

The insertion of a novel retrotransposon in the promoter of a vernalization gene resulted in early flowering in tetraploid wheat

**Chenggen Chu^{1,2}, Chor-Tee Tan³, Guotai Yu²,
Shaobin Zhong², Steven S. Xu⁴ and Liuling
Yan³,**

(1)Heartland Plant Innovations, Inc., Manhattan, KS, USA

(2)North Dakota State University, Fargo, ND, USA

(3)Oklahoma State University, Stillwater, OK, USA

(4)USDA-ARS, Fargo, ND, USA

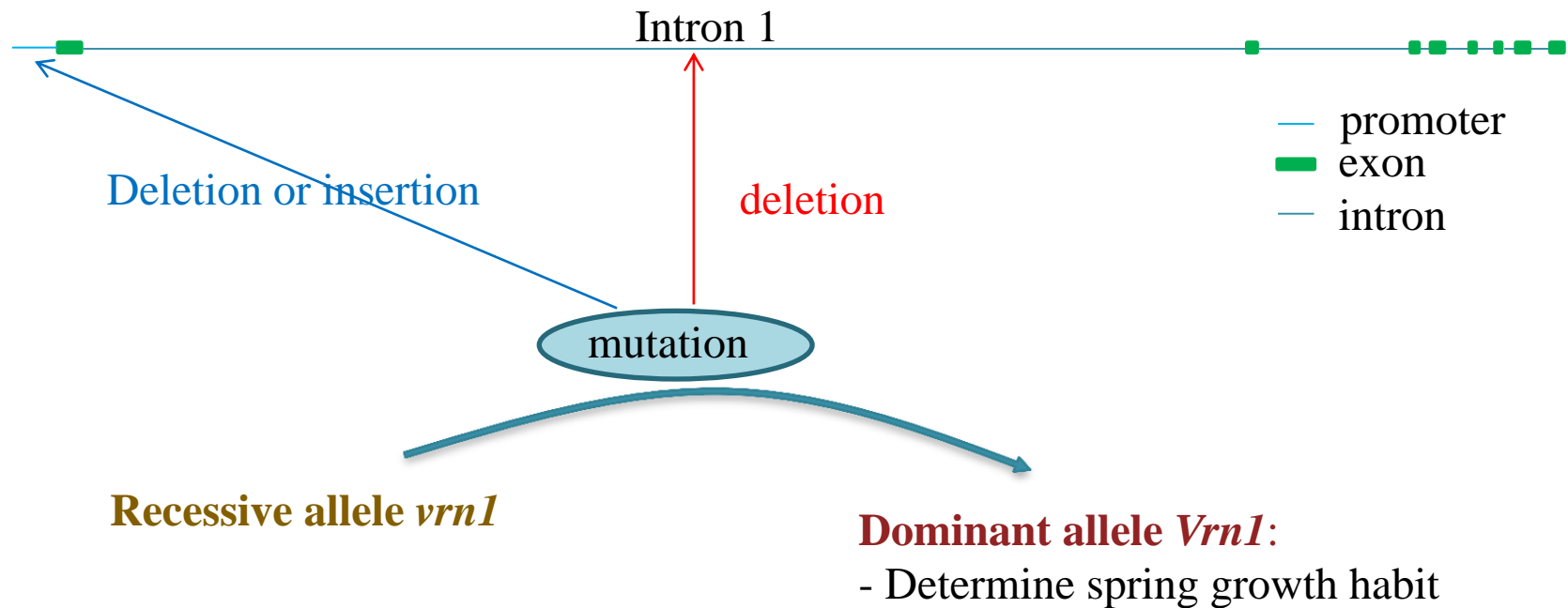
Wheat growth habit

- Wheat (*Triticum* L. sp.) crops are divided into winter and spring wheat.
 - Winter wheat---- requires an exposure to a period of low temperature to induce flowering (Vernalization requirement)
 - Vernalization genes (*VRN* genes) determine Vernalization requirement.
- Three homoeologues of the *VRN1* gene, *VRN-A1*, *VRN-B1*, and *VRN-D1* on chromosome arms 5AL, 5BL, and 5DL, respectively, are major genes determining growth habit in hexaploid wheat (*T. aestivum* L, $2n = 6x = 42$, AABBDD).
- A dominant allele from any of the three genes leads to spring growth habit, only when all three genes were recessive leads to winter growth habit

