Identification and Characterization of *TaZHD1* and *TaZHD10* controlling Leaf Rolling under Moisture stress conditions by modulating Bulliform Cells activity in Bread Wheat

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Introduction/Background

- Leaf rolling is a complex quantitative trait, controlled by myriad of genes in major cereal crops.
- More than 70 QTLs/genes associated with leaf rolling have been extensively studied in rice and maize, but in wheat are scarcely reported.
- Zinc finger-homeodomain (ZHD) class IV family are plant-specific transcription factors, involve in various biological process such as growth, development, signalling and stress responses.
- The information suggested that these genes/TFs might be putatively involved in adaxial or abaxial leaf rolling by modulation of bulliform cells
- However, while the potential regulatory role of ZHD genes/ TFs has been extensively studied in Arabidopsis, rice, maize, and other model crops, but the molecular basis of ZHD genes in leaf rolling and its component trait (drought tolerance) in wheat have not yet been identified.

Materials and Methods

Development of RIL population by crossing parental genotypes, NI5439 and HD2012

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Morphological observation of RILs for leaf rolling traits at three consecutive years (designated as E17, E18 and E19)

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Genotyping using 72 SSR and 3589 SNPs (Axiom 35K Breeder's SNP array)

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QTL mapping using **Ici Mapping v 4.2** package

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Identification and characterization of candidate leaf rolling gene(s) using functional genomics approach

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Morphological observation of contrasting RILs for leaf rolling under drought-induced condition

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Histological observation of flag leaf tissues for bulliform cells activity in leaf rolling

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Differential expression profiling of candidate ZHD (*TaZHD1* and *TaZHD10*) genes using qRT-PCR methods

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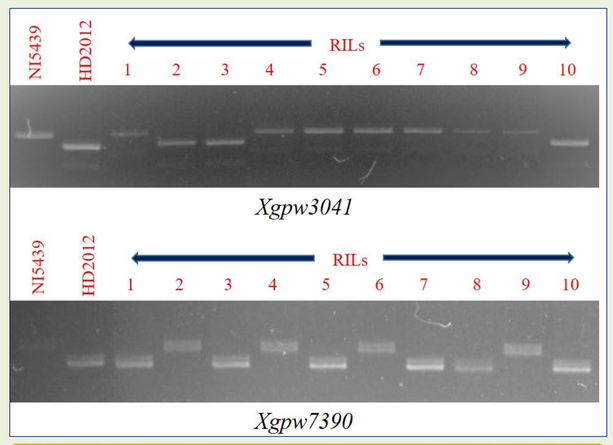
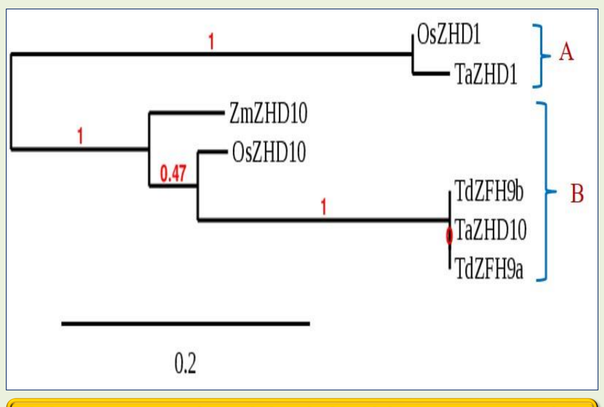
Development of metabolic and gene regulatory network underlying leaf rolling in wheat under severe drought-stress

Results and Discussion

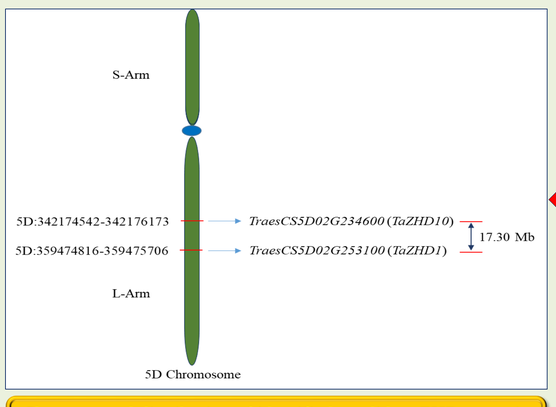
Phenotypic evaluation for leaf rolling across the E17, E18, and E19



