

Development and Application of Fast Immunological Selection Methods for High Molecular Weight Glutenin Subunits in Wheat Breeding

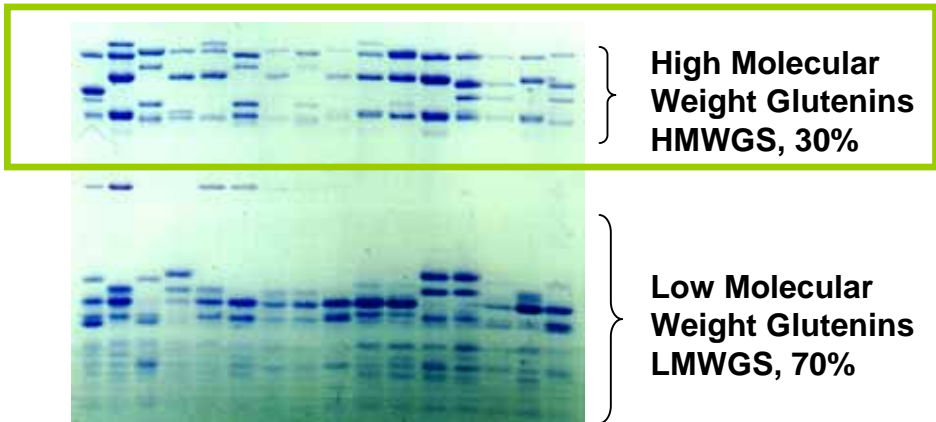
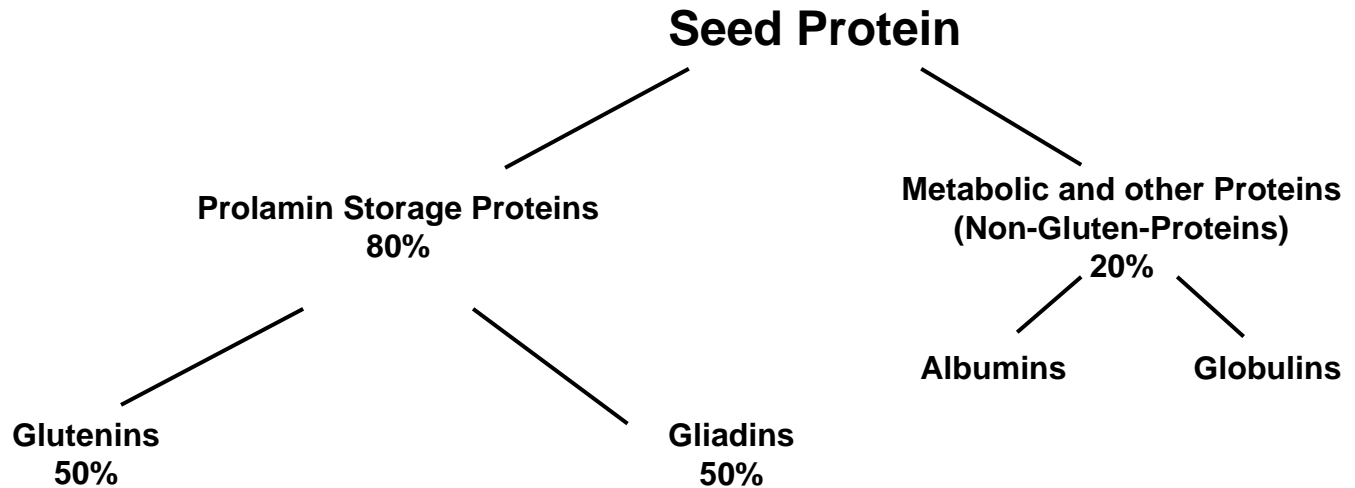
Helga Gruber and Berta Killermann

Bavarian State Research Center of Agriculture

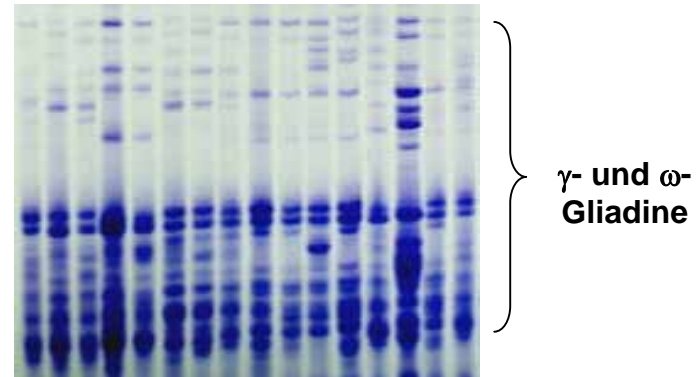
Institute for Crop Science and Plant Breeding

D-85354 Freising, Germany

Proteins in wheat endosperm



→ Dough Elasticity



→ Dough Plasticity, Viscosity

Immunological features of HMW subunits

- Comparison of HMW subunit amino acid sequences (AAS)
- High degree of homology in their AAS (up to 95 %)
- Extensive structural homologies between HMW subunits

Immunological features of HMW subunits

- Comparison of HMW subunit amino acid sequences (AAS)
- High degree of homology in their AAS (up to 95 %)
- Extensive structural homologies between HMW subunits



Approach for monoclonal antibody development

- Search for unique AAS-motifs
- Search for small structural epitopes characteristic for single HMW subunits
- Use of synthetic peptides as immunogen

ELISA-development for detection of HMW subunit 1 (allele *Glu-A1a*)

AAS of HMW subunit 1 (precursor)

tr | X61 MTKRLVLF¹⁰AAVVVALV²⁰ALTA³⁰AE³⁵GEASGQL⁴⁰QC⁴⁵ERELQE⁵⁰---HSLKA⁵⁵CRQVVDQQLRDVSP⁶⁰E

N-Term.

