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# Genetic analysis of important loci in the winter wheat backbone parent Aimengniu-V

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**Abstract**

To determine the genetic effect of winter wheat backbone parent Aimengniu-V (hereafter denoted as AMN-V), two $F_2$ populations (Aifeng 3//Mengxian 201/Neuzucht and Mengxian 201//Aifeng 3/Neuzucht) were developed to detect yield-related quantitative trait loci (QTL) in AMN-V. Eight markers (*Xwmc336*, *Xmag905*, *Xmag912*, *Xgwm124*, *Xgwm261*, *Xgwm260*, *Barc1118*, and *Xmag1884*) which were specific for AMN-V were used for single marker QTL analysis in the two $F_2$ populations of Aifeng 3//Mengxian 201/Neuzucht and Mengxian 201//Aifeng 3/Neuzucht. Of these markers, three specific loci of AMN-V (*Xgwm261*, *Xgwm124*, and *Xwmc336*) were linked with QTL for yield-related traits. Nine QTL for yield-related traits such as fertile spikelet number per spike, spike density, plant height, spike length, spikelet number per spike, 100-kernel weight, kernel weight per plant, heading day, and kernel number per spike were detected near *Xgwm261*. Four QTL linked with spikelet number per spike, fertile spikelet number per spike, kernel weight per spike, and plant height were detected near *Xwmc336*. Near *Xgwm124*, six QTL for the spike number per plant, spike length, kernel number per spike, kernel weight per plant, sterile spikelet number per spike, and spike density were detected. Two other $F_2$ populations (AMN-V/Jimai 22 and AMN-V/Tainong 18) were simultaneously developed to detect yield-related QTL in AMN-V. Eight specific markers of AMN-V were also used for single marker QTL analysis in AMN-V/Jimai 22 and AMN-V/Tainong 18. Of these, *Xgwm261* was linked with QTL for yield-related traits such as 100-kernel weight, plant height, spike length, spike density, kernel weight per spike and spike number per plant. The $F_2$ populations of AMN-V/Jimai 22 and AMN-V/Tainong 18 were used to detect yield-related QTL in chromosome 1RS of AMN-V. The results showed that the 1RS chromosome was linked with QTL for increasing the spikelet number per spike, kernel weight per spike, fertile spikelet number per spike, and 100-kernel weight. The 1RS chromosome and specific loci (*Xgwm261*, *Xgwm124*, and *Xwmc336*) of AMN-V may provide information for understanding the genetic basis that AMN-V became a backbone parent.

**Keywords:** backbone parent; specific loci; QTL analysis; genetic effect analysis; yield-related traits.
**Abbreviations:** AMN-Aimengniu; QTL-quantitative trait loci ; RIL-recombinant inbred line; LOD-logarithm of odds; PH-plant height; SL-spike length; KNPS-kernel number per spike; SNPP-spike number per plant; KWPP-kernel weight per plant; HKW-100-kernel weight; TKW -1000-kernel weight; SPN-spikelet number per spike; SD-spike density; FSPN-fertile spikelet number per spike; HD-heading day; SSPN-sterile spikelet number per spike; KWPS-kernel weight per spike.

## Introduction

The well-known Chinese winter wheat germplasm Aimengniu (hereafter denoted as AMN) is derived from the three-way crosses of Aifeng 3//Mengxian 201/Neuzucht (A//M/N). And the parent Neuzucht originating from Petkus is 1B/1R substitution line. According to some important agronomic traits such as plant height (PH), heading date (HD), spike type, awn type, and so on, AMN has been divided into 10 types (sib-lines). Types I to VII (AMN-I to AMN-VII) are commonly used in wheat breeding programs. AMN, which contains the synthetically advanced character of high yield, multiple disease resistance, and reduced height, has high general combining ability, high heritability, and carries many advanced dominant genes (Li et al., 1998). Roughly estimated, by the year 1998, more than 13 varieties, 78 advanced lines and 97 new germplasms had been developed from the elite germplasm AMN. From 1983 to 1996, the planting area added up to 2.060 million ha, and the yield was increased as much as 10.752 billion kilogram (Li et al., 1998). The backbone parent AMN-V is a famous authorised cultivar (Lumai 1) that has been popularized for as long as 20 years (Li et al., 1998; Ge et al., 2009). AMN-V is the leading variety of Huanghuai Wheat Area of China. Extensive studies on the seven AMN types have been reported (Qi et al. 2001a, b and 2004; Zhao et al., 2009a, 2009b; Cui et al., 2010; Zhao et al., 2011). However, the establishment of AMN-V as a backbone parent lacks genetic bases. A previous study (Zhao et al., 2011) showed that AMN-V has better general characteristics than that of the other sib-lines, and that it contains the 1RS chromosome. Backbone parents play important roles in renewing varieties. Knowing the effects of important chromosomal loci of backbone parents is very important. Molecular marker technology have been widely used in determining the effects of chromosomal loci on backbone parents (Lorenzen et al., 1995; Han et al., 2009; Si et al., 2009; Li et al., 2009; Yuan et al., 2010). For example, Li et al. (2009) revealed that three important loci of the backbone parent Orofen have high transference frequencies in the derived offspring. These loci are also related to important agronomic traits. Hence, they are thought to be important genetic bases for

