Li et al., 2007)⁴. We used a walking speed of 1.0 cM for all of the QTL calculations, and a stepwise regression probability (*P*-value inclusion threshold) of 0.001. We considered a QTL to be present if the limit of detection (LOD) was >2.5 in the NAM population, and >2.0 in at least one RIL population.

RESULTS

Phenotypic Variation

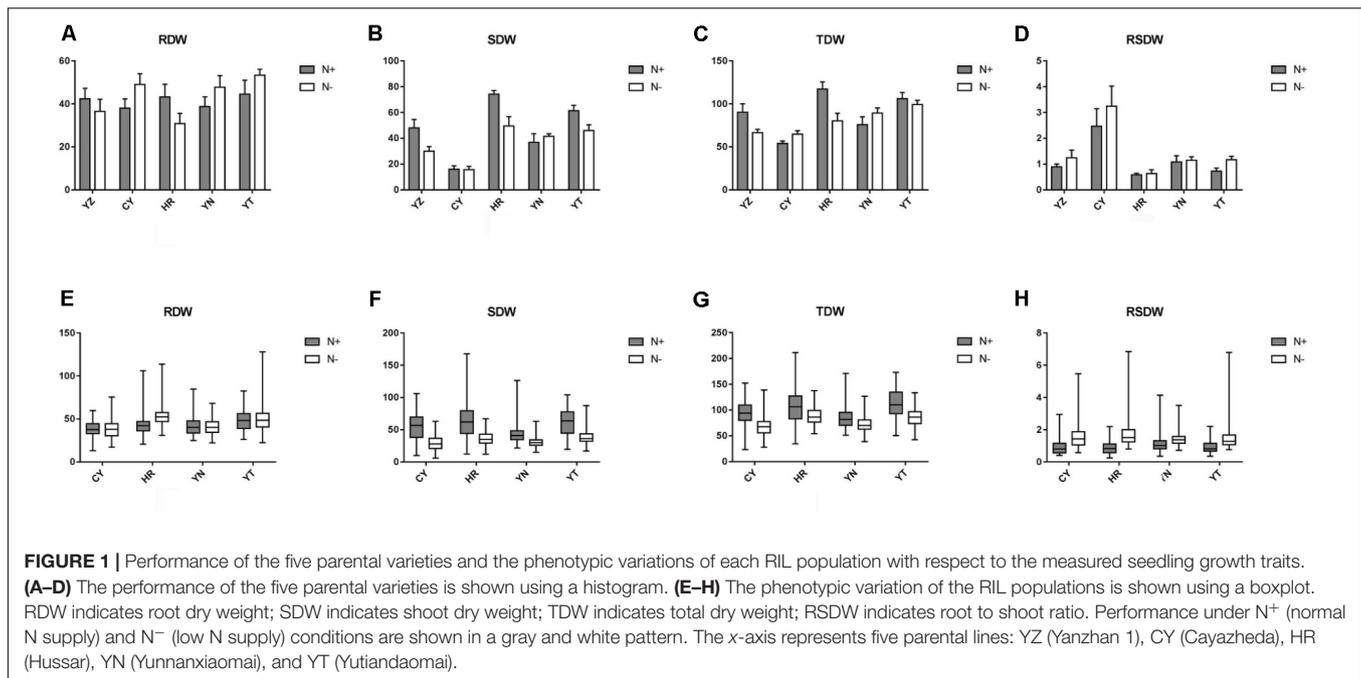
The traits of the five parents differed substantially under both N^+ and N^- conditions across all of the treatments and environments, and exhibited distinctly different responses to N deficiency (**Figures 1A–D** and **Supplementary Table S2**). For instance, under N^- conditions, both the RDW and SDW of the common parent YZ were reduced, but RDW increased in YT and SDW increased in YN, suggesting that these parental species possessed different levels of N-deficiency tolerance (**Figures 1A–D** and **Supplementary Table S2**). Strong transgressive segregation was observed in all of the RIL populations, indicating that favorable alleles were distributed among the parents. Considerable continuous variation was observed in all of the measured traits across all of the populations (**Figures 1E–H** and **Supplementary Tables S2, S3**). For each parent and RIL population, the differences in SDW between the N^+ and N^- conditions were significantly greater than the differences in RDW (one-way ANOVA, $P < 0.001$, **Figure 1**; **Supplementary Table S3**). The estimated h^2 of all of the traits ranged from 18.5% for RSDW to 74.0% for TDW. The h^2 of TDW, RDW, and SDW was high (mostly $> 40\%$) under both N^+ and N^- conditions. The h^2 of RSDW was lower, however, ranging from 18.5 to 47.8%. For each trait, h^2 varied between populations. Our results suggested that all of the measured traits were affected not only by

¹<http://www.kyazma.nl/>

²<http://www.biometris.nl/uk/Software/MapChart/>

³<http://en.wikipedia.org/wiki/SPSS>

⁴<http://www.isbreeding.net/>



genotype, environment, and GEI, but also by genetic background (**Supplementary Table S3**). The phenotypic pairwise correlations between the measured traits were similar under both N⁺ and N⁻. RDW and SDW were positively correlated with each other and with TDW. RSDW was positively correlated with RDW (as expected), but negatively correlated with SDW (**Table 1**).

The Novel Genetic Map

We selected several polymorphic markers distributed across all 21 chromosomes for linkage analysis: 548 for CY, 1,127 for YN, 1,514 for YT, and 1,595 for HR. We mapped 2,059 loci, including 34 linkage groups, to our integrated genetic map (**Figure 2, Table 2, and Supplementary Table S4**), with a total coverage of 2,355.75 cM and an average marker spacing of 1.13 cM. Our integrated map included three genomes: the A genome was 887.67 cM (38.0%), and contained 946 loci (45.89%); the B genome was 955.34 cM (40.90%), and contained 979 loci (47.55%); and the D genome was 492.74 cM (21.1%), and contained 135 loci (6.06%). The chromosome sizes ranged from 0.61 cM (chromosome 4D) to 186.86 cM (chromosome 1B). Chromosome 2B had the most loci (202), while chromosome 3D had the least (4). We obtained good coverage for the A and B genomes, but few polymorphic loci were identified for the D genome. Our integrated linkage map had greater genome coverage, more markers, and lower average maker distance than the individual maps (**Table 2**).

QTL Mapping for Seedling Growth Traits

We detected 67 QTLs affecting seedling growth traits, including 31 QTLs identified only under N⁺ treatment, 22 only under N⁻ treatment, and 14 detected under both (**Table 3, Supplementary Tables S5, S6, and Supplementary Figure S1**). The 67 QTLs were distributed across all 21 chromosomes, except 2D, 3D, 4B, 4D, 5D,

and 7D. The phenotypic variance (PVE) explained by these QTLs ranged from 2.3% (SDW in E5N⁺) to 38.0% (SDW in E3N⁺). The 44 QTLs with PVEs greater than 10% (identified as “primary QTLs”) were mainly concentrated on chromosomes 1B, 2A, 2B, and 3A. Statistics of the favorable QTLs donated by parents are shown in **Figure 3** and **Supplementary Table S4**. Twenty-three favorable QTLs were donated by the domesticated cultivar of YZ, in which 15 were detected only under N⁺ conditions, and four were detected only under N⁻ conditions. The semi-wild cultivars CY and YN contributed eight and nine favorable QTLs detected only under N⁻ conditions.

