



Research Article

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Composition and Analysis of Vernalization and Photoperiod Genes in Wheat



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Abstract

To understand the vernalization and photoperiod gene composition of wheat in Henan's wheat region, 12 common wheat varieties widely planted in the Henan wheat region were used as experimental materials. Using molecular markers from previous studies, the allelic variations and gene compositions of vernalization genes (*Vrn-A1*, *Vrn-B1*, *Vrn-D1*, and *Vrn-B3*) and photoperiod genes (*Ppd-A1*, *Ppd-B1*, *Ppd-D1*) were detected. The results of this study indicate that the wheat production area in Henan is mainly planted with winter wheat varieties and predominantly photoperiod-insensitive wheat varieties. This fully demonstrates that the Henan wheat region is a transitional zone from winter wheat to spring wheat, with winter wheat being the main crop planted, and some areas also planting spring wheat. This research is beneficial for providing a scientific basis for the rational utilization of wheat varieties, studying the composition of vernalization and photoperiod genes, and has guiding significance for wheat introduction and ecological breeding.

Keywords: Wheat; Vernalization; Vernalization gene; Photoperiod gene

Introduction

Wheat (*Triticum aestivum* L.) is one of the most important cereal crops in the world, with a wide cultivation area; it accounts for about one-third of the total cultivated area of cereal crops and is one of the essential foods for human survival [1]. Henan Province is a major wheat-producing area in China, serving as a transitional zone from winter wheat to spring wheat, with winter wheat being the primary crop cultivated, and some areas also planting spring wheat. Temperature and light are two critical ecological factors that affect the growth, yield, and quality of wheat, regulated through the vernalization process (low-temperature treatment) and the photoperiod response (day and night light duration changes), which in turn affect its growth and development [2]. The differences in temperature and light conditions across regions lead to a variety of developmental ecological types of wheat [3].

Vernalization refers to the process where wheat must undergo continuous low-temperature treatment to flower normally, transitioning from vegetative to reproductive growth; it is an important qualitative change in the wheat development process, directly affecting the planting range and cultivation methods of

wheat [4-7]. Current research shows that wheat vernalization is mainly controlled by vernalization genes such as *VRN-1*, *VRN-2*, *VRN-3*, and *VRN-4* [8], among which the *VRN-1* gene has the most significant effect [9,10]. It contains three alleles (*Vrn-A1*, *Vrn-B1*, and *Vrn-D1*), located on the long arms of wheat chromosomes 5A, 5B, and 5D, respectively [11-14]. The *VRN-3* gene is mainly regulated by vernalization and long-day conditions, playing a significant role in promoting wheat flowering. *Vrn-B3* is a partial homolog of *VRN-3* and has been located on wheat chromosome 7B.

The photoperiod response is also an important physiological characteristic of wheat, which, together with vernalization, affects the ecological adaptability of wheat. Studies have shown that the wheat photoperiod response is mainly influenced by photoperiod genes such as *Ppd-A1*, *Ppd-B1*, and *Ppd-D1* [15-17]. When the gene loci are dominant (*Ppd-A1a*, *Ppd-B1a*, and *Ppd-D1a*), wheat is not sensitive to the photoperiod response, while the recessive allelic variants (*Ppd-A1b*, *Ppd-B1b*, and *Ppd-D1b*) are sensitive. The three allelic genes have different responses to the

photoperiod, with the Ppd-D1a gene being the most insensitive to the photoperiod, followed by Ppd-B1a, and Ppd-A1a being the weakest. Currently, the four main vernalization genes of wheat, Vrn-A1, Vrn-B1, Vrn-D1, and Vrn-B3, and the photoperiod gene Ppd-D1 locus have all been cloned, and corresponding functional markers have been developed. These molecular markers can be used to detect and analyze the relevant functional genes in wheat materials [10,18].

