# Functional analysis and breeding application of *Gli-D2* gliadin loci in commercial wheat

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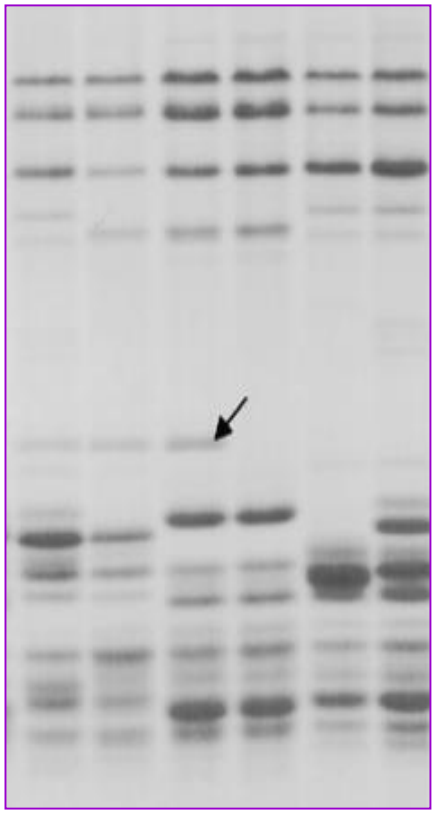
# Outline of presentation

- Background on wheat gluten and gliadins
- Main approaches
- Sequencing *Gli-A2*, *-B2* and *–D2* loci
- Identifying full-length gliadin transcripts
- Correspondence between full-length transcripts and the gliadins in the grains
- Analyzing the effects of *Gli-2* deletions
- Future prospects

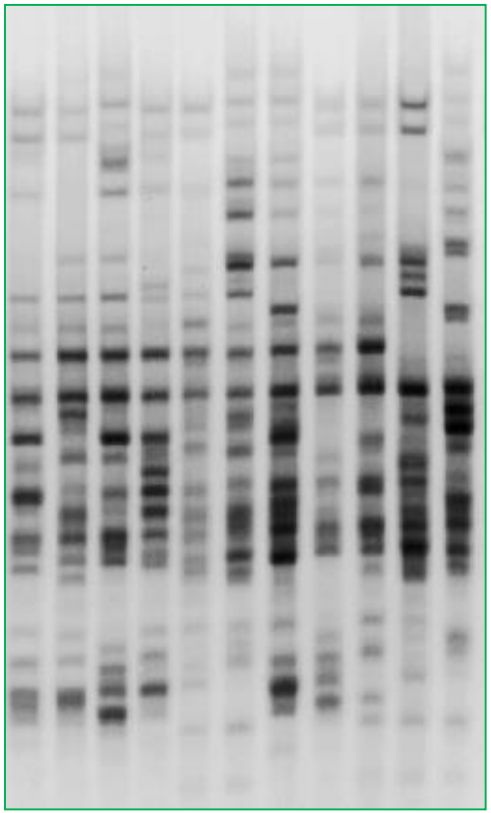
**Gluten proteins:** major components of gluten complex, synthesized during wheat grain development, and belonging to three complex groups (HMW-GS, LMW-GS and gliadin)



Glutenins in different cultivars

**High-molecular-weight glutenin subunits:** 3-5 proteins expressed per cultivar from three loci

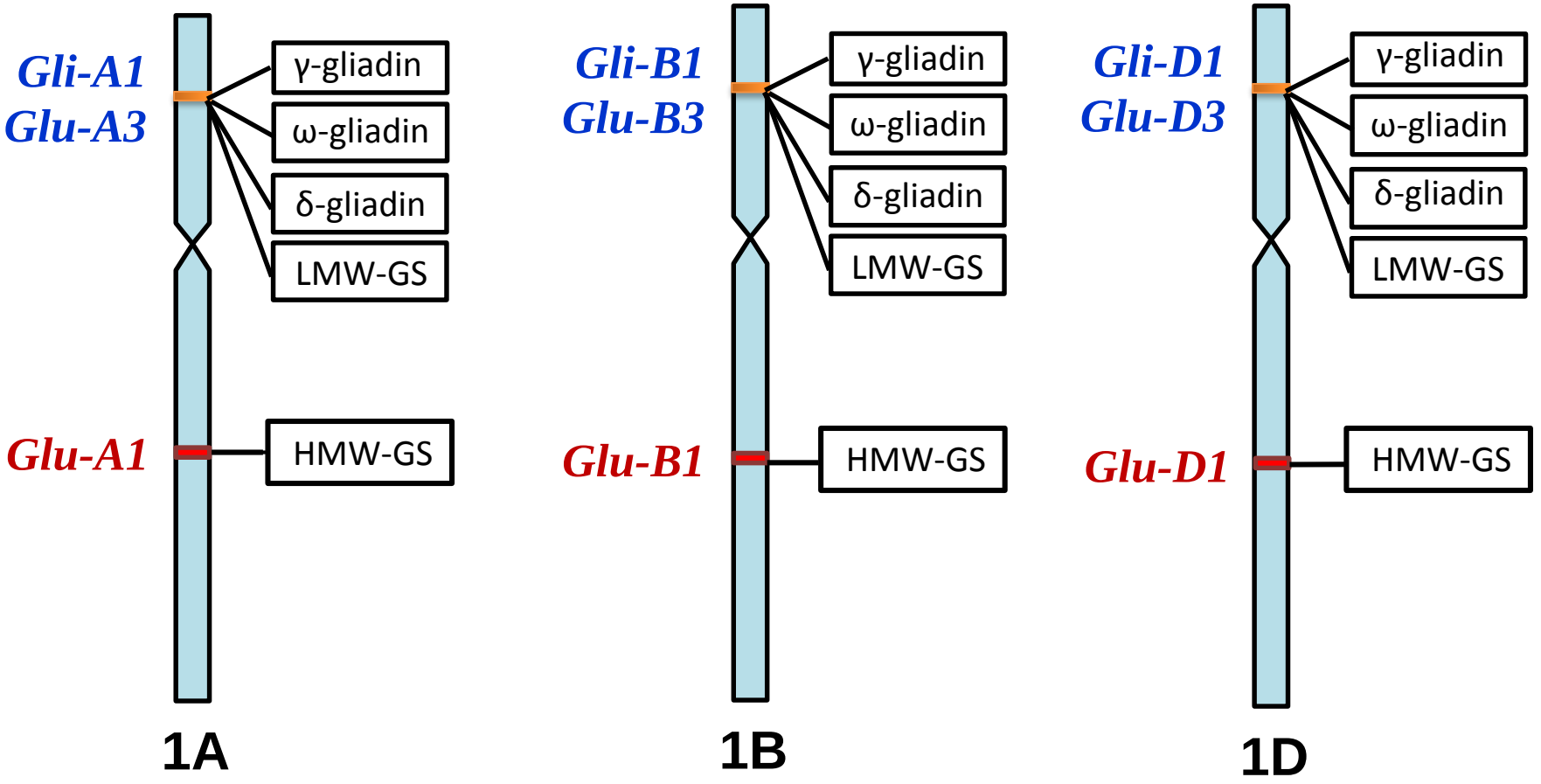
**Low-molecular-weight glutenin subunits:** 7-15 proteins expressed per cultivar from three loci



