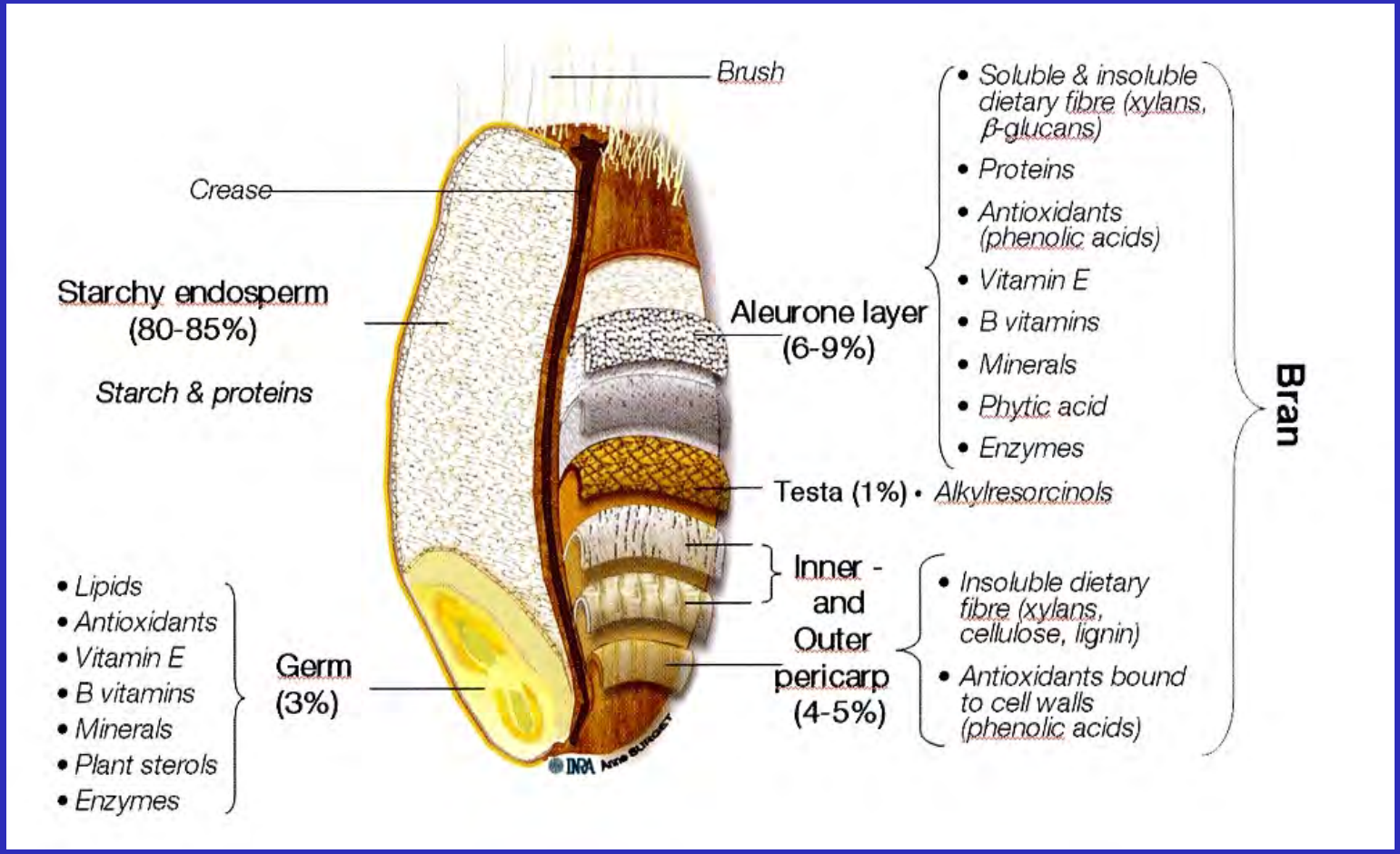
Modifying protein and starch composition of wheat kernel for technological and nutritional improvement

