

Interaction of Thaumatin Like-Proteins with β -glucan in Barley

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McGill



Barley: A Major Cereal



- Fourth Major Cereal worldwide
- Barley is mainly used as animal feed and for producing malt
- Barley is also used for human consumption due to its health benefits such as lowering blood cholesterol

Beta-Glucan: A Major Dietary Fiber

- Non-starch carbohydrates are major components of dietary fibers accumulated in barley endosperm's cell wall
- The cell walls of barley endosperm are composed of $\approx 75\%$ β -(1,3)(1,4)-glucan, 20% arabinoxylan, 5% protein and traces of cellulose
- (1,3;1,4)- β -glucan is an important non-starch carbohydrate determining the grain use
- Effective degradation of β -glucan is essential for quality malt synthesis and prevention of brewing complications
- High (1,3;1,4)- β -glucan content is desirable for human nutrition and health

Beta-glucan and Malting?

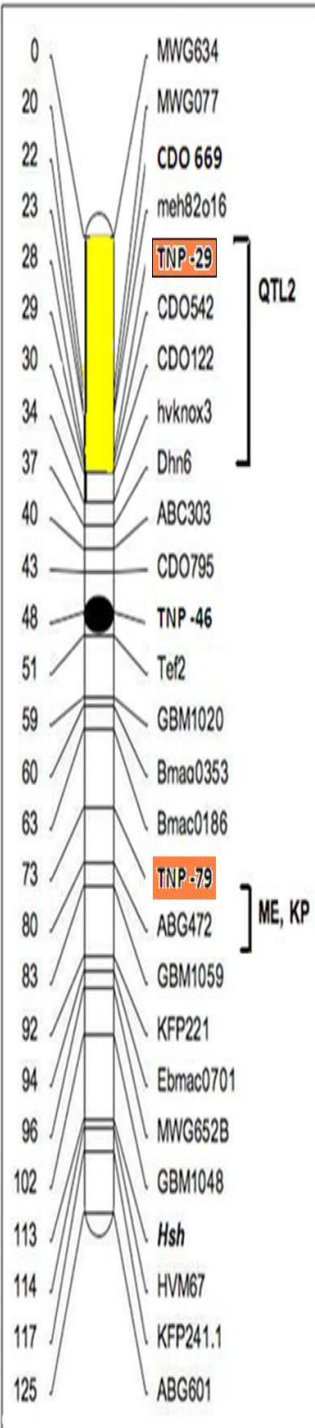
- Excessive amount of β -glucan during malting leads to problems like:
 - highly viscous wort
 - diminished rate of wort filtration
 - haze formation in beer
- Amount of β -glucan is an important index for determining the overall malting quality.

Genetics of Malting Quality

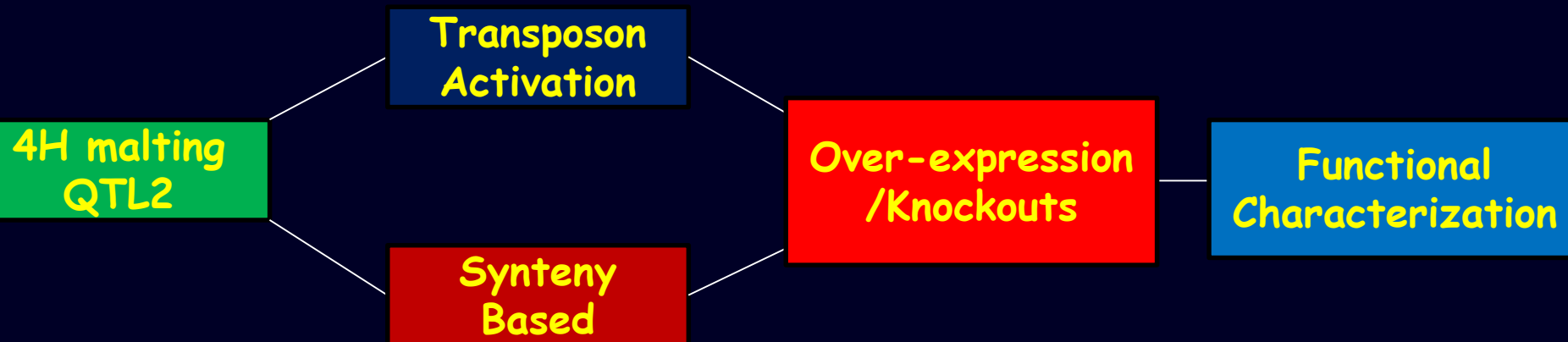
- Over 250 malting quality QTLs spread on all 7 barley chromosomes
- Two major QTLs affecting large variation in malt quality have been identified
- These QTLs are located on chromosome 7H and 4H and termed QTL1 and QTL2 respectively

Malting Quality QTL2

- Originally identified by Han et al, 1997 on Chromosome 4H
- Contributes 29% and 38% of the variation for malt β -glucan and malt extract
- Fine mapped by Gao et al, 2004
- Further investigations by Singh Lab