tr | X61 ⁶⁰CPVGGG⁷⁰PVARQY⁸⁰EQVVVPPK⁹⁰GSFY¹⁰⁰PGETTP¹¹⁰PQQLQ¹¹⁵QSILWGIPALLRRYYLSVTS¹²⁰PQ

tr | X61 ¹²⁰QVSYYPGQAS¹³⁰SQRPGQ¹⁴⁰QPPGQ¹⁵⁰QOE-----YYLTS¹⁶⁰PQ¹⁷⁰SGQWQ¹⁷⁵QPPGQ¹⁸⁰QAGYYPTSP¹⁸⁵Q

tr | X61 ¹⁸⁰QSGQE¹⁹⁰QPGYYPTSP²⁰⁰WQPELQ²¹⁰QPTQ²²⁰GQ²³⁰RRQ²³⁵QPPG²⁴⁰QQLRQ²⁴⁵GQ²⁵⁰QSGQ²⁵⁵QGGQ²⁶⁰PPRY²⁶⁵YPTSS²⁷⁰Q

tr | X61 ²⁴⁰QPGQL²⁵⁰QQLAQ²⁶⁰QGGQ²⁷⁰QPPER²⁸⁰GQ²⁸⁵QGGQ²⁹⁰QSGQ²⁹⁵QGGQ³⁰⁰QGY³⁰⁵YPI³¹⁰SP

tr | X61 ³⁰⁰QQLGQ³¹⁰Q---QQ³²⁰SGQ³³⁰GLGY³³⁵YPTSP³⁴⁰QSGQ³⁴⁵QSGY³⁵⁰YPTSA³⁵⁵QQ³⁶⁰PGQL³⁶⁵QOST³⁷⁰QEQ³⁷⁵QLG³⁸⁰QEQD

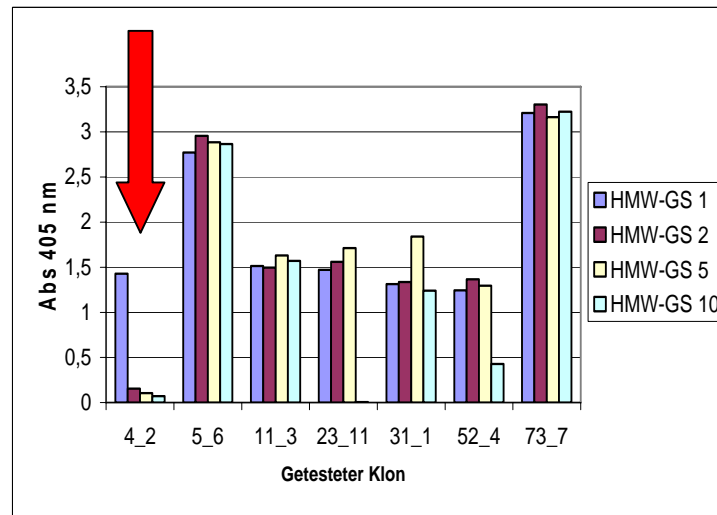
tr | X61 ³⁵⁰QSGQ³⁶⁰GRGQ³⁷⁰QSGQ³⁸⁰RQDQ³⁹⁰QSGQ⁴⁰⁰QPPG⁴⁰⁵QRQ⁴¹⁰PGY⁴¹⁵YSTSP⁴²⁰QQLG⁴²⁵QGP⁴³⁰PRYY⁴³⁵YPTSP⁴⁴⁰QPPG⁴⁴⁵QE