the establishment of Orofen as a backbone parent. Quantitative trait loci (QTL) analysis using molecular markers is another significant method used for studying the effect of important chromosomal loci of backbone parents. Several studies have detected QTL related to yield components using populations constructed by different backbone parents. Li et al. (2000) and Tan et al. (2000) detected QTL related to yield, kernel number per spike (KNPS), kernel weight per spike (KWPS), etc., using $F_2$ and recombinant inbred line (RIL) population of the rice backbone parent Minghui 63. Tian et al. (2006) located QTL related to spike length (SL), KNPS, 1000-kernel weight (TKW), and yield using $BC_4F_4$ population of the rice backbone parent Guichao 2. Ma et al. (2007) and Lin et al. (2006) have located QTL related to SL, spikelet number per spike (SPN), spike density (SD), etc., using RIL population of the wheat backbone parent Nanda 2419. Jing et al. (2007) detected the *Yr* gene on chromosome 4BL using the $F_2$ population of the wheat backbone parent Xiaoyan 6. In the present study, the $F_2$ populations of Aifeng 3//Mengxian 201/Neuzucht (A//M/N), Mengxian 201//Aifeng 3/Neuzucht (M//A/N), AMN-V/Jimai 22 (V/J), and AMN-V/Tainong 18 (V/T) were used for the single marker QTL analysis. The purpose was to determine the genetic effect of specific loci and 1RS chromosome of AMN-V, and provide genetic bases for its establishement as a backbone parent.

## Results

### *Phenotypic performance of the four $F_2$ populations*

The variation among the four $F_2$ populations for SL, SPN, KWPS, KNPS, SD, PH, spike number per plant (SNPP), sterile spikelet number per spike (SSPN), fertile spikelet number per spike (FSPN), 100-kernel weight (HKW), kernel weight per plant (KWPP), and HD was shown in Table 1. The phenotypic variations of all the 12 traits among the $F_2$ lines were obvious in all the four populations and segregated continuously. Both absolute values of skewness and kurtosis for PH and KNPS were less than 1.0 in all the four $F_2$ populations, indicating a normal distribution. SL, HKW, and KWPP followed normal distributions in three of the four $F_2$ populations. FSPN showed a good fit to normal distribution in two of the four $F_2$ populations. The four spikelet-related traits, SNPP, SSPN, SPN, and SD, obeyed normal distributions in only one of the four $F_2$ populations, as HD did. However, KWPS did not follow a normal distribution in any of the four $F_2$ populations. The results above indicate that the phenotypic data for all the 12 traits mentioned above are generally suitable for QTL analysis.

### *Specific locus analysis of AMN-V*

A total of 656 polymorphic markers from the three parents were used to analysis the genetic differences between AMN-V and the other six sib-lines. Eight markers (i.e., *Xwmc336*, *Xmag905*, *Xmag912*, *Xgwm124*, *Xgwm261*, *Xgwm260*, *Barc1118*, and *Xmag1884*) showed specific patterns in AMN-V. Hence, they were considered the specific markers of AMN-V. The eight markers were used for single marker QTL analysis in the A//M/N and M//A/N-derived $F_2$ populations. *Xgwm261*, *Xgwm124*, and *Xwmc336* had stable and clear patterns in A//M/N-derived $F_2$ population. *Xgwm261* and *Xwmc336* had stable and clear patterns in M//A/N-derived $F_2$ population. *Xgwm124* showed significant segregation distortion in M//A/N-derived $F_2$ population, and was excluded from QTL analysis (data not shown). The eight specific markers of AMN-V were used for single marker QTL analysis in the V/J and V/T-derived $F_2$ populations. Only one marker, *Xgwm261*, had stable and clear patterns in the two $F_2$ populations.