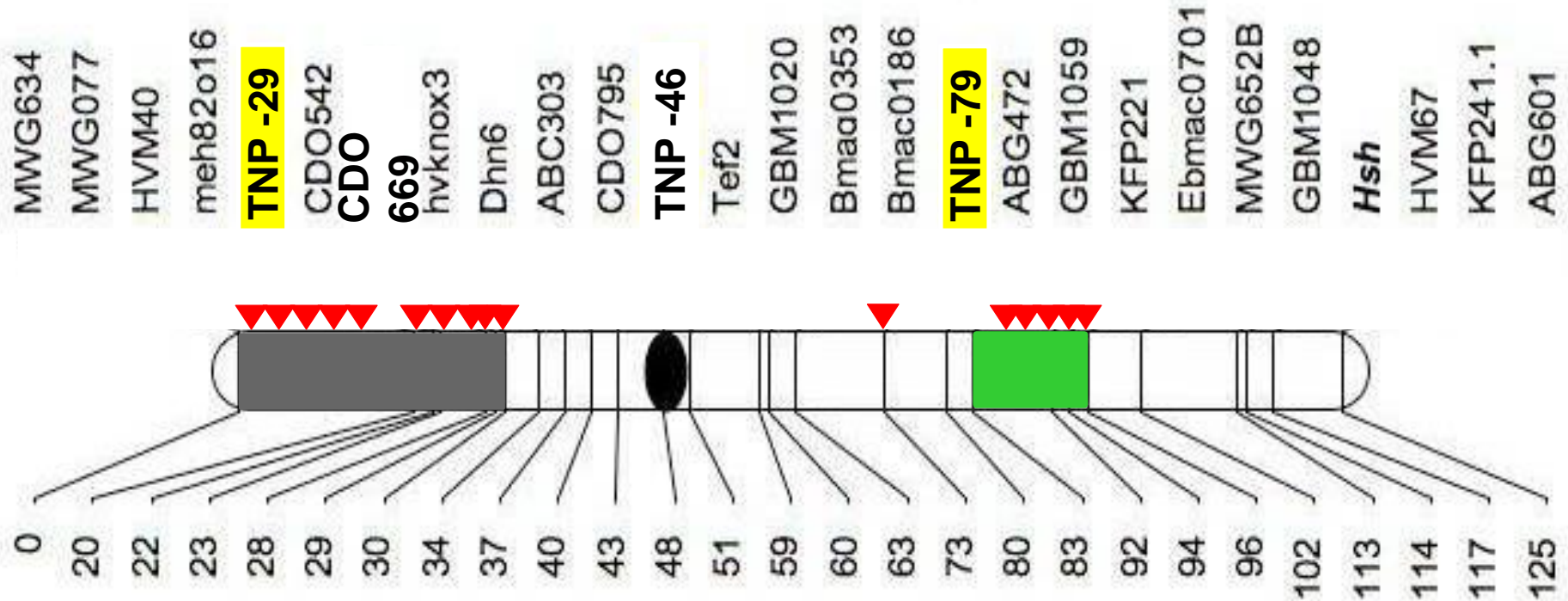
Strategy to Explore QTL2



Targeting Malting Quality QTLs

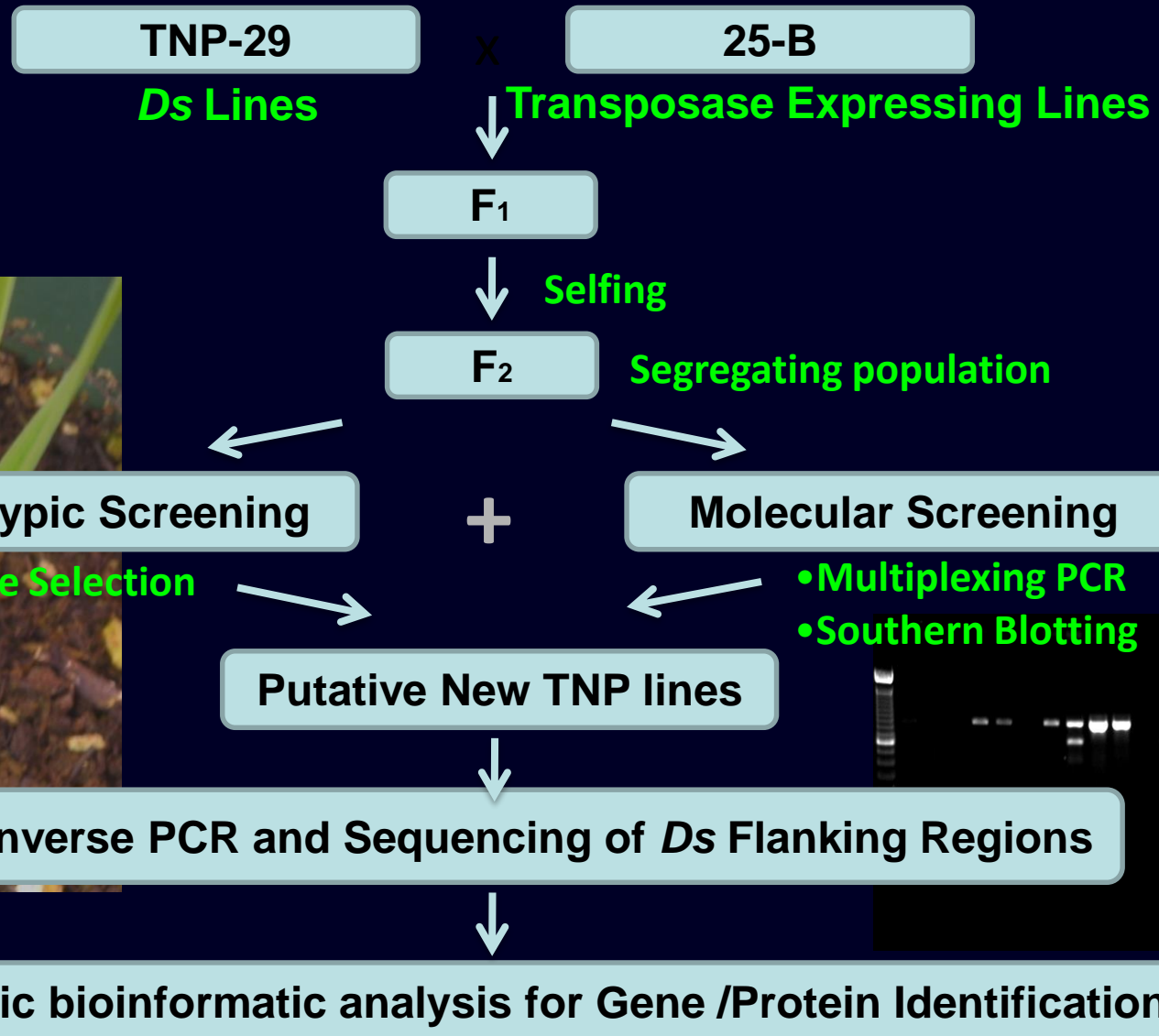
Malting quality
QTL2

Malting quality
QTL



Chromosome 4H Showing Malting Quality QTL 2

General Scheme



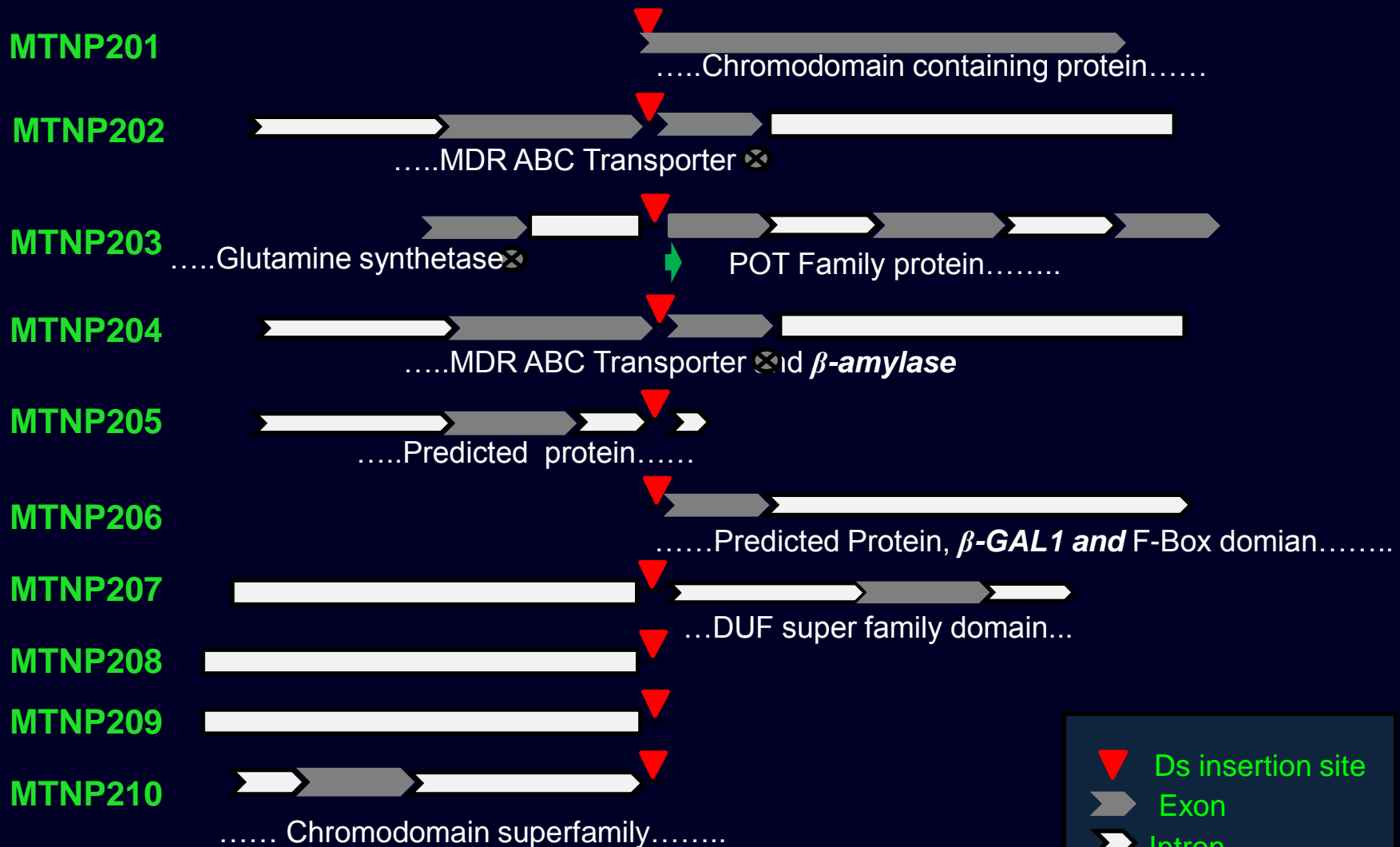
Identification of New Mutants

Population	TNP 29	TNP 79
Plants Screened	680	173
New <i>Ds</i> Lines	64	19
Stable Lines	29	11
Unstable Lines	35	08
Reactivation Frequency	9.2%	11%