DOMENICO LAFIANDRA

Department of Agrobiology & Agrochemistry,
University of Tuscia, Viterbo
Italy

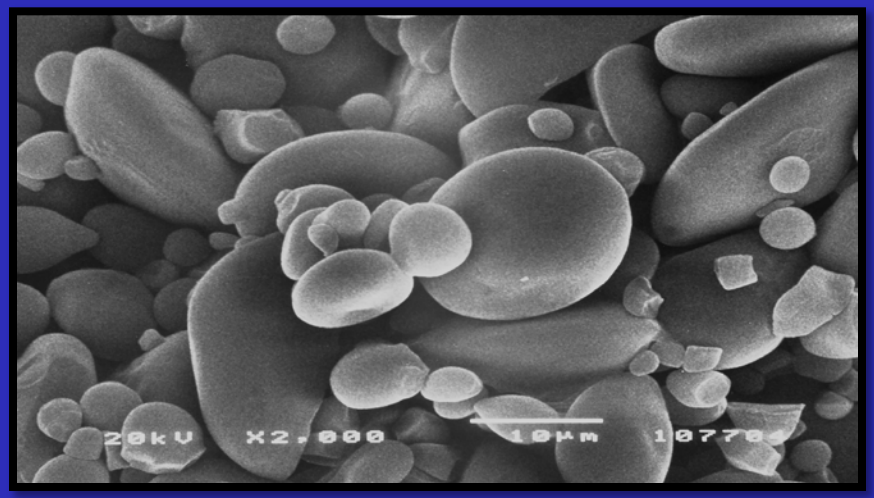
The wheat grain



Modifying wheat kernel composition

- Gluten proteins
  - HMW-glutenin subunits
  - LMW-glutenin subunits

  → Bread-, Pasta-making
- Starch → Low-, high-amylose



Sources of variation

- Natural variation (landraces, wild relatives)
- Mutagenesis
- Transgenesis

GLUTEN PROTEINS

alcohol-soluble

alcohol-insoluble

Gliadins monomers

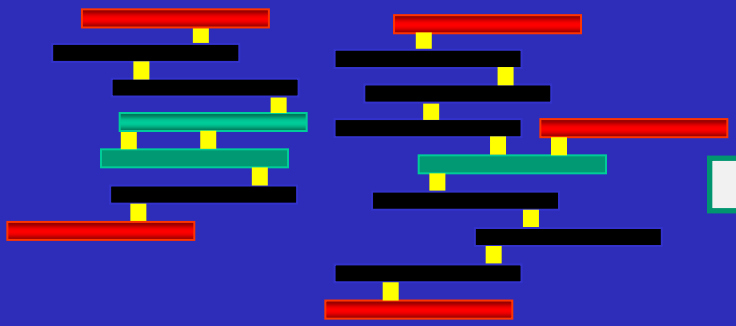
- ω-gliadins — no S-S bonds
- γ-gliadins
- α/β-gliadins — intra-chain S-S bonds