Genotyping of RILs using SSR markers across the E17, E18, and E19



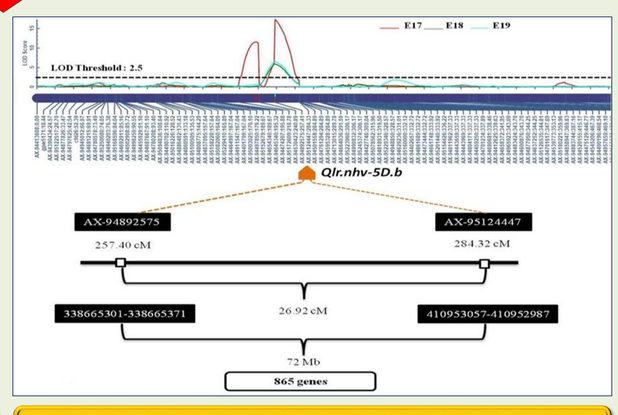
Phylogenetic tree topology of identified candidate ZHD genes in wheat and its closest orthologs



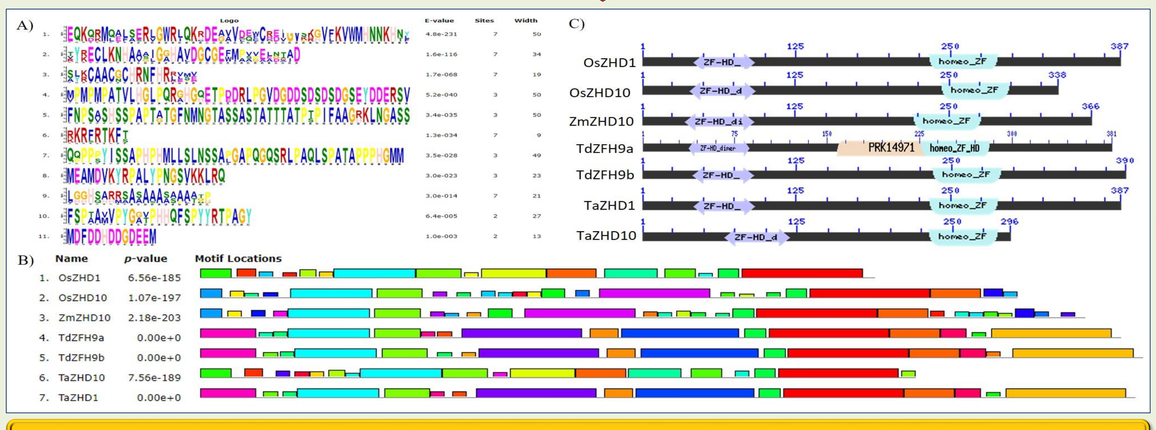
Chromosomal distribution of identified candidate ZHD genes in wheat

