

Fig. S1 Linkage analysis of *WFZP-A*, *WFZP-D* and YM44 SSs phenotype. This linkage analysis was conducted using another batch of individuals in the F₂ population differing from those used in Fig. 2A.

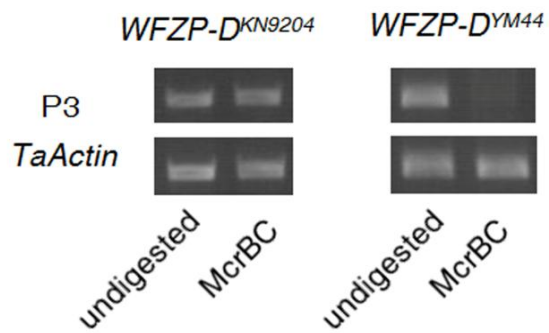


Fig. S2 McrBC analysis of the promoter of *WFZP-D* in the P3 region in the individuals of F₂ crossed by KN9204 and YM44. *WFZP*^{KN9204} indicating individuals with the *WFZP-D* homozygous genotype deriving from KN9204, and *WFZP*^{YM44} indicating individuals with the *WFZP-D* homozygous genotype deriving from YM44.

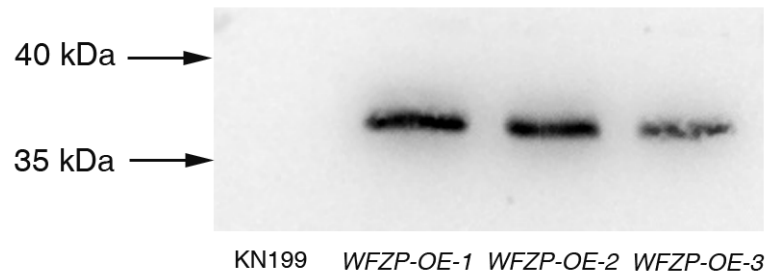


Fig. S3 Western blotting of *WFZP* OE lines expressing the protein of *WFZP* fused with 3 FLAGs.

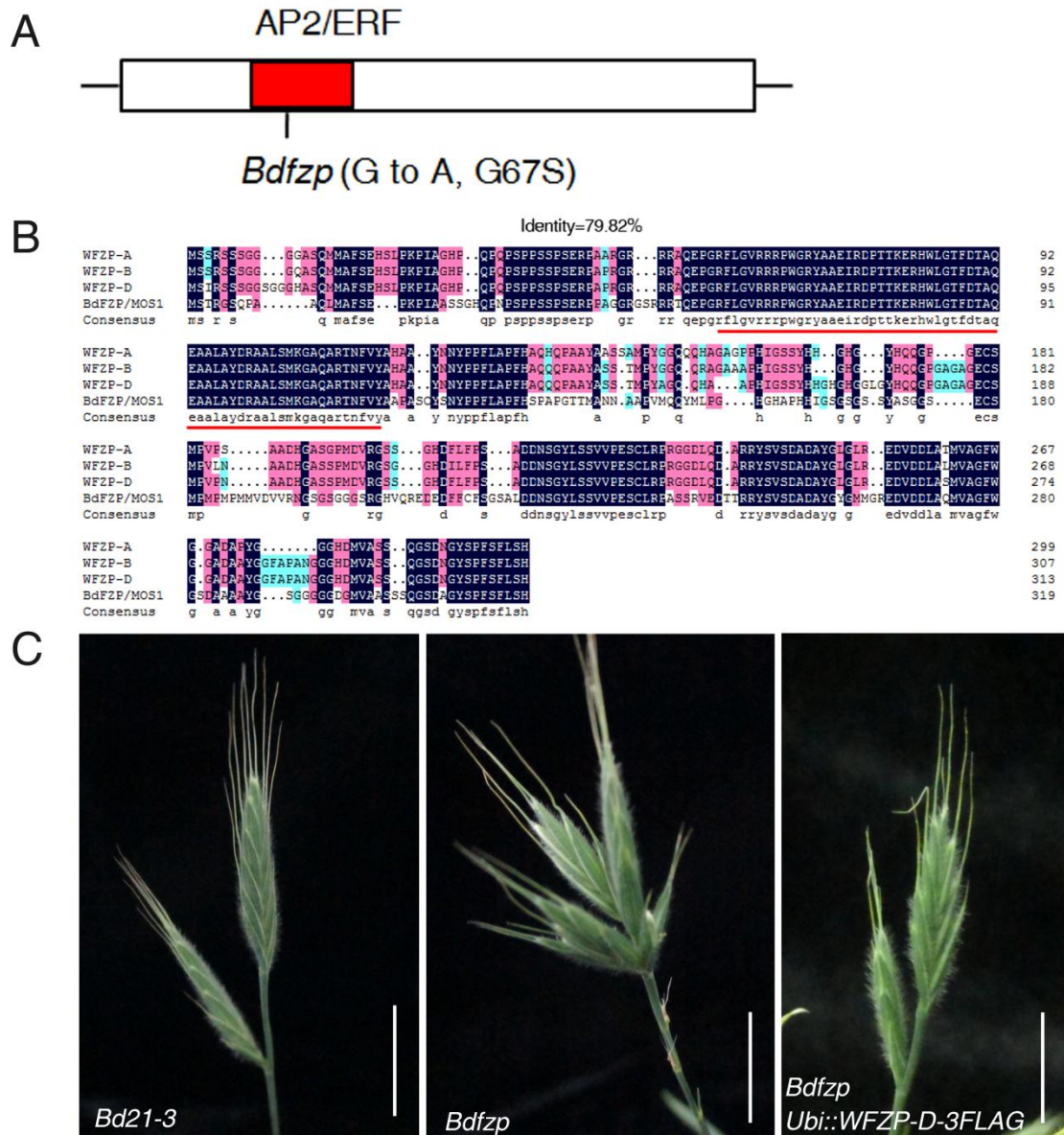


Fig. S4 Functional verification of *WFZP*. (A) The schematic diagram of the sequence of *BdFZP* in *Bdfzp* mutant. A G-to-A mutation occurred in AP2/ERF domain (red color). (B) The protein alignment of *WFZP*-A, B, D and *BdFZP*. (C) The spike of *Bd21-3*, *Bdfzp* and *Ubi::WFZP-D* in *Bdfzp*. Bars = 1cm in C.