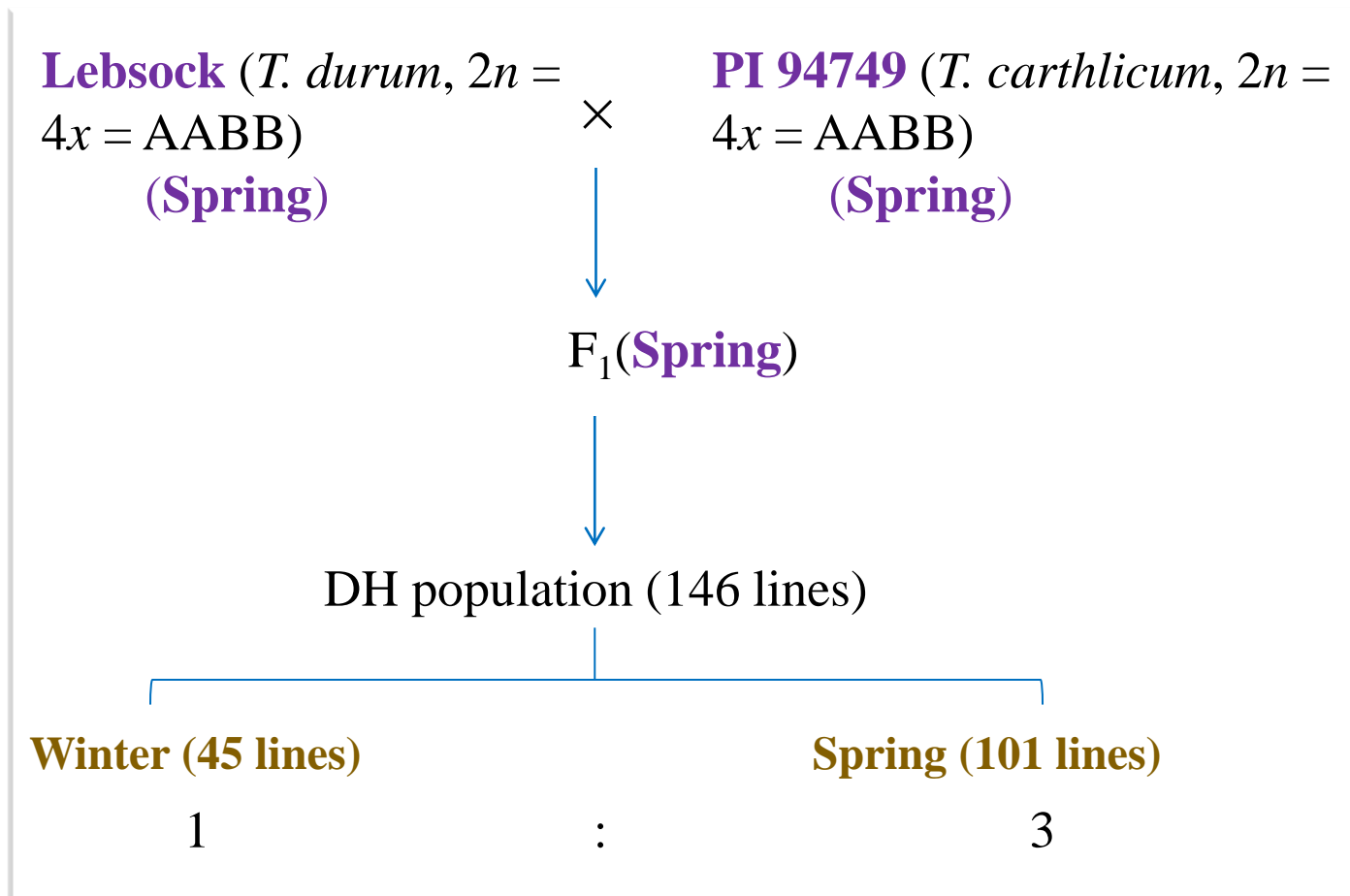
Wheat *VRN1* gene structure (Yan et al. 2003)

Recessive allele *vrn1*:

- Determine winter growth habit
- Complete sequence: ~12 kb
- Eight exons and 7 introns