Wheat is widely planted in different ecological regions of China. According to regional divisions, wheat planting in China can be divided into four areas: the Southwest wheat region, the Northwest wheat region, the North China and Huang-Huai-Hai wheat region, and the Middle and Lower Yangtze River wheat region. Among these, the North China and Huang-Huai-Hai wheat region account for about 267 million mu, which is 79.6% of the total wheat planting area in the country. By analyzing the composition of vernalization and photoperiod genes in wheat varieties from the North China and Huang-Huai-Hai wheat region, we can understand the vernalization and photoperiod characteristics of these varieties. This can further help study the changing trends of wheat vernalization, thus providing a reference for production. Henan Province is a major wheat-planting province in China and is also part of the North China and Huang-Huai-Hai wheat region. Currently, there are few reports on the detection of vernalization and photoperiod genes in wheat varieties in this province. Therefore, this experiment used 12 wheat varieties, both local and improved, that have been widely planted in Henan Province in recent years. By detecting their vernalization and photoperiod genes, we can understand the dominant and recessive composition of these genes and, through analysis, determine the theoretical phenotypes, with the aim of providing a theoretical basis for wheat genetic breeding in Henan Province.

Materials and Methods

Experimental material

The experimental materials consisted of 12 local and improved wheat varieties promoted in Henan Province, selected from the commonly planted wheat varieties in Henan Province in 2021 and 2022. Based on preliminary observations of different maturity times in the experimental fields, early and late-maturing wheat varieties were chosen as test materials. The experiments were conducted from 2020 to 2022 at the Plant Germplasm Resources and Genetics Laboratory of the College of Life Sciences at Henan University. The 12 wheat varieties selected for the experiment, sourced from the Plant Germplasm Resources and Genetics Laboratory of the College of Life Sciences at Henan University, are: Lu Mai 20, Gu Shen 19, Yan Mai Cao 1, Lu Mai 21, Xu Mai 1901, Zhongguo Chun, Jin Mai 1917, Jin Mai 2914, He Da 310, He Da 669, Ji Mai 44, and Zhou Mai 18.

Extraction and purification of DNA

Translation of the DNA extraction procedure using the CTAB

method:

i. Weighing and Grinding: Take approximately 2g of fresh leaves and place them in a 2.0 ml EP tube with grinding beads. Freeze thoroughly with liquid nitrogen and grind to a powder using a grinder at a frequency of 45 shakes per second for 50 seconds.

ii. CTAB Extraction: Quickly add 800 μ L of preheated (65 $^{\circ}$ C) 2 \times CTAB extraction buffer. Invert the tube until the leaf powder is well mixed with the solution. Incubate in a 65 $^{\circ}$ C water bath for 30 minutes, inverting every 10 minutes to ensure full reaction with CTAB (avoid vigorous shaking to prevent genomic DNA from breaking).

iii. Chloroform-Isoamyl Alcohol Treatment: Remove the EP tube and add 800 μ L (equal volume) of chloroform: isoamyl alcohol (24:1). Invert to mix and then shake at 40 r/min for 10 minutes. Centrifuge at 12,000 rpm for 10 minutes.

iv. DNA Precipitation: After centrifugation, transfer the supernatant to a new 2.0mL EP tube and repeat step 3.

v. Ethanol Precipitation: After centrifugation, transfer the supernatant to a new 1.5mL EP tube. Add double the volume of anhydrous ethanol (stored at -20 $^{\circ}$ C) and place in a -20 $^{\circ}$ C freezer for 2 hours to further precipitate the DNA.

vi. DNA Washing: Centrifuge the EP tube at 8,000 rpm. Carefully pour out the anhydrous ethanol and wash the DNA pellet twice with 70% ethanol. Invert the tube to dry overnight and then add 100 μ L of sterile water to dissolve the DNA for later use.

vii. DNA Quantification: Measure the DNA concentration and dilute with ddH₂O to 100 ng/ μ L.

viii. Replication for Reliability: Extract DNA from three samples of each variety as biological replicates to ensure the reliability of the results.