### *QTL analysis of the three-way cross populations*

Single marker QTL analysis was performed for the three specific loci (*Xgwm261*, *Xgwm124*, and *Xwmc336*) of AMN-V using the A//M/N and M//A/N-derived $F_2$ populations. These loci were linked with QTL for yield-related traits (Table 2). Near *Xgwm261*, the AMN-V alleles were originated from Aifeng 3 or Mengxian 201. Five QTL for yield-related traits, i.e., *QFspn-2D*, *QSd-2D*, *QPh-2*, *QSl-2D*, and *QSpn-2D*, were detected near *Xgwm261* in both A//M/N and M//A/N-derived $F_2$ populations. These QTL accounted for 3.7/4.5%, 2.5/2.3%, 7.3/11.4%, 6.9/9.0% and 3.0/6.3% of the phenotypic variation, respectively, in the two $F_2$ populations. Alleles of AMN-V near *Xgwm261* had favorable effects on SD and PH, whereas had unfavorable effects on FSPN, SL, and SPN. In addition, QTL for HKW (*QHkw-2D*) and KWPP (*QKwpp-2D*) near *Xgwm261* were detected in the A//M/N-derived $F_2$ population, with favorable alleles increasing HKW and unfavorable alleles reducing KWPP in AMN-V. These two QTL explained 4.5 and 3.3% of the phenotypic variation, respectively. QTL linked with the HD (*QHd-2D*) and KNPS (*QKnps-2D*) near *Xgwm261* were detected in the M//A/N-derived $F_2$ population. Both the unfavorable alleles of the two traits were from AMN-V. These two QTL exhibited 6.3 and 3.1% of the phenotypic variation, respectively. The AMN-V alleles near *Xwmc336* were inherited from Neuzucht. QTL for SPN (*QSpn-1A*) and FSPN (*QFspn-1A*) were detected near *Xwmc336* in the A//M/N-derived $F_2$ population, with favorable alleles increasing SPN and FSPN in AMN-V. They each explained 4.7% of the phenotypic variation. QTL for KWPS, i.e., *QKwps-1A*, was also detected near *Xwmc336* in the A//M/N-derived $F_2$ population, explaining 4.4% of the phenotypic variation. Alleles of *QKwps-1A* in AMN-V had unfavorable effects on KWPS. In addition, QTL for SPN (*QSpn-1A*) and PH (*QPh-1A*) were detected near *Xwmc336* in the M//A/N-derived $F_2$ population. They accounted for 2.3 and 2.5% of the phenotypic variation, respectively. The QTL alleles increasing these traits were contributed by Neuzucht, i.e., with favorable alleles of increasing SPN and unfavorable effects of increasing PH in AMN-V. Near *Xgwm124*, QTL for SNPP (*QSnpp-1B*), SL (*QSl-1B*), KNPS (*QKnps-1B*), KWPP (*QKwpp-1B*), SSPN (*QSspn-1B*), and SD (*QSd-1B*) were detected in the A//M/N-derived $F_2$ population. *QSnpp-1B* and *QSd-1B* explained 6.5 and 5.2% of the phenotypic variation, with increased alleles from Aifeng 3 or Mengxian 201. *QSl-1B*, *QKnps-1B*, *QKwpp-1B*, and *QSspn-1B* explained 3.7, 2.7, 6.7, and 3.7% of the phenotypic variation, respectively, with increased alleles from Neuzucht. Due to that AMN-V alleles near *Xgwm124* were inherited from Aifeng 3, AMN-V alleles of *Xgwm124* showed favorable effects on increasing SNPP and SD and reducing SSPN, whereas showed unfavorable effects of reducing SL, KNPS and KWPP.