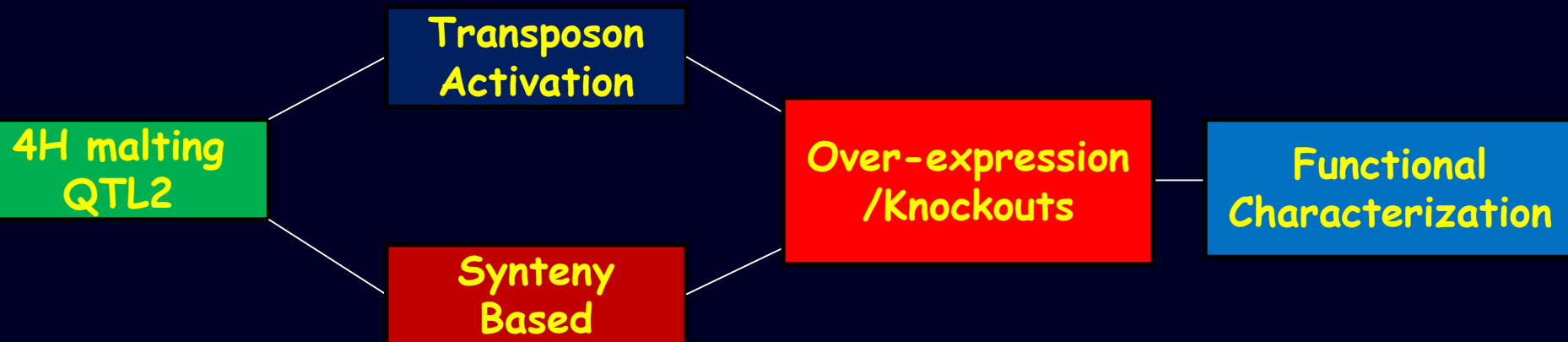
Saturation of Important Genomic Regions with Transposons