In multiple environments, we repeatedly detected 18 QTLs for TDW on chromosomes 1A, 2A, 2B, 3A, 3B, 4A, 5A, 5B, 6D, 7A, and 7B. These 18 QTLs included eight identified only under the N⁺ treatment, seven only under the N⁻ treatment, and three under both treatments. Most of the favorable alleles (those that increased the value of a given trait) were donated by parent CY. Four QTLs (*QTDW-2A.3*, *QTDW-3A.1*, *QTDW-7A.1*, and *QTDW-7B.1*) were regarded as primary QTLs, as they explained 10.5–31.7% of the phenotypic variation.

We identified 26 QTLs for RDW on chromosomes 1A, 1B, 1D, 2A, 2B, 3A, 3B, 4A, 6A, 6B, and 7A. Of these 26, 16 were only detected under N⁺ treatment, six were only detected under N⁻ treatment, and four were detected under both N⁺ and N⁻ treatments. Most of these QTLs explained more than 10% of the phenotypic variation. Two QTLs detected in the HR population, *QRDW-2A.2* and *QRDW-7A.2*, accounted for 18.0–34.6% and 23.2–31.9% of the phenotype variance, respectively. Most of the favorable alleles were donated by parent YZ.

The QTLs for SDW were detected on chromosomes 1B, 2A, 2B, 3A, 4A, 6B, and 7B. These QTLs included six under N⁺ treatment only, four under N⁻ treatment only, and four under both treatments. The favorable alleles were mainly donated

TABLE 1 | Pairwise phenotypic correlations among the measured seedling traits.

	RDW-N ⁺	RSDW-N ⁺	SDW-N ⁺	TDW-N ⁺	RDW-N ⁻	RSDW-N ⁻	SDW-N ⁻	TDW-N ⁻
RDW-N ⁺		0.184**	0.296**	0.651**	0.282**	0.038	0.226**	0.309**
RSDW-N ⁺			-0.686**	-0.470**	-0.083	0.250**	-0.329**	-0.243**
SDW-N ⁺				0.918**	0.318**	-0.221**	0.563**	0.528**
TDW-N ⁺					0.366**	-0.156**	0.533**	0.542**
RDW-N ⁻						0.440**	0.376**	0.844**
RSDW-N ⁻							-0.518**	-0.028
SDW-N ⁻								0.810**

**Indicates significance at $P \leq 0.01$.

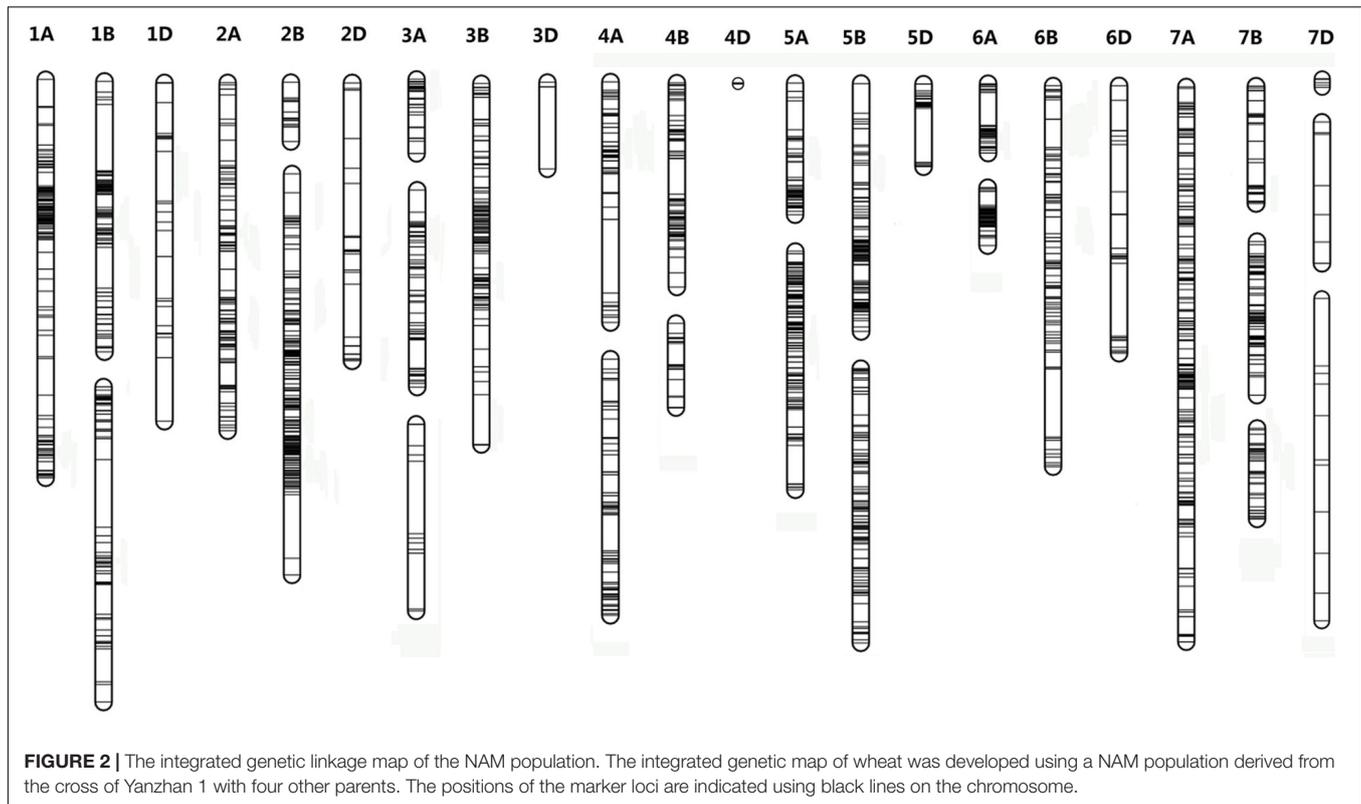


FIGURE 2 | The integrated genetic linkage map of the NAM population. The integrated genetic map of wheat was developed using a NAM population derived from the cross of Yanzhan 1 with four other parents. The positions of the marker loci are indicated using black lines on the chromosome.

by parent YZ. We identified four primary QTLs: *QSDW-2A.1*, *QSDW-2A.3*, *QSDW-2B.1*, and *QSDW-3A.1*.

We identified nine QTLs for RSDW on chromosomes 1A, 1B, 3A, 5A, and 6B. These included two QTLs under N⁺ treatment only, five under N⁻ treatment only, and two under both treatments. Four primary QTLs (*QRSDW-1A.1*, *QRSDW-1B.1*, *QRSDW-6B.1*, *QRSDW-6B.2*) were identified with PVEs ranging from 10.3 to 31.0%.