STS molecular detection of vernalization and photoperiod genes

STS (Sequence Tagged Site) molecular detection involves the use of specifically developed primers for targeted amplification. The primers, synthesized by Shangya Biotechnology Co., Ltd., are used for PCR-specific amplification of target genes. The PCR reaction program is set according to the recommended procedure provided in the mix instructions (Table 1). This is followed by agarose gel electrophoresis and photography using a gel imaging system. The results are then analyzed based on the size of the amplified bands to identify specific genes. The primer sequences for the seven gene loci *VRN-A1*, *VRN-B1*, *VRN-D1*, *VRN-B3*, *Ppd-A1*, *Ppd-B1*, and *Ppd-D1*, which are used for vernalization gene detection, were designed according to Yan et al. [19], Zhang et al. [20], Whittal et al. [21], Nishida et al. [22], and Beales [23] (Table 2).

Table 1: PCR reaction program.

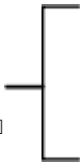
34 cycles of 	Temperature	Times
	94°C	3min
	94°C	30s
	50-65°C	30s
	72°C	1-2min
	72°C	5min
	4°C	Forever

Table 2: PCR primers used to detect vernalizing and photoperiodic genes.

Locus	Allele(s)	primer name	Sequence[5'→3']	Product Size(bp)	Annealing Temp(°C)	Reference
VRN-A1	Vrn-A1a	VRN1AF	GAAAGGAAAAATTCTGCTCG	965(Vrn-A1a)	50	[19]
	Vrn-A1b	VRN1AR	GCAGGAAATCGAAATCGAAG	714(Vrn-A1b)		
	Vrn-A1c			734(Vrn-A1c)		
	vrn-A1			734(vrn-A1)		
	Vrn-A1c	Intr1/A/F	AGCCTCCACGGTTTGAAAGTAA	1170(Vrn-A1c)	65	[19]
		Intr1/A/R	AAGTAAGACAACACGAATGTGAGA			
VRN-B1	Vrn-B1	Intr1/B/F	CAAGTGAACGGTTAGGACA	709(Vrn-B1)	58	[21]
		Intr1/B/R	CTCATGCCAAAAATTGAAGATGA			
	vrn-B1	Intr1/B/F	CAAGTGAACGGTTAGGACA	1149(vrn-B1)	56.4	[21]
		Intr1/B/R2	CAAATGAAAAGGAATGAGAGCA			
VRN-D1	Vrn-D1	Intr1/D/F	GTTGTCTGCCTCATCAAATCC	1671(Vrn-D1)	61	[21]
		Intr1/D/R3	GGTCACTGGTGGTCTGTGC			
	vrn-D1	Intr1/D/F	GTTGTCTGCCTCATCAAATCC	997(vrn-D1)	61	[21]
		Intr1/D/R	AAATGAAAAGGAACGAGAGCG			
	Vrn-D1a	VRN1DF	CGACCCGGGCGGCACGAGTG	631(Vrn-D1a)	65	[20]
		VRN1SNP161CR	AGGATGGCCAGGCCAAAACG			
	Vrn-D1b	VRN1DF	CGACCCGGGCGGCACGAGTG	631(Vrn-D1b)	65	[20]
		VRN1SNP161AR	AGGATGGCCAGGCCAAAACG			
VRN-B3	Vrn-B3	B-INS-F	CATAATGCCAAGCCGGTGTAGTAC	1240(Vrn-B3)	63	[21]
		B-INS-R	ATGTCTGCCAATTAGCTAGC			
	vrn-B3	BNOINS-F	ATGCTTTTCGCTTGCCATCC	1140(vrn-B3)	57	[21]
		BNOINS-R	CTATCCCTACCGCCATTAG			
PPD-A1	Ppd-A1a	TaPpd-A1-F1	CGTACTCCCTCCGTTTCTTT	338(Ppd-A1a)	57	[22]
		TaPpd-A1-R2	AATTTACGGGGACCAAATACC			
	Ppd-A1b	TaPpd-A1-F1	CGTACTCCCTCCGTTTCTTT	299(Ppd-A1b)	57	[22]
		TaPpd-A1-R3	GTTGGGGTCGTTTGGTGGTG			
PPD-B1	Ppd-B1a	TaPpd-B1-F1	ACACTAGGGCTGGTGAAGA	1600(Ppd-B1a)	60	[22]
	Ppd-B1b	TaPpd-B1-R1	CCGAGCCAGTGCAAATTAAC	1292(Ppd-B1b)		
PPD-D1	Ppd-D1a	TaPpd-D1-F1	ACGCCTCCCACTACTG	288vPpd-D1a)	54	[23]
		TaPpd-D1-R1	CACTGGTGGTAGCTGAGATT			
	Ppd-D1b	TaPpd-D1-F1	ACGCCTCCCACTACTG	415(Ppd-D1b)	54	[23]
		TaPpd-D1-R2	TGTTGGTTCAAACAGAGAGC			