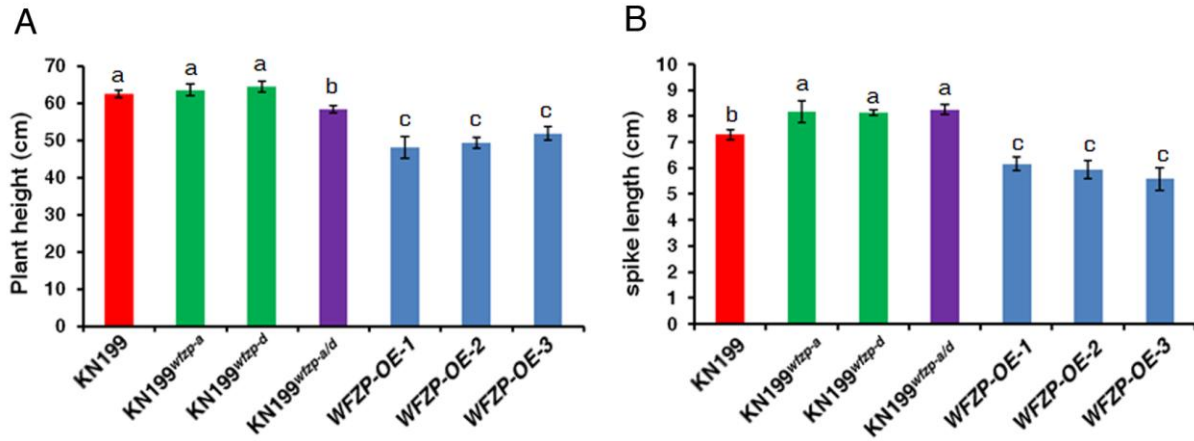


Fig. S5 Statistics comparison of plant height (A) and spike length (B) between KN199, KN199^{wfzp-a}, KN199^{wfzp-d}, KN199^{wfzp-a/d} and WFZP OE lines. The error bars denote ± SE. Different letters mean significant difference at $P < 0.01$.

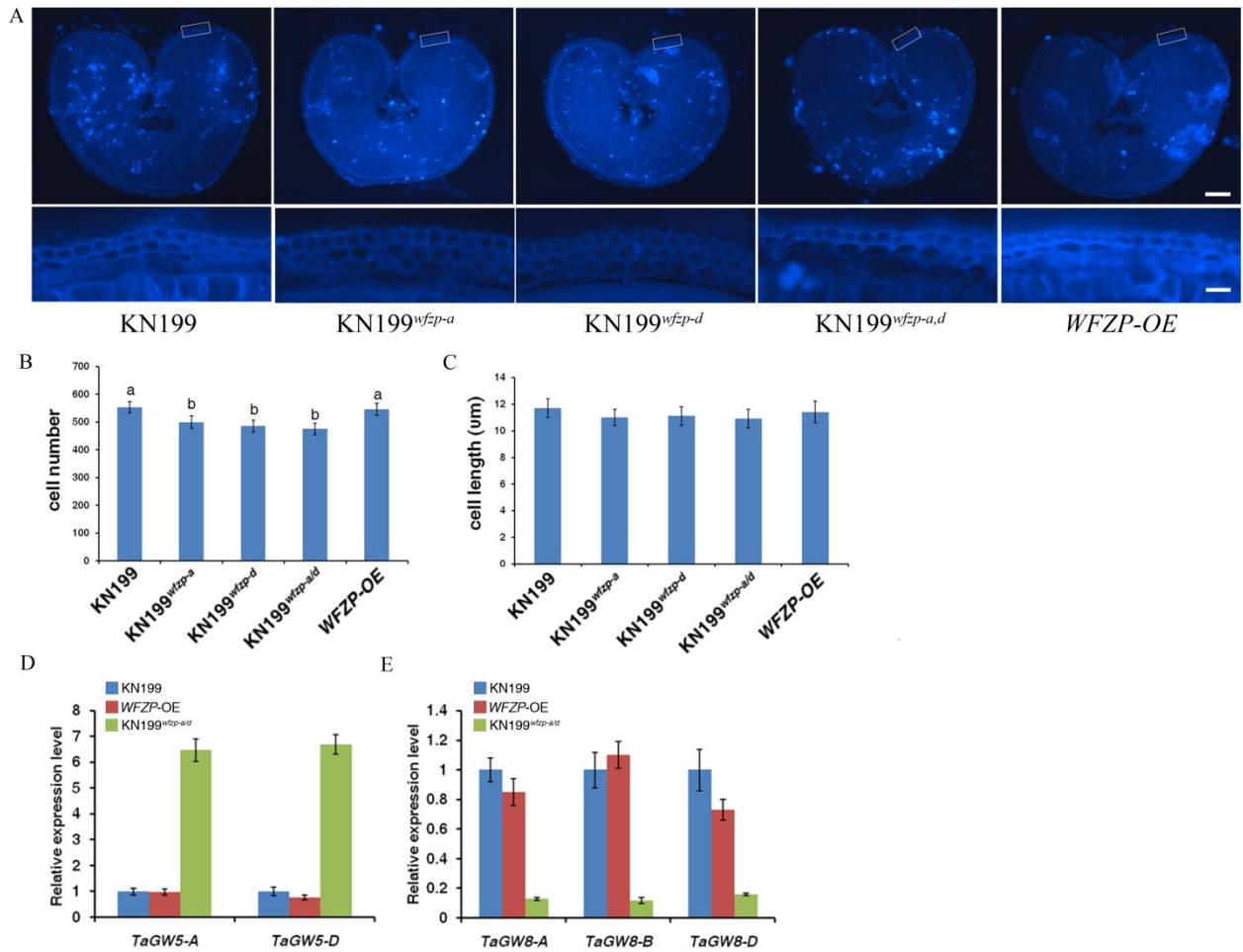


Fig. S6 Effects of *WFZP* on seed coat cell proliferation. (A) Cross sections at the middle of seeds of KN199, KN199^{wfzp-a}, KN199^{wfzp-d}, KN199^{wfzp-a/d} and *WFZP* OE lines stained with Fluorescent Brightener 28. Figures in the bottom part were magnified figures in the pane in the upper part. (B) and (C) Statistics comparison of cell number (B) and cell length (C) in the cross section of the outer seed integuments of KN199, KN199^{wfzp-a}, KN199^{wfzp-d}, KN199^{wfzp-a/d} and *WFZP* OE lines. The error bars denote ± SE. Different letters mean significant difference at $P < 0.01$. (D) and (E) The expression level of *TaGW5* (D) and *TaGW8* (E) in KN199, *WFZP* OE, and KN199^{wfzp-a/d} detected by qPCR. The error bars denote ± SE.