tr | X61 ⁴¹⁰QPPR⁴²⁰LQ⁴³⁰QPE⁴⁴⁰QGGQ⁴⁵⁰QPPQ⁴⁵⁵GG⁴⁶⁰EQ⁴⁶⁵GGQ⁴⁷⁰QPPG⁴⁷⁵QGGQ⁴⁸⁰QPPG⁴⁸⁵QGGQ⁴⁹⁰QPPG⁴⁹⁵QGGQ⁵⁰⁰QPPG⁵⁰⁵QGGQ⁵¹⁰QPPG⁵¹⁵QGGQ⁵²⁰QPPG⁵²⁵QGGQ⁵³⁰QPPG⁵³⁵QGGQ⁵⁴⁰QPPG⁵⁴⁵QGGQ⁵⁵⁰QPPG⁵⁵⁵QGGQ⁵⁶⁰QPPG⁵⁶⁵QGGQ⁵⁷⁰QPPG⁵⁷⁵QGGQ⁵⁸⁰QPPG⁵⁸⁵QGGQ⁵⁹⁰QPPG⁵⁹⁵QGGQ⁶⁰⁰QPPG⁶⁰⁵QGGQ⁶¹⁰QPPG⁶¹⁵QGGQ⁶²⁰QPPG⁶²⁵QGGQ⁶³⁰QPPG⁶³⁵QGGQ⁶⁴⁰QPPG⁶⁴⁵QGGQ⁶⁵⁰QPPG⁶⁵⁵QGGQ⁶⁶⁰QPPG⁶⁶⁵QGGQ⁶⁷⁰QPPG⁶⁷⁵QGGQ⁶⁸⁰QPPG⁶⁸⁵QGGQ⁶⁹⁰QPPG⁶⁹⁵QGGQ⁷⁰⁰QPPG⁷⁰⁵QGGQ⁷¹⁰QPPG⁷¹⁵QGGQ⁷²⁰QPPG⁷²⁵QGGQ⁷³⁰QPPG⁷³⁵QGGQ⁷⁴⁰QPPG⁷⁴⁵QGGQ⁷⁵⁰QPPG⁷⁵⁵QGGQ⁷⁶⁰QPPG⁷⁶⁵QGGQ⁷⁷⁰QPPG⁷⁷⁵QGGQ⁷⁸⁰QPPG⁷⁸⁵QGGQ⁷⁹⁰QPPG⁷⁹⁵QGGQ⁸⁰⁰QPPG⁸⁰⁵QGGQ⁸¹⁰QPPG⁸¹⁵QGGQ⁸²⁰QPPG⁸²⁵QGGQ⁸³⁰QPPG⁸³⁵QGGQ⁸⁴⁰QPPG⁸⁴⁵QGGQ⁸⁵⁰QPPG⁸⁵⁵QGGQ⁸⁶⁰QPPG⁸⁶⁵QGGQ⁸⁷⁰QPPG⁸⁷⁵QGGQ⁸⁸⁰QPPG⁸⁸⁵QGGQ⁸⁹⁰QPPG⁸⁹⁵QGGQ⁹⁰⁰QPPG⁹⁰⁵QGGQ⁹¹⁰QPPG⁹¹⁵QGGQ⁹²⁰QPPG⁹²⁵QGGQ⁹³⁰QPPG⁹³⁵QGGQ⁹⁴⁰QPPG⁹⁴⁵QGGQ⁹⁵⁰QPPG⁹⁵⁵QGGQ⁹⁶⁰QPPG⁹⁶⁵QGGQ⁹⁷⁰QPPG⁹⁷⁵QGGQ⁹⁸⁰QPPG⁹⁸⁵QGGQ⁹⁹⁰QPPG⁹⁹⁵QGGQ¹⁰⁰⁰QPPG¹⁰⁰⁵QGGQ¹⁰¹⁰QPPG¹⁰¹⁵QGGQ¹⁰²⁰QPPG¹⁰²⁵QGGQ¹⁰³⁰QPPG¹⁰³⁵QGGQ¹⁰⁴⁰QPPG¹⁰⁴⁵QGGQ¹⁰⁵⁰QPPG¹⁰⁵⁵QGGQ¹⁰⁶⁰QPPG¹⁰⁶⁵QGGQ¹⁰⁷⁰QPPG¹⁰⁷⁵QGGQ¹⁰⁸⁰QPPG¹⁰⁸⁵QGGQ¹⁰⁹⁰QPPG¹⁰⁹⁵QGGQ¹¹⁰⁰QPPG¹¹⁰⁵QGGQ¹¹¹⁰QPPG¹¹¹⁵QGGQ¹¹²⁰QPPG¹¹²⁵QGGQ¹¹³⁰QPPG¹¹³⁵QGGQ¹¹⁴⁰QPPG¹¹⁴⁵QGGQ¹¹⁵⁰QPPG¹¹⁵⁵QGGQ¹¹⁶⁰QPPG¹¹⁶⁵QGGQ¹¹⁷⁰QPPG¹¹⁷⁵QGGQ¹¹⁸⁰QPPG¹¹⁸⁵QGGQ¹¹⁹⁰QPPG¹¹⁹⁵QGGQ¹²⁰⁰QPPG¹²⁰⁵QGGQ¹²¹⁰QPPG¹²¹⁵QGGQ¹²²⁰QPPG¹²²⁵QGGQ¹²³⁰QPPG¹²³⁵QGGQ¹²⁴⁰QPPG¹²⁴⁵QGGQ¹²⁵⁰QPPG¹²⁵⁵QGGQ¹²⁶⁰QPPG¹²⁶⁵QGGQ¹²⁷⁰QPPG¹²⁷⁵QGGQ¹²⁸⁰QPPG¹²⁸⁵QGGQ¹²⁹⁰QPPG¹²⁹⁵QGGQ¹³⁰⁰QPPG¹³⁰⁵QGGQ¹³¹⁰QPPG¹³¹⁵QGGQ¹³²⁰QPPG¹³²⁵QGGQ¹³³⁰QPPG¹³³⁵QGGQ¹³⁴⁰QPPG¹³⁴⁵QGGQ¹³⁵⁰QPPG¹³⁵⁵QGGQ¹³⁶⁰QPPG¹³⁶⁵QGGQ¹³⁷⁰QPPG¹³⁷⁵QGGQ¹³⁸⁰QPPG¹³⁸⁵QGGQ¹³⁹⁰QPPG¹³⁹⁵QGGQ¹⁴⁰⁰QPPG¹⁴⁰⁵QGGQ¹⁴¹⁰QPPG¹⁴¹⁵QGGQ¹⁴²⁰QPPG¹⁴²⁵QGGQ¹⁴³⁰QPPG¹⁴³⁵QGGQ¹⁴⁴⁰QPPG¹⁴⁴⁵QGGQ¹⁴⁵⁰QPPG¹⁴⁵⁵QGGQ¹⁴⁶⁰QPPG¹⁴⁶⁵QGGQ¹⁴⁷⁰QPPG¹⁴⁷⁵QGGQ¹⁴⁸⁰QPPG¹⁴⁸⁵QGGQ¹⁴⁹⁰QPPG¹⁴⁹⁵QGGQ¹⁵⁰⁰QPPG¹⁵⁰⁵QGGQ¹⁵¹⁰QPPG¹⁵¹⁵QGGQ¹⁵²⁰QPPG¹⁵²⁵QGGQ¹⁵³⁰QPPG¹⁵³⁵QGGQ¹⁵⁴⁰QPPG¹⁵⁴⁵QGGQ¹⁵⁵⁰QPPG¹⁵⁵⁵QGGQ¹⁵⁶⁰QPPG¹⁵⁶⁵QGGQ¹⁵⁷⁰QPPG¹⁵⁷⁵QGGQ¹⁵⁸⁰QPPG¹⁵⁸⁵QGGQ¹⁵⁹⁰QPPG¹⁵⁹⁵QGGQ¹⁶⁰⁰QPPG¹⁶⁰⁵QGGQ¹⁶¹⁰QPPG¹⁶¹⁵QGGQ¹⁶²⁰QPPG¹⁶²⁵QGGQ¹⁶³⁰QPPG¹⁶³⁵QGGQ¹⁶⁴⁰QPPG¹⁶⁴⁵QGGQ¹⁶⁵⁰QPPG¹⁶⁵⁵QGGQ¹⁶⁶⁰QPPG¹⁶⁶⁵QGGQ¹⁶⁷⁰QPPG¹⁶⁷⁵QGGQ¹⁶⁸⁰QPPG¹⁶⁸⁵QGGQ¹⁶⁹⁰QPPG¹⁶⁹⁵QGGQ¹⁷⁰⁰QPPG¹⁷⁰⁵QGGQ¹⁷¹⁰QPPG¹⁷¹⁵QGGQ¹⁷²⁰QPPG¹⁷²⁵QGGQ¹⁷³⁰QPPG¹⁷³⁵QGGQ¹⁷⁴⁰QPPG¹⁷⁴⁵QGGQ¹⁷⁵⁰QPPG¹⁷⁵⁵QGGQ¹⁷⁶⁰QPPG¹⁷⁶⁵QGGQ¹⁷⁷⁰QPPG¹⁷⁷⁵QGGQ¹⁷⁸⁰QPPG¹⁷⁸⁵QGGQ¹⁷⁹⁰QPPG¹⁷⁹⁵QGGQ¹⁸⁰⁰QPPG¹⁸⁰⁵QGGQ¹⁸¹⁰QPPG¹⁸¹⁵QGGQ¹⁸²⁰QPPG¹⁸²⁵QGGQ¹⁸³⁰QPPG¹⁸³⁵QGGQ¹⁸⁴⁰QPPG¹⁸⁴⁵QGGQ¹⁸⁵⁰QPPG¹⁸⁵⁵QGGQ¹⁸⁶⁰QPPG¹⁸⁶⁵QGGQ¹⁸⁷⁰QPPG¹⁸⁷⁵QGGQ¹⁸⁸⁰QPPG¹⁸⁸⁵QGGQ¹⁸⁹⁰QPPG¹⁸⁹⁵QGGQ¹⁹⁰⁰QPPG¹