Result

Analysis of allelic variation in wheat vernalization genes

Analysis of the dominance and recessiveness of the *VRN-A1* gene.

The use of primers VRN1AF and VRN1AR for specific amplification analysis of the genomic DNA of 12 wheat varieties

showed that at the *Vrn-A1* locus, all 12 wheat varieties amplified a target fragment of 734bp (Figure 1a), and the genotype belongs to *vrn-A1*. Primers Intr1/A/F and Intr1/A/R were used to detect large fragment deletions in the first intron region of *Vrn-A1* in the 12 wheat varieties, and a 1170bp fragment was not amplified (Figure 1b), so the genotype *Vrn-A1c* does not exist in the 12 wheat varieties. From this, it can be inferred that these materials do not carry the dominant *Vrn-A1* gene, and their *Vrn-A1* gene genotype is the recessive *vrn-A1*.

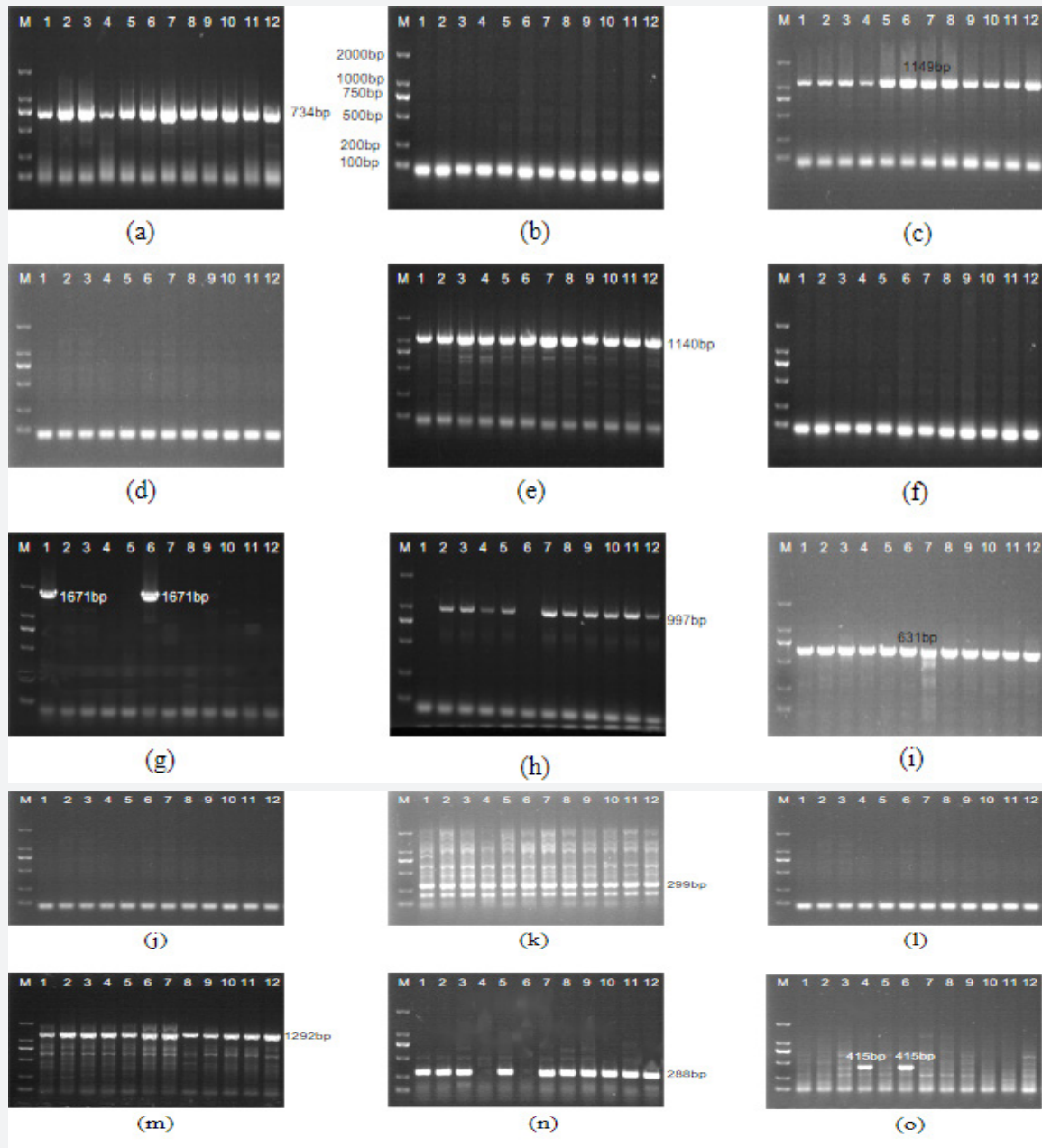


Figure 1: Analysis of allelic variation

1 : Lu Mai 20 ; 2 : Gu Shen 19 ; 3 : Yan Mai Cao 1 ; 4 : Lu Mai 21 ; 5 : Xu Mai 1901 ; 6 : Zhongguo Chun ; 7 : Jin Mai 1917 ; 8 : Jin Mai 2914 ; 9 : He Da 310 ; 10 : He Da 669 ; 11 : Ji Mai 44 ; 12 : Zhou Mai 18 ; M : Marker D 2000.

Analysis of the dominance and recessiveness of the VRN-B1 and VRN-B3 gene