Ds in Malting Related Genes and Beyond

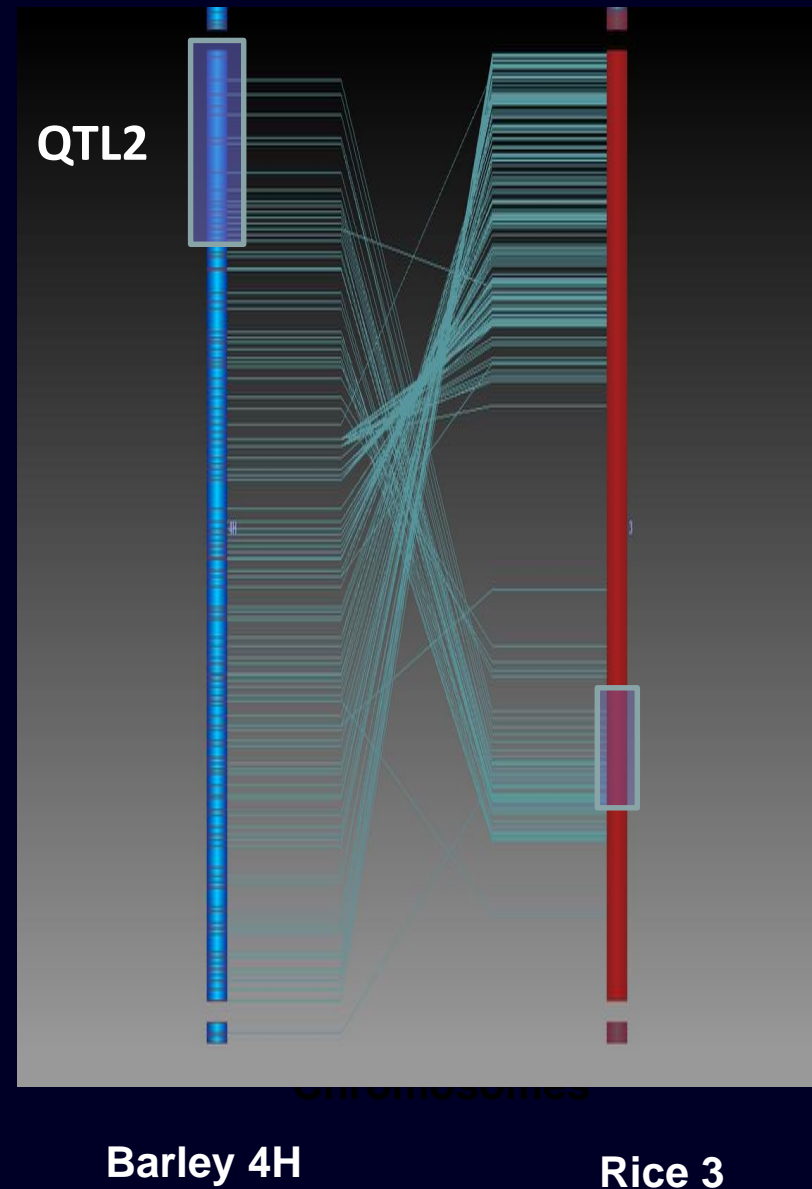


Strategy to Explore QTL2

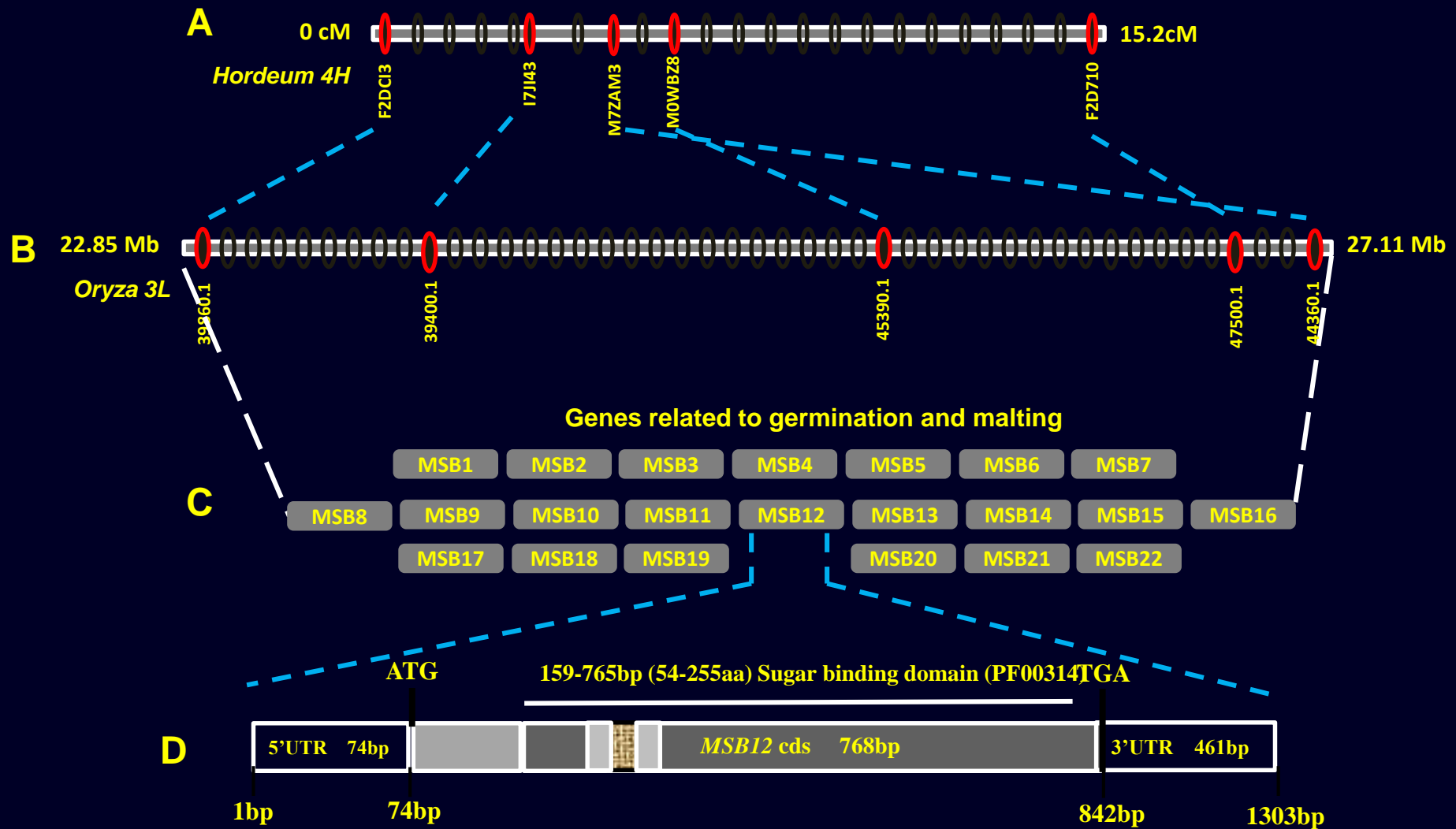


Candidate Gene Hunt

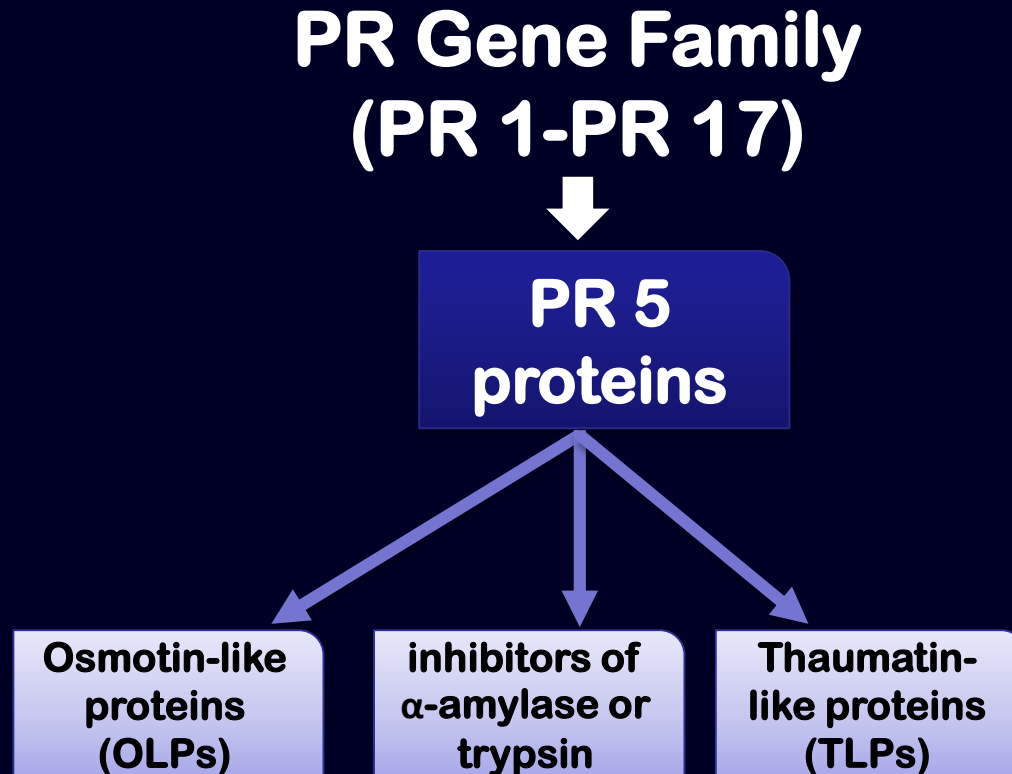
- Synteny based approach:
100+ genes from rice
corresponding barley
QTL2 area.
- Narrowed down to 22
putative candidates
- *MSB12*, hypothesized to be
a major gene in effecting
malting quality.



MSB12-A Candidate Gene



MSB12 belongs to Thaumatin Like Protein (TLP) of Pathogen Related Gene Family



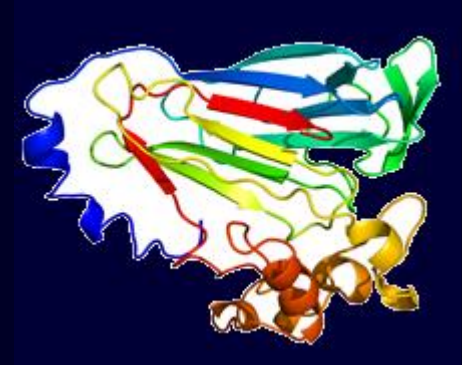
- TLPs exhibited high similarity with thaumatin (sweet tasting protein) isolated from the fruits of *Thaumatococcus danielli*
- TLPs are conserved in plants and their molecular mass is ranged between 21-26 kDa
- 5-8 disulphide bridges formed by 10-16 conserved cysteine residues

Barley TLPs: Chromosome Location and Sequence Information

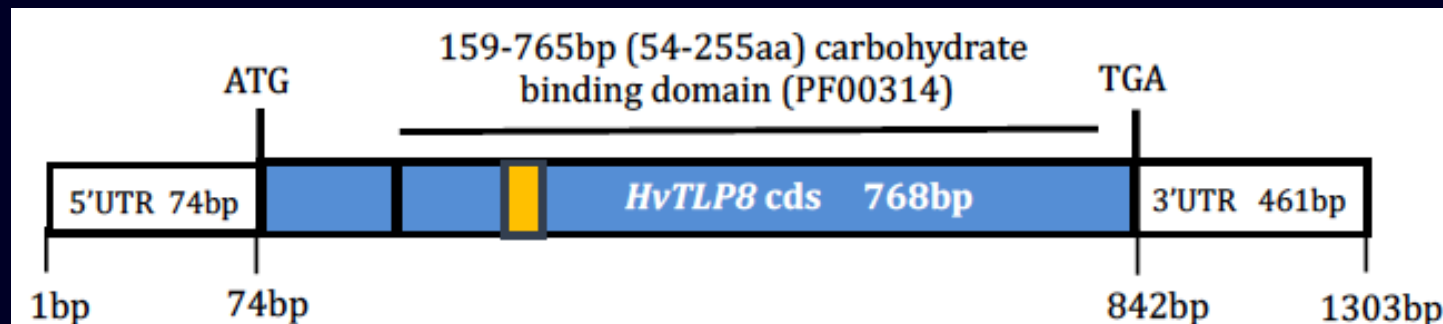
Barley TLP	Gene annotation	Chromosome location and distance (cM)	Cysteine Residues	Amino Acids	Binding Motif	NCBI Accession No.	pI values
TLP1	MLOC_24152.1	7HL, 140.86	10	173	Yes	AY839292	4.33
TLP2	MLOC_77363.1	7HL, 138.83	10	173	yes	AY839293	4.51
TLP3	MLOC_60646.1	5HS, 42.87	10	175	No	AY839294	5.02
TLP4	MLOC_20378.1	5HS,14.23	10	231	No	AF355455	5.69
TLP5	MLOC_2781.1	5HS,13.26	16	236	No	AY839295	6.04
TLP6	MLOC_75397.1	5HS, 14.51	16	226	No	AF355456	7.33
TLP7	MLOC_18451	5HS, 14.79	16	227	No	AF355457	7.36
TLP8	MLOC_39318.1	4HS, 3.47	16	255	Yes	AF355458	8.11

Characteristics of Barley *TLP8*

- 21 to 26 kD protein
- Total 8 in barley and highly conserved
- Signature sugar binding domain and motif