### *QTL analysis of V/J and V/T-derived $F_2$ populations*

Single marker QTL analysis was performed for *Xgwm261* using V/J and V/T-derived $F_2$ populations. Near *Xgwm261*, QTL linked with HKW (*QHkw-2D*), PH (*QPh-2D*), SL (*QSl-2D*), and SD (*QSd-2D*) were detected in both V/J and V/T-derived $F_2$ populations (Table 3). These QTL accounted for 13.5/2.9%, 9.4/4.8%, 8.0/7.0%, and 8.1/7.0% of the phenotypic variation in (V/J)/(V/T)-derived $F_2$ populations,

**Table 1.** Phenotypic values for 11 wheat yield-related traits in the four $F_2$ populations.

| Populations | Items | PH (cm) | SL (cm) | SNPP | SSPN | SPN | FSPN | KNPS | HKW (g) | KWPS (g) | KWPP (g) | SD | HD |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| A//M/N | Mean | 90.69 | 8.79 | 18.03 | 1.85 | 19.32 | 17.48 | 45.07 | 3.72 | 1.14 | 20.55 | 226.39 | 177.08 |
| | S.D. | 12.20 | 1.73 | 8.95 | 1.43 | 2.26 | 2.72 | 10.74 | 0.61 | 0.48 | 11.77 | 43.07 | 1.10 |
| | Min-Max | 51.00–117.00 | 3.50–20.5 | 1.00–53.00 | 0.00–8.00 | 9.00–25.00 | 7.00–23.00 | 13.00–75.00 | 1.98–5.37 | 0.13–4.27 | 2.10–61.37 | 97.56–400.00 | 174.00–180.00 |
| | Skewness | –0.34 | 1.22 | 0.81 | 1.23 | 0.85 | –0.98 | –0.04 | –0.26 | 1.58 | 0.95 | 0.84 | –1.01 |
| | Kurtosis | –0.06 | 9.33 | 0.70 | 2.72 | 2.53 | 1.99 | 0.19 | –0.07 | 9.53 | 0.81 | 2.06 | 0.55 |
| M//A/N | Mean | 87.58 | 7.73 | 13.50 | 1.83 | 20.48 | 18.67 | 40.06 | 3.63 | 1.11 | 15.11 | 274.18 | 179.27 |
| | S.D. | 14.79 | 1.65 | 7.29 | 1.50 | 2.69 | 3.04 | 11.21 | 0.68 | 0.43 | 9.25 | 57.49 | 2.21 |
| | Min-Max | 47.00–122.00 | 3.70–12.50 | 1.00–49.00 | 0.00–7.00 | 12.00–34.00 | 9.00–30.00 | 2.00–71.00 | 1.86–5.33 | 0.11–2.88 | 2.47–43.95 | 163.79–507.46 | 174.00–182.00 |
| | Skewness | –0.15 | 0.37 | 1.00 | 0.90 | 0.40 | 0.22 | –0.38 | –0.27 | 0.54 | 0.85 | 0.81 | –0.46 |
| | Kurtosis | –0.49 | –0.15 | 2.07 | 0.37 | 2.40 | 0.70 | 0.59 | –0.11 | 1.03 | 0.09 | 0.87 | –1.02 |
| V/J | Mean | 68.26 | 9.07 | 13.84 | 0.65 | 17.63 | 16.97 | 44.49 | 4.59 | 1.54 | 21.02 | 198.35 | 176.16 |
| | S.D. | 6.62 | 1.46 | 6.11 | 0.89 | 1.55 | 1.86 | 8.02 | 0.64 | 0.36 | 10.19 | 30.45 | 1.04 |
| | Min-Max | 40.00–86.00 | 5.90–15.00 | 2.00–39.00 | 0.00–5.00 | 13.00–21.00 | 12.00–21.00 | 24.00–77.00 | 2.00–6.40 | 0.47–3.24 | 4.60–70.83 | 116.67–311.48 | 175.00–179.00 |
| | Skewness | –0.37 | 0.27 | 1.03 | 1.47 | –0.23 | –0.19 | 0.27 | –0.53 | 0.26 | 1.40 | 0.66 | 0.59 |
| | Kurtosis | 0.90 | 0.46 | 1.60 | 2.44 | –0.18 | –0.33 | 0.36 | 0.94 | 1.92 | 3.27 | 1.05 | –0.39 |
| V/T | Mean | 69.63 | 8.99 | 14.14 | 0.69 | 18.10 | 17.41 | 48.57 | 4.58 | 1.78 | 24.77 | 205.47 | 175.96 |
| | S.D. | 6.02 | 1.38 | 6.65 | 0.89 | 1.92 | 2.35 | 9.66 | 0.52 | 0.66 | 12.93 | 35.42 | 1.10 |
| | Min-Max | 55.02–90.00 | 4.60–12.50 | 1.00–42.0 | 0.00–4.00 | 13.00–34.00 | 10.00–34.00 | 16.00–75.00 | 2.500–5.60 | 0.19–7.80 | 3.18–78.90 | 144.04–413.05 | 175.00–179.00 |
| | Skewness | 0.25 | –0.02 | 0.77 | 1.22 | 3.30 | 1.75 | –0.07 | –0.88 | 4.57 | 0.78 | 2.15 | 1.09 |
| | Kurtosis | 0.31 | 0.32 | 1.08 | 0.88 | 25.94 | 13.50 | 0.07 | 2.18 | 39.27 | 0.92 | 8.58 | 0.39 |