Specific amplification analysis using primers Intr1/B/F and Intr1/B/R, Intr1/B/F and Intr1/B/R2 on the genomic DNA of 12 wheat varieties showed that all 12 samples amplified a 1149bp band (Figure 1c, Figure 1d), indicating a *vrn-B1* genotype. Specific amplification analysis using primers B-INS-F and B-INS-R, BNOINS-F and BNOINS-R on the genomic DNA of 12 wheat varieties showed that only a 1140bp band was amplified (Figure 1e, Figure 1f), indicating a *vrn-B3* genotype

Analysis of the dominance and recessiveness of the VRN-D1 gene

Specific amplification analysis using primers Intr1/D/F and Intr1/D/R3, Intr1/D/F and Intr1/D/R on the genomic DNA of 12 wheat varieties showed that at the *VRN-D1* locus, Lu Mai 20 and Zhongguo Chun amplified a 1671bp fragment, indicating a dominant *Vrn-D1* genotype (Figure 1g). The remaining 10 materials amplified a 997bp fragment (Figure 1h), indicating a *vrn-D1* genotype. Testing the 12 materials with primers VRN1DF and VRN1SNP161CR, VRN1DF and VRN1SNP161AR revealed no mutation in the promoter region of the dominant *Vrn-D1* (Figure 1i, Figure 1j), indicating that Lu Mai 20 and Zhongguo Chun have a *Vrn-D1a* genotype.