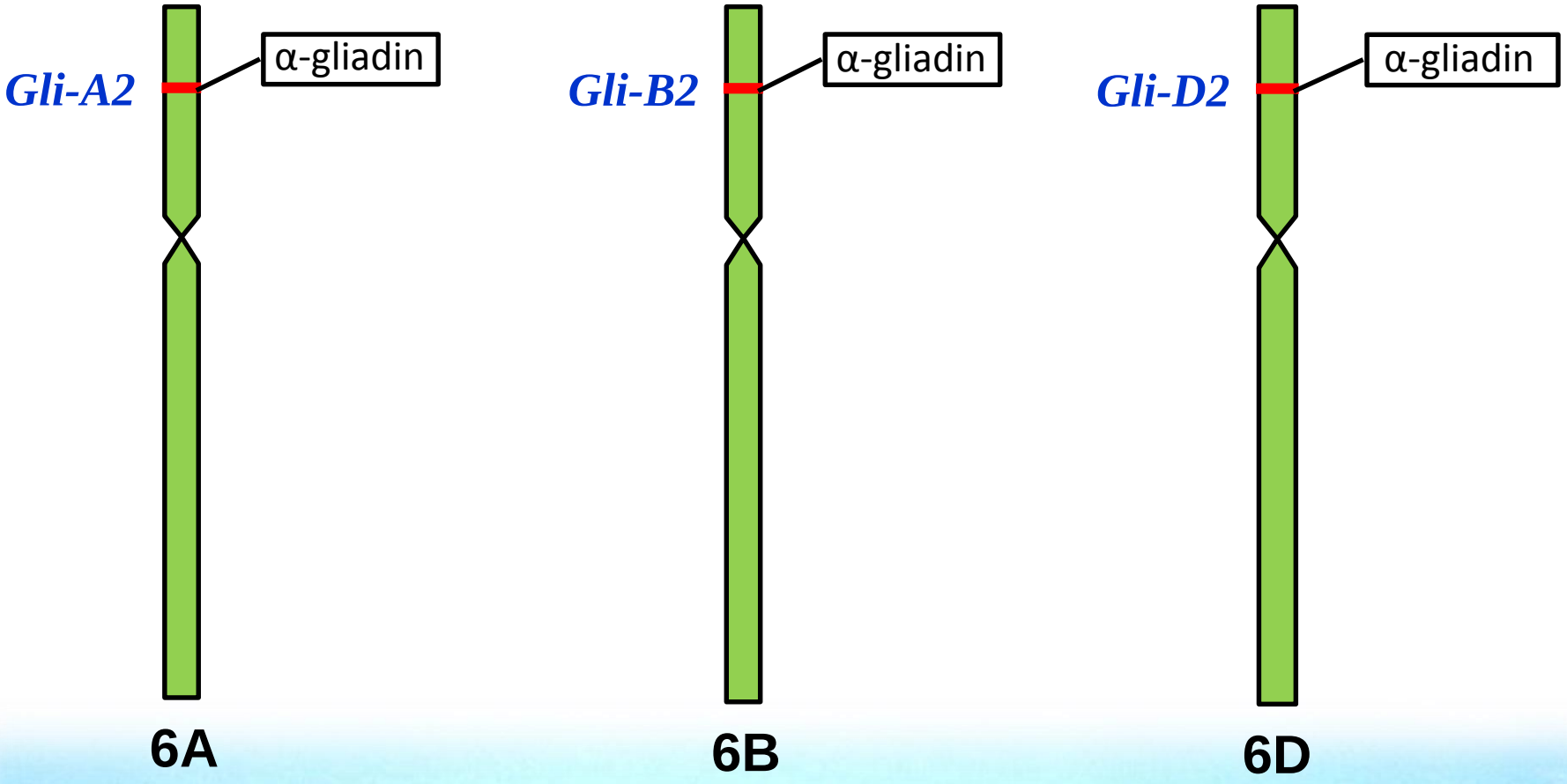
Gliadins in different cultivars

**Gliadins:** at least 50-80 proteins detected per cultivar from six loci

# Main gluten gene loci: *Gli-1/Glu-3* and *Glu-1*

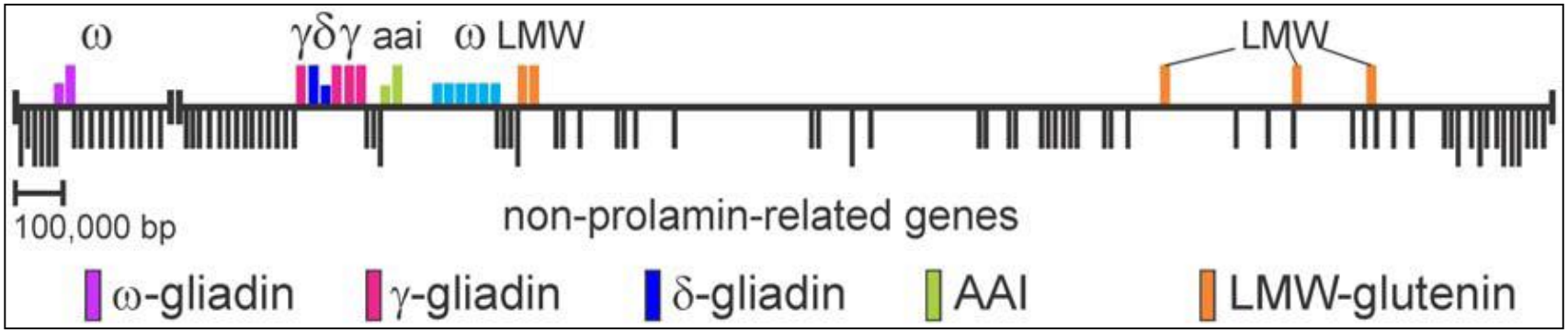


# Main gluten gene loci: *Gli-2*



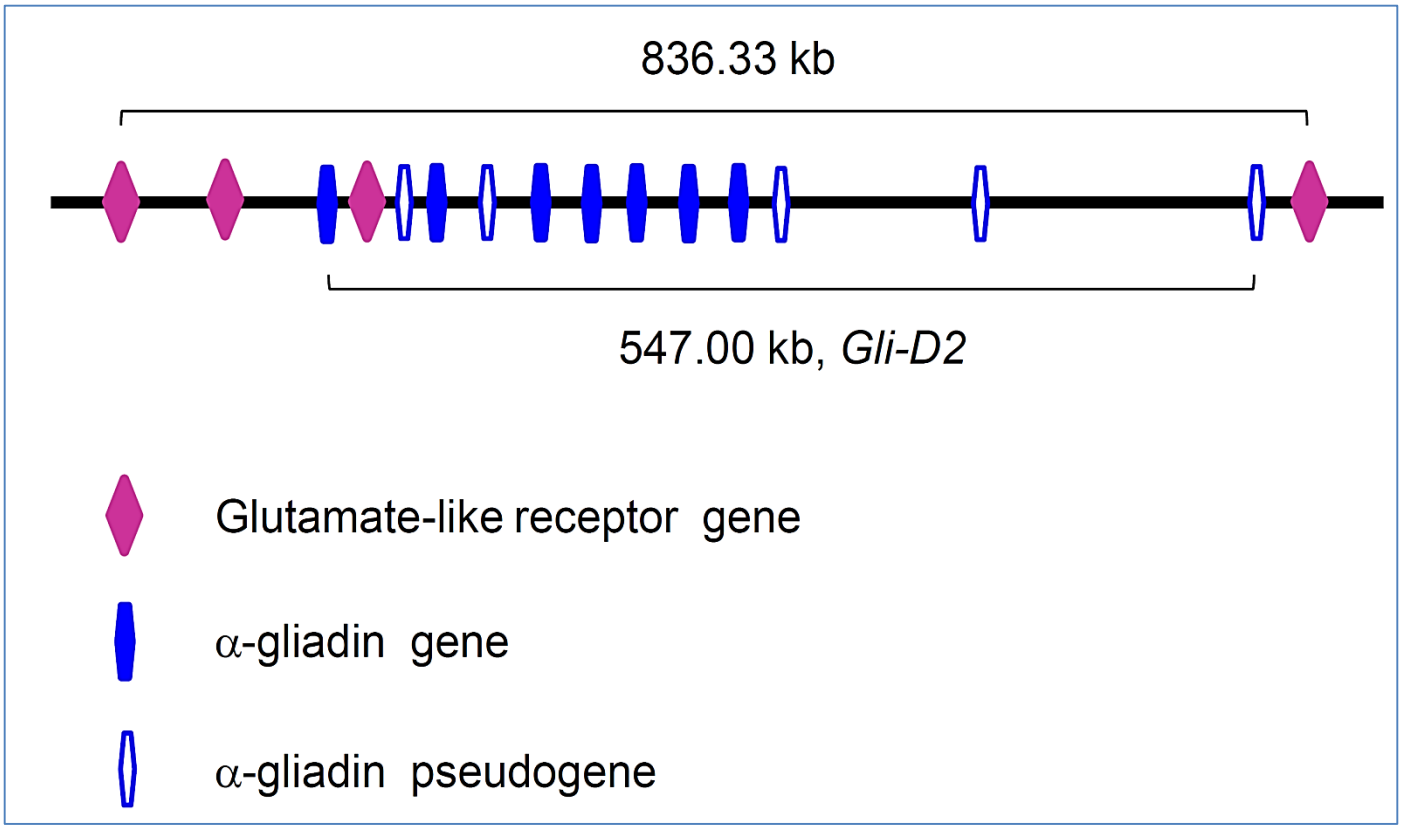
# The *Gli-1/Glu-3* loci are very large and complex

## *Gli-1/Glu-3* locus of *Aegilops tauschii*: ~ 3100 kb sequenced



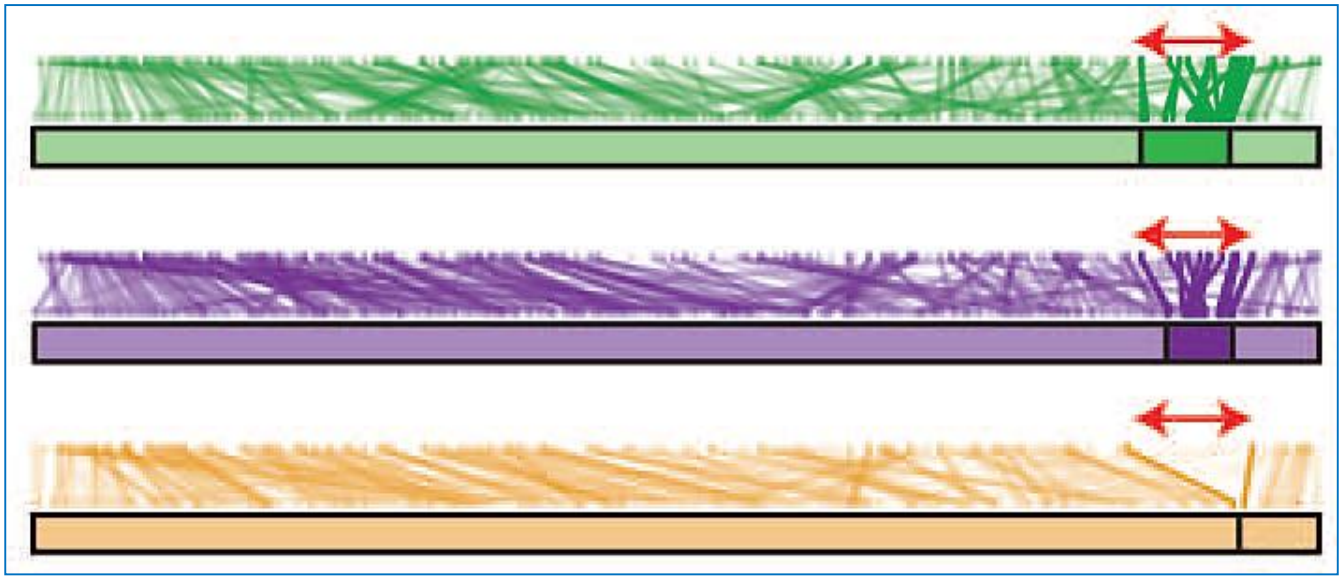

**Eight ω-gliadin, four γ-gliadin and two δ-gliadin genes are found in a genomic DNA segment of about 888.5 kb (Dong et al., 2016. Plant J. 87, 495 – 506)**

# *Gli-2* locus of *Aegilops tauschii*: ~ 550 kb




**A total of 12 α-gliadin genes are found in a genomic DNA segment of about 550 kb (Huo et al., 2017. Plant J. 92, 571-583)**

- **The draft sequence of *Gli-2* loci in Chinese Spring is still very fragmented**
- **No *Gli-D2* was present in the sequenced CS genotype**




Pfeifer et al., 2013, Science 345, 1250091

- **Several RNAi studies have shown that reducing gliadin expression is beneficial for gluten and dough functionality**