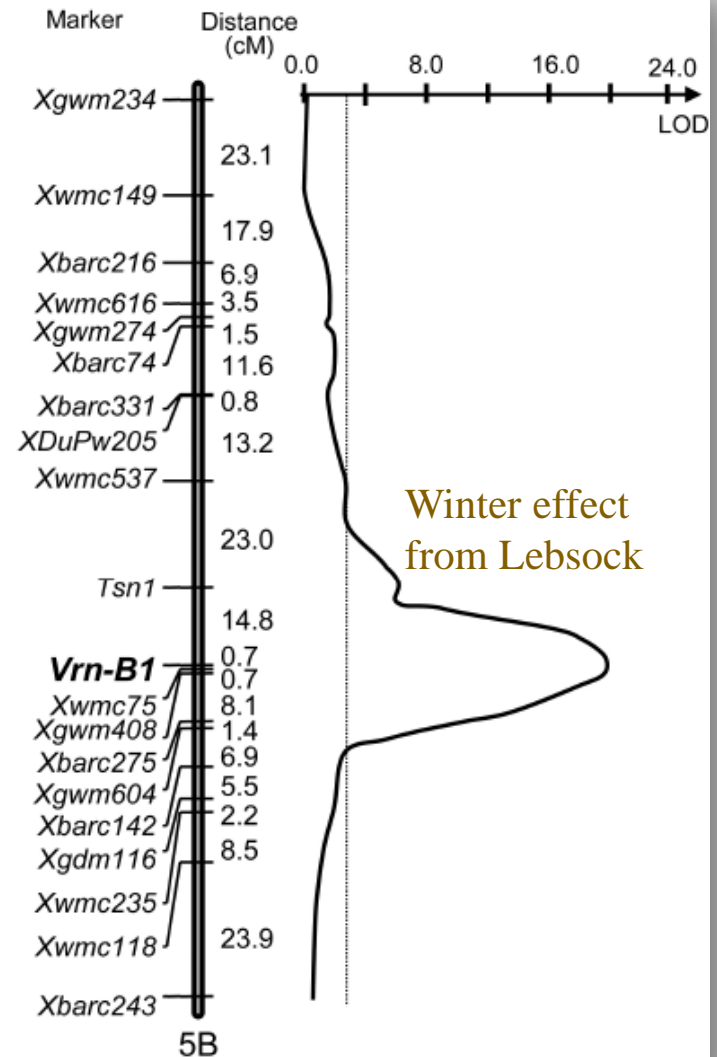
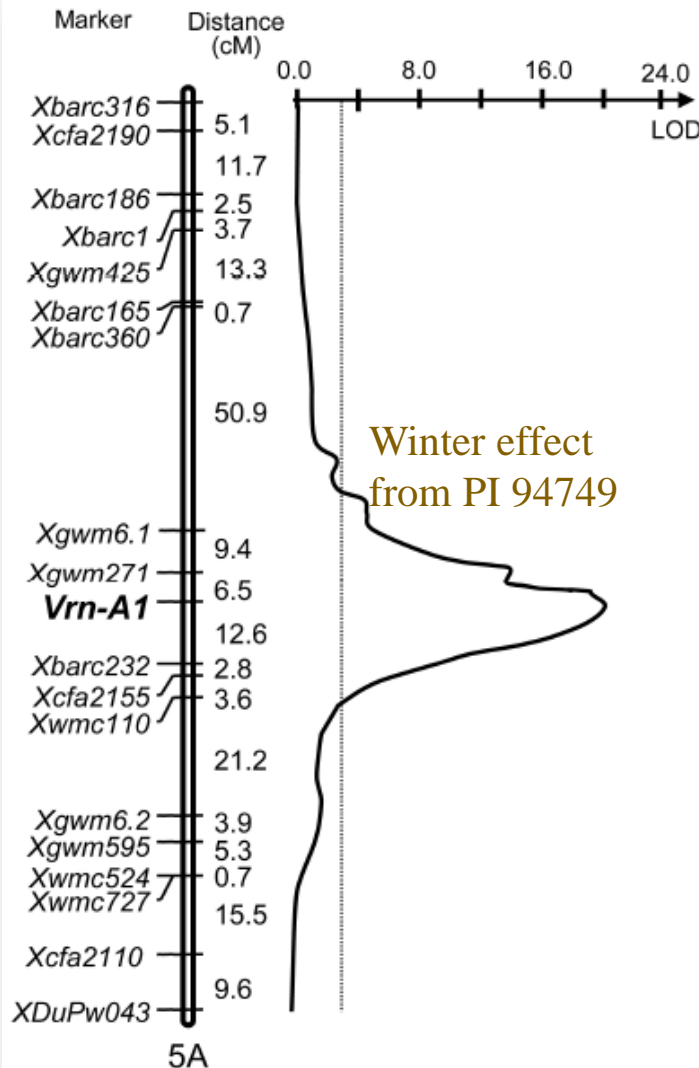


Growth habit segregation in a tetraploid wheat doubled haploid (DH) population



Chu C, Tan C., Yu G., Zhong S., Xu S. and Yan L. (2011) G3: Genes, Genomes, Genet. 1:637-645.

QTL analysis for growth habit in DH population



Analysis in F₂ and two BC₁F₂ populations

Population	No. of plants			
	Total	Winter	Spring	
F ₂	200	13	187	(15:1)
Lebsock/PI 94749//Lebsock	100	0	100	
Lebsock/PI 94749//PI 94749	100	0	100	



Lebsock: *Vrn-A1Vrn-A1vrn-B1vrn-B1*

PI 94749: *vrn-A1vrn-A1Vrn-B1Vrn-B1*

Allelic variation on *VRN-A1*

vrn-A1

Intron 1

PI	GAAAGGAAAAAATTCTGCTCGTTTTTTTTTCTCTGTGGTGTGTGTTTGTGGCGAGAGAGAAAAAT
LB	GAAAGGAAAAAATTCTGCTCGTTTTTTTTTCTCTGTGGTGTGTGTTTGTGGCGAGAGAGAAAAAT

PI	GATTTGGGGGAAAGCAAAATCCGGAGATTTCGCACGTACGATCGTTTCGACACGTTCGACGCCC
LB	GATTTGGGGGAAAGCAAAATCCGGAGATTTCGCACGTACGATCGTTTCGACACGTTCGACGCCC

PI	GGCGGGCCCCGGGGTGGGGCATCGTGTGGCTGCAGGACCGCGGGGGCCCCGCAAAGCGGGGCC
LB	GGCGGGCCCCGGGGTGGGGCATCGTGTGGCTGCAGGACCGCGGGGGCCCCGCAAAGCGGGGCC

PI	GGGCCAATGGGTGCTCGACAGCGGGCTATGCTCCAGACCAGCCCGGTATTGCATACCGCGCG
LB	GGGCCAATGGGTGCTCGACAGCGGGCTATGCTCCAGACCAGCCCGGTATTGCATACCGCGCG

PI	TCGGGGGCCAGATCCCTTTTAAAAACCCCTCCCCCCCCTGCCGGAATCCTCGTTTTTGGCCTGG
LB	TCGGGGGCCAGATCCCTTTTAAAAACCCCTCCCCCCCCTGCCGGAATCCTCGTTTTTGGCCTGG