DISCUSSION

NAM Population and the Novel Integrated Genetic Map

The five parents of the NAM population in this study constitute local adaptable varieties of different origins, and exhibit high phenotypic and genetic diversity. The common parent of YZ was

domesticated with the features of a short lifecycle and high yield (Yao et al., 2010). HR (Squadron/Rendezvous) is a British dwarf cultivar developed by Cambridge Plant Breeders (Cambridge, United Kingdom) and Syngenta (formerly Imperial Chemical Industries) that is resistant to many wheat diseases, but was domesticated with a longer lifecycle (Wilde et al., 2008). The other three semi-wild parents are wheat germplasm resources unique to Western China, and possess many morphological characteristics that differ significantly from common wheat, such as late-flowering, brittle rachis when naturally mature, hard glumes, high protein content, and barren tolerance (Sun et al., 1998; Chen et al., 2007; Zeng et al., 2010; Guo and Han, 2014). The use of this NAM population increased the number of QTLs identified and enhanced the mapping resolution in comparison to the bi-parental population analyses. We constructed an integrated map of the NAM population using 2,059 SNP markers with an average marker spacing distance of 1.13 cM. The novel integrated

TABLE 2 | The novel integrated genetic linkage map of the NAM population.

Chromosome	CY			HR			YN			YT			Integrated map		
	Coverage (cM)	Markers No.	Average spacing (cM)	Coverage (cM)	Markers No.	Average spacing (cM)	Coverage (cM)	Markers No.	Average spacing (cM)	Coverage (cM)	Markers No.	Average spacing (cM)	Coverage (cM)	Markers No.	Average spacing (cM)
1A	65.7	22	2.99	91.77	31	2.96	77.8	29	2.68	103.24	105	0.98	126.41	145	0.87
1B	52.54	25	2.1	64.99	25	2.6	95.52	54	1.77	78.51	46	1.71	186.86	139	1.34
1D	38.93	4	9.73	81.28	18	4.52	12.34	6	2.06	8.81	6	1.47	107.51	24	4.48
2A	87.5	24	3.65	135.49	48	2.82	78.6	32	2.46	70.76	30	2.36	110.61	96	1.15
2B	6.54	5	1.31	92.17	77	1.2	132.27	72	1.84	77.51	84	0.92	147.90	212	0.70
2D	3.51	2	1.75	53.48	11	4.86	11.08	5	2.22	38.67	13	2.97	86.92	27	3.22
3A	30.12	13	2.32	69.6	45	1.55	158.83	51	3.11	19.43	16	1.21	150.92	96	1.57
3B	163.64	39	4.2	76.43	32	2.39	91.67	53	1.73	49.97	6	8.33	115.48	107	1.08
3D	-	-	-	28.73	3	9.58	1.68	3	0.56	-	-	-	30.32	4	7.58
4A	6.73	3	2.24	86.63	38	2.28	98.37	35	2.81	76.52	45	1.7	158.20	107	1.48
4B	0.65	2	0.32	90.37	53	1.71	78.52	35	2.24	19.9	16	1.24	95.38	89	1.07
4D	-	-	-	0.6	5	0.12	0.62	2	0.31	-	-	-	0.61	5	0.12
5A	112.87	39	2.89	108.23	57	1.9	93.64	47	1.99	65.89	80	0.82	121.59	186	0.65
5B	57.73	8	7.22	130.48	48	2.72	136.46	80	1.71	189.31	104	1.82	167.19	212	0.79
5D	4.7	6	0.78	28.41	15	1.89	5.78	6	0.96	1.62	9	0.18	28.58	25	1.14
6A	36.21	10	3.62	9.83	29	0.34	15.68	21	0.75	41.4	68	0.61	45.13	113	0.40
6B	8.51	6	1.42	103.48	28	3.7	99	37	2.68	58.97	27	2.18	121.05	86	1.41
6D	29.8	9	3.31	21.23	5	4.25	44.55	8	5.57	41.3	9	4.59	85.35	28	3.05
7A	61.87	10	6.19	141.9	80	1.77	202.47	68	2.98	128.69	82	1.57	174.82	202	0.87
7B	22.5	15	1.5	118.61	67	1.77	74.79	33	2.27	55.25	47	1.18	121.47	134	0.91
7D	5.81	4	1.45	114.25	15	7.62	47.04	7	6.72	4.58	29	0.16	153.45	22	6.98
Total	795.85	246	3.24	1647.92	730	2.26	1556.74	684	2.28	1130.31	822	1.38	2355.75	2059	1.13

TABLE 3 | Stable additive QTLs for seedling traits in the NAM population.

Chromosome	Position	Right maker	Left maker	QTL detected for N ⁺ , N ⁻ treatments			Comparison with previous studies	
				N ⁺	N ⁻	N ⁺ and N ⁻	QTL	References
1A	115	BobWhite_c12305_959	wspn_Ex_c11939_19147790		QRDW-1A.1	QRDW-1A.1	QTDW-1A.1	Sun et al., 2013
1A	120	BS00062759_51	BS00063847_51		QRDW-1A.1	QRDW-1A.1	QTDW-1, QRDW-1	Guo et al., 2012
1B	37	Tdurum_config20299_368	BobWhite_c48550_198		QRSDW-1B.1 QSDW-1B.1	QRSDW-1B.1 QSDW-1B.1		
1B	42	wspn_Ex_c5098_9047611	BS00063092_51		QRSDW-1B.2	QRSDW-1B.2	SDW-H, NUP-H, RDW-H,	An et al., 2006
1B	44	tplb0048b10_1365	Ku_c28580_432	QRDW-1B.1	QRSDW-1B.3	QRSDW-1B.3		
1B	50	RAC875_c1785_366	BS00082071_51	QSDW-1B.2				
1B	52	wspn_Ex_c1440_2764269	BS00072289_51	QRDW-1B.2				
1B	58	wspn_Ex_c21559_30710510	Kukri_c44191_452			QSDW-1B.3	TN-H cTRL	An et al., 2006 Laperche et al., 2006
1B	1	Kukri_c8390_1102	BS00080212_51			QSDW-1B.4		
1B	55	Ex_c29452_302	BobWhite_c20073_382			QRSDW-1B.4		
1D	26	BS00042197_51	RAC875_c62_1514		QRDW-1D.1		Qsnp-1D RFW, RDW R-GS	Xu et al., 2014 Zhang et al., 2013 Fontaine et al., 2009
2A	31	IAAV7468	Tdurum_config66015_346		QRDW-2A.1		QTKW-2A.1, QKI-2A.1	Cui et al., 2014
2A	48	RAC875_c20700_853	RAC875_c13116_943	QTDW-2A.1		QSDW-2A.1	QTKW-2A.2	Cui et al., 2014
2A	52	Tdurum_config47258_1039	wspn_Ex_c22645_31845564	QRDW-2A.2	QSDW-2A.2 QTDW-2A.2		QKnpS-2A.2	Cui et al., 2014
2A	72	Ra_c26702_797	Excalibur_c96_619	QRDW-2A.3			NS%, GPC	Laperche et al., 2007
2A	75	BobWhite_c16923_64	RAC875_c104160_61			QRDW-2A.4		
2A	76	Tdurum_config60205_806	Excalibur_rep_c66618_87	QSDW-2A.3				
2A	81	Ku_c13700_1196	BS00107804_51	QRDW-2A.5				
2A	84	IAAV2861	wspn_Ex_rep_c70299_69243401	QRDW-2A.6	QTDW-2A.4			Zhang et al., 2013
2A	94	BobWhite_c17783_174	CAPT_c1527_136	QRDW-2A.7				
2A	97	Tdurum_config47508_250	BS00053834_51	QRDW-2A.8				
2B	10	BS00099658_51	Excalibur_c17250_592	QSDW-2B.1			QGY-2B R-GS, A-GS, A-DTH LA, cTDM, tADM	Xu et al., 2014 Fontaine et al., 2009 Laperche et al., 2006