Gil-Humanes et al., 2010, Proc. Natl. Acad. Sci. USA 107, 17023 – 17028

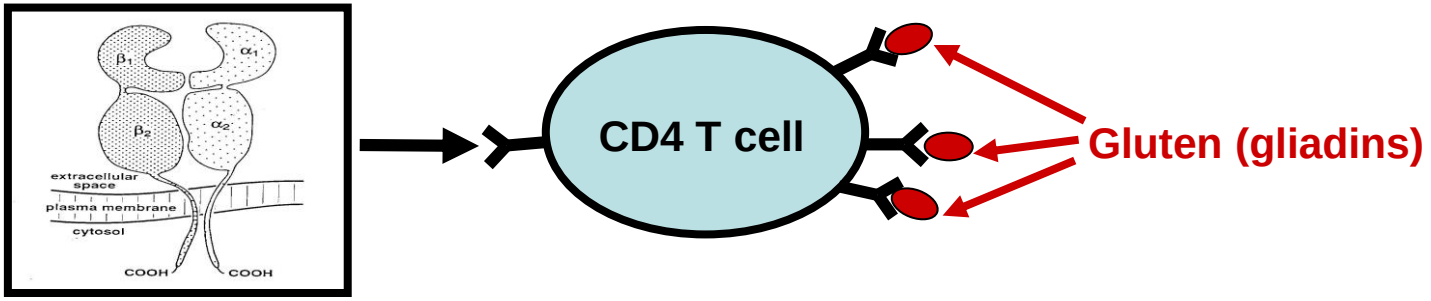
Altenbach et al., 2014, BMC Plant Biol. 14, 393

**The expression and accumulation of gliadins in bread wheat grains are still not well understood;**

**The role of different types of gliadins in wheat end-use quality control has not been systematically studied using defined mutants**

- **Gliadins are also the dominant carriers of celiac disease epitopes**
- **The α-gliadins encoded by 6D carry the most celiac-toxic epitopes**

- **Celiac disease occurs widely, with high incidence in Western countries (1%). It causes diarrhea, malabsorption, and disease lesion in the small intestine**
- **Affecting genetically susceptible individuals with the DQ2 and/or DQ8 positive HLA haplotype**




Source: Alessio Fasano and Carlo Catassi, UM, USA

**Combining genomic, transcriptomic, proteomic, mutational genetic and bioinformatic approaches to understand:**

1) **The physical structure of *Gli-A2*, *-B2* and *-D2* loci**
2) **Unique full-length gliadin transcripts in the developing grains**
3) **Correspondence between transcripts and accumulated gliadins**
4) **Presence of CD epitopes in different gliadins**
5) **Effects of *Gli-A2*, *-B2* and *-D2* deletions on end-use quality related parameters**

The main material used in our work is Xiaoyan 81, an elite winter-type bread wheat cultivar

## Sequencing *Gli-2* locus

- **58 BAC clones, 29, 21 and 8 for *Glu-A2*, *-B2* and *-D2*, respectively, were sequenced using 2nd and 3rd generation sequencing platforms**

| Locus | Number of contigs assembled | Number of gaps in the contigs | Total length (kb) | Annotated gliadin genes | Total number of glaidin genes |
|---|---|---|---|---|---|
| *Gli-A2* | 7 | 0 | 1283.349 | 10 active, 11 inactive | 28 active, 22 pseudo members |
| *Gli-B2* | 6 | 2 | 870.230 | 11 active, 8 inactive | |
| *Gli-D2* | 4 | 0 | 421.091 | 7 active, 3 inactive | |

## ➢ Identifying full-length transcripts

| α-gliadin gene | FLT | Protein (aa) | Location | α-gliadin gene | FLT | Protein (aa) | Location |
|---|---|---|---|---|---|---|---|
| *α1* | √ | 293 | 6A | *α15* | √ | 313 | 6B |
| *α2* | √ | 293 | 6A | *α16* | √ | 313 | 6B |
| *α3* | √ | 287 | 6A | *α17* | √ | 297 | 6B |
| *α4* | √ | 287 | 6A | *α18* | √ | 312 | 6B |
| *α5* | √ | 286 | 6A | *α19* | √ | 282 | 6D |
| *α6* | √ | 289 | 6A | *α20* | √ | 286 | 6D |
| *α7* | √ | 287 | 6A | *α21* | √ | 293 | 6D |
| *α8* | √ | 296 | 6A | *α22* | √ | 308 | 6D |
| *α9* | √ | 325 | 6B | *α23* | √ | 309 | 6D |
| *α10* | √ | 299 | 6B | *α24* | √ | 291 | 6D |
| *α11* | √ | 293 | 6B | *α25* | √ | 299 | 6D |
| *α12* | √ | 293 | 6B | *α26* | - | 289 | 6A |
| *α13* | √ | 296 | 6B | *α27* | - | 296 | 6A |
| *α14* | √ | 296 | 6B | *α28* | - | 296 | 6B |

## ➢ Developing *Gli* locus deletion mutants

| Deletion line | *Gli* locus deleted | Size of the deletion (cM) | Location of the deletion | Diagnostic microsatellite marker (missed in the deletion line) |
|---|---|---|---|---|
| DLGliA1 | *Gli-A1* | > 40.4 | 1AS | *Xgdm33*, *Xcfa2153*, *Xgpw7072*, *Xgpw2276*, and *Xwmc24* |
| DLGliB1 | *Gli-B1* | > 23.2 | 1BS | *Xpsp3000*, *Xwmc406*, *Xwmc230*, and *Xgpw4069* |
| DLGliD1 | *Gli-D1* | > 12.5 | 1DS | *Xwmc147*, *Xgpw7082*, *Xwmc432*, *Xcfd15*, and *Xwmc336* |
| DLGliA2 | *Gli-A2* | > 51.5 | 6AS | *Xgwm334*, *Xgpw2082*, *Xgpw7073*, *Xgpw7076*, and *Xgpw7592* |
| DLGliB2 | *Gli-B2* | > 7 | 6BS | *Xgdm113*, *Xbarc14*, *Xpsp3009*, *Xwmc494*, and *Xgwm508* |
| DLGliD2 | *Gli-D2* | > 23.5 | 6DS | *Xcfd132*, *Xcfd33*, and *Xgdm127* |

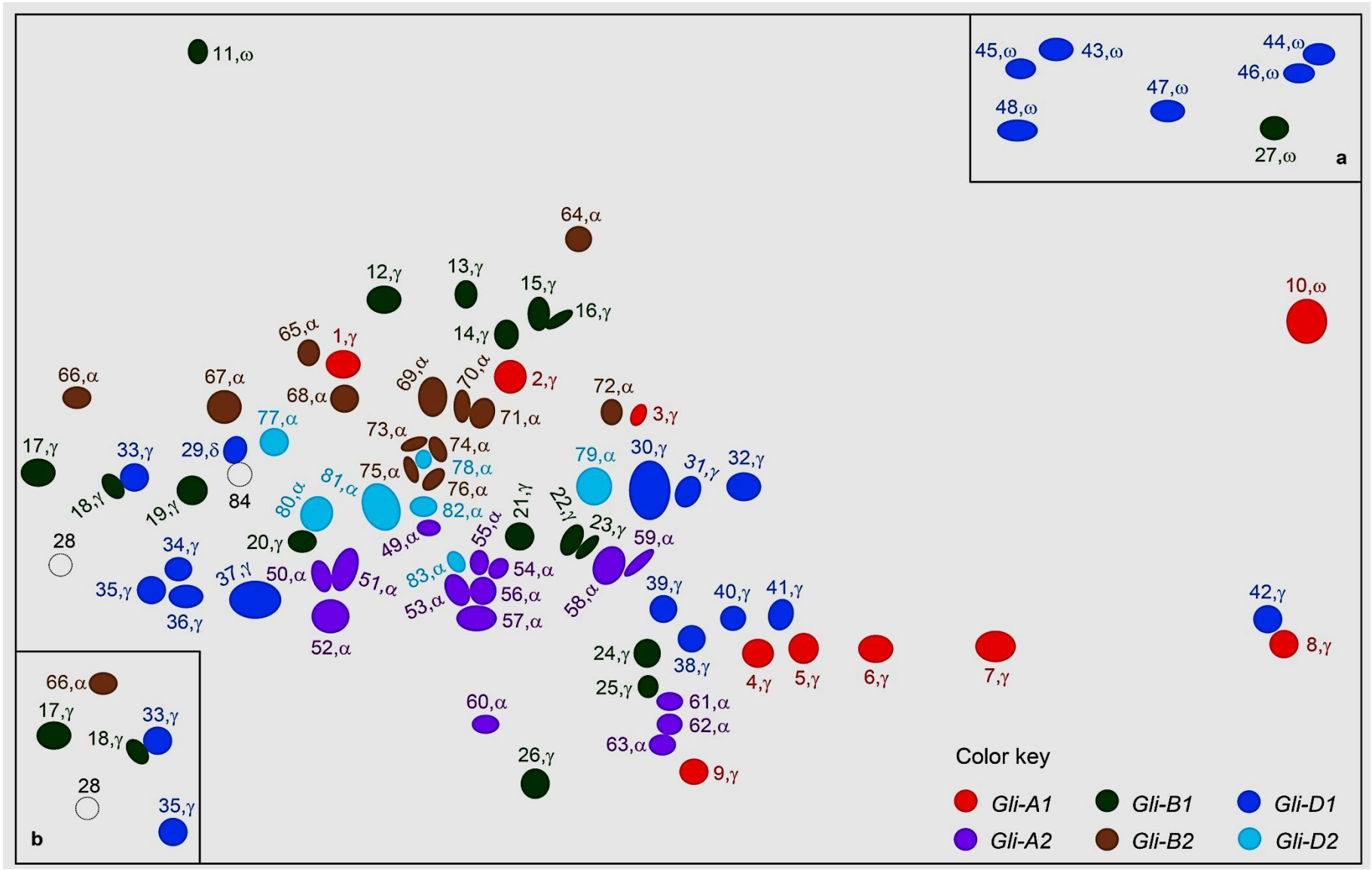
## Resolving gliadin 2-DE spots of Xiaoyan 81

- Of the 84 gliadin 2-DE spots of Xiaoyan 81 mature grains, 82 were well resolved, 35 for α-gliadin, 37 for γ-gliadin, 9 for ω-gliadin, and 1 for δ -gliadin.
- The 82 spots were assigned to individual *Gli* locus using the six deletion mutants.

| **Deletion line (*Gli* locus deleted)** | **Gliadin spot lacked** | | ***Gli* locus assignment of the spot** |
|---|---|---|---|
| | **2-DE spot** | **Total** | |
| **DLGliA1 (*Gli-A1*)** | **1 - 10** | **10** | ***Gli-A1*** |
| **DLGliB1 (*Gli-B1*)** | **11 - 27** | **17** | ***Gli-B1*** |
| **DLGliD1 (*Gli-D1*)** | **29 - 48** | **20** | ***Gli-D1*** |
| **DLGliA2 (*Gli-A2*)** | **49 - 63** | **15** | ***Gli-A2*** |
| **DLGliB2 (*Gli-B2*)** | **64 - 76** | **13** | ***Gli-B2*** |
| **DLGliD2 (*Gli-D2*)** | **77 - 83** | **7** | ***Gli-D2*** |