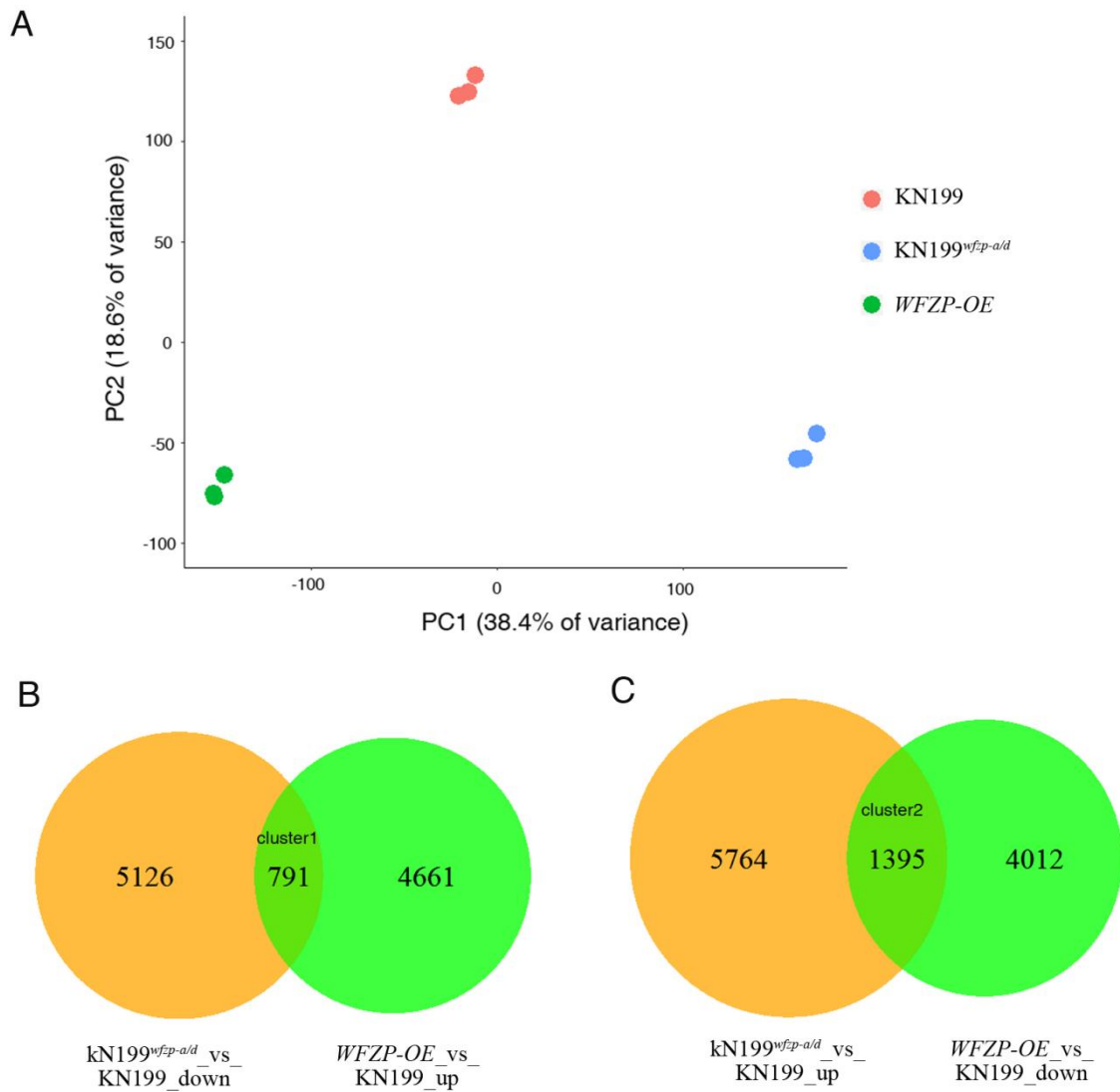


Fig. S7 The overall view of RNA-seq data. (A) The PCA result of RNA-seq data of different samples. (B) The overlapped DEGs between KN199^{wfp-a/d}_vs_KN199 down-regulated genes and WFZP-OE_vs_KN199 up-regulated genes. (C) The overlapped DEGs between KN199^{wfp-a/d}_vs_KN199 up-regulated genes and WFZP-OE_vs_KN199 down-regulated genes.

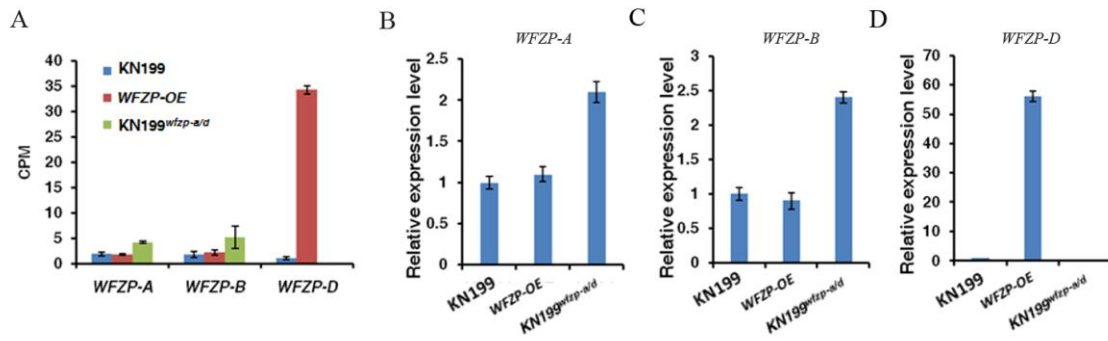


Fig. S8 The expression level of *WFZP-A*, *B* and *D* in KN199, *KN199^{wfzp-a/d}* and *WFZP OE* lines detected by RNA-seq (A) and qPCR (B to D). The error bars denote \pm SE.