⁹⁰⁵QGGQ¹⁹¹⁰QPPG¹⁹¹⁵QGGQ¹⁹²⁰QPPG¹⁹²⁵QGGQ¹⁹³⁰QPPG¹⁹³⁵QGGQ¹⁹⁴⁰QPPG¹⁹⁴⁵QGGQ¹⁹⁵⁰QPPG¹⁹⁵⁵QGGQ¹⁹⁶⁰QPPG¹⁹⁶⁵QGGQ¹⁹⁷⁰QPPG¹⁹⁷⁵QGGQ¹⁹⁸⁰QPPG¹⁹⁸⁵QGGQ¹⁹⁹⁰QPPG¹⁹⁹⁵QGGQ²⁰⁰⁰QPPG²⁰⁰⁵QGGQ²⁰¹⁰QPPG²⁰¹⁵QGGQ²⁰²⁰QPPG²⁰²⁵QGGQ²⁰³⁰QPPG²⁰³⁵QGGQ²⁰⁴⁰QPPG²⁰⁴⁵QGGQ²⁰⁵⁰QPPG²⁰⁵⁵QGGQ²⁰⁶⁰QPPG²⁰⁶⁵QGGQ²⁰⁷⁰QPPG²⁰⁷⁵QGGQ²⁰⁸⁰QPPG²⁰⁸⁵QGGQ²⁰⁹⁰QPPG²⁰⁹⁵QGGQ²¹⁰⁰QPPG²¹⁰⁵QGGQ²¹¹⁰QPPG²¹¹⁵QGGQ²¹²⁰QPPG²¹²⁵QGGQ²¹³⁰QPPG²¹³⁵QGGQ²¹⁴⁰QPPG²¹⁴⁵QGGQ²¹⁵⁰QPPG²¹⁵⁵QGGQ²¹⁶⁰QPPG²¹⁶⁵QGGQ²¹⁷⁰QPPG²¹⁷⁵QGGQ²¹⁸⁰QPPG²¹⁸⁵QGGQ²¹⁹⁰QPPG²¹⁹⁵QGGQ²²⁰⁰QPPG²²⁰⁵QGGQ²²¹⁰QPPG²²¹⁵QGGQ²²²⁰QPPG²²²⁵QGGQ²²³⁰QPPG²²³⁵QGGQ²²⁴⁰QPPG²²⁴⁵QGGQ²²⁵⁰QPPG²²⁵⁵QGGQ²²⁶⁰QPPG²²⁶⁵QGGQ²²⁷⁰QPPG²²⁷⁵QGGQ²²⁸⁰QPPG²²⁸⁵QGGQ²²⁹⁰QPPG²²⁹⁵QGGQ²³⁰⁰QPPG²³⁰⁵QGGQ²³¹⁰QPPG²³¹⁵QGGQ²³²⁰QPPG²³²⁵QGGQ²³³⁰QPPG²³³⁵QGGQ²³⁴⁰QPPG²³⁴⁵QGGQ²³⁵⁰QPPG²³⁵⁵QGGQ²³⁶⁰QPPG²³⁶⁵QGGQ²³⁷⁰QPPG²³⁷⁵QGGQ²³⁸⁰QPPG²³⁸⁵QGGQ²³⁹⁰QPPG²³⁹⁵QGGQ²⁴⁰⁰QPPG²⁴⁰⁵QGGQ²⁴¹⁰QPPG²⁴¹⁵QGGQ²⁴²⁰QPPG²⁴²⁵QGGQ²⁴³⁰QPPG²⁴³⁵QGGQ²⁴⁴⁰QPPG²⁴⁴⁵QGGQ²⁴⁵⁰QPPG²⁴⁵⁵QGGQ²⁴⁶⁰QPPG²⁴⁶⁵QGGQ²⁴⁷⁰QPPG²⁴⁷⁵QGGQ²⁴⁸⁰QPPG²⁴⁸⁵QGGQ²⁴⁹⁰QPPG²⁴⁹⁵QGGQ²⁵⁰⁰QPPG²⁵⁰⁵QGGQ²⁵¹⁰QPPG²⁵¹⁵QGGQ²⁵²⁰QPPG²⁵²⁵QGGQ²⁵³⁰QPPG²⁵³⁵QGGQ²⁵⁴⁰QPPG²⁵⁴⁵QGGQ²⁵⁵⁰QPPG²⁵⁵⁵QGGQ²⁵⁶⁰QPPG²⁵⁶⁵QGGQ²⁵⁷⁰QPPG²⁵⁷⁵QGGQ²⁵⁸⁰QPPG²⁵⁸⁵QGGQ²⁵⁹⁰QPPG²⁵⁹⁵QGGQ²⁶⁰⁰QPPG²⁶⁰⁵QGGQ²⁶¹⁰QPPG²⁶¹⁵QGGQ²⁶²⁰QPPG²⁶²⁵QGGQ²⁶³⁰QPPG²⁶³⁵QGGQ²⁶⁴⁰QPPG²⁶⁴⁵QGGQ²⁶⁵⁰QPPG²⁶⁵⁵QGGQ²⁶⁶⁰QPPG²⁶⁶⁵QGGQ²⁶⁷⁰QPPG²⁶⁷⁵QGGQ²⁶⁸⁰QPPG²⁶⁸⁵QGGQ²⁶⁹⁰QPPG²⁶⁹⁵QGGQ²⁷⁰⁰QPPG²⁷⁰⁵QGGQ²⁷¹⁰QPPG²⁷¹⁵QGGQ²⁷²⁰QPPG²⁷²⁵QGGQ²⁷³⁰QPPG²⁷³⁵QGGQ²⁷⁴⁰QPPG²⁷⁴⁵QGGQ²⁷⁵⁰QPPG²⁷⁵⁵QGGQ²⁷⁶⁰QPPG²⁷⁶⁵QGGQ²⁷⁷⁰QPPG²⁷⁷⁵QGGQ²⁷⁸⁰QPPG²⁷⁸⁵QGGQ²⁷⁹⁰QPPG²⁷⁹⁵QGGQ²⁸⁰⁰QPPG²⁸⁰⁵QGGQ²⁸¹⁰QPPG²⁸¹⁵QGGQ²⁸²⁰QPPG²⁸²⁵QGGQ²⁸³⁰QPPG²⁸³⁵QGGQ²⁸⁴⁰QPPG²⁸⁴⁵QGGQ²⁸⁵⁰QPPG²⁸⁵⁵QGGQ²⁸⁶⁰QPPG²⁸⁶⁵QGGQ²⁸⁷⁰QPPG²⁸⁷⁵QGGQ²⁸⁸⁰QPPG²⁸⁸⁵QGGQ²⁸⁹⁰QPPG²⁸⁹⁵QGGQ²⁹⁰⁰QPPG²⁹⁰⁵QGGQ²⁹¹⁰QPPG²⁹¹⁵QGGQ²⁹²⁰QPPG²⁹²⁵QGGQ²⁹³⁰QPPG²⁹³⁵QGGQ²⁹⁴⁰QPPG²⁹⁴⁵QGGQ²⁹⁵⁰QPPG²⁹⁵⁵QGGQ²⁹⁶⁰QPPG²⁹⁶⁵QGGQ²⁹⁷⁰QPPG²⁹⁷⁵QGGQ²⁹⁸⁰QPPG²⁹⁸⁵QGGQ²⁹⁹⁰QPPG²⁹⁹⁵QGGQ³⁰⁰⁰QPPG³⁰⁰⁵QGGQ³⁰¹⁰QPPG³⁰¹⁵QGGQ³⁰²⁰QPPG³⁰²⁵QGGQ³⁰³⁰QPPG³⁰³⁵QGGQ³⁰⁴⁰QPPG³⁰⁴⁵QGGQ³⁰⁵⁰QPPG³⁰⁵⁵QGGQ³⁰⁶⁰QPPG³⁰⁶⁵QGGQ³⁰⁷⁰QPPG³⁰⁷⁵QGGQ³⁰⁸⁰QPPG³⁰⁸⁵QGGQ³⁰⁹⁰QPPG³⁰⁹⁵QGGQ³¹⁰⁰QPPG³¹⁰⁵QGGQ³¹¹⁰QPPG³¹¹⁵QGGQ³¹²⁰QPPG³¹²⁵QGGQ³¹³⁰QPPG³¹³⁵QGGQ³¹⁴⁰QPPG³¹⁴⁵QGGQ³¹⁵⁰QPPG³¹⁵⁵QGGQ³¹⁶⁰QPPG³¹⁶⁵QGGQ³¹⁷⁰QPPG³¹⁷⁵QGGQ³¹⁸⁰QPPG³¹⁸⁵QGGQ³¹⁹⁰QPPG³¹⁹⁵QGGQ³²⁰⁰QPPG³²⁰⁵QGGQ³²¹⁰QPPG³²¹⁵QGGQ³²²⁰QPPG³²²⁵QGGQ³²³⁰QPPG³²³⁵QGGQ³²⁴⁰QPPG³²⁴⁵QGGQ³²⁵⁰QPPG³²⁵⁵QGGQ³²⁶⁰QPPG³²⁶⁵QGGQ³²⁷⁰QPPG³²⁷⁵QGGQ³²⁸⁰QPPG³²⁸⁵QGGQ³²⁹⁰QPPG³²⁹⁵QGGQ³³⁰⁰QPPG³³⁰⁵QGGQ³³