3D Structure

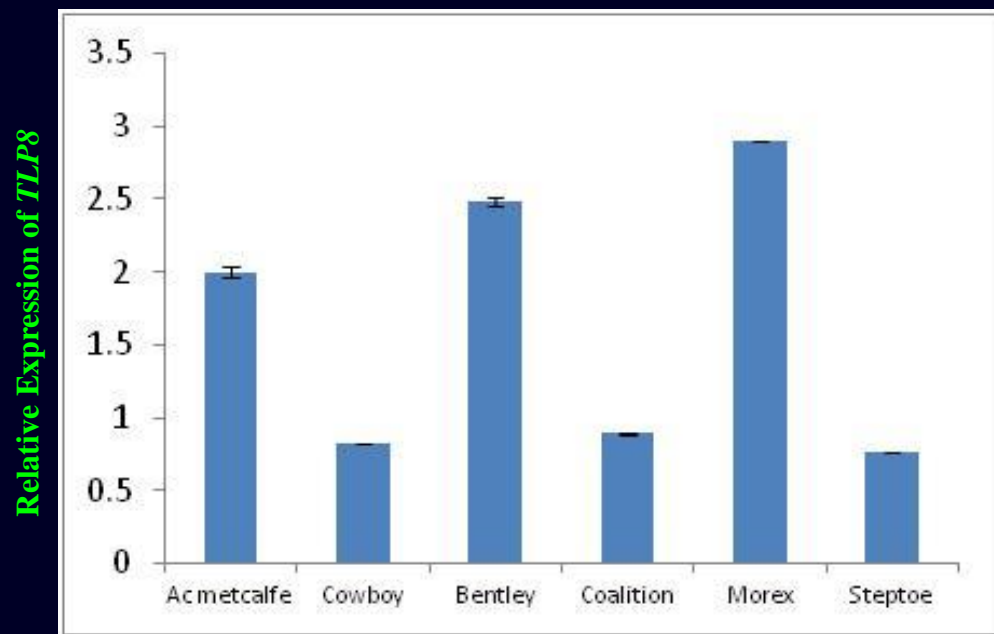
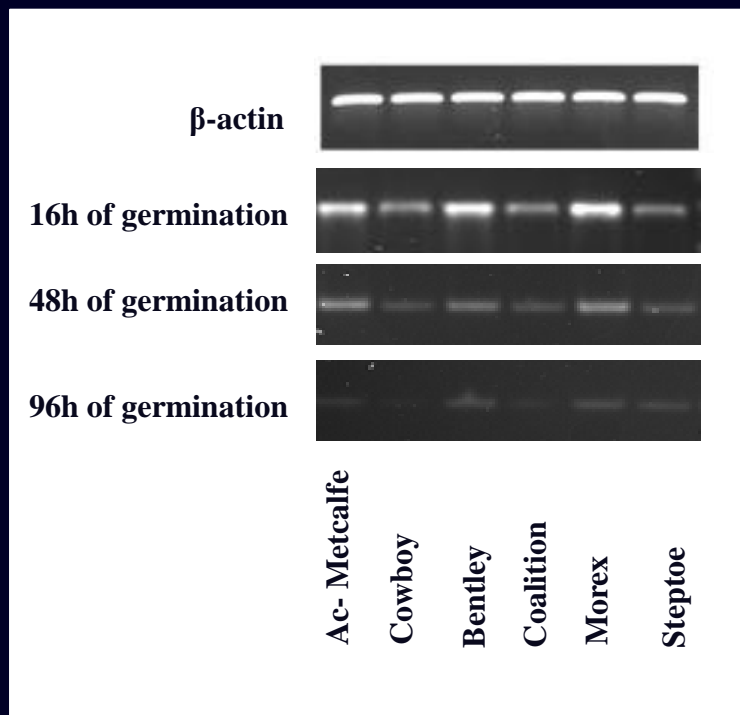


- Anti-parallel beta sheets
- 8 disulfide bridges
- 16 conserved cysteine residues



Binding Motif (CQTDGCGG)