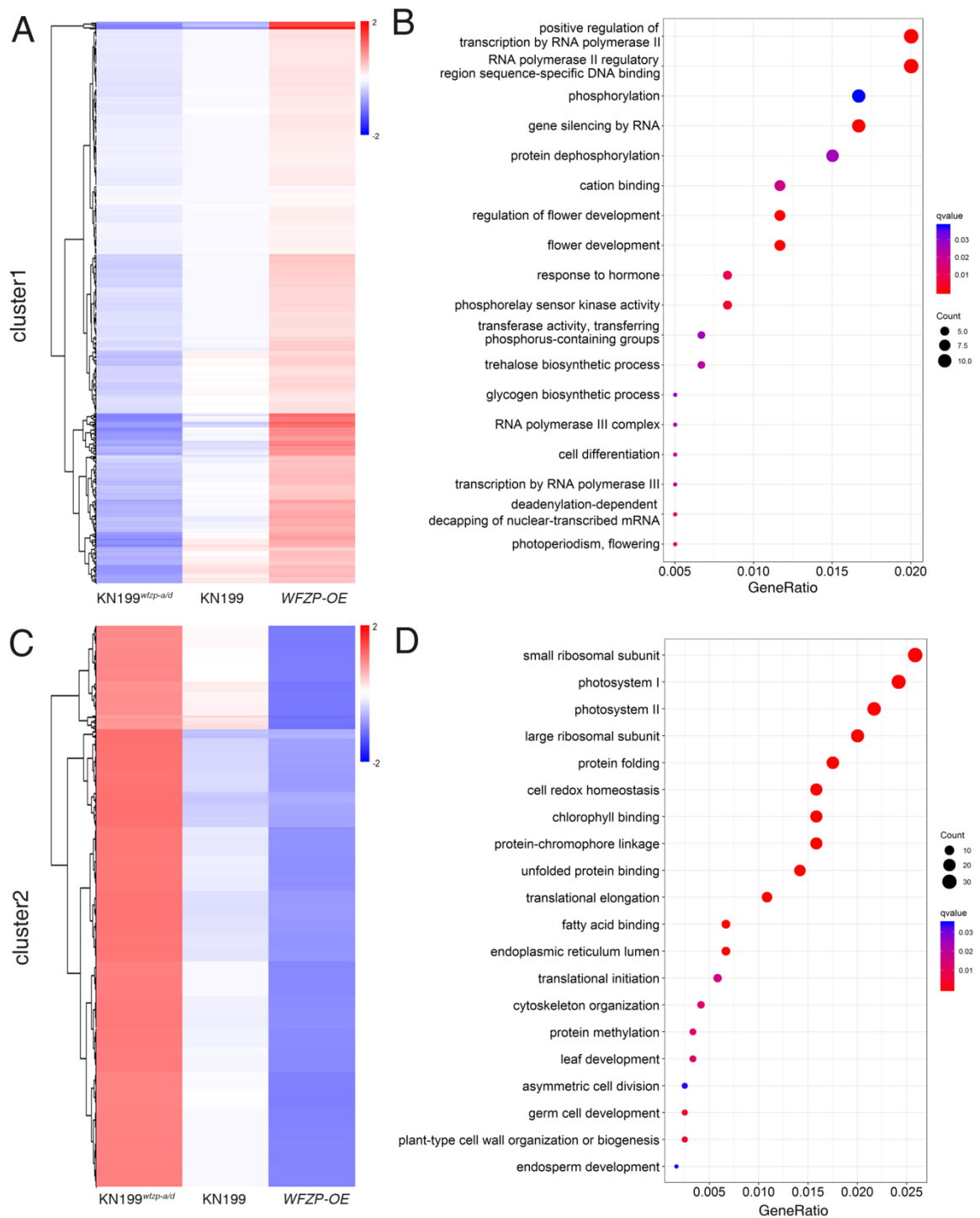


Fig. S9 GO enrichment analysis of genes in the overlapped DEGs of *KN199^{wfp-a/d}* up-regulated/down-regulated genes and *WFZP-OE* down-regulated/up-regulated genes. (A) Heatmap of genes down-regulated in *KN199^{wfp-a/d}* and up-regulated in *WFZP-OE* line. (B) GO enrichment analysis of genes down-regulated in *KN199^{wfp-a/d}* and up-regulated in *WFZP-OE* line. (C) Heatmap of genes up-regulated in *KN199^{wfp-a/d}* and down-regulated in

WFZP-OE line. (D) GO enrichment analysis of genes up-regulated in *KN199^{wfzp-a/d}* and down-regulated in *WFZP-OE* line.