Continued

TABLE 3 | Continued

Chromosome	Position	Right maker	Left maker	QTL detected for N ⁺ , N ⁻ treatments			Comparison with previous studies	
				N ⁺	N ⁻	N ⁺ and N ⁻	QTL	References
2B	40	Ex_c12051_875	BS00041585_51		QSDW-2B.2			
2B	73	RFL_Config4849_702	BS00066545_51	QRDW-2B.1				
2B	77	RFL_Config1987_3440	Tdurum_contig26542_457	QTDW-2B.1				Fontaine et al., 2009
2B	78	Excalibur_rep_c66577_159	RFL_Config4718_1269	QRDW-2B.2				Laperche et al., 2006
2B	80	GENE-4029_80	w SNP_BE490763B_Ta_2_1			QRDW-2B.3	R-GDH, R-NH4	
2B	91	Tdurum_contig28795_322	w SNP_Ex_c45468_51254832		QTDW-2B.2			
2B	93	RAC875_c35778_201	BS00094578_51		QTDW-2B.3			
2B	94	BS00022374_51	BS00009060_51	QRDW-2B.4				
2B	98	Excalibur_c42146_266	BS00026432_51		QTDW-2B.4			
2B	108	BS00060618_51	w SNP_RFL_Config2324_1803878	QRDW-2B.5				
3A	4	Excalibur_c34889_526	Jagger_c6722_104	QRDW-3A.1				
3A	7	RAC875_c787_431	Ku_c17560_91	QRDW-3A.2				
3A	10	BS0006492_51	BS00022129_51		QRSDW-3A.1	QSDW-3A.1 QRDW-3A.3 QTDW-3A.1	TN-H, RDW-H	An et al., 2006
3B	63	w SNP_Ku_c29102_39008953	w SNP_Ex_c64005_62986957	QTDW-3B.1				
3B	115	IAAV8892	BS00071041_51	QRDW-3B.1				Laperche et al., 2007
4A	17	BobWhite_rep_c66057_98	w SNP_Ex_c5690_9994305			QRDW-4A.1		
4A	21	Ra_c7973_1185	IAAV4609					
4A	22	Ku_c11865_406	w SNP_Ex_c31508_40288653	QSDW-4A.1				Xu et al., 2014
5A	24	BS00047242_51	Excalibur_rep_c95828_165			QTDW-5A.1	QRFW/L.3B	Zhang et al., 2013
5A	30	w SNP_Ex_c18941_27840714	Tdurum_contig10601_289					An et al., 2006
5B	30	RAC875_c29907_115	BS00015136_51	QTDW-5B.1		QRSDW-5A.1	NUP-HN1	An et al., 2006
6B	10	w SNP_Ku_c24391_34351602	Kukri_c23491_274	QSDW-6B.1				
6B	19	CAPT1_c7959_386	w SNP_Ex_c7191_12352173	QRSDW-6B.1		QRDW-6B.1		An et al., 2006
6B	48	w SNP_Ra_c20409_29673950	BS00033629_51	QRSDW-6B.2				
6D	85	BS00022787_51	IACX5958	QTDW-6D.1				
7A	7	Tdurum_contig5646_929	Excalibur_c20311_240		QRDW-7A.1		NS%-7A1	Laperche et al., 2007
							SFW	Zhang et al., 2013
							QTKw-7A.2	Cui et al., 2014

Continued

TABLE 3 | Continued

Chromosome	Position	Right maker	Left maker	QTL detected for N ⁺ , N ⁻ treatments			Comparison with previous studies	
				N ⁺	N ⁻	N ⁺ and N ⁻	QTL	References
7A	90	RFL_Contig5285_365	BS00108184_51			QTDW-7A.1	QSLFW.WY.7A	Zhang et al., 2013
7A	105	Excalbur_c47990_159	w SNP_KU_c10202_16937059	QRDW-7A.2		QTDW-7A.2	NUP-H	An et al., 2006
7A	113	CAP7_c7296_88	BobWhite_c5125_258				NUR	Laperche et al., 2006
7B	0	w SNP_Ex_c7934_13467460	Tdurum_contig55961_526			QSDW-7B.2QTDW-7B.1	NUP	Laperche et al., 2006
7B	35	BobWhite_c10448_80	Jagger_c9314_100	QSDW-7B.1			NS%-7B1	Laperche et al., 2007
							cLRN_PRL	Laperche et al., 2006

^aQTLs marked with a bold typeface represent primary QTLs.

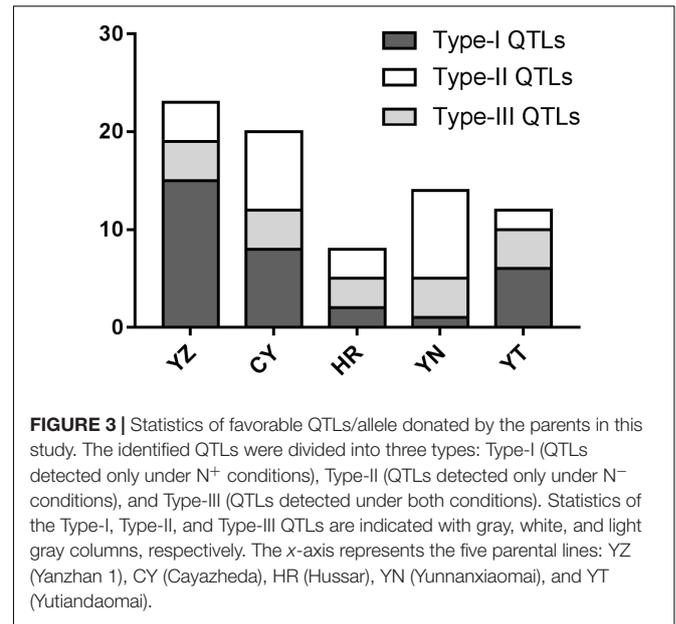


FIGURE 3 | Statistics of favorable QTLs/allele donated by the parents in this study. The identified QTLs were divided into three types: Type-I (QTLs detected only under N⁺ conditions), Type-II (QTLs detected only under N⁻ conditions), and Type-III (QTLs detected under both conditions). Statistics of the Type-I, Type-II, and Type-III QTLs are indicated with gray, white, and light gray columns, respectively. The x-axis represents the five parental lines: YZ (Yanzhan 1), CY (Cayazheda), HR (Hussar), YN (Yunnanxiaomai), and YT (Yutiandaomai).

genetic map shows good genome coverage, high density, and good collinearity with physical maps, and is thus more suitable for genetic research than the four individual genetic maps.