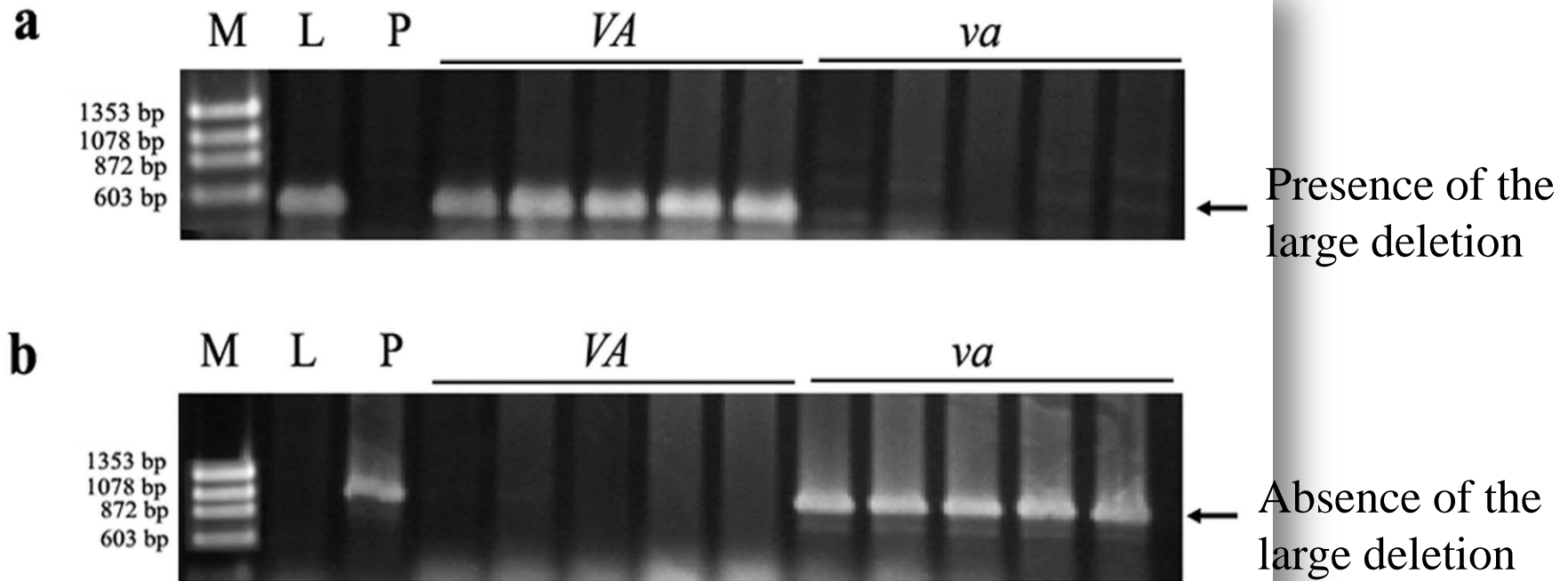
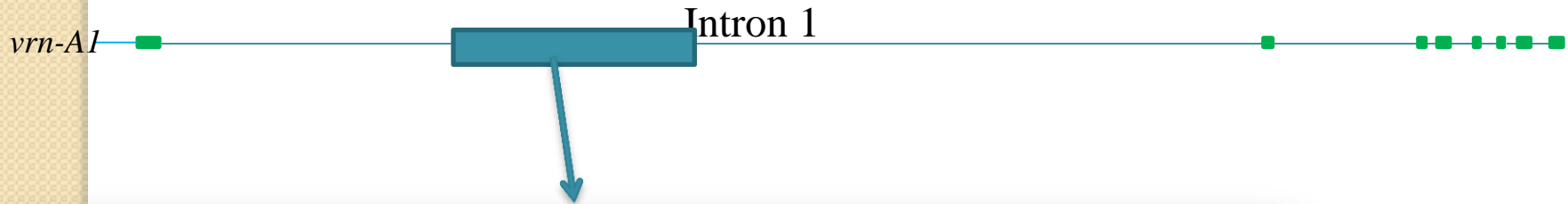
PI	CCATCCTCCCTCTCCTCCCCTCTCTTCCACCTCACGTCCTCACCCAACCACCTGATAGCC
LB	CCATCCTCCCTCTCCTCCCCTCTCTTCCACCTCACGTCCTCACCCAACCACCTGATAGCC

PI	ATGGCTCCGCCGCCTCGCCTCCGCCTGCGCCAGTCGGAGTAGCCGTCGCGGTCTGCCGGT
LB	ATGGCTCCGCCGCCTCGCCTCCGCCTGCGCCAGTCGGAGTAGCCGTCGCGGTCTGCCGGT

PI	GTTGGAGGGGTAGGGGGCGTAGGGTTGGCCCGGTTCTCGAGCGGAGATGGGGCGGGGGGAAGG
LB	GTTGGAGGGGTAGGGGGCGTAGGGTTGGCCCGGTTCTCGAGCGGAGATGGGGCGGGGGGAAGG

PI	TGCA
LB	TGCA

Allelic variation on *VRN-A1*



Allelic variation on *VRN-B1*

Intron 1

vrn-B1



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PI  CAAGTGGAAACGGTTAGGACAGTAATCTCTTGATATTTTTATCTGGCTGGGGATATTTACGTAAAAAATTAT
LB  CAAGTGGAAACGGTTAGGACAGTAATCTCTTGATATTTTTATCTGGCTGGGGATATTTACGTAAAAAATTAT
*****