Analysis of allelic variation in wheat photoperiod genes

Analysis of the dominance and recessiveness of the Ppd-A1 and Ppd-B1 gene

Specific amplification analysis using primers TaPpd-Al-F1 and TaPpd-Al-R2, TaPpd-Al-F1 and TaPpd-Al-R3 on the genomic DNA of 12 wheat varieties showed that the primers TaPpd-Al-F1 and TaPpd-Al-R2 did not amplify any bands; however, primers TaPpd-Al-F1 and TaPpd-Al-R3 amplified a target band of 299bp

(Figure 1k, Figure 1 l), indicating a *Ppd-Alb* genotype. Specific amplification using primers TaPpd-B1-F1 and TaPpd-B1-R1 on the 12 samples resulted in the amplification of a 1292bp band (Figure 1m), indicating a *Ppd-B1b* genotype.

Analysis of the dominance and recessiveness of the Ppd-D1 gene

Specific amplification analysis using primers TaPpd-D1-F1 and TaPpd-D1-R1 on the genomic DNA of 12 wheat varieties showed that only Lu Mai 21 and Zhongguo Chun did not amplify any bands, while the remaining 10 varieties all amplified a 288bp band (Figure 1n), indicating a *Ppd-D1a* genotype. Specific amplification analysis using primers TaPpd-D1-F1 and TaPpd-D1-R2 on the genomic DNA of 12 wheat varieties showed that only Lu Mai 21 and Zhongguo Chun amplified a 415bp band, indicating a *Ppd-D1b* genotype; the remaining 10 varieties did not amplify any bands (Figure 1o).

Composition and developmental characteristics of vernalization and photoperiod genes

Molecular detection of vernalization and photoperiod genes in 12 wheat varieties showed that among them, Lu Mai 20 and Zhongguo Chun carry a dominant vernalization gene *Vrn-D1*, theoretically exhibiting a spring wheat phenotype. The remaining 10 wheat varieties have recessive vernalization genotypes, theoretically exhibiting a winter wheat phenotype. Photoperiod gene detection results indicate that Lu Mai 21 and Zhongguo Chun carry the *Ppd-D1b* gene, theoretically showing sensitivity to the photoperiod; the other 10 wheat varieties carry the *Ppd-D1a* gene, theoretically showing insensitivity to the photoperiod. The composition of vernalization and photoperiod genotypes and developmental characteristics of the 12 wheat materials are as shown in Table 3.

Table 3: Characteristics of vernalization and photoperiodic genotype development.

Cultivar name	Genotypes	Developmental characteristi
Lu Mai 20	<i>vrn-A1 vrn-B1 Vrn-D1a vrn-B3 Ppd-Alb Ppd-B1b Ppd-D1a</i>	Spring photoperiod insensitive
Gu Shen 19	<i>vrn-A1 vrn-B1 vrn-D1 vrn-B3 Ppd-Alb Ppd-B1b Ppd-D1a</i>	Winter photoperiod insensitivity
Yan Mai Cao 1	<i>vrn-A1 vrn-B1 vrn-D1 vrn-B3 Ppd-Alb Ppd-B1b Ppd-D1a</i>	Winter photoperiod insensitivity
Lu Mai 21	<i>vrn-A1 vrn-B1 vrn-D1 vrn-B3 Ppd-Alb Ppd-B1b Ppd-D1b</i>	Winter photoperiod sensitivity
Xu Mai 1901	<i>vrn-A1 vrn-B1 vrn-D1 vrn-B3 Ppd-Alb Ppd-B1b Ppd-D1a</i>	Winter photoperiod insensitivity
Zhongguo Chun	<i>vrn-A1 vrn-B1 Vrn-D1a vrn-B3 Ppd-Alb Ppd-B1b Ppd-D1b</i>	Spring photoperiod sensitivity
Jin Mai 1917	<i>vrn-A1 vrn-B1 vrn-D1 vrn-B3 Ppd-Alb Ppd-B1b Ppd-D1a</i>	Winter photoperiod insensitivity
Jin Mai 2914	<i>vrn-A1 vrn-B1 vrn-D1 vrn-B3 Ppd-Alb Ppd-B1b Ppd-D1a</i>	Winter photoperiod insensitivity
He Da 310	<i>vrn-A1 vrn-B1 vrn-D1 vrn-B3 Ppd-Alb Ppd-B1b Ppd-D1a</i>	Winter photoperiod insensitivity
He Da 669	<i>vrn-A1 vrn-B1 vrn-D1 vrn-B3 Ppd-Alb Ppd-B1b Ppd-D1a</i>	Winter photoperiod insensitivity
Ji Mai 44	<i>vrn-A1 vrn-B1 vrn-D1 vrn-B3 Ppd-Alb Ppd-B1b Ppd-D1a</i>	Winter photoperiod insensitivity
Zhou Mai 18	<i>vrn-A1 vrn-B1 vrn-D1 vrn-B3 Ppd-Alb Ppd-B1b Ppd-D1a</i>	Winter photoperiod insensitivity