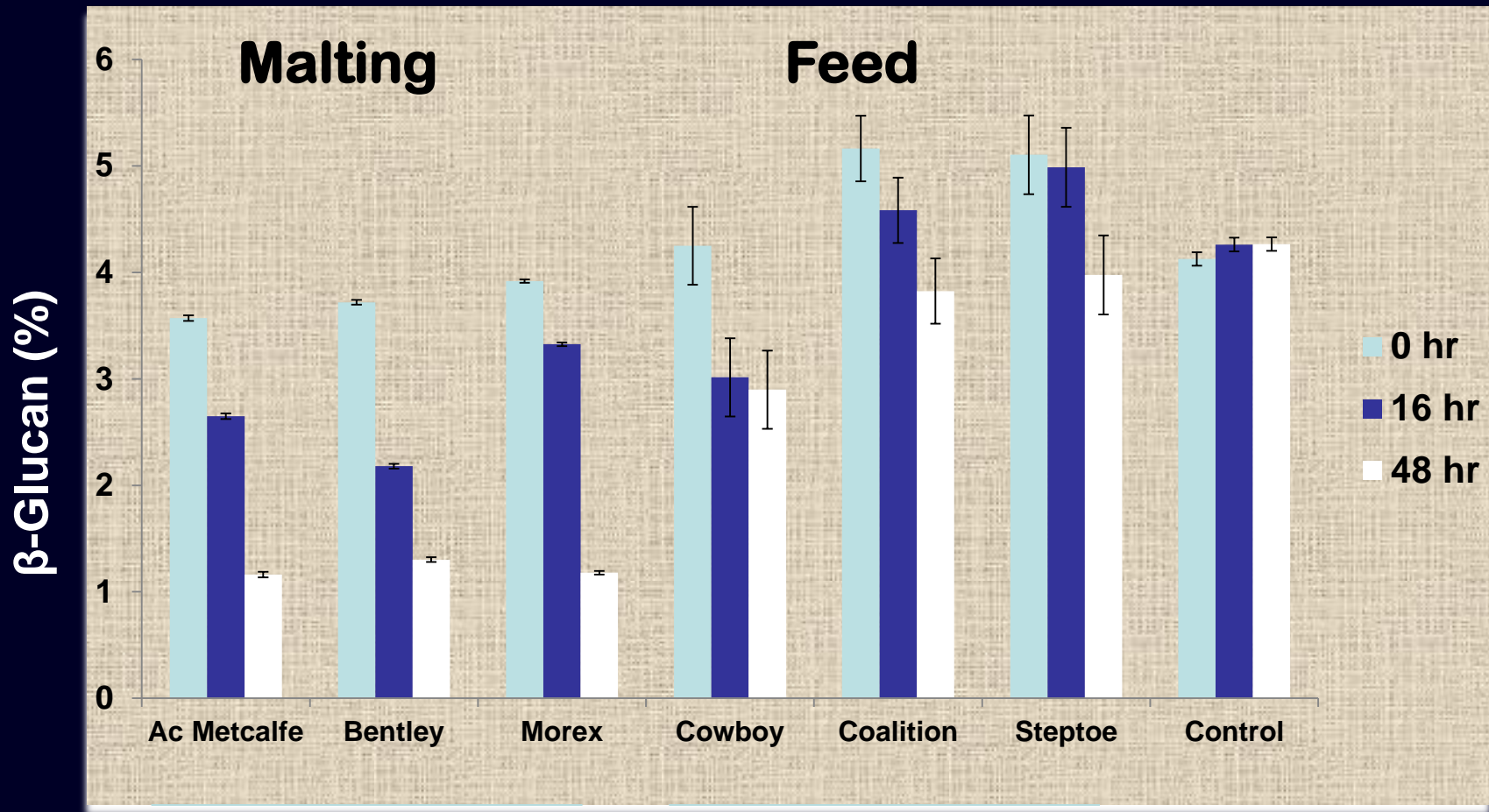
Gene expression of *TLP8*



Quantitative RT-PCR

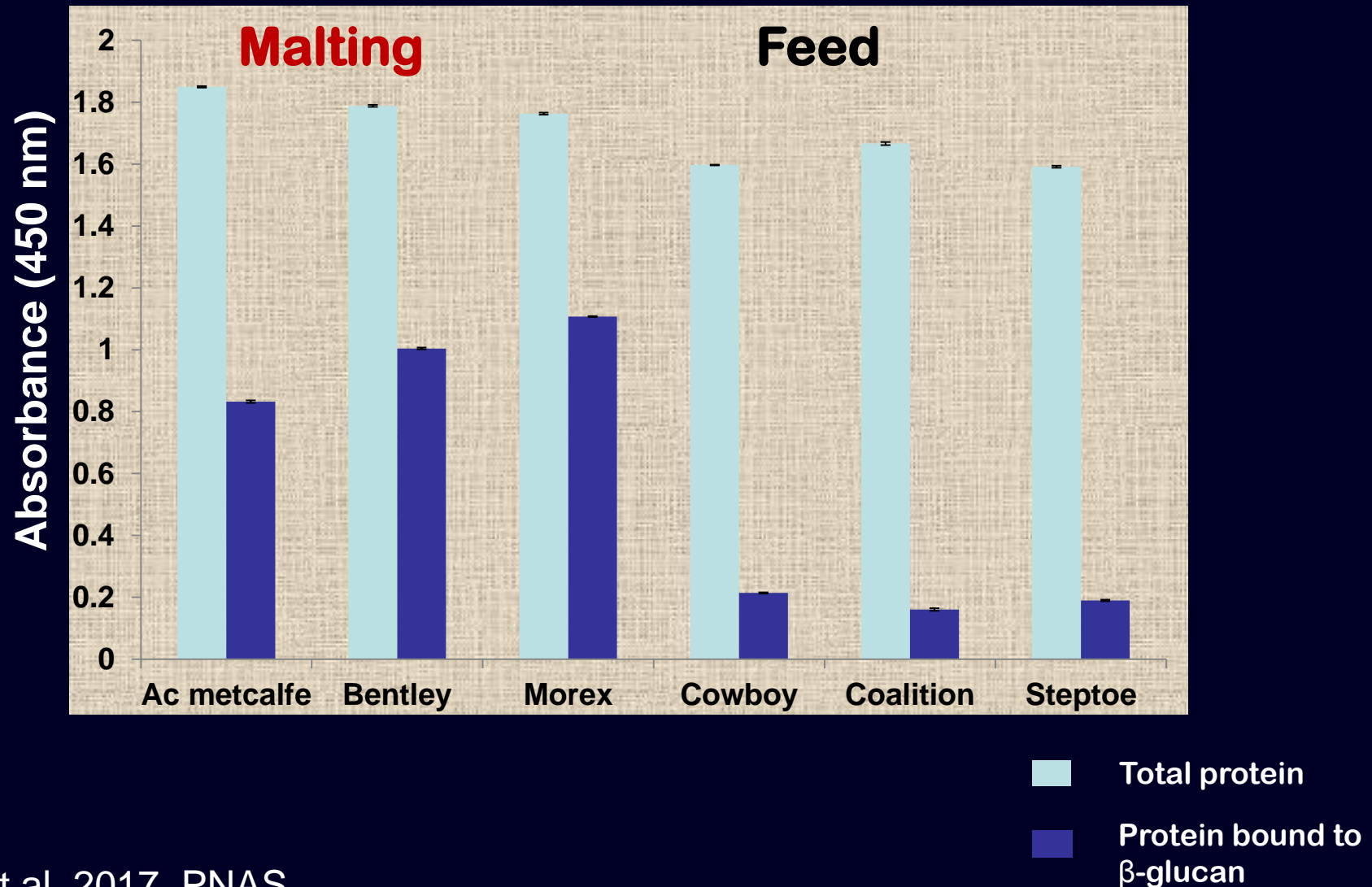
Higher expression of *TLP8* was observed in malting than in feed varieties during initial stages of germination

Levels of β -glucan

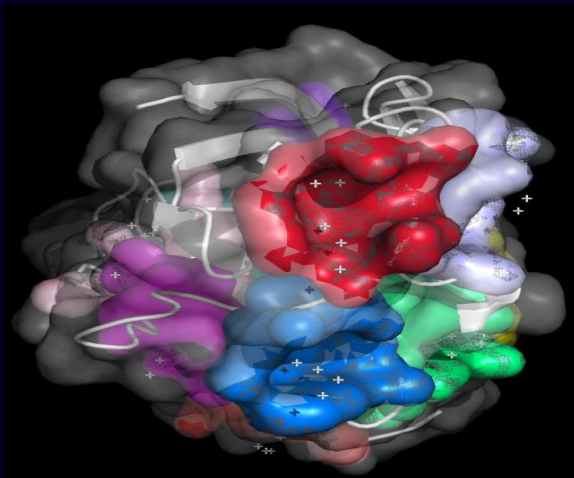


Level of β -glucan content inversely proportional to the degree of *TLP8* expression at different stages of seed germination in different barley varieties.

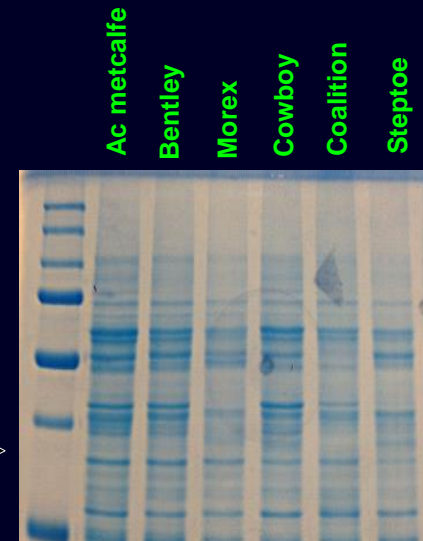
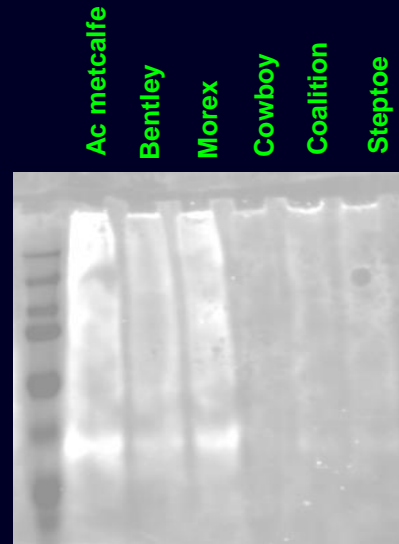
Extent of β -Glucan binding to TLPs as determined by ELISA