S.D., Standard deviation.

**Table 2.** QTL of part-specific loci of AMN-V in A//M/N and M//A/N-derived $F_2$ populations.

| | A//M/N-derived $F_2$ population | | | | | M//A/N-derived $F_2$ population | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Marker | QTL | LOD | $R^2$ (%) | *A* | *D* | QTL | LOD | $R^2$ (%) | *A* | *D* |
| *Xgwm261* | *QFspn-2D* | 1.6 | 3.7 | 0.69 | 0.02 | *QFspn-2D* | 2.4 | 4.5 | 0.88 | –0.10 |
| | *QSd-2D* | 1.2 | 2.5 | –8.08 | –3.76 | *QSd-2D* | 1.3 | 2.3 | –11.97 | 0.87 |
| | *QPh-2D* | 3.2 | 7.3 | 3.40 | 3.05 | *QPh-2D* | 6.4 | 11.4 | 6.88 | –0.45 |
| | *QSl-2D* | 3.1 | 6.9 | 0.61 | 0.01 | *QSl-2D* | 4.9 | 9.0 | 0.67 | –0.25 |
| | *QSpn-2D* | 1.3 | 3.0 | 0.51 | 0.02 | *QSpn-2D* | 3.4 | 6.3 | 0.91 | –0.37 |
| | *QHkw-2D* | 1.6 | 4.5 | –0.10 | –0.18 | *QHd-2D* | 3.5 | 6.3 | 0.75 | –0.30 |
| | *QKwpp-2D* | 1.2 | 3.3 | 2.69 | 0.68 | *QKnps-2D* | 1.6 | 3.1 | 2.61 | 0.61 |
| *Xwmc336* | *QSpn-1A* | 1.9 | 4.7 | 0.61 | –0.22 | *QSpn-1A* | 1.3 | 2.3 | 0.06 | –0.91 |
| | *QKwps-1A* | 1.7 | 4.4 | –0.16 | 0.03 | *QPh-1A* | 1.3 | 2.5 | 1.44 | –5.90 |
| | *QFspn-1A* | 1.8 | 4.7 | 0.75 | –0.23 | | | | | |
| *Xgwm124* | *QSnpp-1B* | 2.9 | 6.5 | –1.07 | 4.53 | | | | | |
| | *QSl-1B* | 1.6 | 3.7 | 0.33 | 0.40 | | | | | |
| | *QKnps-1B* | 1.2 | 2.7 | 0.51 | 3.40 | | | | | |
| | *QKwpp-1B* | 2.5 | 6.7 | 0.78 | 5.91 | | | | | |
| | *QSspn-1B* | 1.6 | 3.7 | 0.09 | –0.56 | | | | | |
| | *QSd-1B* | 2.3 | 5.2 | –6.79 | –15.5 | | | | | |

$R^2$: Phenotypic variation explained. *A*: Additive effect, wherein positive values indicate that the alleles increasing the phenotypic values are from Neuzucht. *D*: Dominant effect.

respectively. The favorable alleles of HKW, and SL were contributed by AMN-V in both V/J and V/T-derived $F_2$ populations, whereas AMN-V had unfavorable effects on PH and SD in the two $F_2$ populations. QTL linked with KWPS (*QKwps-2D*) and SNPP (*QSnpp-2D*) near this locus were also detected in the V/J and V/T-derived $F_2$ populations, respectively. AMN-V had favorable effects on KWPS and unfavorable effects on SNPP, accounting for 5.8 and 4.7% of the phenotypic variation, respectively.