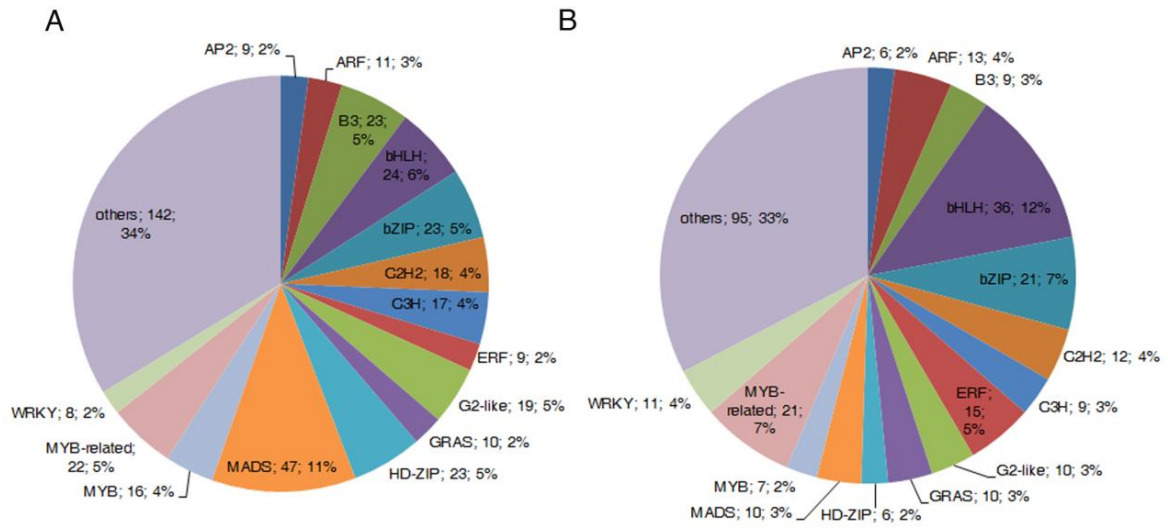


Fig. S10 Transcription factors in the KN199^{wfzp-a/d}_vs_KN199 down-regulated genes (A) and KN199^{wfzp-a/d}_vs_KN199 up-regulated genes (B).

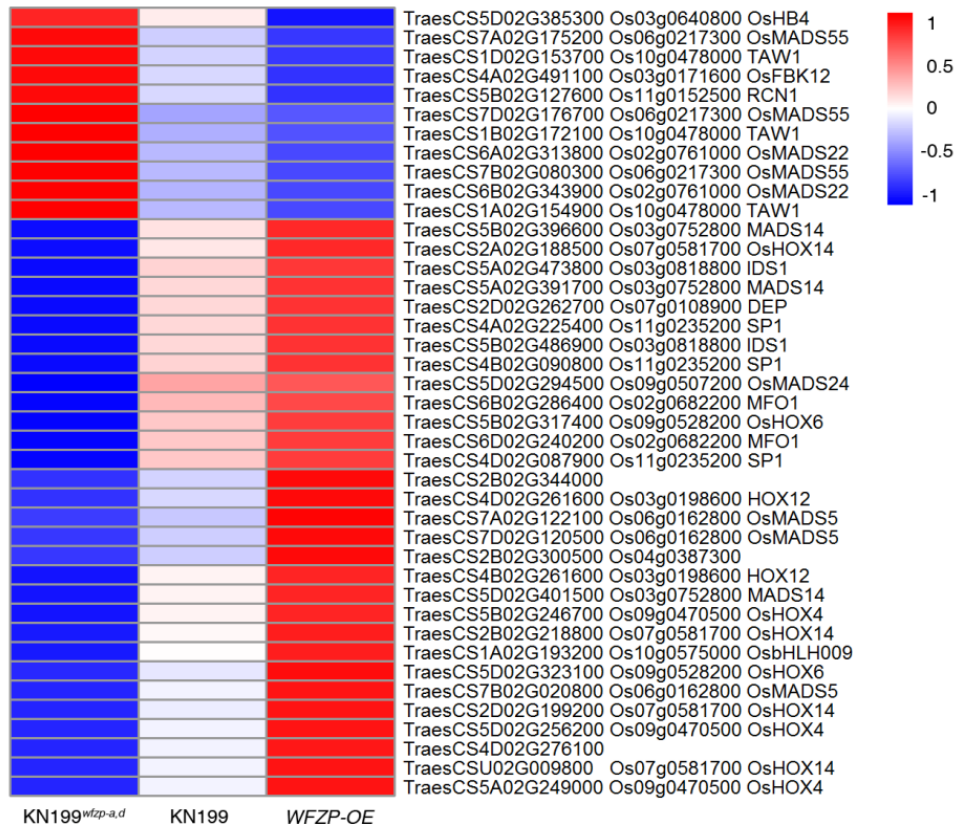


Fig. S11 Heatmap of MADS family TFs, HD-ZIP family TFs and other development related genes in the overlapped DEGs of KN199^{wfp-a/d} up-regulated/down-regulated genes and WFZP-OE down-regulated/up-regulated genes.

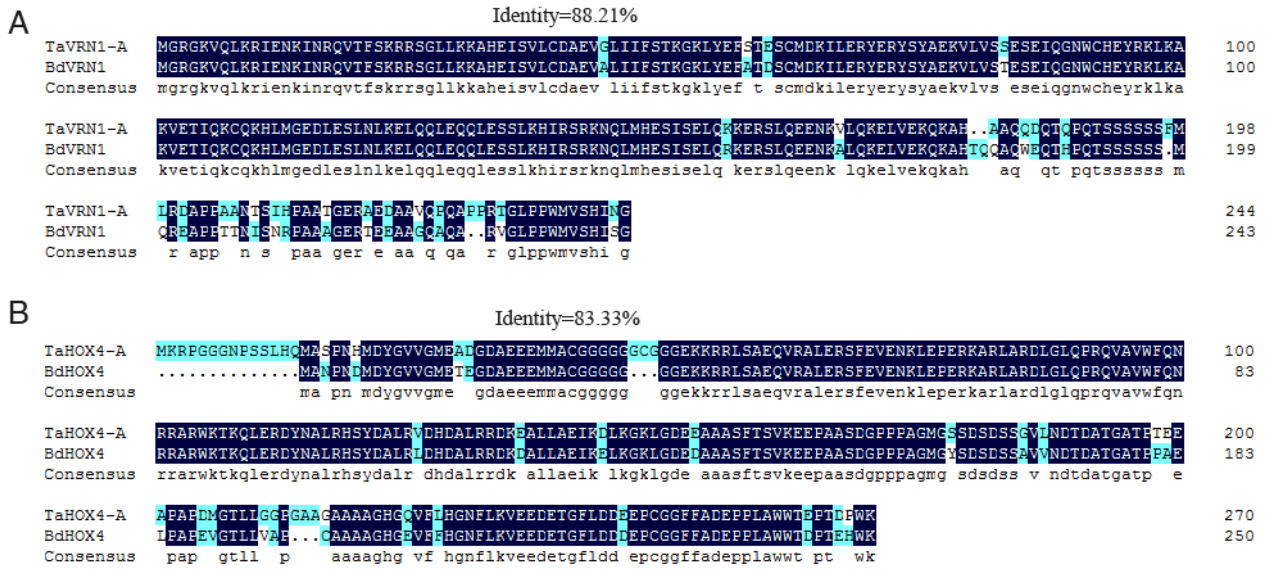


Fig. S12 The protein alignment of TaVRN1-A and BdVRN1 (A) and TaHOX4-A and BdHOX4 (B).