# Resolving gliadin 2-DE spots of Xiaoyan 81

## 2-DE gliadin spots of Xiaoyan 81 mature grains

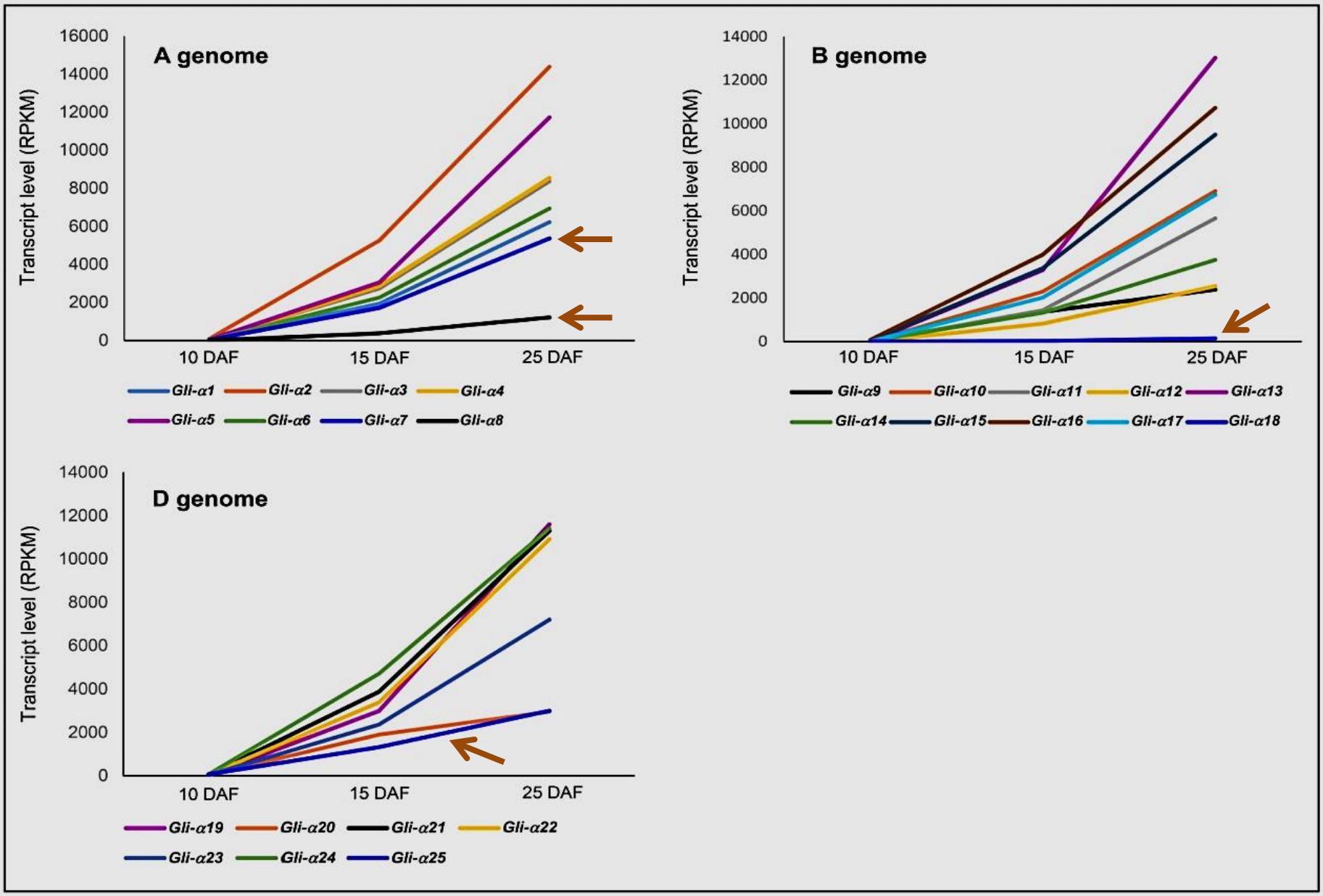


## ➢ Correpodence among gliadin genes, FLTs and proteins

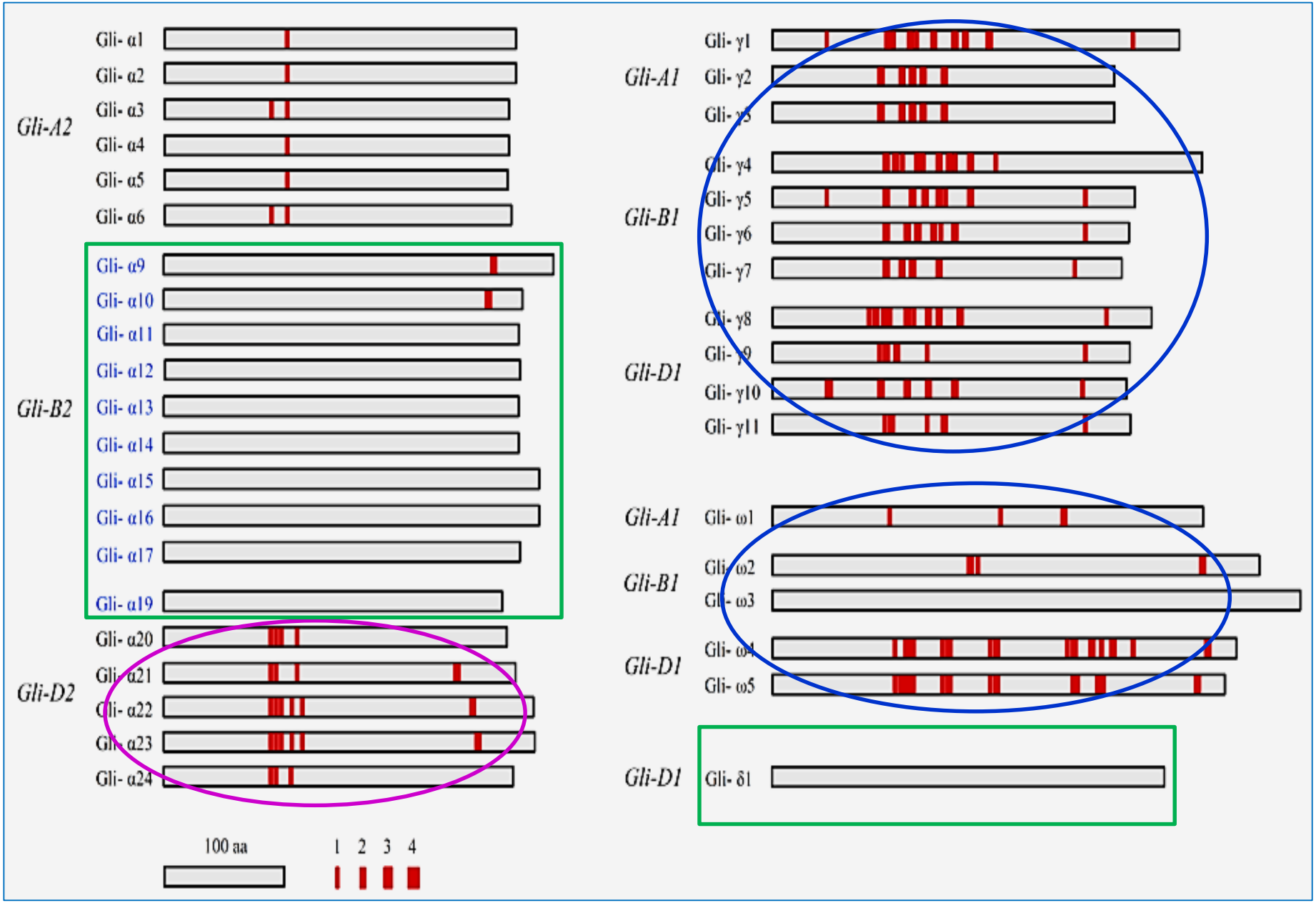
- The 35 α-gliadin 2-DE spots were matched to 21 active α-gliadin gene members.
- The 37 γ-gliadin 2-DE spots were matched to 11 active γ-gliadin gene members.
- The 1 δ-gliadin 2-DE spot was matched to 1 δ-gliadin gene.
- The 9 ω-gliadin 2-DE spots were matched to 5 ω-gliadin gene members.

- ✓ **In Xiaoyan 81 mature grains, 38 gliadin proteins are accumulated, including 21 α-gliadins, 11 γ-gliadins, 5 ω-gliadins and 1 δ-gliadin.**
- ✓ **Seven active α-gliadin genes are transcribed, but their proteins are not detected in mature grains.**

# Correpodence among gliadin genes, FLTs and proteins

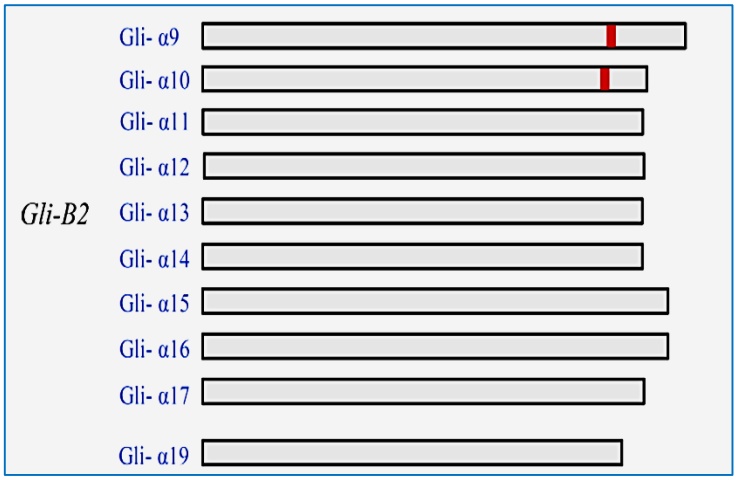


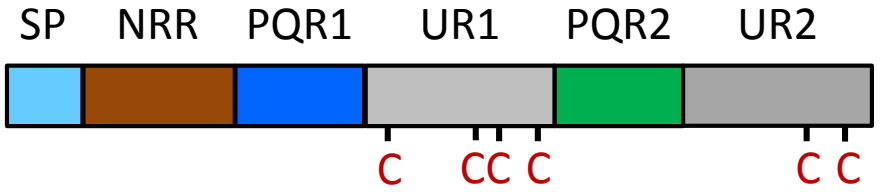
# ➢ Analyzing CD epitopes in 38 accumulated glaidins