¹⁰QPPG³³¹⁵QGGQ³³²⁰QPPG³³²⁵QGGQ³³³⁰QPPG³³³⁵QGGQ³³⁴⁰QPPG³³⁴⁵QGGQ³³⁵⁰QPPG³³⁵⁵QGGQ³³⁶⁰QPPG³³⁶⁵QGGQ³³⁷⁰QPPG³³⁷⁵QGGQ³³⁸⁰QPPG³³⁸⁵QGGQ³³⁹⁰QPPG³³⁹⁵QGGQ³⁴⁰⁰QPPG³⁴⁰⁵QGGQ³⁴¹⁰QPPG³⁴¹⁵QGGQ³⁴²⁰QPPG³⁴²⁵QGGQ³⁴³⁰QPPG³⁴³⁵QGGQ³⁴⁴⁰QPPG³⁴⁴⁵QGGQ³⁴⁵⁰QPPG³⁴⁵⁵QGGQ³⁴⁶⁰QPPG³⁴⁶⁵QGGQ³⁴⁷⁰QPPG³⁴⁷⁵QGGQ³⁴⁸⁰QPPG³⁴⁸⁵QGGQ³⁴⁹⁰QPPG³⁴⁹⁵QGGQ³⁵⁰⁰QPPG³⁵⁰⁵QGGQ³⁵¹⁰QPPG³⁵¹⁵QGGQ³⁵²⁰QPPG³⁵²⁵QGGQ³⁵³⁰QPPG³⁵³⁵QGGQ³⁵⁴⁰QPPG³⁵⁴⁵QGGQ³⁵⁵⁰QPPG³⁵⁵⁵QGGQ³⁵⁶⁰QPPG³⁵⁶⁵QGGQ³⁵⁷⁰QPPG³⁵⁷⁵QGGQ³⁵⁸⁰QPPG³⁵⁸⁵QGGQ³⁵⁹⁰QPPG³⁵⁹⁵QGGQ³⁶⁰⁰QPPG³⁶⁰⁵QGGQ³⁶¹⁰QPPG³⁶¹⁵QGGQ³⁶²⁰QPPG³⁶²⁵QGGQ³⁶³⁰QPPG³⁶³⁵QGGQ³⁶⁴⁰QPPG³⁶⁴⁵QGGQ³⁶⁵⁰QPPG³⁶⁵⁵QGGQ³⁶⁶⁰QPPG³⁶⁶⁵QGGQ³⁶⁷⁰QPPG³⁶⁷⁵QGGQ³⁶⁸⁰QPPG³⁶⁸⁵QGGQ³⁶⁹⁰QPPG³⁶⁹⁵QGGQ³⁷⁰⁰QPPG³⁷⁰⁵QGGQ³⁷¹⁰QPPG³⁷¹⁵QGGQ³⁷²⁰QPPG³⁷²⁵QGGQ³⁷³⁰QPPG³⁷³⁵QGGQ³⁷⁴⁰QPPG³⁷⁴⁵QGGQ³⁷⁵⁰QPPG³⁷⁵⁵QGGQ³⁷⁶⁰QPPG³⁷⁶⁵QGGQ³⁷⁷⁰QPPG³⁷⁷⁵QGGQ³⁷⁸⁰QPPG³⁷⁸⁵QGGQ³⁷⁹⁰QPPG³⁷⁹⁵QGGQ³⁸⁰⁰QPPG³⁸⁰⁵QGGQ³⁸¹⁰QPPG³⁸¹⁵QGGQ³⁸²⁰QPPG³⁸²⁵QGGQ³⁸³⁰QPPG³⁸³⁵QGGQ³⁸⁴⁰QPPG³⁸⁴⁵QGGQ³⁸⁵⁰QPPG³⁸⁵⁵QGGQ³⁸⁶⁰QPPG³⁸⁶⁵QGGQ³⁸⁷⁰QPPG³⁸⁷⁵QGGQ³⁸⁸⁰QPPG³⁸⁸⁵QGGQ³⁸⁹⁰QPPG³⁸⁹⁵QGGQ³⁹⁰⁰QPPG³⁹⁰⁵QGGQ³⁹¹⁰QPPG³⁹¹⁵QGGQ³⁹²⁰QPPG³⁹²⁵QGGQ³⁹³⁰QPPG³⁹³⁵QGGQ³⁹⁴⁰QPPG³⁹⁴⁵QGGQ³⁹⁵⁰QPPG³⁹⁵⁵QGGQ³⁹⁶⁰QPPG³⁹⁶⁵QGGQ³⁹⁷⁰QPPG³⁹⁷⁵QGGQ³⁹⁸⁰QPPG³⁹⁸⁵QGGQ³⁹⁹⁰QPPG³⁹⁹⁵QGGQ⁴⁰⁰⁰QPPG⁴⁰⁰⁵QGGQ⁴⁰¹⁰QPPG⁴⁰¹⁵QGGQ⁴⁰²⁰QPPG⁴⁰²⁵QGGQ⁴⁰³⁰QPPG⁴⁰³⁵QGGQ⁴⁰⁴⁰QPPG⁴⁰⁴⁵QGGQ⁴⁰⁵⁰QPPG⁴⁰⁵⁵QGGQ⁴⁰⁶⁰QPPG⁴⁰⁶⁵QGGQ⁴⁰⁷⁰QPPG⁴⁰⁷⁵QGGQ⁴⁰⁸⁰QPPG⁴⁰⁸⁵QGGQ⁴⁰⁹⁰QPPG⁴⁰⁹⁵QGGQ⁴¹⁰⁰QPPG⁴¹⁰⁵QGGQ⁴¹¹⁰QPPG⁴¹¹⁵QGGQ⁴¹²⁰QPPG⁴¹²⁵QGGQ⁴¹³⁰QPPG⁴¹³⁵QGGQ⁴¹⁴⁰QPPG⁴¹⁴⁵QGGQ⁴¹⁵⁰QPPG⁴¹⁵⁵QGGQ⁴¹⁶⁰QPPG⁴¹⁶⁵QGGQ⁴¹⁷⁰QPPG⁴¹⁷⁵QGGQ⁴¹⁸⁰QPPG⁴¹⁸⁵QGGQ⁴¹⁹⁰QPPG⁴¹⁹⁵QGGQ⁴²⁰⁰QPPG⁴²⁰⁵QGGQ⁴²¹⁰QPPG⁴²¹⁵QGGQ⁴²²⁰QPPG⁴²²⁵QGGQ⁴²³⁰QPPG⁴²³⁵QGGQ⁴²⁴⁰QPPG⁴²⁴⁵QGGQ⁴²⁵⁰QPPG⁴²⁵⁵QGGQ⁴²⁶⁰QPPG⁴²⁶⁵QGGQ⁴²⁷⁰QPPG⁴²⁷⁵QGGQ⁴²⁸⁰QPPG⁴²⁸⁵QGGQ⁴²⁹⁰QPPG⁴²⁹⁵QGGQ⁴³⁰⁰QPPG⁴³⁰⁵QGGQ⁴³¹⁰QPPG⁴³¹⁵QGGQ⁴³²⁰QPPG⁴³²⁵QGGQ⁴³³⁰QPPG⁴³³⁵QGGQ⁴³⁴⁰QPPG⁴³⁴⁵QGGQ⁴³⁵⁰QPPG⁴³⁵⁵QGGQ⁴³⁶⁰QPPG⁴³⁶⁵QGGQ⁴³⁷⁰QPPG⁴³⁷⁵QGGQ⁴³⁸⁰QPPG⁴³⁸⁵QGGQ⁴³⁹⁰QPPG⁴³⁹⁵QGGQ⁴⁴⁰⁰QPPG⁴⁴⁰⁵QGGQ⁴⁴¹⁰QPPG⁴⁴¹⁵QGGQ⁴⁴²⁰QPPG⁴⁴²⁵QGGQ⁴⁴³⁰QPPG⁴⁴³⁵QGGQ⁴⁴⁴⁰QPPG⁴⁴⁴⁵QGGQ⁴⁴⁵⁰QPPG⁴⁴⁵⁵QGGQ⁴⁴⁶⁰QPPG⁴⁴⁶⁵QGGQ⁴⁴⁷⁰QPPG⁴⁴⁷⁵QGGQ⁴⁴⁸⁰QPPG⁴⁴⁸⁵QGGQ⁴⁴⁹⁰QPPG⁴⁴⁹⁵QGGQ⁴⁵⁰⁰QPPG⁴⁵⁰⁵QGGQ⁴⁵¹⁰QPPG⁴⁵¹⁵QGGQ⁴⁵²⁰QPPG⁴⁵²⁵QGGQ⁴⁵³⁰QPPG⁴⁵³⁵QGGQ⁴⁵⁴⁰QPPG⁴⁵⁴⁵QGGQ⁴⁵⁵⁰QPPG⁴⁵⁵⁵QGGQ⁴⁵⁶⁰QPPG⁴⁵⁶⁵QGGQ⁴⁵⁷⁰QPPG⁴⁵⁷⁵QGGQ⁴⁵⁸⁰QPPG⁴⁵⁸⁵QGGQ⁴⁵⁹⁰QPPG⁴⁵⁹⁵QGGQ⁴⁶⁰⁰QPPG⁴⁶⁰⁵QGGQ⁴⁶¹⁰QPPG⁴⁶¹⁵QGGQ⁴⁶²⁰QPPG⁴⁶²⁵QGGQ⁴⁶³⁰QPPG⁴⁶³⁵QGGQ⁴⁶⁴⁰QPPG⁴⁶⁴⁵QGGQ⁴⁶⁵⁰QPPG⁴⁶⁵⁵QGGQ⁴⁶⁶⁰QPPG⁴⁶⁶⁵QGGQ⁴⁶⁷⁰QPPG⁴⁶⁷⁵QGGQ⁴⁶⁸⁰QPPG⁴⁶⁸⁵QGGQ⁴⁶⁹⁰QPPG⁴⁶⁹⁵QGGQ⁴⁷⁰⁰QPPG⁴⁷⁰⁵QGGQ⁴⁷¹⁰QPPG