Chr	QTL	Env	Pos	Flanking markers	LOD	PVE(%)	Add
1B	*Qlr.nhv-1B*	E18	172	*AX-95149749-gwm153*	5.32	15.35	-0.26
2A	*Qlr.nhv-2A*	E17	681	*AX-94942225-AX-95254393*	10.85	12.03	-0.32
		E19	679	*AX-94942225-AX-95254393*	5.64	12.13	-0.27
2B	*Qlr.nhv-2B*	E17	686	*AX-94711931-AX-95260437*	6.86	6.49	-0.23
2D	*Qlr.nhv-2D.1*	E18	430	*AX-94743285-AX-94823535*	3.01	7.44	-0.18
	Qlr.nhv-2D.2	E19	283	*AX-94661194 - AX-94603691*	4.21	15.80	-0.31
3A	*Qlr.nhv-3A*	E17	823	*AX-94598770-AX-94844071*	4.95	4.06	0.18
4A	*Qlr.nhv-4A.1*	E17	482	*AX-94739181-AX-94500554*	14.18	15.26	0.36
	Qlr.nhv-4A.2	E18	80	*AX-95212081-AX-94470023*	5.13	13.43	0.24
		E19	83	*AX-95212081- AX-94470023*	5.06	11.38	0.26
4B	*Qlr.nhv-4B*	E17	262	*AX-94993235-AX-94508980*	5.44	5.43	-0.21
5D	*Qlr.nhv-5D.1*	E17	237	*AX-95172609-AX-94530423*	11.47	14.01	0.35
	Qlr.nhv-5D.2	E17	258	*AX-94892575-AX-95124447*	17.19	20.10	-0.41
		E18	258	*AX-94892575-AX-95124447*	5.84	16.08	-0.26
		E19	258	*AX-94892575 -AX-95124447*	6.45	14.25	-0.29
6B	*Qlr.nhv-6B*	E17	142	*AX-94500240-AX-94980566*	5.31	4.47	-0.19

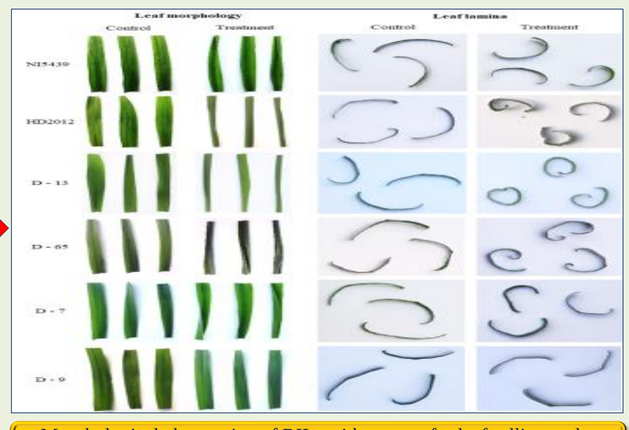
QTLs for leaf rolling in RIL mapping population derived by NI5439 and HD2012



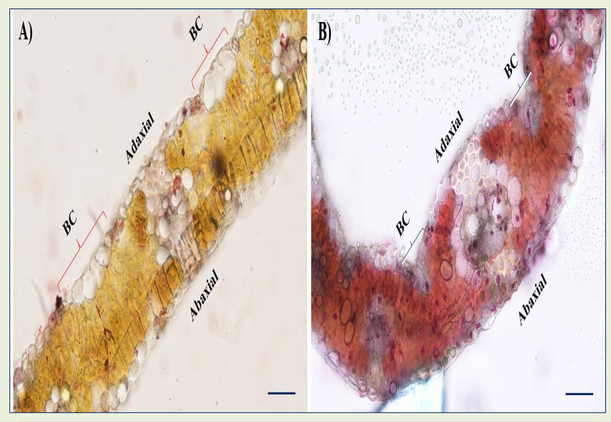
QTL (*Qlr.nhv-5D.2*) on 5D chromosome flanked by markers *AX-94892575- AX-95124447*