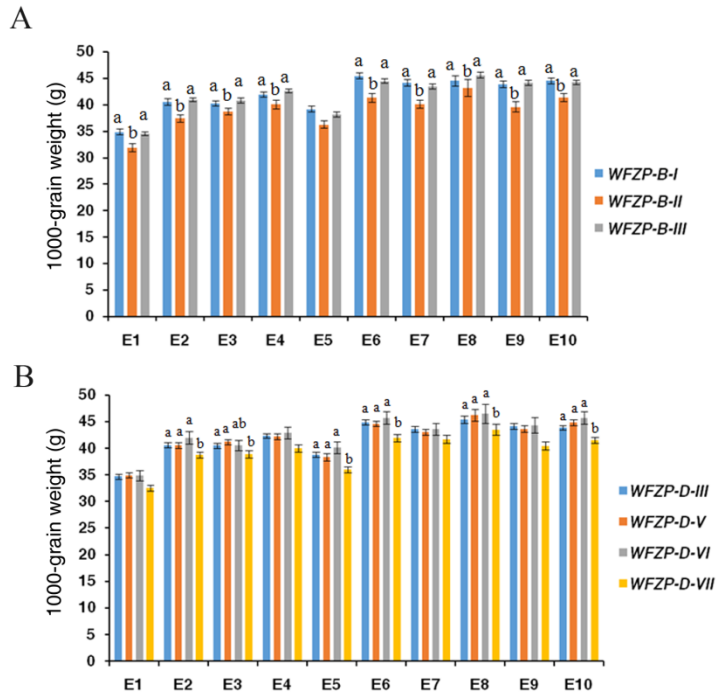


Fig. S13 1000-grain weight of cultivars with different haplotypes of *WFZP-B* (A) and *WFZP-D* (B) in ten growing environments. The error bars denote \pm SE. Different letters mean significant difference at $P < 0.05$. Note that the number of cultivars with *WFZP-D-I*, *II* and *IV* were too few (2, 3 and 2 for each, Supplemental dataset S7), so only the other four haplotypes were analyzed.

Table S1. The polymorphism of *WFZP-B*

No.	Site	Variation type	<i>WFZP-B-I</i>	<i>WFZP-B-II</i>	<i>WFZP-B-III</i>
1	-1987 - -1933	Indel	Del	Del	In
2	-1839	SNP	T	T	C
3	-1809- -1808	SNP	CT	CT	TC
4	-1747	SNP	T	T	C
5	-1726	SNP	C	C	T
6	-1419	SNP	T	T	C
7	-1208	SNP	T	T	C
8	-1187	SNP	A	A	G
9	-867	SNP	C	C	G
10	-785	SNP	A	A	G
11	-752- -750	Indel	Del	Del	In
12	-559	SNP	G	G	A
13	-545	SNP	T	T	C
14	-484	SNP	G	A	G
15	-482	SNP	A	A	G
16	-469	SNP	C	C	A
17	-424- -412	Indel	Del	Del	In
18	-392	SNP	T	T	C
19	-374	SNP	C	C	T
20	-362	SNP	A	A	G
21	-330- -315	Indel	Del	Del	In
22	-311	SNP	T	T	C
23	-308	SNP	A	A	G
24	-269	SNP	G	G	A
25	-137	SNP	T	T	A
26	144	SNP	T	T	C
27	491	SNP	G	G	A
28	619	SNP	T	T	A

Variations labeled with the same color were linked.

Variations highlighted were selected to develop molecular markers.

Table S2. The polymorphism of *WFZP-D*

No.	Site	Variation type	<i>WFZP-D-I</i>	<i>WFZP-D-II</i>	<i>WFZP-D-III</i>	<i>WFZP-D-IV</i>	<i>WFZP-D-V</i>	<i>WFZP-D-VI</i>	<i>WFZP-D-VII</i>
1	-1974	SNP	G	G	G	C	C	C	G
2	-1425	SNP	G	G	G	A	A	A	G
3	-1410	Indel	In	Del	In	Del	In	Del	Del
4	-1344	SNP	T	T	T	C	C	C	T
5	-1261	SNP	T	T	T	G	G	G	T
6	-1183	SNP	T	T	T	C	C	C	T
7	-1166	SNP	C	C	C	T	T	T	C
8	-1164	SNP	C	T	C	T	C	T	T
9	-1063	SNP	T	C	C	T	T	C	T
10	-908	SNP	T	T	T	A	A	A	T
11	-843	SNP	C	A	C	A	C	A	A
12	-742	SNP	T	T	T	A	A	A	T
13	-528	SNP	A	G	A	G	A	G	G
14	-378	SNP	T	T	T	A	A	A	T
15	552	SNP	C	C	C	G	G	G	C

Variations labeled with the same color were linked.

Variations highlighted were selected to develop molecular markers.

Table S3. Association analysis of *WFZP* with SNS and TGW

environment	Years	location	Water and temperature	P-value					
				<i>WFZP-A</i>		<i>WFZP-B</i>		<i>WFZP-D</i>	
				SNS	TGW	SNS	TGW	SNS	TGW
E1	2015	Shunyi	DS+HS	0.00496*	0.0075*	0.77482	0.03662*	0.96727	0.05439
E2	2015	Shunyi	DS	0.01641*	0.0001376*	0.74611	5.026E-06*	0.33573	0.02965*
E3	2015	Shunyi	WW+HS	0.02615*	0.22213	0.39132	0.03341*	0.92688	0.03128*
E4	2015	Shunyi	WW	0.08337	0.0006261*	0.93637	0.006*	0.74899	0.06958
E5	2016	Shunyi	DS+HS	0.02464*	0.53468	0.07453	0.07095	0.95462	0.02916*
E6	2016	Shunyi	DS	0.00316*	0.00358*	0.14227	5.388E-05*	0.733	0.01924*
E7	2016	Shunyi	WW+HS	0.18959	0.08703	0.18488	5.749E-05*	0.95009	0.66021
E8	2016	Shunyi	WW	0.02339*	6.935E-05*	0.14266	0.00689*	0.61586	0.0176*
E9	2016	Changping	WW	0.00663*	0.07545	0.05396	0.0001063*	0.93546	0.0554
E10	2016	Changping	DS	5.201E-05*	0.00313*	0.31636	0.0002177*	0.19367	0.0003555*

SNS, spikelet number per spike; TGW, thousand grain weight; DS, drought stress; HS, heat stress; WW, well-watered.