PI  ATGGGGTTAAAGTGACATCGCAATTTAGCATGCTACCTCATCTTCTCATTTAGAATCTTACTAGAOGCTACA
LB  ATGGGGTTAAAGTGACATCGCAATTTAGCATGCTACCTCATCTTCTCATTTAGAATCTTACTAGAOGCTACA
*****

PI  ATACCTTGTTGTCTGGGCTCATCAAATCTGTGCTTGCTGCTTGAACAAATGAACCTGCTCATCTCGGTTATTT
LB  ATACCTTGTTGTCTGGGCTCATCAAATCTGTGCTTGCTGCTTGAACAAATGAACCTGCTCATCTCGGTTATTT
*****

PI  CCAGAAATTTTGTTCACAGGCTTCCCTATCATTCGTATTGCTAGCTCCGGGCATGCGGCCTATTTTGTGCTT
LB  CCAGAAATTTTGTTCACAGGCTTCCCTATCATTCGTATTGCTAGCTCCGGGCATGCGGCCTATTTTGTGCTT
*****

PI  GCCTGGAGATACTGTCTACGGCAGCGCAAGGAGAAAAGAGTCACTTGACTAGCTAATGCATGGAATAATTGTC
LB  GCCTGGAGATACTGTCTACGGCAGCGCAAGGAGAAAAGAGTCACTTGACTAGCTAATGCATGGAATAATTGTC
*****

PI  TGCAGCTGATGAAACTCCGGCATGAAGAGTCAAACCAAAAAGTAGAGAGTTCCCTCCAAATATAAAATGAGA
LB  TGCAGCTGATGAAACTCCGGCATGAAGAGTCAAACCAAAAAGTAGAGAGTTCCCTCCAAATATAAAATGAGA
*****

PI  GTTCTGACAGACTTTTCCCTTTCAACCATCATAATTGCTGCTGATATTTGTTGGTGCTGGCGATGGTTCT
LB  GTTCTGACAGACTTTTCCCTTTCAACCATCATAATTGCTGCTGATATTTGTTGGTGCTGGCGATGGTTCT
*****

PI  TGACAAAGTAAAGGAGTCAATAAAATCACGGGAGACTGATCCATTCTTTCCGCCACACGCTGACATTAGTCCA
LB  TGACAAAGTAAAGGAGTCAATAAAATCACGGGAGACTGATCCATTCTTTCCGCCACACGCTGACATTAGTCCA
*****

PI  TGTTAGTTTCCCGTTTCTGCTGCTTCCATAATTCCCGGCCGGCGAAGTACTAGATCAACCTCCACGGTTTC
LB  TGTTAGTTTCCCGTTTCTGCTGCTTCCATAATTCCCGGCCGGCGAAGTACTAGATCAACCTCCACGGTTTC
*****

PI  AAAAAAGTAGGAAATATCATACCATCGGAATGACCGCTGCTTAGTAAATATCCATTGTTGTTTGTAATCTTGC
LB  AAAAAAGTAGGAAATATCATACCATCGGAATGACCGCTGCTTAGTAAATATCCATTGTTGTTTGTAATCTTGC
*****

PI  TGAGAAAGCAACGTTAOCATTTGCCCTCATGGCAAAGACCTGTATGTTGAGGTGCTAAATCTTTTCTAGTTTT
LB  TGAGAAAGCAACGTTAOCATTTGCCCTCATGGCAAAGACCTGTATGTTGAGGTGCTAAATCTTTTCTAGTTTT
*****

PI  GTACACTGAGGGTATGAGTGGGCTAACGGGAAAAGGGTAAAGCAAGTTTGATTGGCTTACCTTCAGCCTCCT
LB  GTACACTGAGGGTATGAGTGGGCTAACGGGAAAAGGGTAAAGCAAGTTTGATTGGCTTACCTTCAGCCTCCT
*****