Mapping of QTLs Involved in N Deficiency Tolerance

N uptake and utilization at the seedling stage are important for accumulating a N reservoir, which then fulfills the N requirements during plant growth until the maturity stage (Lian et al., 2005). Genotype selection based on comprehensive performance under N⁺ and N⁻ conditions would be valuable for evaluating N deficiency tolerance (Fontaine et al., 2009; Wang et al., 2017). In this study, we tested two different N supply levels, namely, N⁺ (normal nitrogen supplement) and N⁻ (low nitrogen supplement), under hydroponic culture conditions. The identified QTLs could be divided into three types: Type-I (QTLs detected only under N⁺ conditions), Type-II (QTLs detected only under N⁻ conditions), and Type-III (QTLs detected under both conditions). We identified 14 Type-III QTLs that were indispensable for constitutive processes, with polymorphisms existing between their parents (Laperche et al., 2007). Thirty-one Type-I QTLs identified in our study were assumed to be associated with high levels of N uptake or utilization. Fifteen favorable alleles of Type-I QTLs were donated by parent YZ. *QRDW-2A.2* was mapped to the chromosomal region associated with NS% (straw nitrogen content) and GPC (grain protein content) reported by Laperche et al. (2007). *QSDW-6B.1* and *QRDW-7A.2* have been reported to affect NUP (root N content; An et al., 2006). We detected 23 Type-II QTLs involved in N-deficiency tolerance. These favorable alleles were mainly donated by parents CY and YN. Type-II QTLs, namely *QRSDW-1B.2*, *QRDW-1D.1*, *QRDW-2A.1*, *QSDW-2A.2*, *QTDW-2A.2*, *QRSDW-5A.1*, *QSDW-7B.2*, and *TDW-7B.1*, have been reported to influence N deficiency tolerance and related

traits in previous studies (An et al., 2006; Laperche et al., 2006, 2007; Habash et al., 2007; Fontaine et al., 2009; Guo et al., 2012; Sun et al., 2013; Zhang et al., 2013; Cui et al., 2014; Xu et al., 2014). The remaining common QTLs/genome regions are listed in **Table 3**. The coincidence of the QTLs across different genetic backgrounds not only implies the reliability of the QTLs detected in this study, but also highlights the importance of the chromosomal region.

The “global” interaction variable has previously been used to characterize plant responses to stress (Yan et al., 1999; Yadav et al., 2003; Lian et al., 2005; Laperche et al., 2006, 2007). In this study, we compared two QTL sets detected under the two N levels, from which 23 Type-II QTLs were discovered to be involved in N deficiency tolerance. To further distinguish the QTLs specifically involved in the adaptation of wheat to N deficiency, the “global” interaction variable of $(N_y^- - N_y^+)/N_y^+$ was alternatively used for QTL detection. We hypothesized that the QTLs identified both by the “global” interaction variable and by the N^- treatments constituted high confidence QTLs involved in N deficiency tolerance. Four QTLs (*QRSDW-1B.2*, *QRDW-2A.1*, *QRSDW-5A.1*, and *QRDW-7A.1*) were identified that met these criteria, and have previously been reported to influence several traits (**Table 3**), including SDW, NUP (root N content), RDW (An et al., 2006), NUP, and NS% (Laperche et al., 2007), R-GS (glutamine synthetase activity, Fontaine et al., 2009), and TKW (thousand kernel weight, Cui et al., 2014).

Implications for Breeding

The size and topology of the root system determines the N uptake ability of the plant (Lea and Morot-Gaudry, 2001). When N is limited or deficient, wheat responds by increasing root growth and proliferation at the expense of the shoots, leading to high root/shoot ratios (Ericsson, 1995; Ameziane et al., 1997). As N uptake during the vegetative stage plays an important role in plant growth even into maturity, breeders select wheat genotypes that perform well under both N^+ and N^- conditions (Fontaine et al., 2009). In the NAM population, the phenotypic variation of the parents resulted in a rich allelic variation in response to N fluctuation. RDW, SDW, and TDW were high in the parents YZ, HR, and YT under N^+ conditions, but these traits decreased substantially under N^- conditions. In comparison, RDW, SDW, and TDW were lower in parents CY and YN under N^+ conditions, but increased under N^- conditions. Many RILs had greater RDW, SDW, and TDW under N^- conditions than under N^+ conditions; for instance, RDW, SDW, and TDW were greater in many RILs under N^- conditions in comparison to their parents. This can be explained as the pyramiding of favorable alleles from both parents, which is valuable for the breeding of wheat varieties tolerant of low N levels.

To develop wheat varieties adapted to limited or deficient N conditions, direct selection for favorable QTLs specifically detected under N^- condition is effective. We identified eight primary QTLs (*QRDW-1A.1*, *QTDW-1A.1*, *QSDW-1B.1*, *QRDW-1D.1*, *QTDW-2B.2*, *QTDW-2B.3*, *QTDW-2B.4*, and *QRDW-6A.1*), all of which are probably involved in N-deficiency tolerance. These QTLs are of value in wheat breeding programs designed to increase N deficiency tolerance. Moreover, N uptake

or utilization traits have been considered as indirect selection criteria for the improvement of N-deficiency tolerance (Lian et al., 2005; Fontaine et al., 2009; Wang et al., 2017). In this study, we also identified 25 primary QTLs implicated in N uptake and utilization, and 11 primary QTLs associated with constitutive process (**Table 3**). For instance, *QTDW-3A.1* showed multiple effects on biomass, grain number and yield in the mature periods; *QTDW-5A.1* was also mapped to chromosomal region affecting thousand kernel weight in the mature periods (**Supplementary Table S7**). The QTLs also could be used in breeding programs by pyramiding the different types of QTLs or by using pleiotropic QTLs through MAS. Thus, the mapped QTL interval markers could be used in MAS after being converted into high-throughput KASP (Kompetitive Allele Specific PCR) markers.