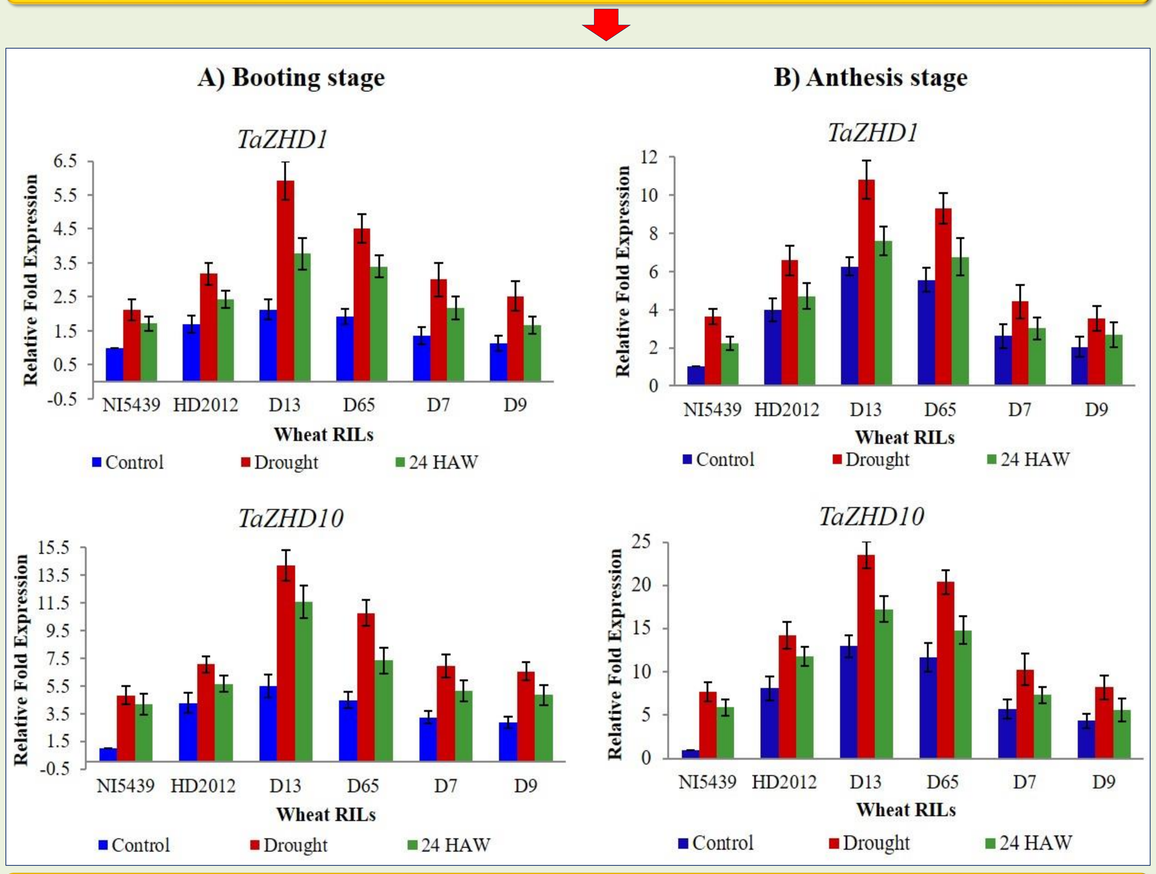
Comparative analysis of conserved motifs and domains of identified ZHD proteins in wheat and its closest orthologs