Glycosylation of TLP8



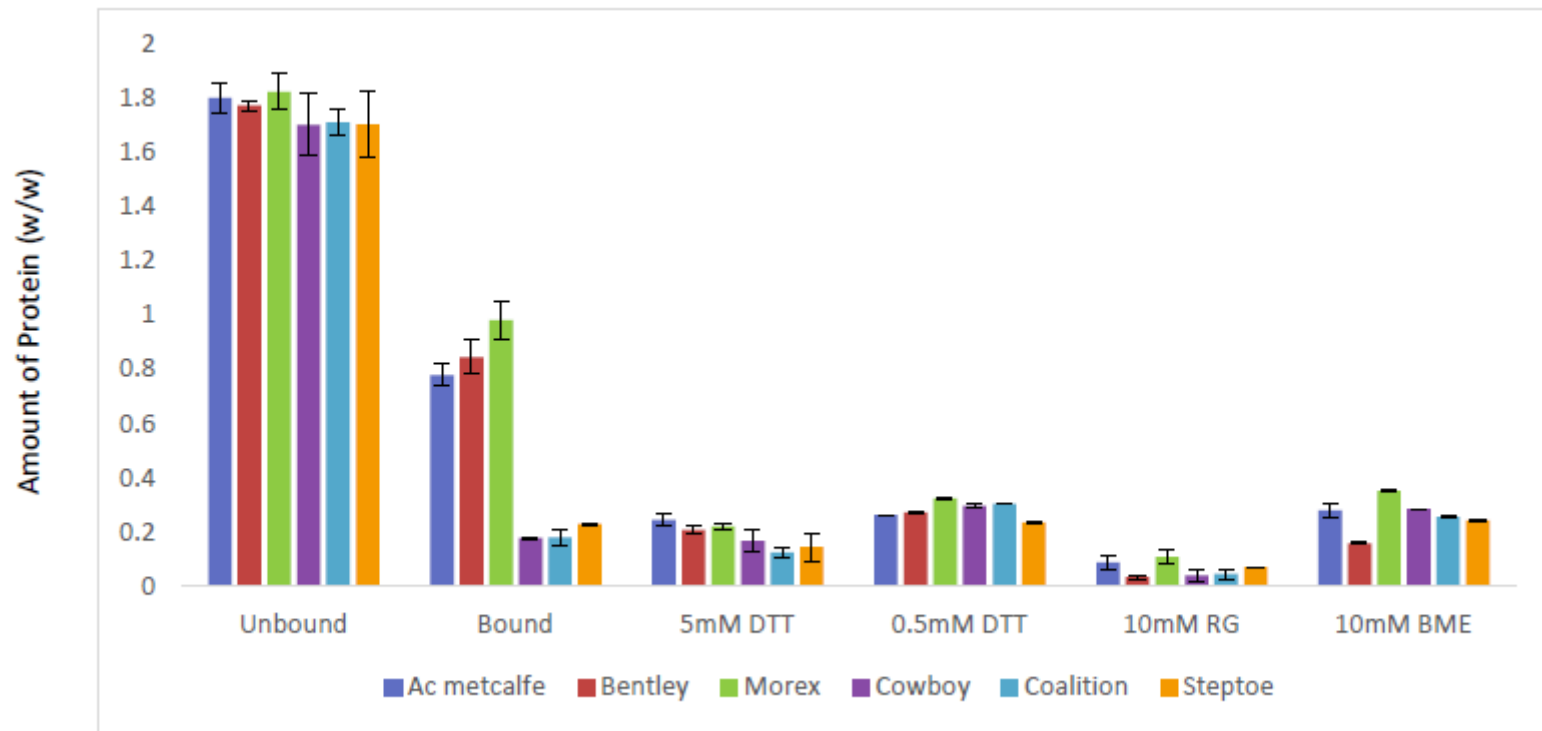
TLP8



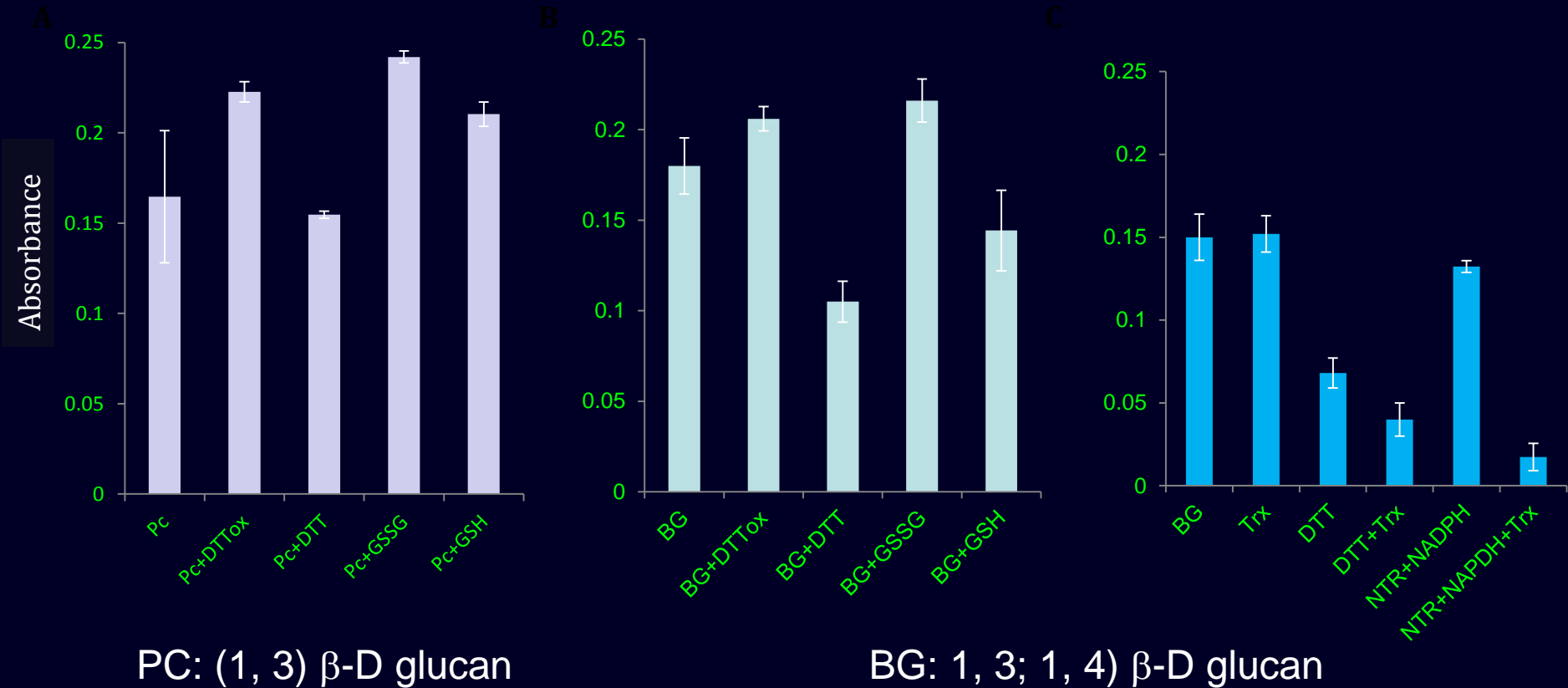
Probable site of glycosylation
in **the acidic pocket**

TLP8 is glycosylated in
malting varieties

Interaction of β -glucan and TLPs enriched Protein Fractions from different Barley Cultivars