# ➢ Analyzing CD epitopes in 38 accumulated gliadins

## The CSTT group of α-gliadins




UR2

```
Gli-α1
Gli-α2   PSSQVSFQQPLQQYPLGQGSFRPSQQNPQAQGSVQPQQLPQFEEIRNLALQTLPAMCNVYIPPYC--TMAPFGIFGTN  293
Gli-α3   PSSQVSFQQPLQQYPLGQGSFRPSQQNPQAQGSVQPQQLPQFEEIRNLALQTLPAMCNVYIPPYC--TIAPFGIFGTN  293
Gli-α4   PSSQVSFQQPLQQYPLGQGSFRPSQQNPQAQGSVQPQQLPQFEEIRNLALQTLPAMCNVYIPPYC--TIAPFGIFGTN  287
Gli-α5   PSSQVSFQQPLQQYPLGQGSFRPSQQNPQAQGSVQPQQLPQYEEIRNLALQTLPAMCNVYIPPYC--TIAPFGIFGTN  287
Gli-α6   LSSQVSFQQPQQQYPLGQGSFRPSQQNSQAQGSVQPQQLPQFEEIRNLALQTLPAMCNVYIPPYC--TIAPFGIFGTK  286
Gli-α7   LSSQVSFQQPQQQYPLGQGSFRPSQQNPQAQGSVQPQQLPQFEEIRNLALQTLPAMCNVYIPPYC--TIAPFGIFGTN  289
Gli-α8   PSSQVSFQQPLQQYPLGQGSFRPSQQNPQAQGSVQPQQLPQFEEIRNLALQTLPAMCNVYIPPYC--TIAPFGIFGTN  287
Gli-α9   PSSQVSLQQPQQQYPSGQGFFQPSQQNPQAQGSVQPQQLPQFEEIRNLALQTLPRMCNVYIPPYCSTTIAPFGIFGTN  296
Gli-α10  PSSQVSYQQPQQQYPSGQGSFQPSQQNPQAQASVQPQQLPQFEEIRNLARQTLPAMCNVYIPPYCSTTIAPSGIFGTN  325
Gli-α11  PSSQVSYQQPQQQYPSGQGSFQPSQQNPQAQGFVQPQQLPQFEEIRNLALQTLPAMCNVYIPPYCSTTIAPFGIMSTN  299
Gli-α12  PSSQVSYQQPQQQYPSGQGFFQPSQQNPQAQGFVQPQQLPQFEEIRNLALQTLPAMCNVYIPPYCSTTIAPFGIMSTN  293
Gli-α13  PSSQVSYQQPQQQYPSGQGFFQPSQQNPQAQGFVQPQQLPQFEEIRNLALQTLPAMCNVYIPPYCSTTIAPFGIMSTN  293
Gli-α14  PSSQVSYQQPQQQYPSGQGFFQPSQQNPQAQGFVQPQQLPQFEEIRNLALQTLPAMCNVYIPPYCSTTIAPFGIMSTN  296
Gli-α15  PSSQVSYQQPQQQYPSGQGFFQPSQQNPQAQGFVQPQQLPQFEEIRNLALQTLPAMCNVYIPPYCSTTIAPFGIMSTN  296
Gli-α16  PSSQVSFQQPQQQYPSSQVSFQPSQLNPQAQGSVQPQQLPQFAEIRNLALQTLPAMCNVYIPPHCSTTIAPFGIFGTN  313
Gli-α17  PSSQVSFQQPQQQYPSSQVSFQPSQLNPQAQGSVQPQQLPQFAEIRNLALQTLPAMCNVYIPPHCSTTIAPFGIFGTN  313
Gli-α18  PSSQVSYQQPQQQYPSGQGFFQPSQQNPQAQGFVQPQQLPQFEEIRNLALQTLPAMCNVYIPPYCSTTIAPFGIMSTN  297
Gli-α19  PSSQVSFQQPQQQYPSSQVSFQPSQLNPQAQGSVQPQQLPQFAEIRNLALQTLPAMCNVYIPPHCSTTIAPFGIFGTN  312
Gli-α20  PSSQVSYQQPQEQYPSGQGSFQSSQQNPQAQGSVQPQQLPQFQEIRNLALQTLPAMCNVYIPPYCSTTIAPFGIFGTN  282
Gli-α21  PLTQVSFQQPQQQYPSGQGSFQPSQQNPQAQGSVQPQQLPQFEEIRNLALETLPAMCNVYIPPYC--TIAQVGIFGTN  286
Gli-α22  PLSQVSFQQPQQQYPSGQGSFQPSQQNPQAQGSVQPQQLPQFEEIRNLALETLPAMCNVYIPPYC--TIAPVGFFGTN  293
Gli-α23  PLSQVSFQQPQQQYPSGQGSFQPSQQNPQAQGSVQPQQLPQFEEIRNLALETLPAMCNVYIPPYC--TIAPVGIFGTN  308
Gli-α24  PLSQVSFQQPQQQYPSGQGSFQPSQQNPQAQGSVQPQQLPQFEEIRNLALETLPAMCNVYIPPYC--TIAPVGIFGTN  309
Gli-α25  PLSQVSFQQPQQQYPSGQGFFQPSQQNPQAQGSFQPQQLPQFEAIRNLALQTLPAMCNVYIPPYC--TIAPFGIFGTN  291
         PLSQVCFQQSQQQYPSGQGSFQPSQQNPQAQGSVQPQQLPQFEEIRNLALETLPAMCNVYIPPYC--TIAPVGIFGTN  299
```

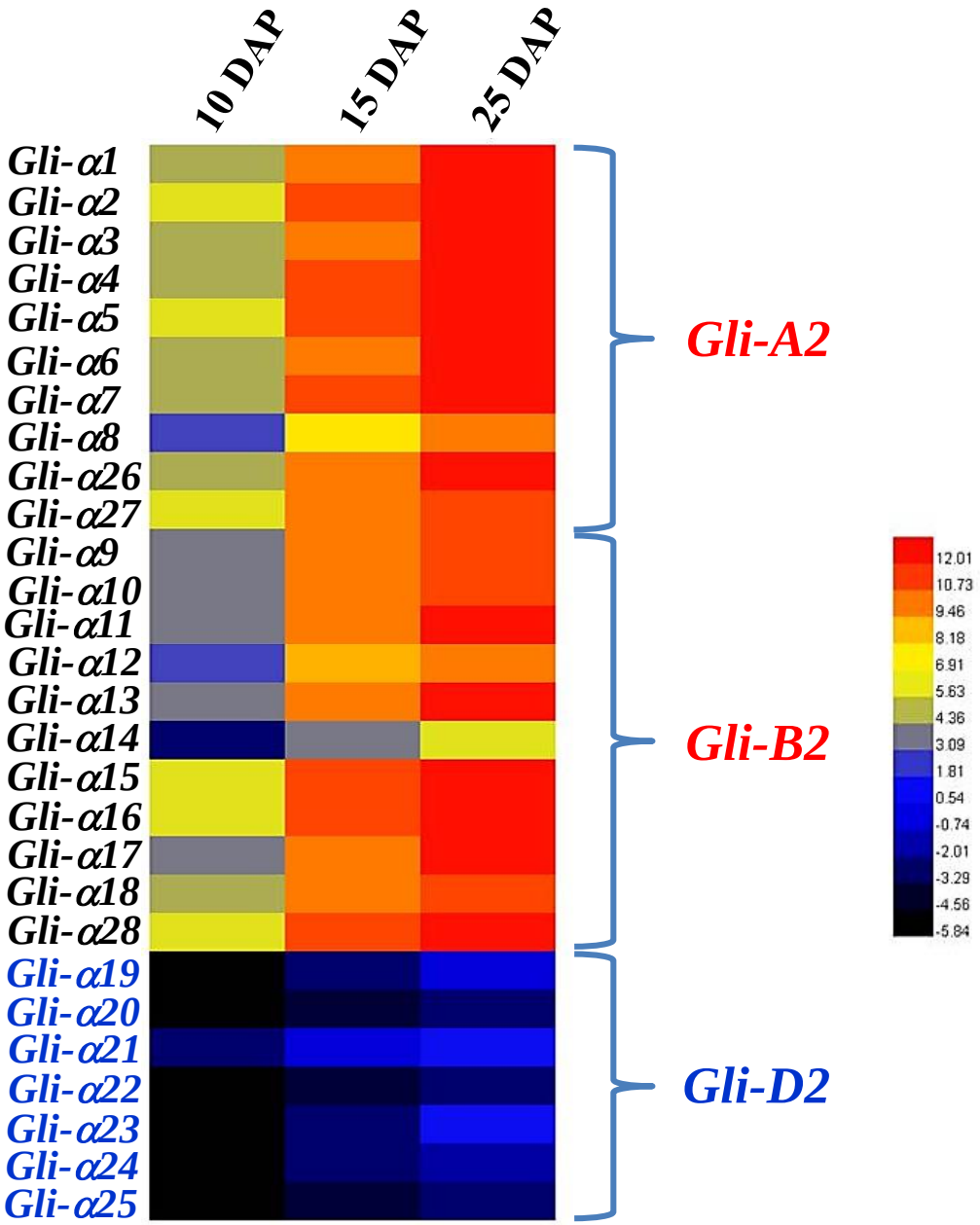
## Analyzing the effects of *Gli-2* deletions

- **We focused on analysing the effects of *Gli-2* locus deletion mutants.**
- **DLGliB2 is partially sterile; DLGliA2 and DLGLiD2 do not differ significantly from WT progenitor.**
- **DLGliD2 exhibits improved gluten and dough functionalties and breadmaking quality, and has a reduced CD epitope level.**
- **DLGliA2 is less superior than DLGliD2.**

**Xiaoyan 81** **DLGliA2** **DLGliB2** **DLGliD2**

## Analysing the effects of *Gli-D2* deletion

The transcripts of the seven active *Gli-D2* gliadin genes could not be detected in DLGliD2 grains