Table S4. Primers used in this study

Primer name	Sequence	Aim and introduction	Reference
Primers for construction			
WFZP-2DOE-F	CGACTCTAGAGGATCCTCAGTTCTGCCATGAGCATC	Clone <i>WFZP-D</i> into pTCK303-3FLAG vector	
WFZP-2DOE-R	GCTTGGCGCGACTAGTGTTGTTTCTGTGGGAGAGGAAG		
VRN1-OE-F	CGACTCTAGAGGATCCATGGGGCGGGGAAGGT	Clone <i>VRN1-A</i> into pTCK303-3FLAG vector	
VRN1-OE-R	GCTTGGCGCGACTAGTCCCGTTGATGTGGCTCACC		
TaHOX4-OE-F	CGACTCTAGAGGATCCATGAAGCGGCCCGGCGG	Clone <i>TaHOX4-A</i> into pTCK303-3FLAG vector	
TaHOX4-OE-R	GCTTGGCGCGACTAGTCTTCCAGGGATCCGTCGG		
WFZP-pMN6-F	TTGACTGTATCGCCCGGATGAGCATCCGCAGCAGCA	Clone <i>WFZP-D</i> into pMN6 vector	
WFZP-pMN6-R	GGAAATTCGAGCTCGGTACCTCAGTGGGAGAGGAAGCTGAA		
WFZP-A-P-F3	GACCCATAAAGCCATAGATTAGG	Clone <i>WFZP-A</i> promoter into ENTR1A-T vector	
WFZP-A-P-R	GGCAGAAGTGAAGTGAGGTTGG		
LUC-F1-KpnI	CGGGGTACCCCGATGGAAGACGCCAAAAACATA	Clone <i>LUC</i> into the downstream of <i>WFZP-A</i> promoter	
LUC-R1-NotI	ATTTGCGGCCGCTTTATTACAATTTGGACTTTCCGCC		
RLUC-F-SalI	ACGCGTCGACGTGATGACTTCGAAAGTTTATGATCC	Clone <i>R-LUC</i> into ENTR1A-T vector	
RLUC-R-EcoRI	CGGAATTCCGTTATTGTTCAATTTTTGAGAACTCG		

Primers for genotyping			
Xwmc522-F	AAAAATCTCACGAGTCGGGC	SSR marker for linkage analysis	https://wheat.pw.usda.gov/GG3
Xwmc522-R	CCCGAGCAGGAGCTACAAAT		
Cfd56-F	TTGCATAATTACTTGCCCTCC		
Cfd56-R	CTGGTCCAACCTCCATCCAT		
WFZP-D-P-F3	GACCCCATAAAGCCATGGATTAGG	Linkage analysis of <i>WFZP-D</i> and SSs. PCR product was digested using <i>EcoNI</i> .	
WFZP-D-P-R2	CGTCTGAGATAACGCTTTTAGCTG		
WFZP_F3	GCTCACAGTCTCAGCAACCA	Linkage analysis of <i>WFZP-D</i> and SSs. WFZP_F3 + WFZP_2A_R3 used for first round PCR.	(Dobrovolskaya et al., 2015)
WFZP_2A_R3	CACTGGGCACCGGCATGGAA		
WFZP-A-indel-F2	AGCCGCAGCCGTCCCCG		
WFZP-A-indel-R2	AAGCGCCCGGGCTCCTG		
Me-4F	GCAGGCATCTTTACACCATCTTA	dCAPs primer of <i>WFZP-A</i> at -387 loci, Me-4F+2A-R2 used for first round PCR, 387F+387R used for second round PCR, using <i>HinfI</i> to digest the PCR product	
WFZP-2A-R2	TGGCAGAAGTGAAGTGAGGT		(Dobrovolskaya et al., 2015)
WFZP-A-387F	GCAAGGATTGTGGCATGCA		
WFZP-A-387R	CAAAGAGATGAGGAGAATGTTGAG		
WFZP-B-P-F1	GCGGATTAACCTTTGACCTCTG	Indel marker for <i>WFZP-B</i> at -1987 - -1933 loci, F1+Indel-R used for first round PCR, F5+Indel-R used for second round PCR	
WFZP-B-P-F5	GAGCATCTTCAACAGGCGTCA		
WFZP-B-Indel-R	GGGAGCGACGAAGAGGTTT		

WFZP-B-484F	CATTCCCTCCTTTTACTCCT	dCAPs primer of <i>WFZP-B</i> at -484 loci, 484F+P-R used for first round PCR, 484F+484R used for second round PCR, using <i>ScaI</i> to digest the PCR product	
WFZP-B-484R	TATGGTCTTATAGGGACTTATAAGTAC		
WFZP-AB-P-R	GGCAGCGAATGCTCCGAGAAG		
WFZP-D-P-F3	GACCCCATAAAGCCATGGATTAGG	dCAPs primer of <i>WFZP-D</i> at -1974 loci, P-F3+Me1R used for first round PCR, 1974F+1974R used for second round PCR, using <i>Sall</i> to digest the PCR product	
Me1R	GTATCATCAGCGGTCAAAGTTAACG		
WFZP-D-1974F	TGATCCTTGTCCAAGCATTTGTCGA		
WFZP-D-1974R	CTCTCACATGAGGGGAGGGC		
Me-3F	GCCTGCAAATTCTGGGTG	dCAPs primer of <i>WFZP-D</i> at -1063 loci, Me3F+Me3R used for first round PCR, 1063F+1063R used for second round PCR, using <i>ApaI</i> to digest the PCR product	
Me-3R	CTTCAATTATATGGCCCTACATGT		
WFZP-D-1063F	TCTCCTATGTTTATTCTAGTGTGCA		
WFZP-D-1063R	GATACATGCCAAAATGCTACAC		
Me-3F	GCCTGCAAATTCTGGGTG	dCAPs primer of <i>WFZP-D</i> at -843 loci, Me3F+Me3R used for first round PCR, 843F+843R used for second round PCR, using <i>HinfI</i> to digest the PCR product	
Me-3R	CTTCAATTATATGGCCCTACATGT		
WFZP-D-843F	GGCAAGCTCTCTTCTTTTCGATT		
WFZP-D-843R	GCCTTGCATGCTTTGATGAT		
Primers for qPCR or ChIP-PCR			
WFZP_F3	GCTCACAGTCTCAGCAACCA	qPCR of <i>WFZP-A</i>	(Dobrovolskaya et al., 2015)
WFZP_2A_R2	TGGCAGAAGTGAAGTGAGGT		