Binding of Purified TLP8 with Beta-Glucan is redox regulated

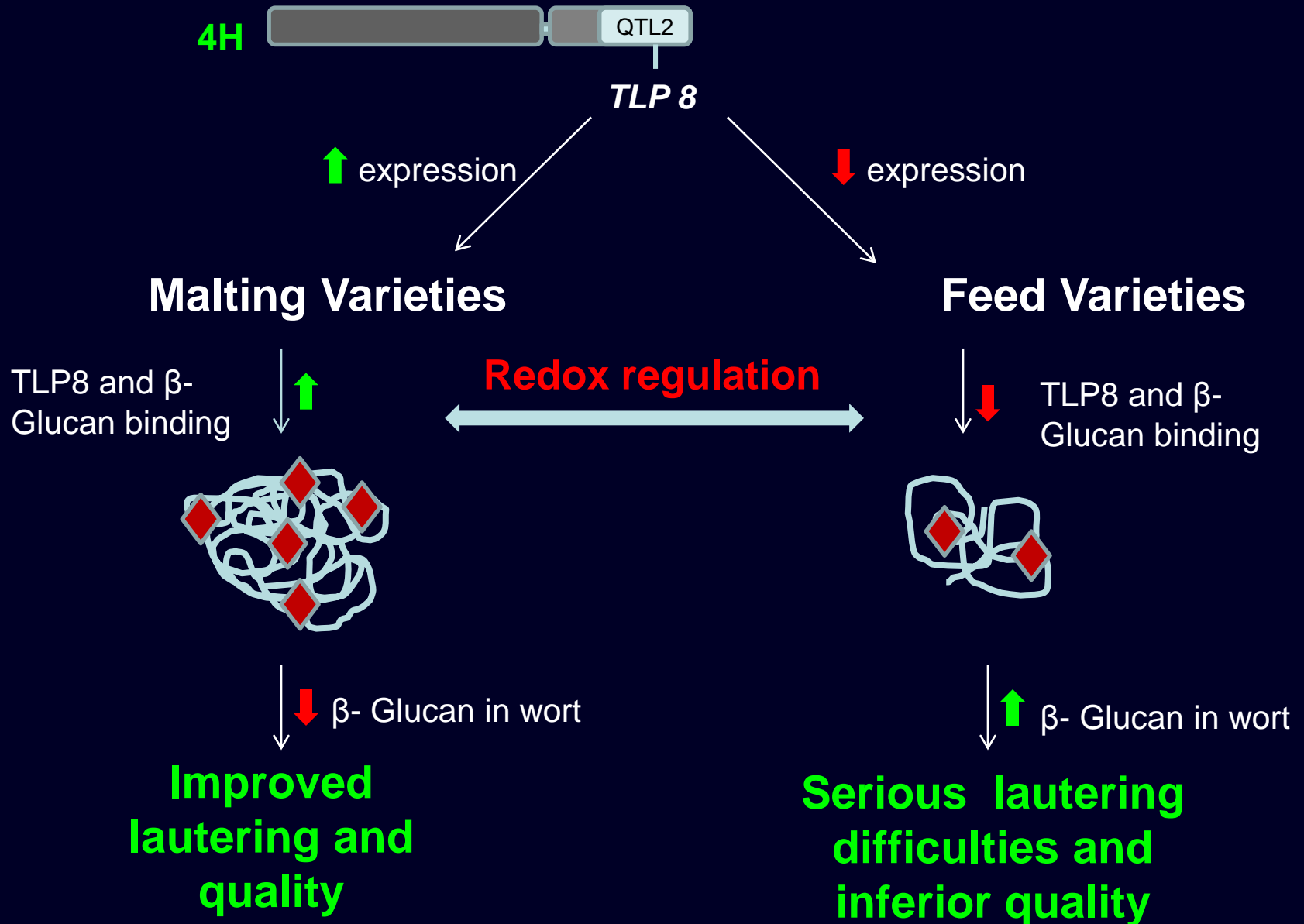


Effect of Redox Agents on Binding of Barley Recombinant TLP8 to (1, 3;1, 4)- β -Glucan

Treatments	% Binding	Treatments	% Binding
Reducing System		Oxidizing System	
Control (No Redox)	38.26	Control (No Redox)	38.26
TRX	38.70	Dehydroascorbate	47.25
DTT	17.34*	H ₂ O ₂	44.50
TRX+DTT	10.20**	DTT _{oxidized}	43.56
NTR + NADPH	33.67	GSSG	43.27
NTR + NADPH + TRX	4.33**	Sodium tetrathionate	37.13
GSH	33.40	DTT: 1,4-Dithiothreitol; Trx: Thioredoxin; NTR: NADP-thioredoxin reductase; GR: Glutathione reductase; GSH: Reduced glutathione; GSSG: Oxidized glutathione; H ₂ O ₂ : Hydrogen peroxide	
GSH + NADPH + GR	18.97		
β -mercaptoethanol	18.03		
DTT, 5-X	15.94**		
Sodium ascorbate	38.64		

*P-value ≤ 0.05 ; **P-value ≤ 0.01 (Student's t-test)

Malting Process Hypothesis



Conclusions

- TLP8

ox-dep
rotein and p
f barley

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β- Gluc
activity or by
further investigat

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ough binding is

β - Glucan either the activity or by sedimentation

Further investigation

Acknowledgements



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Acknowledgements

Singh Lab Crew

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Rajvinder Kaur (McGill)

An aerial photograph of a town situated along a river. The town features a mix of residential buildings with red-tiled roofs, green lawns, and trees. A bridge crosses the river in the upper left. In the background, there are industrial structures, including tall silos. A large, bold, yellow 3D-style text "THANKS" is superimposed over the center of the image.

THANKS

Effect of Redox Agents on Binding of Barley Recombinant TLP8 to (1, 3;1, 4)- β -Glucan

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NTR + NADPH	33.67	GSSG	43.27
NTR + NADPH + TRX	4.33**	Sodium tetrathionate	37.13
GSH	33.40	The control system contained recombinant protein (TLP8), (25 μ g); 1, 3;1, 4- β -D glucan, (2.5mg). Other components: Trx, 5 μ g; DTT, 1 mM; DTT _{oxidized} 5mM; NTR, 7.5 μ g; NADP, 0.25mM, GSH, 10mM; H ₂ O ₂ , 20mM; GSSG, 25mM; glutathione reductase, 2.25 μ g.	
GSH + NADPH + GR	18.97		
β -mercaptoethanol	18.03		
DTT, 5-X	15.94**		
Sodium ascorbate	38.64		
		*P-value ≤ 0.05 ; **P-value ≤ 0.01 (Student's t-test)	

DTT: 1,4-Dithiothreitol; Trx: Thioredoxin; NTR: NADP-thioredoxin reductase; GR: Glutathione reductase
 GSH: Reduced glutathione; GSSG: Oxidized glutathione; H₂O₂: Hydrogen peroxide

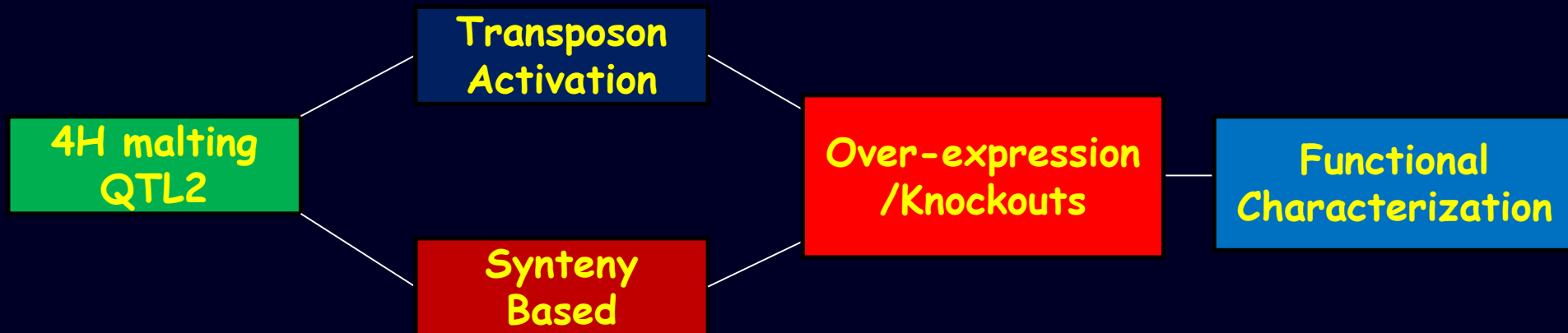
Pathogenesis Related Proteins

- PR-5 proteins have a significant role in protection against fungal attacks during dormancy and germination
- Some PR-5 proteins share sequence similarity with thaumatin, a highly sweet-tasting protein originally found in the fruit of a shrub *Thaumatococcus daniellii*
- Important role in defense and developmental processes in plants, fungi, *C. elegans*, insects, mites and animals

Thaumatococcus-Like Proteins

- TLPs exhibited high similarity with thaumatin (sweet tasting protein) isolated from the fruits of *Thaumatococcus danielli*
- TLPs are conserved in plants and their molecular mass is ranged between 21-26 kDa
- TLPs are rich in cysteine residues and form many disulphide bridges
- TLPs are classified into acidic, basic and neutral categories.

Strategy to Explore QTL2



Malting quality of barley

- Malting quality is a complex trait and controlled by quantitative trait loci (QTL).
- Malting quality is determined by factors like:
 - Kernel plumpness
 - β -glucan content
 - Seed dormancy
 - Diastatic power (α , β - amylase)
 - Malt extract yield



Malting Process