***Genetic effects of 1RS on V/J and V/T-derived $F_2$ populations***

To detect the separation of 1BS and 1RS in V/J and V/T-derived $F_2$ populations, primers for *Glu-B3*, *Gli-B1*, and *SEC-1b* were used for multiplex PCR. Single marker QTL analysis was performed for 1RS using the V/J and V/T-derived $F_2$ populations. QTL linked with the SPN (*QSpn*) and KWPS (*QKwps*) were detected in the V/J-derived $F_2$ population, exhibiting 4.1 and 2.0% of the phenotypic variation, respectively (Table 3). Both QTL alleles increasing the phenotypic values were from 1RS. QTL for FSPN (*QFspn*) and HKW (*QHkw*) were detected in the V/T-derived $F_2$ population, exhibiting 3.1 and 4.8% of the phenotypic variation, respectively. Both QTL alleles increasing the phenotypic values were from 1RS.

**Discussion**

***Important QTL near AMN-V-specific loci***

QTL related to FSPN (*QFspn-2D*), SD (*QSd-2D*), PH (*QPh-2D*), SL (*QSl-2D*), and SPN (*QSpn-2D*) were detected in both A//M/N and M//A/N-derived $F_2$ populations. QTL linked with HKW (*QHkw-2D*), PH (*QPh-2D*), SL (*QSl-2D*), and SD (*QSd-2D*) was detected in both V/J and V/T-derived $F_2$ populations. The combination of the four $F_2$ populations conferred high authenticity and accuracy to the results. According to the information on genes and QTL located in the wheat genome, numerous genes and QTL were found related to important agronomic traits near AMN-V-specific loci (Ma et al., 2007; Zhao et al., 2011; Korzun et al., 1998; Narasimhamoorthy et al., 2006; Kumar et al., 2007; Zhang et al., 2009). In the present study, QTL related to yield-related traits were detected near the abovementioned AMN-V-specific loci in the four $F_2$ populations. These results are in good agreement with those of previous studies. Therefore, the specific loci of AMN-V might account for the genetic bases for its establishment as a backbone parent. QTL linked with the PH (*QPh-2D*) were detected near *Xgwm261*. The dwarf gene of Aifeng 3 can be traced back to Shuiyuan 86 (containing *Rht1* and *Rht2*) and Crimson wheat (containing *Rht8* and *Rht9*). Korzun et al. (1998) have found an *Xgwm261* allele linked to *Rht8* (0.6 cM). Therefore, AMN-V might contain *Rht8*. Yuan et al. (2010) reported that *Xgwm261* is one marker that distinguishes Bima 4 from its sib-lines. The transmission frequency of its preponderant alleles was up to 70% in four generations with an 80.3% rate of genetic contribution. They speculated that the chromosomal region near this locus might be strongly selected by wheat breeders. Zhang et al. (2006a) have reported that the preponderant alleles near *Xgwm261* can be found in many varieties in China. Many studies have surveyed wheat cultivars for the presence of *Rht8* using the "diagnostic" 192 bp allele in the *Xgwm261* locus, either to determine its prevalence in worldwide wheat cultivars (Chebotar et al., 2001; Worland et al., 2001; Ahmad and Sorrells, 2002; Liu et al., 2005; Ganeva et al., 2005; Zhang et al., 2006b) or to ascertain the effect of *Rht8* on other agronomic traits (Rebetzke and Richards, 2000; Bai et al., 2004). Hence, the locus *Xgwm261* plays an important role in wheat-breeding programs.