In this study, 23 favorable QTLs were donated by the domesticated cultivar of Yanzhan 1, in which 15 were Type-I (detected only in N^+ conditions) and only four were Type-II (detected only in N^- conditions). In contrast, the semi-wild cultivars contributed more favorable Type-II QTLs, including eight favorable QTLs from CY and nine from YN. Seven Type-II favorable QTLs (*QRSDW-1B.1*, *QSDW-1B.1*, *QRSDW-1B.3*, *QTDW-2B.2*, *QTDW-2B.3*, *QTDW-2B.4*, and *QRDW-6B.1*), donated by CY and YN, are novel QTLs that have not been reported in previous studies. The modern variety (YZ) possessed more favorable QTLs/genes for N uptake and utilization under N^+ conditions, while the semi-wild wheat varieties were more likely to have favorable QTLs/genes for N-deficiency tolerance (**Figure 3** and **Supplementary Table S8**). This indicates that a domesticated selection might have occurred in the breeding process. Modern domesticated varieties are supplied with adequate N during yield experiments, and therefore lines that use more N to increase yield are more likely to be selected for cultivation. The semi-wild wheat varieties were from wilderness areas with limited N, and are thus subject to strong evolutionary pressure to maintain N-deficiency tolerance. Semi-wild wheat varieties are therefore an important genetic resource that can be used to improve the N-deficiency tolerance of modern varieties.

The “global” interaction variable identified *QRSDW-1B.2*, *QRSDW-5A.1*, and *QRDW-7A.1* as high confidence QTLs involved in N stress adaptation, with favorable alleles donated by the semi-wild wheat YN. *QRSDW-5A.1* with positive alleles increased the RSDW value from 18.9% to 22.7%, indicating tremendous potential for its application in wheat breeding programs designed to increase N-deficiency tolerance. We predicted that the candidate genes for *QRSDW-5A.1* might be within the 0.7 cM confidence interval of *w SNP_Ex_c18941_27840714-Tdurum_contig10601_289*. Based on our integrated genetic map, which had high density and good collinearity with the physical map, we further compared the overlapping intervals of the collocated QTL peaks with the IWGSC RefSeq Annotations database v 1.0.⁵ The confidence intervals of *w SNP_Ex_c18941_27840714-Tdurum_contig10601_289* spanned 0.8 Mb (5A: 547647367–548503773). This region harbors 12

⁵<https://wheat-urgi.versailles.inra.fr/Seq-Repository/Annotations>

annotated genes in wheat (**Supplementary Table S9**), most notably an auxin responsive gene (*ARF*) cluster (including eight genes), which might be a candidate for *QRSDW-5A.1*. This information provides a reference for the future high-resolution mapping and map-based cloning of *QRSDW-5A.1*.

CONCLUSION

A NAM population comprised of four RIL populations was used for QTL mapping. An integrated genetic map of wheat, with high density and good collinearity with the physical maps, was developed. The NAM population was highly variable for all of the measured traits. Many RILs tolerant of N deficiency exhibited high RDW, SDW, and TDW under the N⁻ treatment. We detected 31 QTLs under N⁺ conditions that are possibly involved in N uptake or utilization, with favorable alleles mainly donated by the modern parent YZ. We detected 23 QTLs under N⁻ conditions, possibly associated with N-deficiency tolerance, with most of the favorable being alleles donated by the semi-wild parents CY and YN. Four QTLs detected under N⁻ conditions were identified as high confidence QTLs involved in N-deficiency tolerance. A domesticated selection might have occurred during the breeding process. Semi-wild wheat varieties constitute an important genetic resource that can be used to improve the N-deficiency tolerance of modern varieties.

AUTHOR CONTRIBUTIONS

DR and XF designed the experiments. GZ and JH created the mapping population. DR, QM, WC, and SL carried out phenotypic experiments. DR analyzed experimental results. XF and PJ analyzed Illumina 90K assay sequencing data. XM assisted

with Illumina sequencing. DR, XW, HW, and LK wrote the manuscript.

FUNDING

This work was supported by the 973 Program of China (2014CB138100) and National Natural Science Foundation of China (31171553, 31520103911 and 31471488).

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fpls.2018.00845/full#supplementary-material>

FIGURE S1 | Overlap of QTLs detected for all of the traits under N⁺ and N⁻ treatment. **(A–E)** Statistical overlap of the QTLs detected for all of the traits under N⁺ and N⁻ conditions are shown by Venn diagrams. The gray circle indicates the QTLs detected only under N⁺ conditions; the white circle indicates QTLs detected only under N⁻ conditions; and the light gray circle indicates the QTLs detected for both conditions.

TABLE S1 | Nutrient solution ingredients for wheat seedling growth.

TABLE S2 | Phenotype distribution of the parents and individual population.

TABLE S3 | ANOVA analysis.

TABLE S4 | The integrative genetic map.

TABLE S5 | QTL detected for N⁺, N⁻, and G × N.

TABLE S6 | Distribution of QTLs detected for growth traits in our study.

TABLE S7 | QTL detected for yield traits in mature periods.

TABLE S8 | Favorable QTLs/alleles donated by parents in this study.

TABLE S9 | Candidate genes.

REFERENCES

- Alboresi, A., Gestin, C., Leydecker, M. T., Bedu, M., Meyer, C., and Truong, H. N. (2005). Nitrate, a signal relieving seed dormancy in *Arabidopsis*. *Plant Cell Environ.* 28, 500–512. doi: 10.1111/j.1365-3040.2005.01292.x
- Ameziane, R., Deleens, E., Noctor, G., Morot-Gaudry, J. F., and Limami, M. A. (1997). Stage of development is an important determinant in the effect of nitrate on photoassimilate (l3C) partitioning in chicory (*Cichorium intybus*). *J. Exp. Bot.* 48, 25–33. doi: 10.1093/jxb/48.1.25
- An, D. G., Su, J. Y., Liu, Q. Y., Zhu, Y. G., Tong, Y. P., Li, J. M., et al. (2006). Mapping QTLs for nitrogen uptake in relation to the early growth of wheat (*Triticum aestivum* L.). *Plant Soil* 284, 73–84. doi: 10.1007/s11104-006-0030-3
- Bryman, A., and Cramer, D. (2012). Quantitative data analysis with IBM SPSS 17, 18 and 19: a guide for social scientists. *Int. Stat. Rev.* 80, 334–335. doi: 10.1111/j.1751-5823.2012.00187_14.x
- Chen, F., Yu, Y. X., Xia, X. C., and He, Z. H. (2007). Prevalence of a novel puroindoline b allele in Yunnan endemic wheats (*Triticum aestivum* ssp. *yunnanense* King). *Euphytica* 156, 39–46. doi: 10.1007/s10681-006-9347-5
- Cui, F., Fan, X. L., Chen, M., Zhang, N., Zhao, C. H., Zhang, W., et al. (2016). QTL detection for wheat kernel size and quality and the responses of these traits to low nitrogen stress. *Theor. Appl. Genet.* 129, 469–484. doi: 10.1007/s00122-015-2641-7
- Cui, F., Zhao, C. H., Ding, A. M., Li, J., Wang, L., Li, X. F., et al. (2014). Construction of an integrative linkage map and QTL mapping of grain yield-related traits using three related wheat RIL populations. *Theor. Appl. Genet.* 127, 659–675. doi: 10.1007/s00122-013-2249-8
- Dong, W., Cui, K. H., Ye, G. Y., Pan, J. F., Xiang, J., Huang, J. L., et al. (2014). QTL mapping for nitrogen use efficiency and nitrogen deficiency tolerance traits in rice. *Plant Soil* 359, 281–295.
- Doyle, J. J., and Doyle, J. L. (1987). A rapid DNA isolation procedure for small quantities of fresh leaf tissue. *Phytochem. Bull.* 19, 11–15.
- Ericsson, T. (1995). Growth and shoot : root ratio of seedlings in relation to nutrient availability. *Plant Soil* 168, 205–214. doi: 10.1007/BF00029330
- FAOSTAT (2015). *FAO Statistical Databases. Food and Agriculture Organization (FAO) of the United Nations*. Rome: FAOSTAT.
- Fontaine, J. X., Ravel, C., Pageau, K., Heumez, E., Dubois, F., Hirel, B., et al. (2009). A quantitative genetic study for elucidating the contribution of glutamine synthetase, glutamate dehydrogenase and other nitrogen-related physiological traits to the agronomic performance of common wheat. *Theor. Appl. Genet.* 119, 645–662. doi: 10.1007/s00122-009-1076-4
- Gaju, O., Allard, V., Martre, P., Snape, J. W., Heumez, E., LeGouis, J., et al. (2011). Identification of traits to improve the nitrogen-use efficiency of wheat genotypes. *Field Crop Res.* 123, 139–152. doi: 10.1016/j.fcr.2011.05.010
- Gallais, A., and Hirel, B. (2004). An approach to the genetics of nitrogen use efficiency in maize. *J. Exp. Bot.* 55, 295–306. doi: 10.1093/jxb/erh006