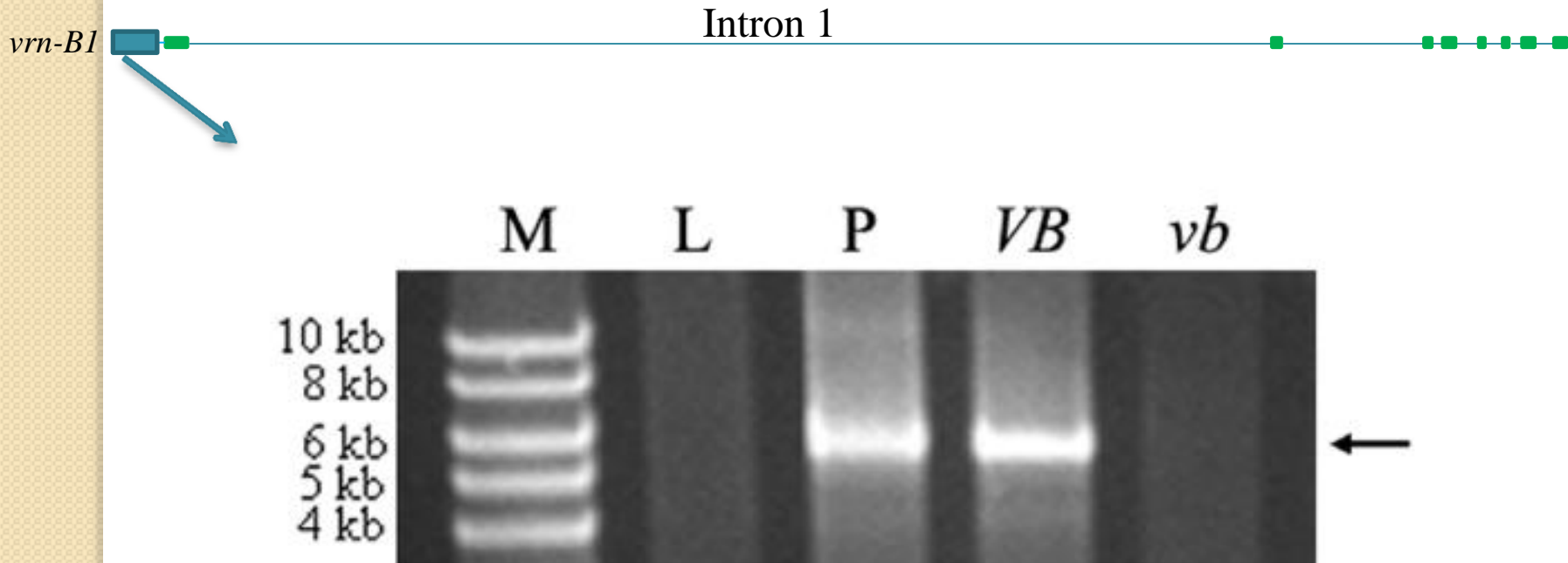
PI  TGTTTGTGTTGAAGCATAGGTGCTTGATGCTATGATCAAGCTGGTCAAGTGATGAAAACGCGTAAGAATCAA
LB  TGTTTGTGTTGAAGCATAGGTGCTTGATGCTATGATCAAGCTGGTCAAGTGATGAAAACGCGTAAGAATCAA
*****

PI  AGTCAGTTAAATTAAGATATAAACAGATGCAAGTCAATTTTAAGCTAGTGCTGCACTGTGAACCTTCAGTATC
LB  AGTCAGTTAAATTAAGATATAAACAGATGCAAGTCAATTTTAAGCTAGTGCTGCACTGTGAACCTTCAGTATC
*****

PI  TCAGATCAAAGAATTGAATAATGCTACCCCTGTGTTCTGCGCTGTTTCATTGGGAAAAGACTGTCATGAACAT
LB  TCAGATCAAAGAATTGAATAATGCTACCCCTGTGTTCTGCGCTGTTTCATTGGGAAAAGACTGTCATGAACAT
*****

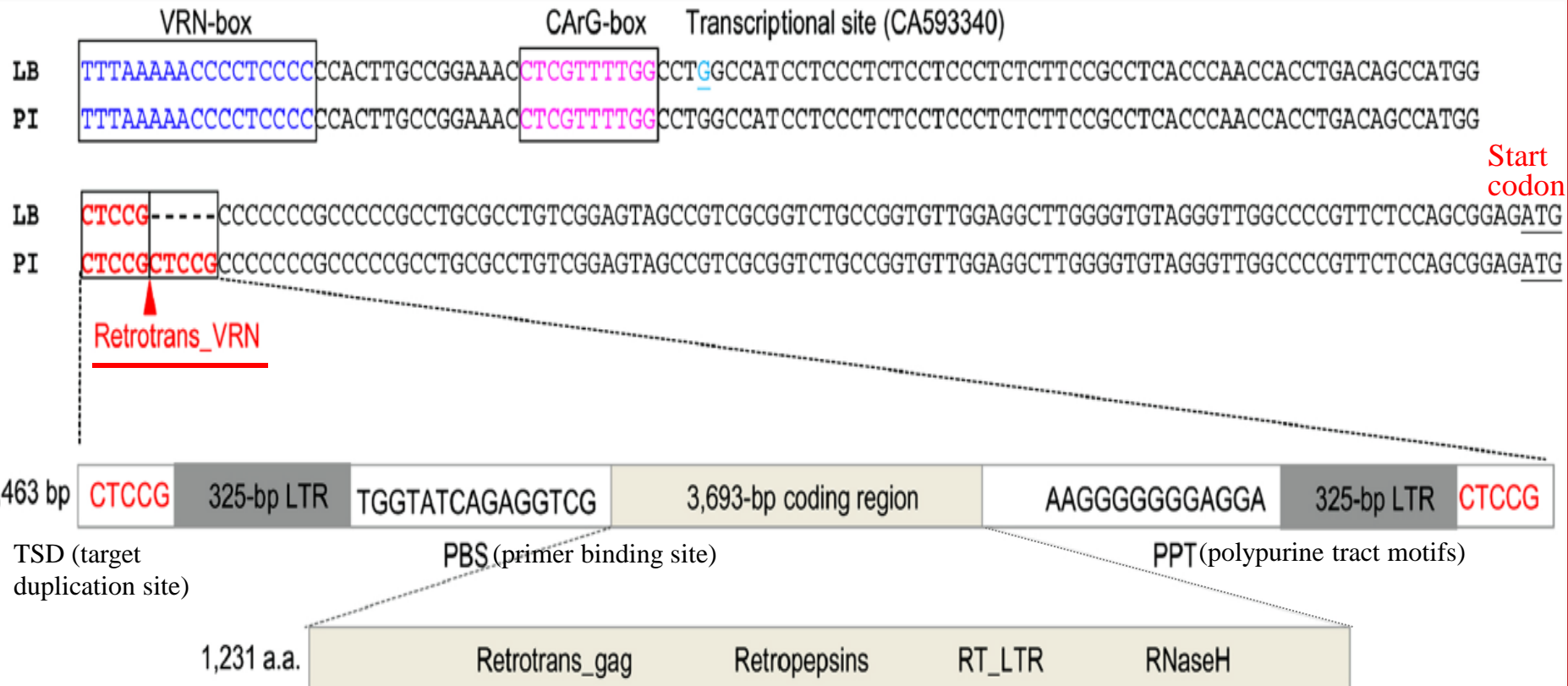
PI  CCTAATGGTAGCATGCATTATCAGCTTGCCGGCTTTATTTCTTTGCTCTCATTCCTTTTCATTG
LB  CCTAATGGTAGCATGCATTATCAGCTTGCCGGCTTTATTTCTTTGCTCTCATTCCTTTTCATTG
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Allelic variation on *VRN-B1*