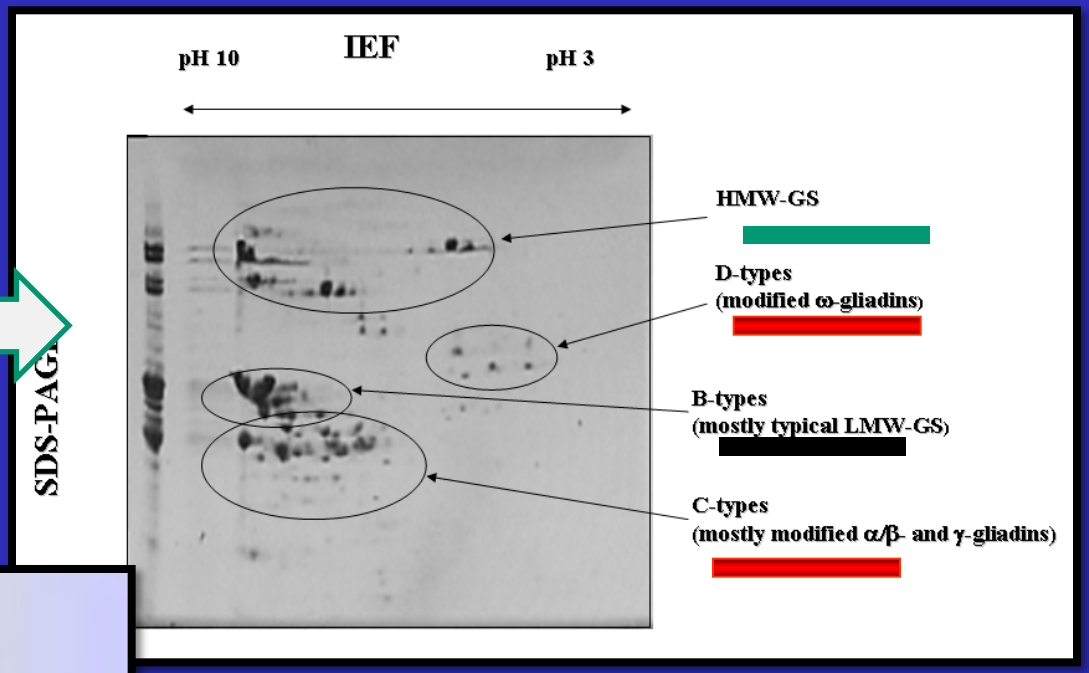
Glutenins polymers

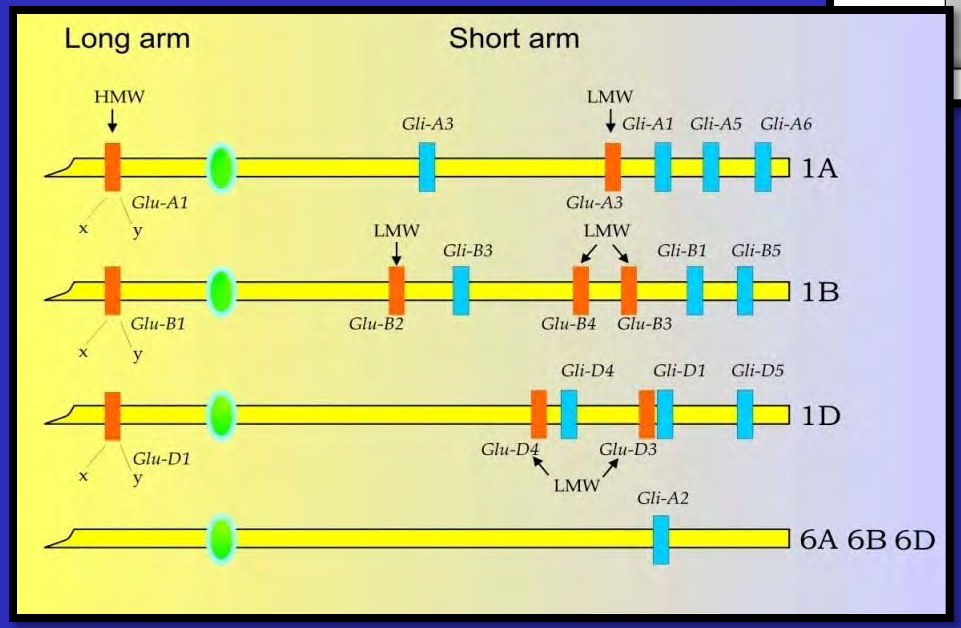
- LMW subunits
- HMW subunits

Inter-chain S-S bonds

Variation in amount and type of gluten proteins is associated with bread- and pasta-making quality of bread and durum wheat







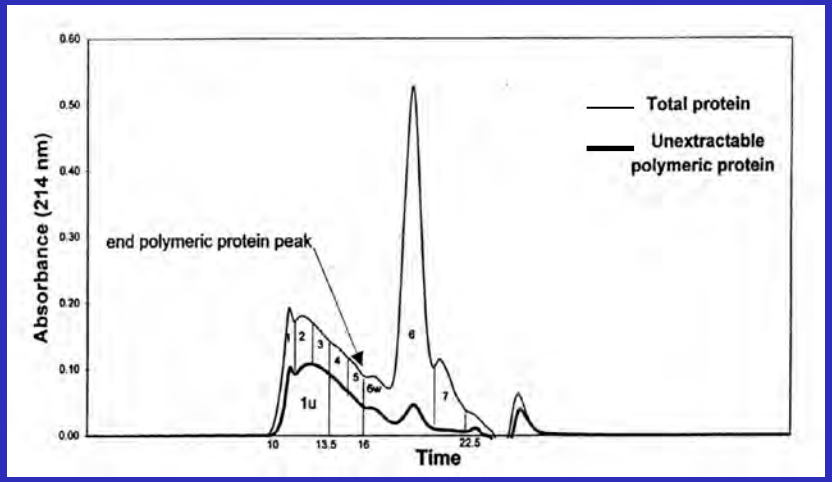
Positive correlation between amount of glutenin subunits, size of the glutenin polymer, and technological properties