- Gan, Y., Filleur, S., Rahman, A., Gotensparre, S., and Forde, B. G. (2005). Nutritional regulation of *ANR1* and other root-expressed MADS-box genes in *Arabidopsis thaliana*. *Planta* 222, 730–742. doi: 10.1007/s00425-005-0020-3
- Guo, X., and Han, F. P. (2014). Asymmetric epigenetic modification and elimination of rDNA sequences by polyploidization in Wheat. *Plant Cell* 26, 4311–4327. doi: 10.1105/tpc.114.129841
- Guo, Y., Kong, F. M., Xu, Y. F., Zhao, Y., Liang, X., Wang, Y. Y., et al. (2012). QTL mapping for seedling traits in wheat grown under varying concentrations of N, P and K nutrients. *Theor. Appl. Genet.* 124, 851–865. doi: 10.1007/s00122-011-1749-7
- Habash, D. Z., Bernard, S., Schondelmaier, J., Weyen, J., and Quarrie, S. A. (2007). The genetics of nitrogen use in hexaploid wheat: N utilisation, development and yield. *Theor. Appl. Genet.* 114, 403–419. doi: 10.1007/s00122-006-0429-5
- Hirel, B., Bertin, P., Quillere, I., Bourdoncle, W., Attagnant, C., Delley, C., et al. (2001). Towards a better understanding of the genetic and physiological basis for nitrogen use efficiency in maize. *Plant Physiol.* 125, 1258–1270. doi: 10.1104/pp.125.3.1258
- Hoagland, D. R., and Arnon, D. I. (1950). The water culture method for growing plants without soil. *Calif. Agric. Exp. Stn. Circ.* 347, 357–359.
- Kiba, T., and Krapp, A. (2016). Plant nitrogen acquisition under low availability: regulation of uptake and root architecture. *Plant Cell Physiol.* 57, 707–714. doi: 10.1093/pcp/pcw052
- Kosambi, D. D. (1944). The estimation of map distances from recombination values. *Ann. Eugen.* 12, 172–175. doi: 10.1111/j.1469-1809.1943.tb02321.x
- Krouk, G., Lacombe, B., and Bielach, A. (2010). Nitrate-regulated auxin transport by NRT1.1 defines a mechanism for nutrient sensing in plants. *Dev. Cell* 18, 927–937. doi: 10.1016/j.devcel.2010.05.008
- Krouk, G., Tillard, P., and Gojon, A. (2006). Regulation of the high-affinity NO₃-uptake system by NRT1.1-mediated NO₃-demand signaling in Arabidopsis. *Plant Physiol.* 142, 1075–1086. doi: 10.1104/pp.106.087510
- Laperche, A., Brancourt, H. M., Heumez, E., Gardet, O., Hanocq, E., Devienne, B. F., et al. (2007). Using genotype × nitrogen interaction variables to evaluate the QTL involved in wheat tolerance to nitrogen constraints. *Theor. Appl. Genet.* 115, 399–415. doi: 10.1007/s00122-007-0575-4
- Laperche, A., Devienne, B. F., Maury, O., Le, G. J., and Ney, B. (2006). A simplified conceptual model of carbon/nitrogen functioning for QTL analysis of winter wheat adaptation to nitrogen deficiency. *Theor. Appl. Genet.* 113, 1131–1146. doi: 10.1007/s00122-006-0373-4
- Laperche, A., Le, G. J., Hanocq, E., and Brancourt, H. M. (2008). Modelling nitrogen stress with probe genotypes to assess genetic parameters and genetic determinism of winter wheat tolerance to nitrogen constraint. *Euphytica* 161, 259–271. doi: 10.1007/s10681-007-9433-3
- Lea, P. J., and Morot-Gaudry, J. F. (2001). *Plant nitrogen*, ed. J. Lea (Berlin: Springer Verlag), 362–374. doi: 10.1007/978-3-662-04064-5
- Li, C., Li, Y., Sun, B., Peng, B., Liu, C., and Liu, Z. (2013). Quantitative trait loci mapping for yield components and kernel-related traits in multiple connected RIL populations in maize. *Euphytica* 193, 303–316. doi: 10.1007/s10681-013-0901-7
- Li, S. S., Jia, J. Z., Wei, X. Y., Zhang, X. C., Li, L. Z., Chen, H. M., et al. (2007). A intervarietal genetic map and QTL analysis for yield traits in wheat. *Mol. Breed.* 20, 167–178. doi: 10.1007/s11032-007-9080-3
- Lian, X., Xing, Y., Yan, H., Xu, C., Li, X., and Zhang, Q. (2005). QTLs for low nitrogen tolerance at seedling stage identified using a recombinant inbred line population derived from an elite rice hybrid. *Theor. Appl. Genet.* 112, 85–96. doi: 10.1007/s00122-005-0108-y
- Liu, Q. P. (2017). Spatio-temporal changes of fertilization intensity and environmental safety threshold in China. *Trans. Chin. Soc. Agric. Eng.* 33, 214–221.
- Marchive, C., Roudier, F., Castaings, L., Brehaut, V., Blondet, E., Colot, V., et al. (2013). Nuclear retention of the transcription factor *NLP7* orchestrates the early response to nitrate in plants. *Nat. Commun.* 4:1713. doi: 10.1038/ncomms2650
- McMullen, M. D., Kresovich, S., Villeda, H. S., Bradbury, P., Li, H., and Sun, Q. (2009). Genetic properties of the maize nested association mapping population. *Science* 325, 737–740. doi: 10.1126/science.1174320
- Ogut, F., Bian, Y., Bradbury, P. J., and Holland, J. B. (2015). Joint-multiple family linkage analysis predicts within-family variation better than single-family analysis of the maize nested association mapping population. *Heredity* 114, 552–563. doi: 10.1038/hdy.2014.123
- Peng, M., Hannam, C., Gu, H., Bi, Y. M., and Rothstein, S. J. (2007). A mutation in *NLA*, which encodes a RING-type ubiquitin ligase, disrupts the adaptability of Arabidopsis to nitrogen limitation. *Plant J.* 50, 320–337. doi: 10.1111/j.1365-313X.2007.03050.x
- Peng, S. B., Tang, Q. Y., and Zou, Y. B. (2009). Current status and challenges of rice production in China. *Plant Prod. Sci.* 12, 3–8. doi: 10.1626/pp.s.12.3
- Quarrie, S. A., Steed, A., Calestani, C., Semikhodskii, A., Lebreton, C., Chinoy, C., et al. (2005). A high-density genetic map of hexaploid wheat *Triticum aestivum* L. from the cross Chinese Spring 9 × SQ1 and its use to compare QTLs for grain yield across a range of environments. *Theor. Appl. Genet.* 110, 865–880. doi: 10.1007/s00122-004-1902-7
- Sandrine, R., Alain, G., and Laurence, L. (2014). Signal interactions in the regulation of root nitrate uptake. *J. Exp. Bot.* 65, 5509–5517. doi: 10.1093/jxb/eru321
- Sophie, B., Marcus, O., Sandeep, M., Brian, W., Ram, P., Tesfaye, T., et al. (2015). Power and resolution of QTL mapping in sorghum using a nested association mapping population and diversity panels. *Genet* 206, 573–585.
- Sun, J. J., Guo, Y., Zhang, G. Z., Gao, M. G., Zhang, G. H., Kong, F. M., et al. (2013). QTL mapping for seedling traits under different nitrogen forms in wheat. *Euphytica* 191, 317–331. doi: 10.1007/s10681-012-0834-6
- Sun, Q. X., Ni, Z. F., Liu, Z. Y., Gao, J. W., and Huang, T. C. (1998). Genetic relationships and diversity among Tibetan wheat, common wheat and European spelt wheat revealed by RAPD markers. *Euphytica* 99, 205–211. doi: 10.1023/A:1018316129246
- Van Ooijen, J. W., and Jansen, J. (2013). Genetic mapping in experimental populations. *Camb. Univ. Press* 49, 701–707. doi: 10.1017/CBO9781139003889
- Vatter, T., Maurer, A., Kopahnke, D., Perovic, D., Ordon, F., and Pillen, K. (2017). A nested association mapping population identifies multiple small effect QTL conferring resistance against net blotch (*Pyrenophora teres f. teres*) in wild barley. *PLoS One* 12:0186803. doi: 10.1371/journal.pone.0186803
- Voorrips, R. E. (2002). MapChart: software for the graphical presentation of linkage maps and QTLs. *J. Hered.* 93, 77–78. doi: 10.1093/jhered/93.1.77
- Wang, S. H., Debbie, W. G., Kerrie, F., Alexandra, A., Shaoman, C., Bevan, E. H., et al. (2014). Characterization of polyploid wheat genomic diversity using a high-density 90000 single nucleotide polymorphism array. *Plant Biotechnol.* 12, 787–796. doi: 10.1111/pbi.12183
- Wang, T., Li, C. X., Wu, Z. H., Jia, Y. C., Wang, H., Sun, S. Y., et al. (2017). Abscisic acid regulates auxin homeostasis in rice root tips to promote root hair elongation. *Front. Plant Sci.* 8:1121. doi: 10.3389/fpls.2017.01121
- Wilde, F., Schön, C. C., Korzun, V., Ebmeyer, E., Schmolke, M., Hartl, L., et al. (2008). Marker-based introduction of three quantitative-trait loci conferring resistance to Fusarium head blight into an independent elite winter wheat breeding population. *Theor. Appl. Genet.* 117, 29–35. doi: 10.1007/s00122-008-0749-8
- Xu, Y. F., Wang, R. F., Tong, Y. P., Zhao, H. T., Xie, Q. G., Liu, D. G., et al. (2014). Mapping QTLs for yield and nitrogen related traits in wheat: influence of nitrogen and phosphorus fertilization on QTL expression. *Theor. Appl. Genet.* 127, 59–72. doi: 10.1007/s00122-013-2201-y
- Yadav, R., Bidinger, R. F., Hash, C., Yadav, Y., Yadav, O., Bhatnagar, S., et al. (2003). Mapping and characterisation of QTL × E interactions for traits determining grain and stover yield in pearl millet. *Theor. Appl. Genet.* 106, 512–520. doi: 10.1007/s00122-002-1081-3
- Yan, J., Zhu, J., He, C., Benmoussa, M., and Wu, P. (1999). Molecular marker-assisted dissection of genotype × environment interaction for plant type traits in rice (*Oryza sativa* L.). *Crop Sci.* 39, 538–544. doi: 10.2135/cropsci1999.0011183X003900020039x
- Yao, Q., Zhou, R. H., Pan, Y. M., Fu, T. H., and Jia, J. Z. (2010). Construction of genetic linkage map and QTL analysis of agronomic important traits based on a RIL population derived from common wheat variety Yanzhan 1 and Zaosui 30. *Sci. Agric. Sin.* 43, 4130–4139.