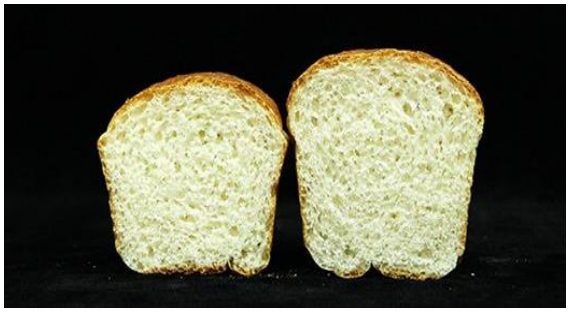


# Improving the breadmaking quality of WT progenitor: Xiaoyan 81

| Env | Line | Water absroption (%) | SDS-SV (mL) | Stability time (min) | Maximum resistance (BU) | Loaf volume (mL) |
|---|---|---|---|---|---|---|
| Beijing | Xiaoyan 81 | 63.10±0.62 | 32.67± 0.24 | 2.79 ± 0.11 | 82.83 ± 14.92 | 580.00 ± 20.00 |
| | DLGliD2 | 63.60±0.42 | 40.27±0.19*** | 4.81 ± 0.44** | 129.33 ± 1.31* | 676.67 ± 37.04* |
| Zhaoxian | Xiaoyan 81 | 63.10±0.36 | 32.40±0.28 | 3.05 ± 0.12 | 82.17 ± 8.99 | 631.67 ± 15.46 |
| | DLGliD2 | 63.83±0.46 | 38.27±0.19*** | 4.74 ± 0.57* | 138.50 ± 16.28* | 728.33 ± 22.48* |
| Xinxiang | Xiaoyan 81 | 64.40±0.14 | 36.37±0.52 | 3.38 ± 0.12 | 84.00 ± 9.91 | 595.00 ± 14.72 |
| | DLGliD2 | 65.10±0.29 | 42.10±0.14*** | 5.97 ± 0.38** | 104.33 ± 5.04 | 698.33 ± 16.50** |

Xiaoyan 81 DLGliD2

Xiaoyan 81 DLGliD2

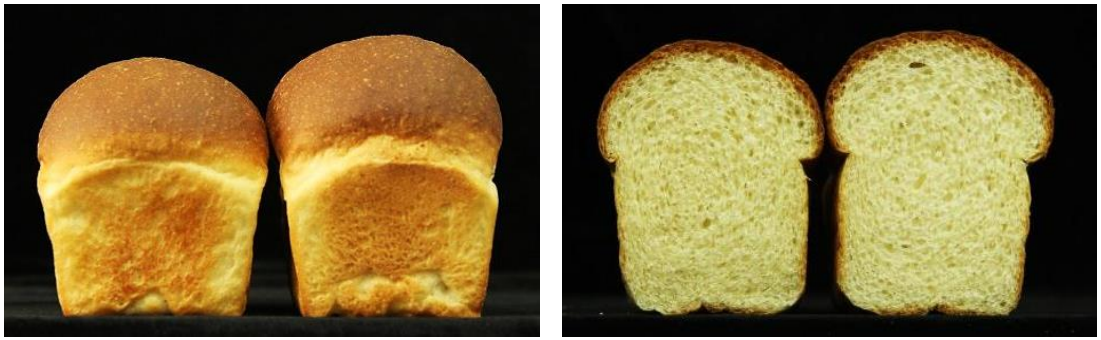


**Loaf voulme increased by 15.37- 17.31%**

# Improving the breadmaking quality of an elite cultivar: Zhengmai 366

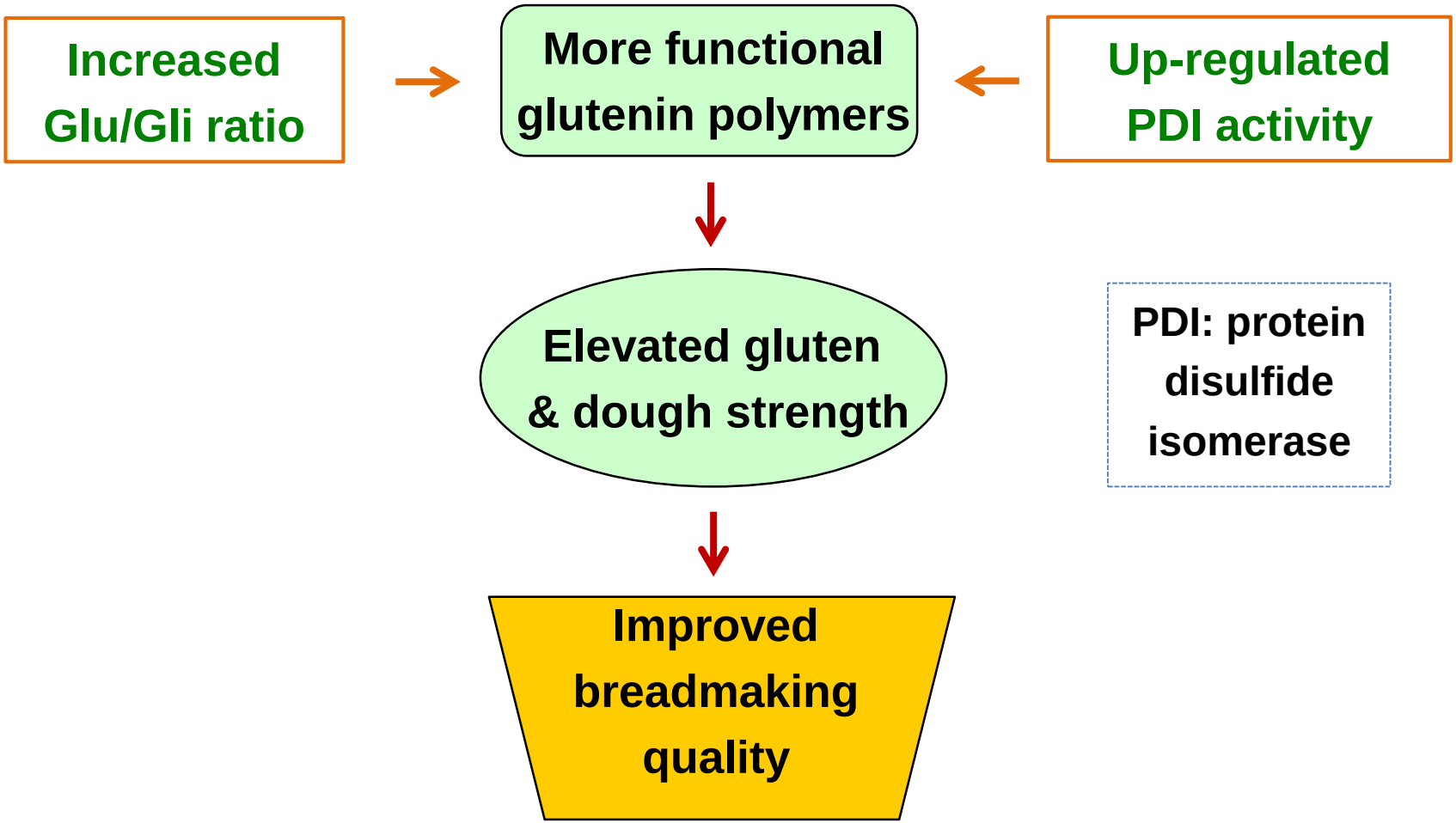
| Env | Line | Water absorption (%) | SDS-SV (mL) | Stability (min) | Loaf volume (mL) |
|---|---|---|---|---|---|
| Zhaoxian | Zhengmai 366 | 66.45±0.05 | 36.73 ± 0.38 | 5.69 ± 0.33 | 745.00 ± 18.71 |
| | ZDLGliD2 | 66.00±0.08 | 44.53 ± 0.45*** | 10.68 ± 0.82** | 833.33 ± 40.28* |
| Xinxiang | Zhengmai 366 | 66.80±0.36 | 40.13 ± 0.19 | 8.59 ± 1.15 | 808.33 ± 6.24 |
| | ZDLGliD2 | 66.80±0.36 | 47.00 ± 0.00*** | 10.77 ± 0.74 | 855.00 ± 20.41* |

ZM366 ZDLGliD2 ZM366 ZDLGliD2

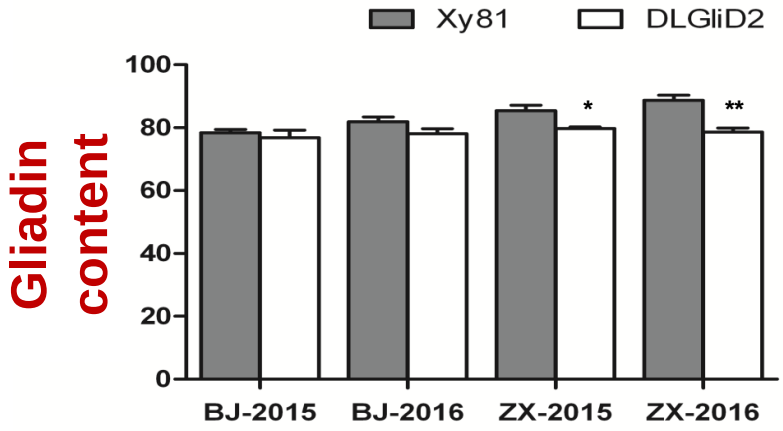


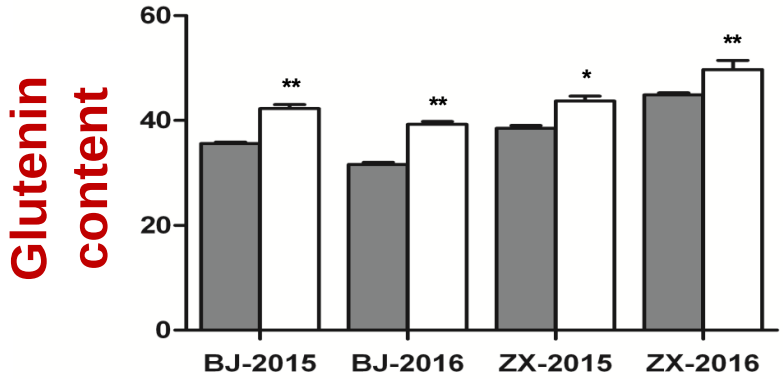
**Loaf voulme increased by 5.82- 11.80%**