Results – HMW subunit 1

Screening of hybridoma cell culture supernatants

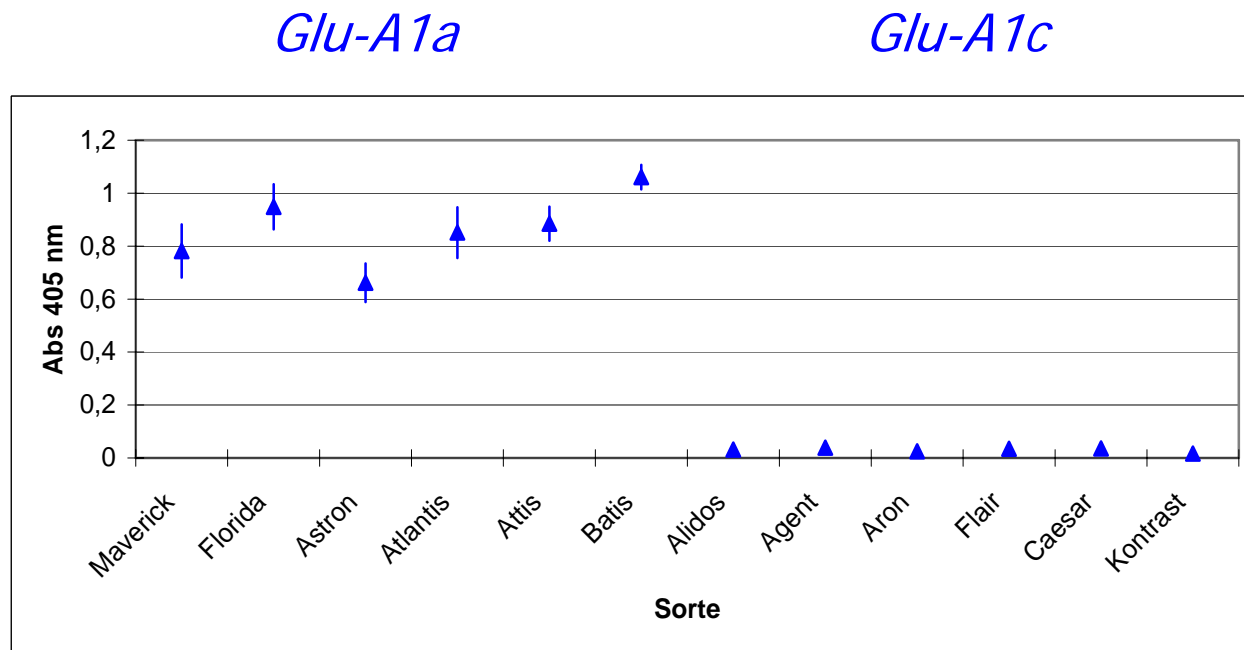
Immunogen: Peptide 592



Coating: HMW subunits 10 µg/ml in 2 M Urea, 50 mM DTT 50 mM Carbonate pH 9,6 1.
antibody: Cell culture supernatant 1:2 dilution

Results – HMW subunit 1

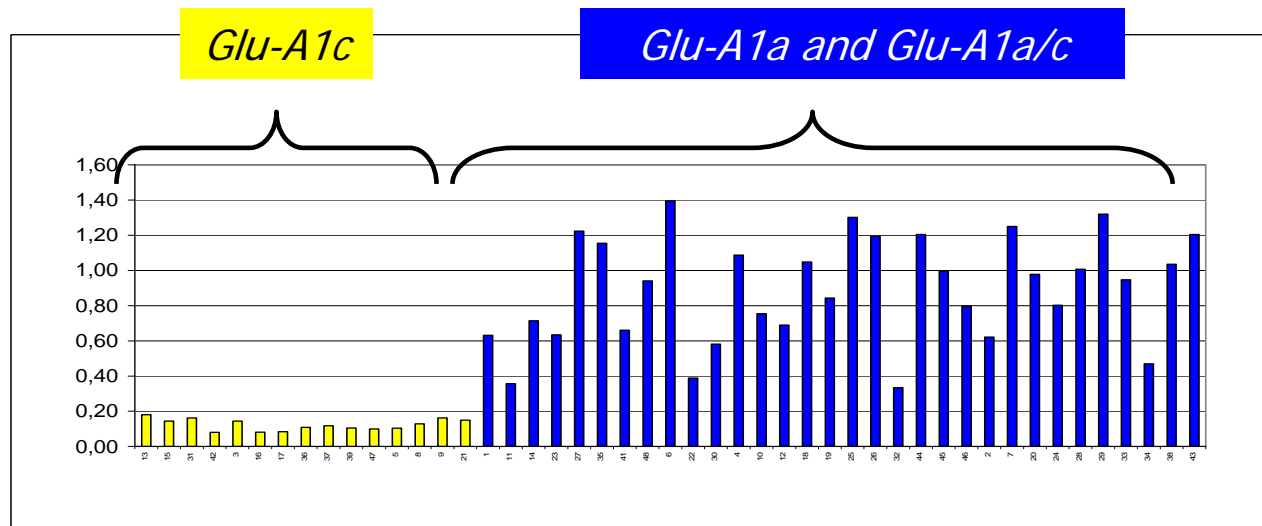
Application of mAb Antipep592 for the detection of HMW subunit 1 in varieties



Reaction of mAb Antipep592 in the *Glu-A1*-Assay: Data are means and standard deviations from 5 kernels per variety

Results – HMW subunit 1

Application of mAb Antipep592 for the detection of HMW subunit 1 in F2 population W04801