***The important role of 1RS chromosome of AMN-V***

Aside from the specific loci, the 1RS chromosome is also an important component of AMN-V. The 1RS chromosome reportedly enhances adaptation, stress tolerance, and yield potentials in bread wheat (Carver and Rayburn, 1994; Schlegel and Meinel, 1994; Moreno-Sevilla et al., 1995a, b; Villareal et al., 1995). It also plays a significant role in international wheat breeding programs. The present study showed that the 1RS of AMN-V increased agronomic traits such as SPN, KWPS, FSPN, and HKW. Therefore, it was speculated that the 1RS chromosome plays an important role in the establishment of backbone parent AMN-V. The creation and utilization of backbone parents can improve breeding efficiency. Consequently, the genetic bases of backbone parents need to be elucidated. In the present study, we specified the genetic effect of AMN-V by detecting the QTL related to yield-related traits near the specific loci as well as 1RS. The results provided important information for a deeper understanding the genetic basis of backbone parents.

**Materials and methods**

***Plant materials***

Aifeng 3, Mengxian 201, Neuzucht, AMN-V as well as its six sib-lines, Jimai 22, and Tainong 18, were used in the present study. These materials were provided by the Subcenter of National Wheat Improvement Center, Tai'an, Shandong Province, China. Four $F_2$ populations derived from crosses of A//M/N, M//A/N, V/J, and V/T, including 197, 245, 248, and 185 individual lines, respectively, were used. AMN-V is a 1BL/1RS translocation line. Both Tainong 18 and Jimai 22 contain the common 1B chromosome. All materials were planted in an experimental field of Shandong Agricultural University in rows 2.0 m long and 30.0 cm apart. The average distance between adjacent plants was about 10 cm. Normal agricultural practices were applied for disease and weed control.

***Agronomic evaluation***

Traits examined included the PH in centimeters, measured as the distance from the ground level to spike tip, excluding awns; the SL in centimeters, measured from the base of the rachis to the top of the uppermost spikelet, excluding the awns; SNPP; SPN and SSPN. The FSPN was estimated by subtracting SSPN from SPN. The above traits were measured in the maturity stage before harvest. The KNPS, HKW, KWPS, and KWPP were measured after harvest. One spike from the leading tillers was harvested with a view to count KNPS, and weigh KWPS; all spikes from one plant were harvested to weigh KWPP. HKW was evaluated in grams after harvest by weighing two samples of 100 kernels. Seed was thoroughly cleaned and all non-wheat materials and broken kernels were removed before trait evaluation. The HD was recorded according to Li et al. (2005). The SD or compactness was calculated as SD=100×SPN/SL.

***Analysis of molecular markers***

Genomic DNA of each material was extracted from young

**Table 3.** QTL of locus *Xgwm261* and genetic effect of 1RS/1BS in V/J and V/T-derived $F_2$ populations.

| | V/J-derived $F_2$ population | | | | | V/T-derived $F_2$ population | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Marker | QTL | LOD | $R^2$ (%) | *A* | *D* | QTL | LOD | $R^2$ (%) | *A* | *D* |
| *Xgwm261* | *QHkw-2D* | 7.7 | 13.5 | 0.35 | 0.23 | *QHkw-2D* | 1.2 | 2.9 | 0.14 | 0.02 |
| | *QPh-2D* | 5.3 | 9.4 | 3.14 | 0.92 | *QPh-2D* | 2.0 | 4.8 | 2.11 | 0.16 |
| | *QSl-2D* | 4.5 | 8.0 | 0.63 | 0.08 | *QSl-2D* | 2.8 | 7.0 | 0.54 | –0.13 |
| | *QSd-2D* | 4.5 | 8.1 | –13.3 | –2.73 | *QSd-2D* | 2.8 | 7.0 | –13.00 | 5.31 |
| | *QKwps-2D* | 3.2 | 5.8 | 0.13 | 0.02 | *QSnpp-2D* | 2.0 | 4.7 | –0.88 | –3.04 |
| *Glu-B3*, *Gli-B1*, | *QSpn* | 2.2 | 4.1 | 0.40 | 0.29 | *QFspn* | 1.2 | 3.1 | 0.56 | 0.02 |
| *SEC-1b* | *QKwps* | 1.1 | 2.0 | 0.03 | –0.09 | *QHkw* | 1.8 | 4.8 | 0.13 | –0.15 |

$R^2$: Phenotypic variation explained. *A*: Additive effect, wherein positive values indicate that the alleles increasing the phenotypic values are from AMN-V or 1RS. *D*: Dominant effect.