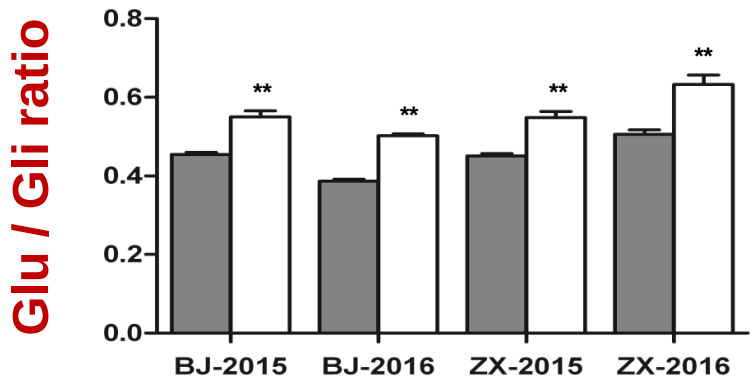
# Mechanism underlying the enhancement of breadmaking quality by *Gli-D2* deletion



## ➢ Analysing the effects of *Gli-D2* deletion

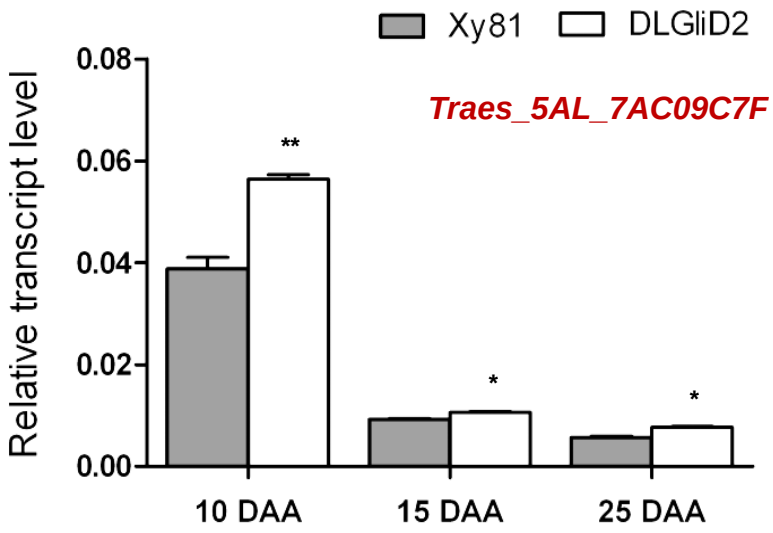


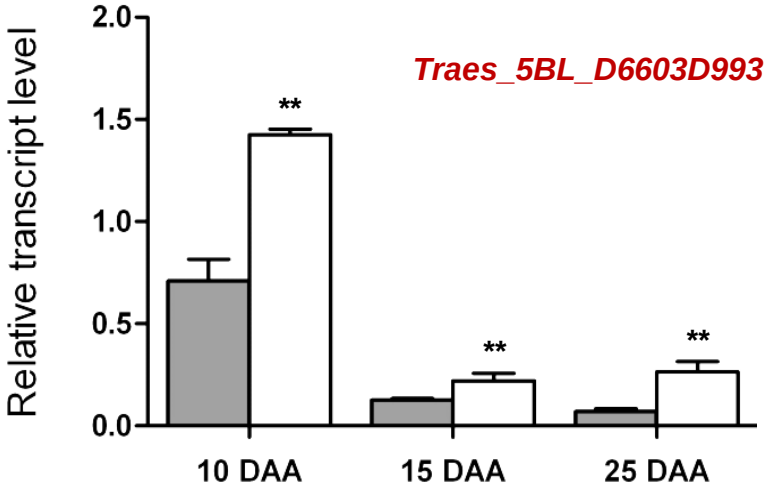



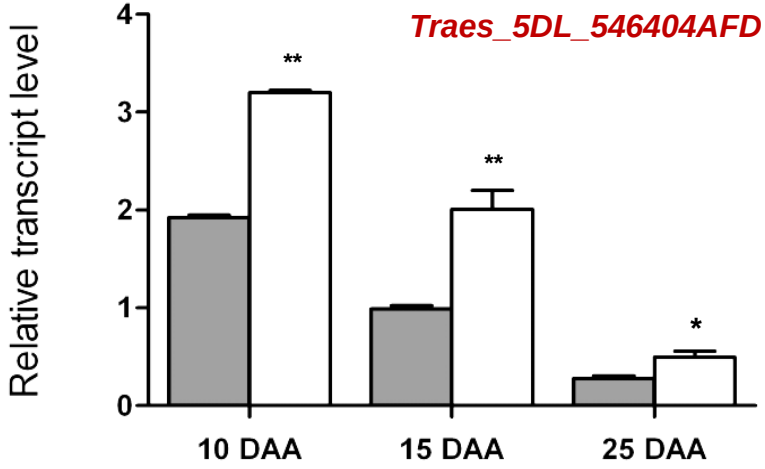



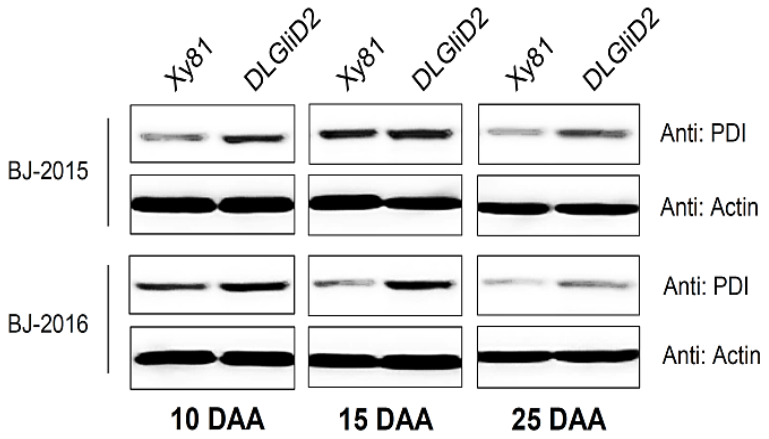

**DLGliD2 grains had reduced gliadins, but increased glutenins and thus Glu/Gli ratio**

# Up-regulated expression of PDI genes in DLGliD2 grains



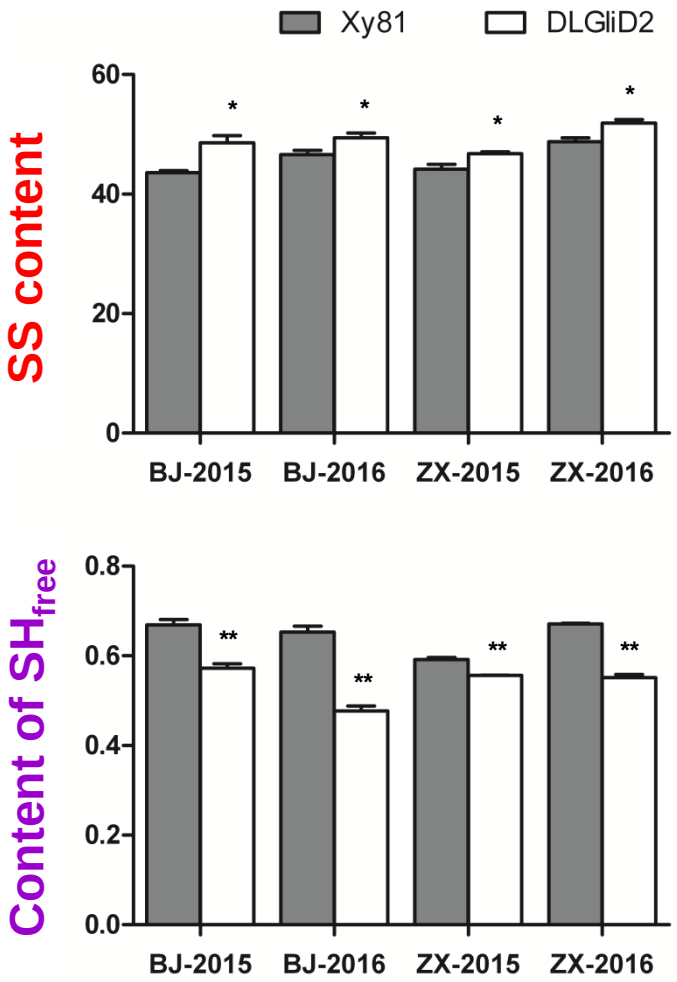



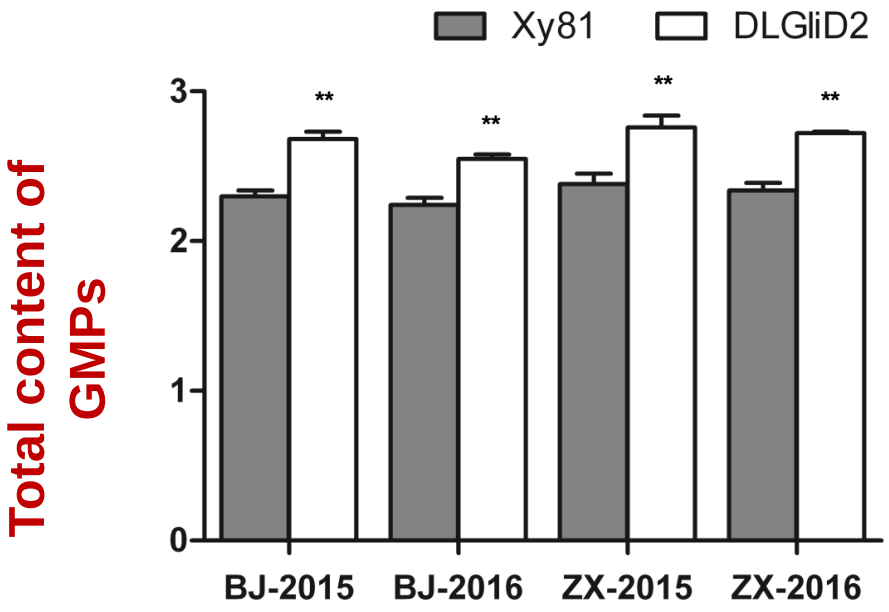
➢ Analysing the effects of *Gli-D2* deletion