- Yu, J., Holland, J. B., McMullen, M. D., and Buckler, E. S. (2008). Genetic design and statistical power of nested association mapping in maize. *Genet* 178, 539–551. doi: 10.1534/genetics.107.074245
- Yuan, S., Zhang, Z. W., Zheng, C., Zhao, Z. Y., Wang, Y., Feng, L. Y., et al. (2016). Arabidopsis cryptochrome 1 functions in nitrogen regulation of flowering. *Proc. Natl. Acad. Sci. U.S.A.* 113, 7661–7666. doi: 10.1073/pnas.1602004113
- Zeng, X. Q., Wang, Y. G., Li, W. Y., Wang, C. Y., Liu, X. L., and Ji, W. Q. (2010). Comparison of the genetic diversity between *Triticum aestivum* ssp. tibetanum Shao and Tibetan wheat landraces (*Triticum aestivum* L.) by using intron-splice junction primers. *Genet. Resour. Crop Evol.* 57, 1141–1150. doi: 10.1007/s10722-010-9553-9
- Zhang, H., Cui, F., Wang, L., Li, J., Ding, A. M., Zhao, C. H., et al. (2013). Conditional and unconditional QTL mapping of drought-tolerance-related traits of wheat seedling using two related RIL populations. *Genet* 92, 213–231. doi: 10.1007/s12041-013-0253-z
- Conflict of Interest Statement:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.
- Copyright © 2018 Ren, Fang, Jiang, Zhang, Hu, Wang, Meng, Cui, Lan, Ma, Wang and Kong. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.