5.5-kb insertion was identified in promoter region of the dominant *Vrn-B1* in PI 94749

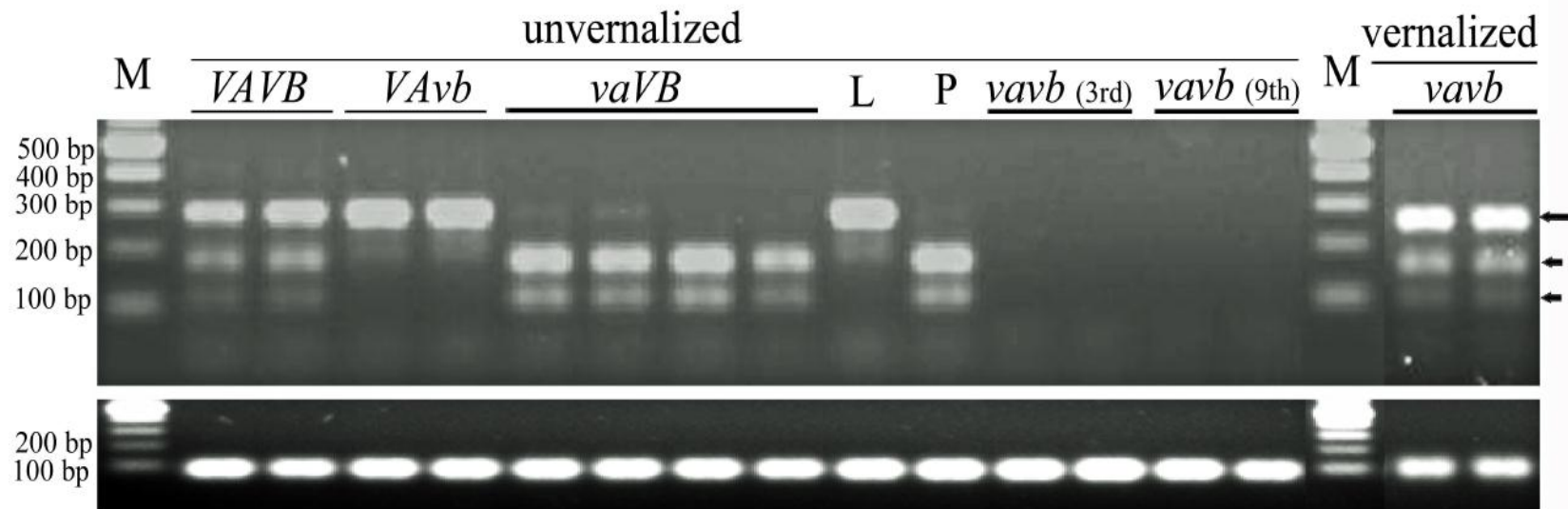
Sequence analysis of 5.5-kb insertion in *Vrn-B1* (**Retrotrans_VRN**)



Sequence analysis of 5.5-kb insertion in *Vrn-B1* (**Retrotrans_VRN**)

Sequence analysis of the complete *VRN-B1* gene in PI 94749 (*Vrn-B1*) and Lebsack (*vrn-B1*) found the Retrotrans_VRN insertion is the only difference between *Vrn-B1* in PI 94749 and *vrn-B1* in Lebsack.

Differential expression of *VRN1* genes between the dominant and recessive alleles