Discussion

Palomino's research indicates that varieties exhibiting spring growth habits carry at least one dominant *VRN-1* allelic gene; varieties with winter growth habits carry recessive allelic combinations of the *VRN-1* gene [24]. This study extracted DNA from experimental materials and conducted STS molecular detection, analyzing the dominance and recessiveness of vernalization genotypes. The dominant *Vrn-D1a* allele was less common, found only in Lu Mai 20 and Zhongguo Chun wheat varieties; the recessive *VRN-1* alleles were more common, found in 10 wheat varieties. This suggests that recessive vernalization genotypes are more prevalent in wheat cultivation in Henan Province, indicating that winter wheat varieties are predominant. This is consistent with Chen's findings, which collected 198 wheat varieties from China's Huang-Huai wheat region for growth habit research, showing that winter varieties are most common in the Huang-Huai wheat region, reflecting the current production status of winter wheat varieties in Henan; *VRN-1* plays a major role in controlling vernalization and photoperiod responses in this region [25]. Mohammed's research also shows that the need for vernalization in wheat is controlled by vernalization genes [26]. This study follows previous research methods to judge the developmental characteristics of 12 experimental materials, and the results are consistent with previous research.

Photoperiod-insensitive genes can promote early maturation of wheat, avoiding adverse late-stage environments (such as hot, dry winds and rainy weather), and increase the double-cropping index. In central and southern Europe, the application of the photoperiod-insensitive gene *Ppd-D1a* has increased wheat yields by 15% to 35%, but in the colder climate of western Europe, the *Ppd-D1a* gene can cause a reduction in wheat yield [27]. Photoperiod-sensitive genes delay wheat maturity, avoiding early low-temperature damage, so *Ppd-D1b* is mainly distributed in areas with higher latitudes and lower temperatures, such as Canada, the northwestern United States, and northeastern China. Grogan's research indicates that an increase in the number of varieties carrying photoperiod-insensitive alleles contributes to greater adaptability to specific environments [28]. This study found that only two varieties in the Henan region carry the photoperiod-sensitive gene *Ppd-D1b*; besides Zhongguo Chun, only Lu Mai 21 is a newly selected material, indicating that the photoperiod-sensitive gene *Ppd-D1b* was eliminated early in the development of modern breeding in the Henan region. The introduction of the photoperiod-insensitive gene *Ppd-D1a* is significant for increasing wheat production and income in Henan, consistent with Yang et al.'s research [29].

Henan Province is a major wheat-producing area in China and a transitional zone from winter wheat to spring wheat. The main production is winter wheat, with some areas also planting spring wheat. As seed resources continue to be exchanged worldwide,

wheat genotypes are becoming more diverse. Researching different temperature-light combination types has significant scientific value for elucidating wheat temperature-light response characteristics. This is one of the important theoretical bases for increasing wheat yield, and the results of this study are beneficial for the regional distribution, introduction, and cultivation of wheat varieties, clarifying the growth and development patterns of wheat vernalization and photoperiod genotypes. These findings will be used for variety improvement and breeding in future research, providing a reference for the rational utilization of wheat varieties.

Conclusion

In the wheat production area of Henan province, winter wheat varieties were the main varieties and photoperiod insensitive wheat varieties were the main varieties. It is fully explained that the wheat area of Henan province is the transition zone from winter wheat to spring wheat, and winter wheat is mainly planted in production, and spring wheat is also planted in some areas.

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