leaves according to SDS-phenol method (Liu et al., 2006). PCR amplification was carried out in a PCR thermal cycler (TakaRa, Dalian, China). The reaction system was 25 μL in volume containing 2 mmol $L^{-1}$ $MgCl_2$, 1×PCR buffer, 200 mmol $L^{-1}$ dNTPs each, 1 U *Taq* DNA polymerase, 10 ng of each primer (25ng $\mu L^{-1}$), and 90 ng of genomic DNA template. The amplification procedure initiated with one cycle of pre-denaturation at 94°C for 5 min; followed by 15 cycles of touchdown amplification at 95°C for 45 s, 65°C (1°C reduction per cycle) for 1 min, and 72°C for 1 min; 15 cycles of normal PCR at 95°C for 45 s, 55°C for 50 s, and 72°C for 1 min; and finally extended at 72°C for 10 min, and PCR products were saved at 10°C. PCR products were mixed with bromophenol blue (5:1) and then separated in 6% non-denaturing polyacrylamide gels (39:1) running under 120 V. The amplification bands were visualized and photographed by Tanon Gis-2010 (Shanghai Tanon Technology Limited Company) after silver staining. In A//M/N and M//A/N-derived $F_2$ populations, all the 197, 245 lines, respectively, and the parental lines, were genotyped with specific markers of AMN-V. Banding patterns from Neuzucht were marked 2, whereas those from the other two parents were marked 0; heterozygotic bands of Neuzucht, Aifeng 3, and Mengxian201 were marked 1. Specific markers of AMN-V were used to detect polymorphisms between AMN-V and Jimai 22, and between AMN-V and Tainong 18. The polymorphic markers were used to genotype the 248 and 185 lines respectively, of the V/J and V/T-derived $F_2$ populations. The banding patterns from AMN-V were marked 2, whereas those from the other two parents were marked 0; the heterozygotic bands of AMN-V and Jimai 22/Tainong18 were marked 1. To detect the separation of 1BS and 1RS in V/J and V/T-derived $F_2$ populations, the STS-polymerase chain reaction (PCR) primer for *Glu-B3*, SSR primer for *Gli-B1*, and STS-PCR primer for *SEC-1b* were used for multiplex PCR. The primer information and the sequences of the three markers were according to Zhang et al. (2003) as follows:

*Glu-B3*

F：GGTACCAACAACAACAACCC

R：GTTGCTGCTGAGGTTGGTTC

*Gli-B1*

F：GCAGACCTGTGTCATTGGTC

R：GATATAGTGGCAGCAGGATACG

*SEC-1b*

F：G TTTGCTGGGGAATTA TTTG

R：TCCTCATC TTTGTCCTCGCC

All primers were synthesized by the Sangon Biotech Co., Ltd. The reaction system of multiplex PCR was 25 μL in volume containing 1×PCR buffer, 2 mmol $L^{-1}$ $MgCl_2$, 300 mmol $L^{-1}$ dNTPs each, 1 U *Taq* DNA polymerase, 6 ng of each primer (25 ng $\mu L^{-1}$), and 110 ng of genomic DNA template. The amplification procedure of multiplex PCR was the same as that of normal PCR.

Plants that presented the patterns of *Glu-B3* and *Gli-B1* were homozygous 1BS chromosomes, and marked as 0. Plants that presented the patterns of *SEC-1b* were homozygous 1RS chromosomes, and marked as 2. Plants that presented the patterns of the three markers were heterozygous chromosomes of 1BS and 1RS, and marked as 1.

### *QTL analysis*

In each individual population, phenotypic data and molecular marker scores were combined. Subsequently, single marker analysis was conducted using IciMapping V3.1, and the threshold logarithm of odds (LOD) scores were calculated using 1000 permutations.

## Conclusions

Four $F_2$ populations (A//M/N, M//A/N, V/J, and V/T) were developed to detect yield-related QTL near three AMN V-specific loci (*Xgwm261, Xgwm124*, and *Xwmc336*). QTL linked with yield-related traits, such as PH, SL, and SD, were detected. The results showed that 1RS chromosome was linked with QTL for increasing the SPN, KWPS, FSPN, and HKW. The 1RS chromosome and specific loci of AMN-V may provide information to understand the genetic bases for its establishment as a backbone parent in wheat breeding, and to distinguish it from other types at the genomic level.

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