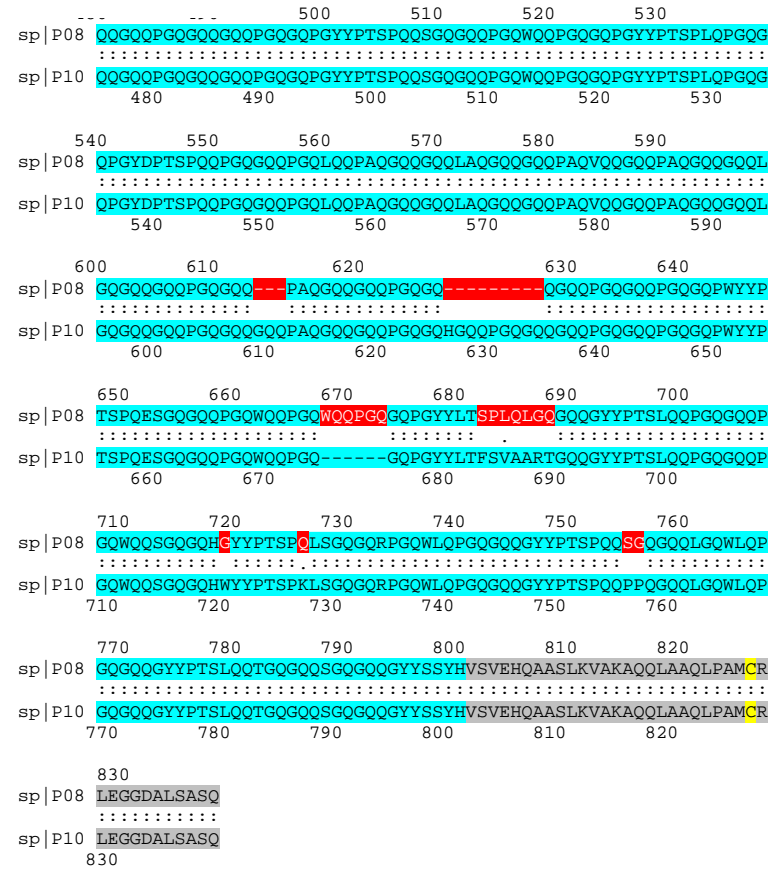
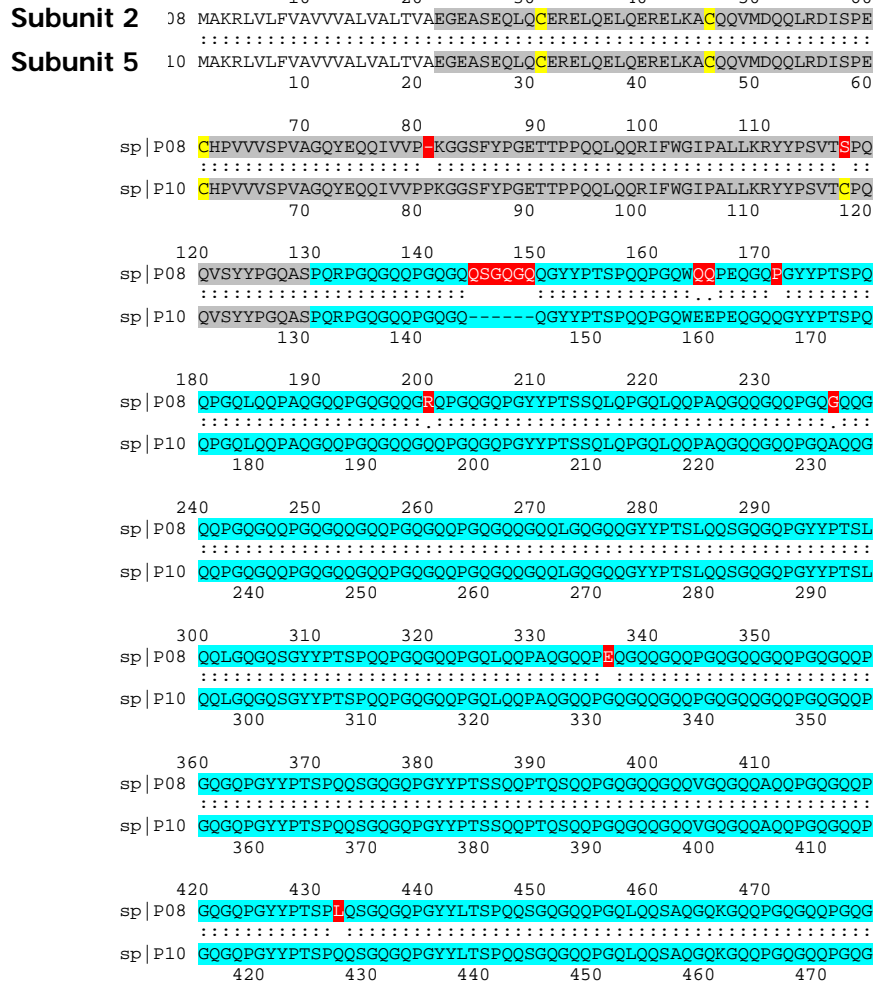


Glu-A1-allele analysis of F2-population. Coating: Melas-extract from half-kernels in A-Page-AP, 1:32 dilution. Examination of *Glu-1*-alleles: SDS-PAGE.

ELISA-development for detection of HMW subunit 5 (allele *Glu-D1d*)

AAS-Alignment of HMW subunit 2 vs. HMW subunit 5

94.8% identity in 851 aa overlap; score: 5759 E(10,000): 0



Signalsequence

N- and C-terminal domain

Central repetitive domain

Peptide design - HMW subunit 5

HMWGS 2 EGEASEQLQCERELQELQERELKACQQVMDQQLRDISPECHPVVSPVAGQYEQQIVVP-KGGSFYPPGETTPPQQL
HMWGS 5 EGEASEQLQCERELQELQERELKACQQVMDQQLRDISPECHPVVSPVAGQYEQQIVVPPKGSFYPPGETTPPQQL

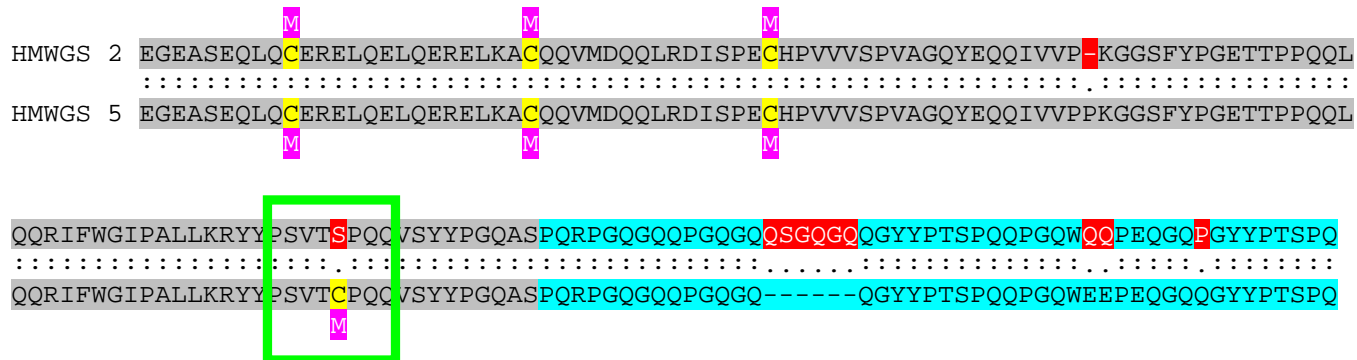
QQRIFWGIPALLKRYYP SVT S PQQ VSYYPGQAS PQRPGQGQQPGQGQ QSGQGQ QGYIPTSPQQPGQW QQPEQGQ P GYYPTSPQ
QQRIFWGIPALLKRYYP SVT C PQQ VSYYPGQAS PQRPGQGQQPGQGQ -----QGYIPTSPQQPGQWEEPEQGQ QGYIPTSPQ