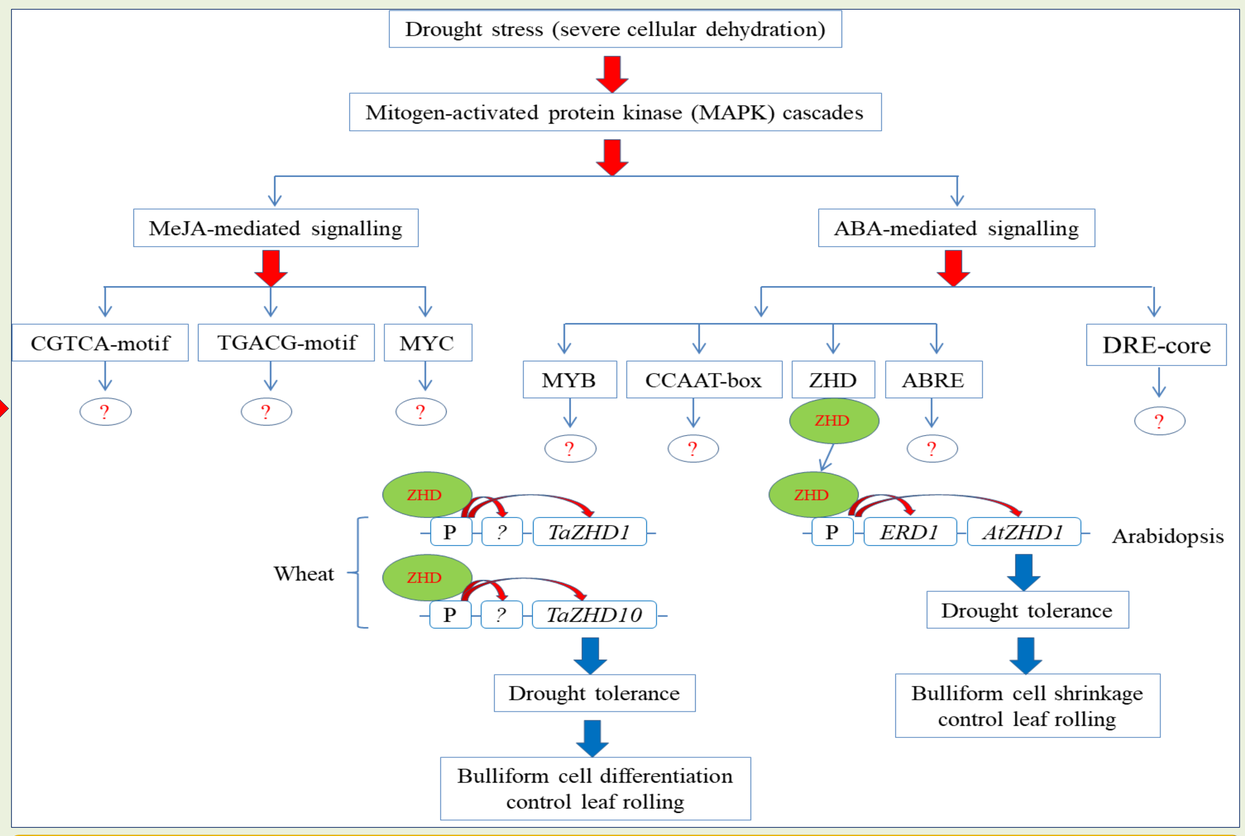
Morphological observation of RILs with parents for leaf rolling under stress-induced condition



Histological observation of leaf tissues for bulliform cells activity in leaf rolling under control and stress condition in D-65 RIL



qRT-PCR based expressions profiling of identified candidate leaf rolling ZHD genes at two different developmental stages in flag leaf tissues



Proposed metabolic and gene regulatory models of leaf rolling under severe drought-stress condition in wheat

Conclusion and Future Prospects

- With compared to parents, significant variation in leaf rolling index (LRI) was observed in all 96 RILs under drought stress condition across the E17, E18 and E19, respectively.
- A stable QTL putatively associated with leaf rolling, *Qlr.nhv-5D.2* on the 5D chromosome flanked with markers *AX-94892575* and *AX-95124447* at 338665336 and 410953022 intervals, respectively, was detected.
- Two candidate genes, namely *TaZHD1* and *TaZHD10* was identified within the marker intervals are the closest orthologs of rice *OsZHD1* and *OsZHD10* genes and may play key role in leaf rolling.
- Functional annotation of identified *TaZHD1* and *TaZHD10* genes revealed that the genes are Zinc finger-homeodomain (ZHD) class IV family plant-specific transcription factors.
- Morphological and histological analysis revealed the significant patterns of bulliform cells while defining leaf rolling under stress and control conditions in flag leaf.
- Relative expression profiling of both genes in flag leaf tissues revealed significant upregulation under drought-induced condition while significantly down-regulated after 24 HAW, at both the developmental stages suggested their functional redundancy and pin-point importance in leaf rolling by regulating bulliform cells differentiations.
- Overall, the results increase our understanding of *TaZHD* genes and provide valuable information as robust candidate genes for future functional genomics research aiming for the breeding of wheat varieties tolerant to leaf rolling.

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