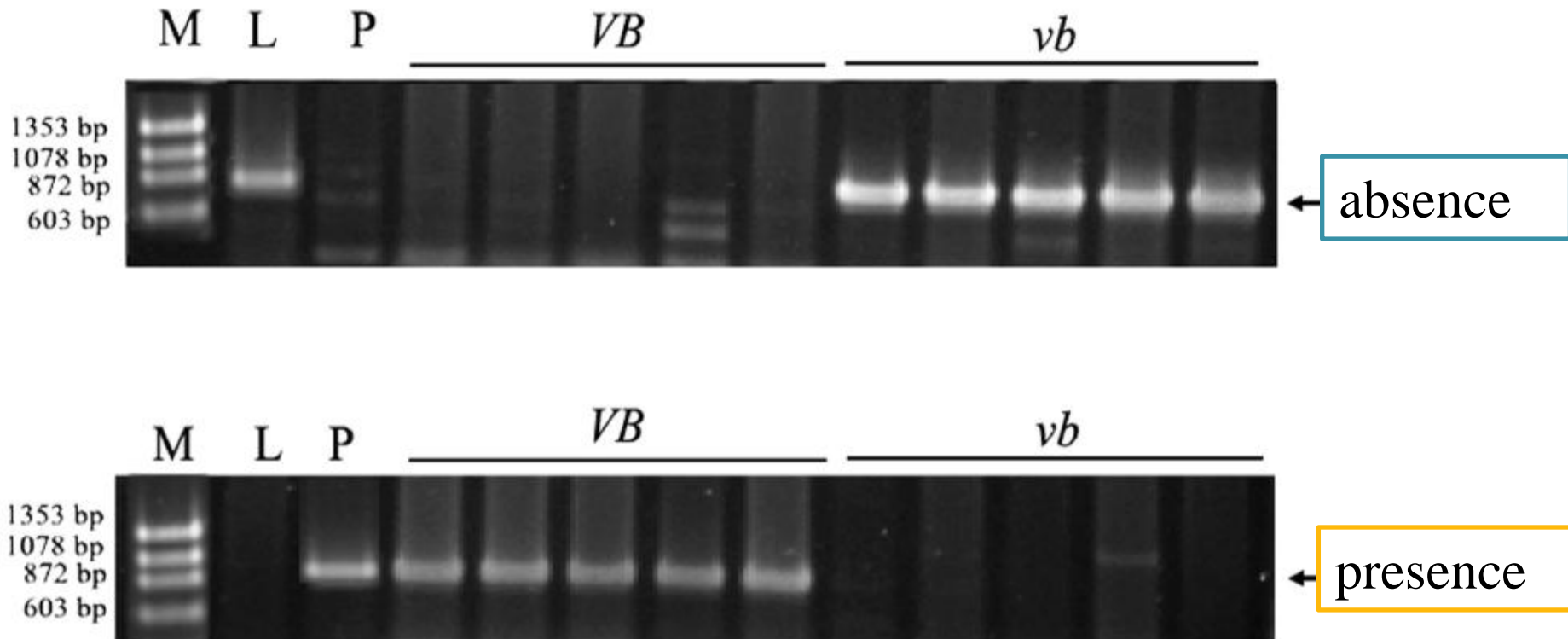
Upper gel: transcripts of *VRN-A1* (313 bp) (indicated by long arrow) and *VRN-B1* (173 bp + 110 bp) (indicated by short arrows).

Lower gel: Actin was expressed at the same level in each of the lines carrying different *VRN-1* alleles.

The 3rd and 9th mean leaf samples were taken at the third and ninth leaf stages, respectively

Frequency of the *Vrn-B1* allele containing retrotrans_VRN in tetraploid wheat

– Markers for detecting retrotrans_VRN



Frequency of the *Vrn-B1* allele containing retrotrans_VRN in tetraploid wheat

Subspecies	Number of Accessions/Lines		
	Total	Presence of retrotrans_VRN	Absence of retrotrans_VRN
<i>T. turgidum</i> subsp. <i>durum</i>	33	0	33
<i>T. turgidum</i> subsp. <i>carthlicum</i>	22	19	3
<i>T. turgidum</i> subsp. <i>dicoccum</i>	30	3	27
<i>T. turgidum</i> subsp. <i>polonicum</i>	20	0	20
<i>T. turgidum</i> subsp. <i>turanicum</i>	30	0	30
<i>T. turgidum</i> subsp. <i>turgidum</i>	19	0	19

Frequency of the *Vrn-B1* allele containing retrotrans_VRN in tetraploid wheat

Subspecies	Number of Accessions/Lines		
	Total	Presence of retrotrans_VRN	Absence of retrotrans_VRN
<i>T. turgidum</i> subsp. <i>durum</i>	33	0	33
<i>T. turgidum</i> subsp. <i>carthlicum</i>	22	19	<div>Have exactly the same sequence</div>
<i>T. turgidum</i> subsp. <i>dicoccum</i>	30	3	
<i>T. turgidum</i> subsp. <i>polonicum</i>	20	0	20
<i>T. turgidum</i> subsp. <i>turanicum</i>	30	0	30
<i>T. turgidum</i> subsp. <i>turgidum</i>	19	0	19

Conclusions

- The winter lines (*vrn-A1vrn-B1*) presented in DH population derived from Lebsock×PI 94749 was due to the homoeoallelic recombination, this demonstrates the decisive role of *VRN1* gene for growth habit of tetraploid wheat.

- The variation in *VRN-B1* was due to the novel retrotrans_VRN insertion in promoter of *vrn-B1*, a position near CArG-box and VRN-box, the regulatory sites of *VRN1* in diploid and hexaploid wheat

- The retrotrans_VRN insertion in the critical promoter region may disrupt repressor binding, which allowed the transcription of *VRN* gene.

- Most insertions have deleterious effects on expression of nearby genes. However, this study provides an example of an insertion that resulted in the liberation of gene expression. Mutation of the *VRN-B1* gene is the mechanism by which tetraploid wheat evolved from the winter wild type to spring type.

- A survey of 154 spring-type accessions of six tetraploid wheat subspecies indicated that the insertion event in the dominant *Vrn-B1* allele might have occurred during the evolution of tetraploid wheat and may have contributed to adaptation to certain environments.

Acknowledgments

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*Thank you
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Questions?*