WFZP_F5	ACGACATGGTCGCCTCGT	qPCR of <i>WFZP-B</i>	(Dobrovolskaya et al., 2015)	
WFZP_2B_R3	CGGTGCATTTGCTTCAGTGT			
WFZP_F5	ACGACATGGTCGCCTCGT	qPCR of <i>WFZP-D</i>	(Dobrovolskaya et al., 2015)	
WFZP_2D_R5	CTGGCTGGTGCATTTGTTG			
WFZP-D-OE-q-F	CACATTGGCAGCTCGTACCA	qPCR of <i>WFZP-D</i> in <i>WFZP</i> overexpression lines		
WFZP-D-OE-q-R	TGGGGAAGAGGAAGTCGTG			
VRN1-q-F	GATCAAACCTCAGCCTCAAACCA	qPCR of selected genes		
VRN1-q-F	CCGCATCCTCTGCCCTCT			
TaHOX4-q-F	TGGCCAGCCCAATCATAT			
TaHOX4-q-R	GGCTCCAGCTTGTTCTCCA			
BdVRN1-q-F	GCTCTGCAGAAGGAACCTTGTTG			
BdVRN1-q-R	CTAGTTTGCGGGTGTGTTTGCTC			
BdHOX4-q-F	GAGATCAAGGAGCTGAAGGG			
BdHOX4-q-R	CTGGAGTCGCTGTCGGAATA			
TaGW5-A-F	CACGGTTCATGGCAGTGAG			
TaGW5-A-R	CACATGATTGATCACAACGATG			
TaGW5-D-F	GTGCAACCGGGTGGAGGA			
TaGW5-D-R	TCACCACCTCCTCTGCAAGG			
				(Ream et al., 2014)

TaGW8-7A-RTF	GCAGGCAGCATTTTGGTGTA		(Ma et al., 2019)	
TaGW8-7A-RTR	TGGGGATGTGTTCACTCTGC			
TaGW8-7B-RTF	CTGACTCCAGTGCTGGACTCA			
TaGW8-7B-RTR	GTTGCTCATTTTCCCCACA			
TaGW8-7D-RTF	ACAGGCAGCATTTTGGTCTC			
TaGW8-7D-RTR	TCTGGCCAACATCGATACCG			
VRN1-ChIP-F1	GGAAACCAAGTAATCACTAACTTG	ChIP-PCR of certain regions of selected genes		
VRN1-ChIP-R1	TGCGGTGTATCTCCAAGAATG			
VRN1-ChIP-F2	GACCTAGCCAGCCAGCATT			
VRN1-ChIP-R2	GAAGGGAAAGAGCGGAGTT			
VRN1-ChIP-F3	CCTCACCCAACCACCTGA			
VRN1-ChIP-R3	CAACCCTACGCCCTACC			
TaHOX4-ChIP-F1	CTATGGGAGATGTCCTAAACC			
TaHOX4-ChIP-R1	ACATTACACTCCAGCAAAGAA			
TaHOX4-ChIP-F2	CTCTTCTCGCAGTAATCGG			
TaHOX4-ChIP-R2	GGAGGGAGGAGGGGTTT			
Primers for amplifying templates of in situ probes				
WFZP- FSP6	GATTTAGGTGACACTATAGAATACATGAGCATCCGCAGCAGCAG		To amplify templates of in situ	

WFZP- RT7	TGTAATACGACTCACTATAGGGCGATCAGTGGGAGAGGAAGCTG	probes of selected genes	
VRN1-FSP6	GATTTAGGTGACACTATAGAATACATGGGGCGGGGGAAGGT		
VRN1-RT7	TGTAATACGACTCACTATAGGGCGACCCGTTGATGTGGCTCACC		
TaHOX4-FSP6	GATTTAGGTGACACTATAGAATACATGAAGCGGCCCGGGCGG		
TaHOX4-RT7	TGTAATACGACTCACTATAGGGCGACTTCCAGGGATCCGTCGG		
Primers for McrBC analysis			
Me-1F	GACCCCATAAAGCCATGGATTAGG	McrBC analysis of certain region in the promoter of <i>WFZP-D</i>	
Me-1R	GTATCATCAGCGGTCAAAGTTAACG		
Me-2F	AGTCTGAGTGGTCAAGACGCG		
Me-2R	AAACACGGCACCCAATCC		
Me-3F	GCCTGCAAATTCTGGGTG		
Me-3R	CTTCAATTATATGGCCCTACATGT		
Me-4F	GCAGGCATCTTTACACCATCTTA		
Me-4R	GGATGAGATGGCGTGATAGG		
Me-5F	GACTGGCACAACATTCTCCTCC		
Me-5R	TGCTGCTGCGGATGCTCA		
Primers for SAAB			

SAAB-oligonucleotide	GAGAGGATCCAGTCAGCATGNNNNNNNNNNNNNNNNNNNNNNCT CAGCCTCGAGAATTCCAA		(Smith et al., 2002)
SAAB-F	GGGCTGGCAAGCCACGTTTGGTG		
SAAB-R	CCGGGAGCTGCATGTGTGTCAGAGG		

Reference

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