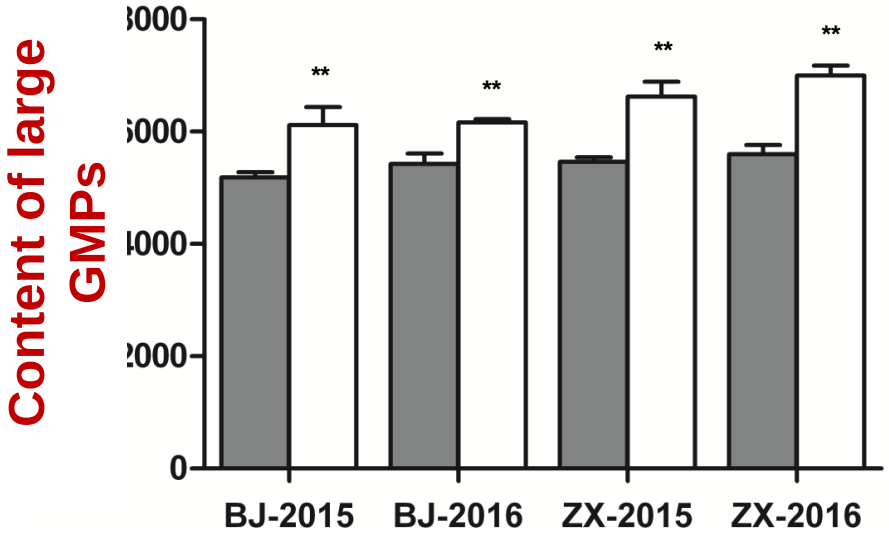
# Indicators of up-regulated activity of PDI enzymes




**The content of disulfide, but not that of free sulfhydryl, was significantly up-regulated in DLGliD2 grains in all four environments**

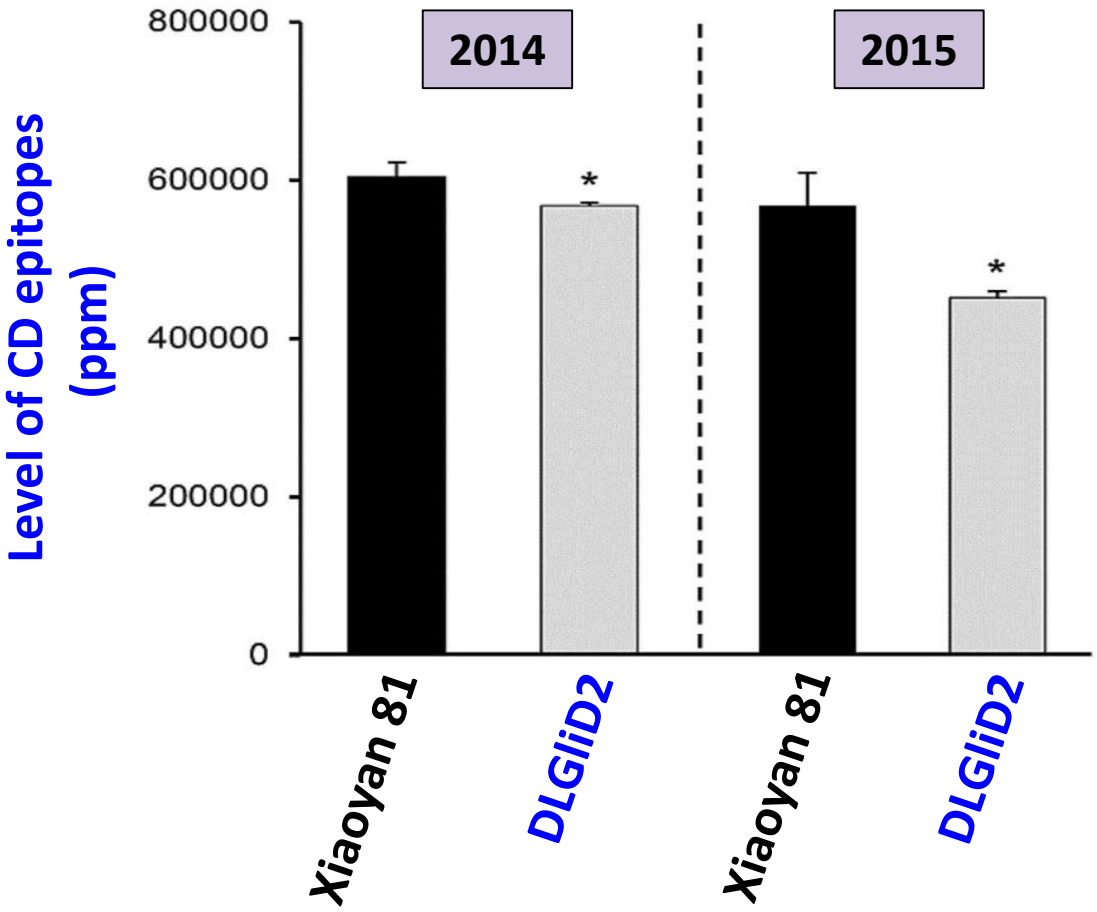
## ➢ Analysing the effects of *Gli-D2* deletion



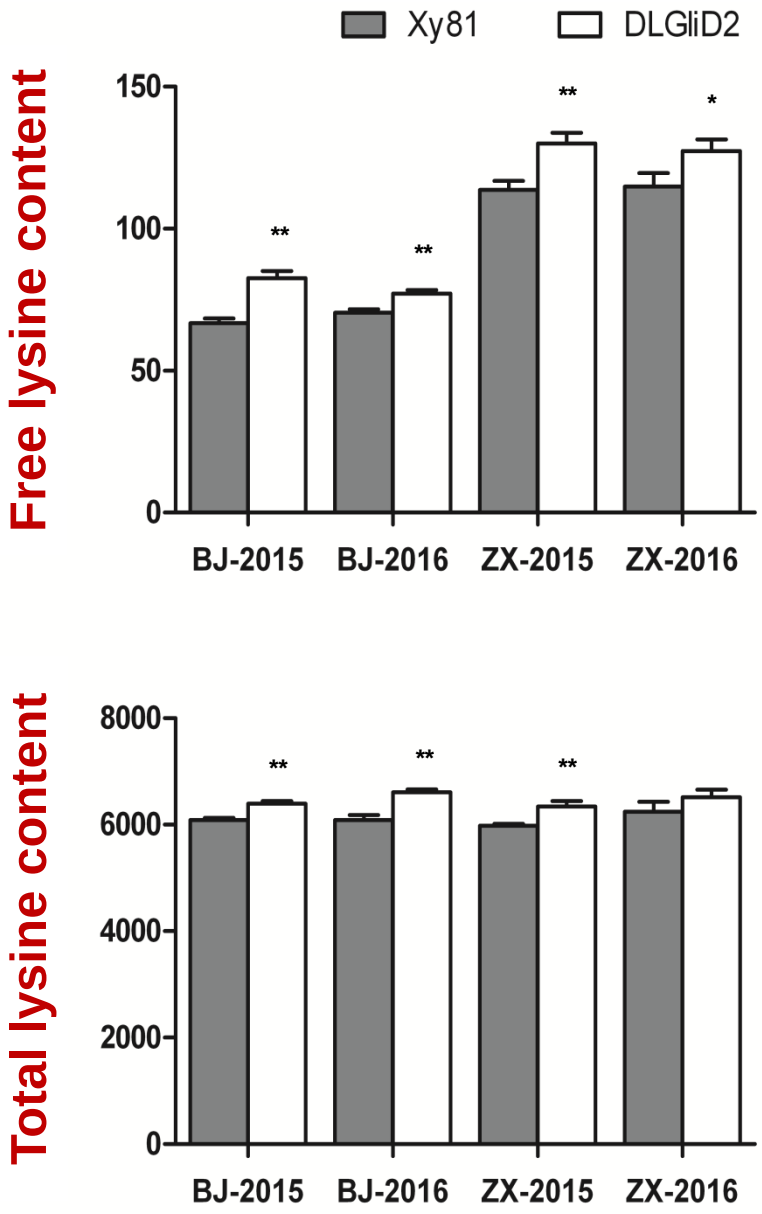




**More functional GMPs were detected in DLGliD2 grains in four environments**

# DLGliD2 grains had reduced level of CD epitopes



## ➢ Analysing the effects of *Gli-D2* deletion




**Free and total lysine contents were elevated in DLGliD2 grains in four environments**

# Pyrimiding newly created alleles with known elite alleles in elite genetic background

**DNA markers were used to introduce newly created alleles into two high-yielding cultivars**

***Gli-D2-null*: lacking Gli-D2**

***Glu-D1d*: encoding 1Dx5 and 1Dy10**

***Sec-1-null:* 1BL/1RS translocation lacking *Sec-1* encoded secalins**

**HG35: nationally certified, with 1BL/1RS but lacking 1Dx5 and 1Dy10**

**ZM7698: nationally certified, expressing 1Dx5 and 1Dy10**

## ☐ Breeidng application of *Gli-D2* deletion (*Gli-D2-null*)

PLHG-BC4F5 HG35

**PLHG-BC4F5 carries *Gli-D2-null* and *Sec-1-null* and expresses 1Dx5 and 1Dy10**

**Its insoluble glutenin (large glutenin polymer) content was 40.91% higher than that of HG35**

## ☐ Breeidng application of *Gli-D2* deletion (*Gli-D2-null*)

PLZM-BC4F5 ZM7698

**PLZM-BC4F5 carries *Gli-D2-null* and *Sec-1-null* and expresses 1Dx5 and 1Dy10**

**Its insoluble glutenin (large glutenin polymers) content was 9.77% higher than that of ZM7698**

## ➢ Summary and future perspectives

- The *Gli-2* locus sequence information is likely useful for further studying the structure, expression, function and evolution of these loci.
- The unique full-length transcripts of gliadin genes and the gliadins accumulated in the grains revealed by us encompass different types of gliadins known to exist in different wheat species and genotypes. They may be used as basic reference materials for future investigations.
- DLGliD2 should be useful for further refining the end-use quality traits of common wheat.
- Our insight on the distribution of CD epitopes in different gliadins and the significant reduction of celiac-toxic α-gliadins in DLGliD2 may stimulate more efforts for developing the wheat lines with enhanced health benefits.

Wang DW, et al., 2017. Genome-wide analysis of complex wheat gliadins, the dominant carriers of celiac disease epitopes. Sci Rep. 7: 44609. doi: 10.1038/srep44609.

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# Thank you !