AAS-position 97:
Cys-Ser exchange

C-terminal protein fragments of HMW subunit 2 and HMW subunit 5

Peptide design - HMW subunit 5

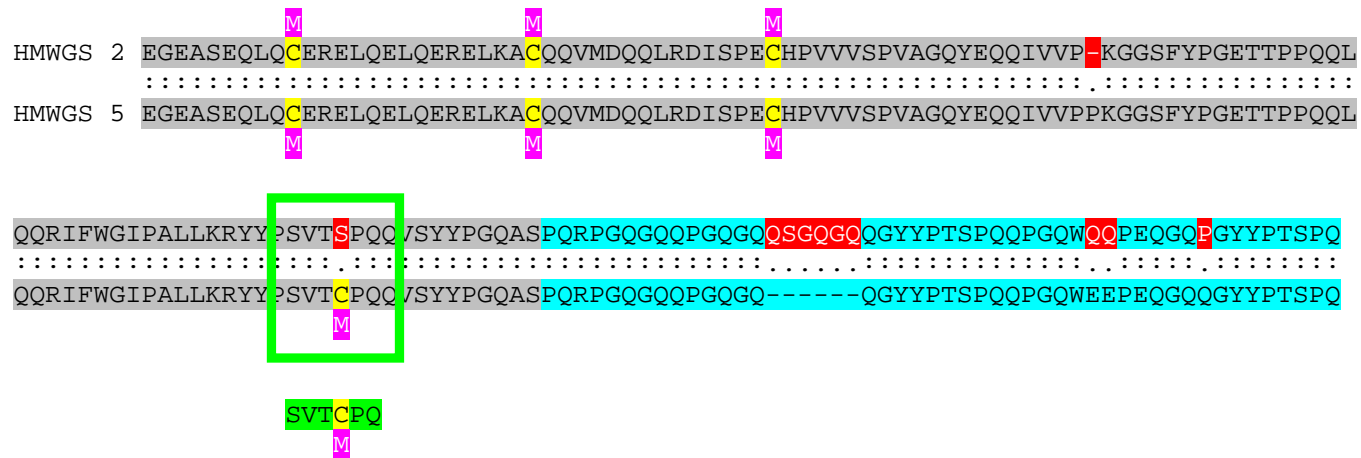
M=Vinylpyridine



Cysteine-specific modification of the proteins with **Vinylpyridine (VP)** for structural enhancement of the Serine – Cysteine exchange at AAS-position 97

Peptide design - HMW subunit 5

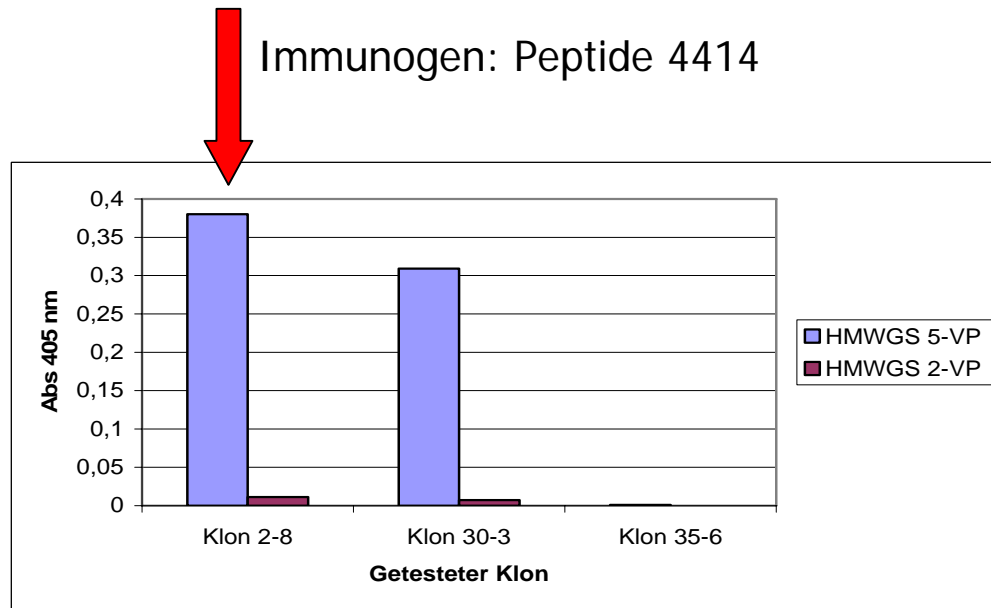
M = Vinylpyridin



Design of a cysteine modified synthetic peptide as immunogen for the development of mAbs

Results – HMW subunit 5

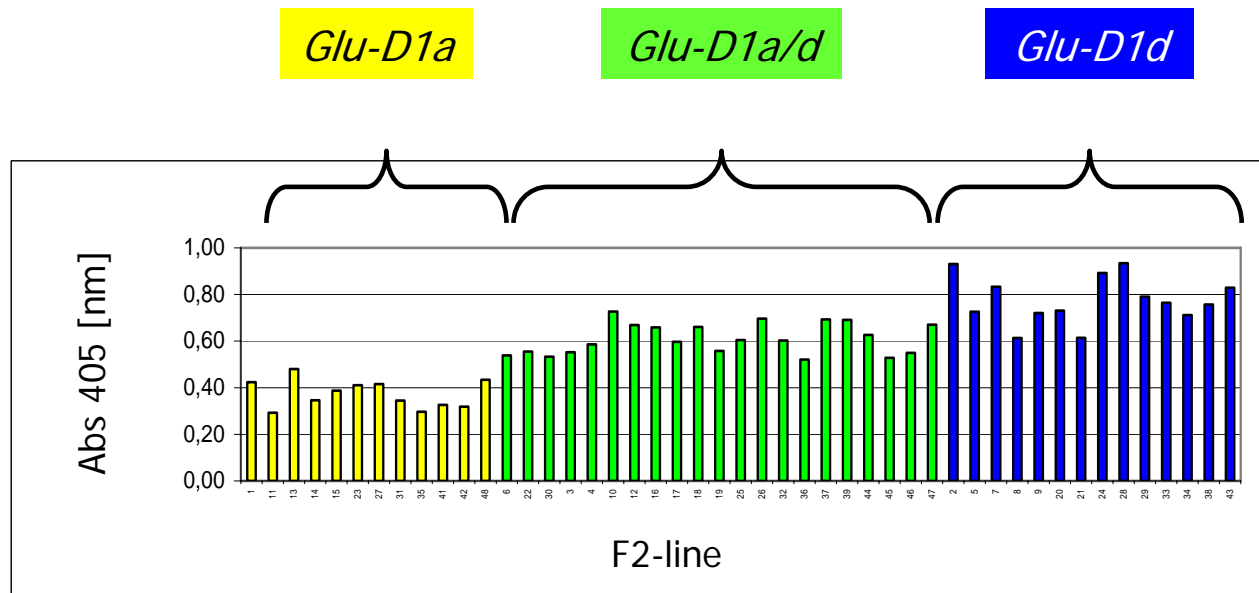
Screening of hybridoma cell culture supernatants



Coating: HMW subunit-VP 50 $\mu\text{g/ml}$ in 2 M urea 50 mM carbonate pH 9,6;
1. antibody: Cell culture supernatants, undiluted

Results – HMW subunit 5

Application of mAb Antipep4414 for the detection of the *Glu-D1d*-allele in F2-population W04801



Glu-D1-allele analysis of F2-population. Coating: Melas-extract from half-kernels in A-Page-AP, 1:32 dilution. Examination of *Glu-1*-alleles: SDS-PAGE.

Conclusion

- **Standardized assay for easy and fast detection of the alleles of the *Glu-A1*-gen locus**
- **Assay for detection of the allele *Glu-D1d***
- **Practical application:** Variety testing
Selection in F2-populations
Differentiation of homozygous and heterozygous lines regarding the *Glu-A1a* allele in F2-populations

Future prospects

Development of an overall *Glu-ABD1*-Assay, for determination of the most important HMW subunit alleles

Quantitative analyses of amounts and proportions of HMW subunits using ELISA methods

- Further elucidation of the influence of HMW subunit proportions on wheat quality
- Introduction of the knowledge into the breeding process (low protein varieties)

Further proteins of interest

- Gliadins
- Wheat proteins responsible for allergic reactions

Acknowledgment

Gert Daniel

Gerhard Zimmermann

Georg Mahlknecht

Monika Sedlmeier

GFP, BayStMLF
and BMELV

DH-populations

wheat material

cell culture

technical assistance

financial support