High amount of glutenin subunits

↓

Large glutenin polymer size

↓

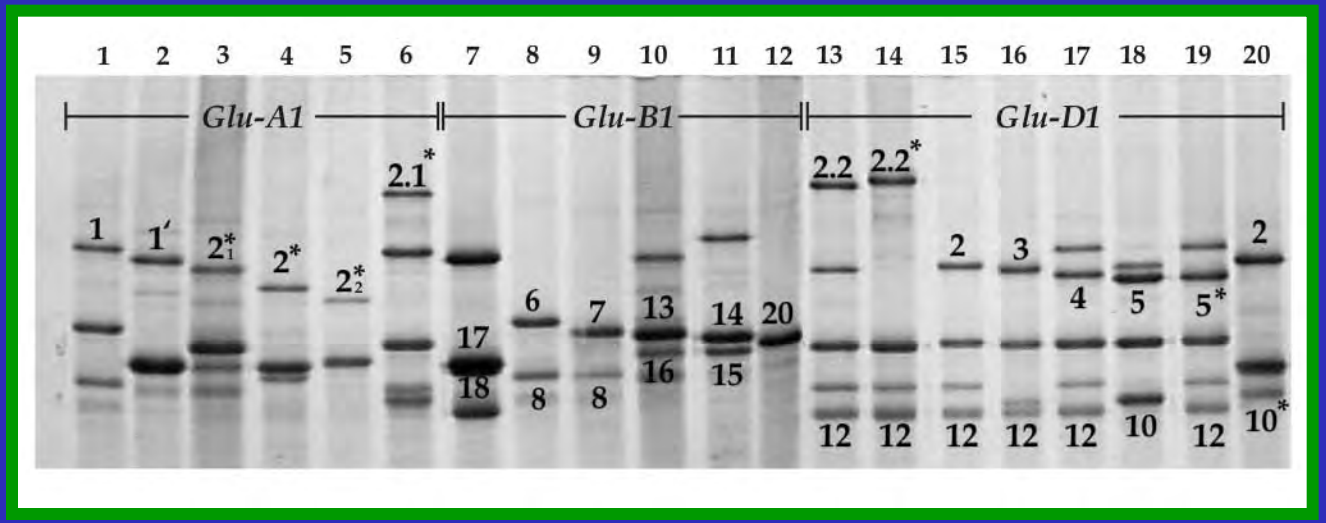


Allelic variation occurs in the expression of HMW subunit genes and the properties of the encoded proteins in bread wheat

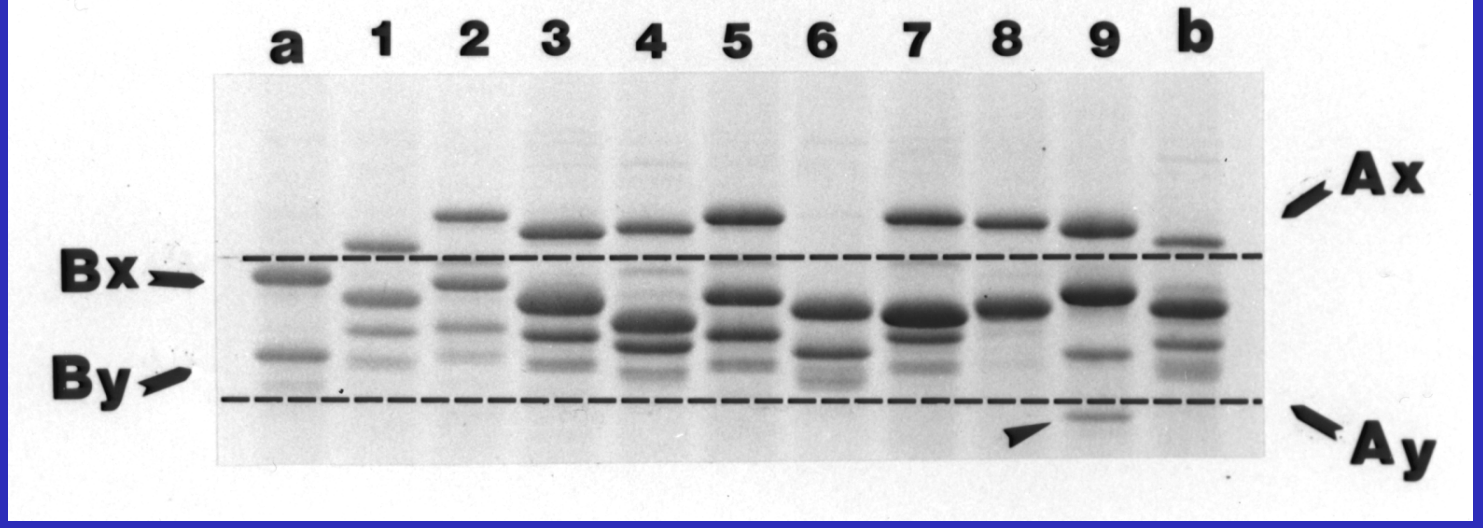
Expression		
1Ax	sometimes	3 to 5
1Ay	never	expressed
1Bx	usually	subunits
1By	sometimes	in total
1Dx	usually	in bread
1Dy	usually	wheat

what

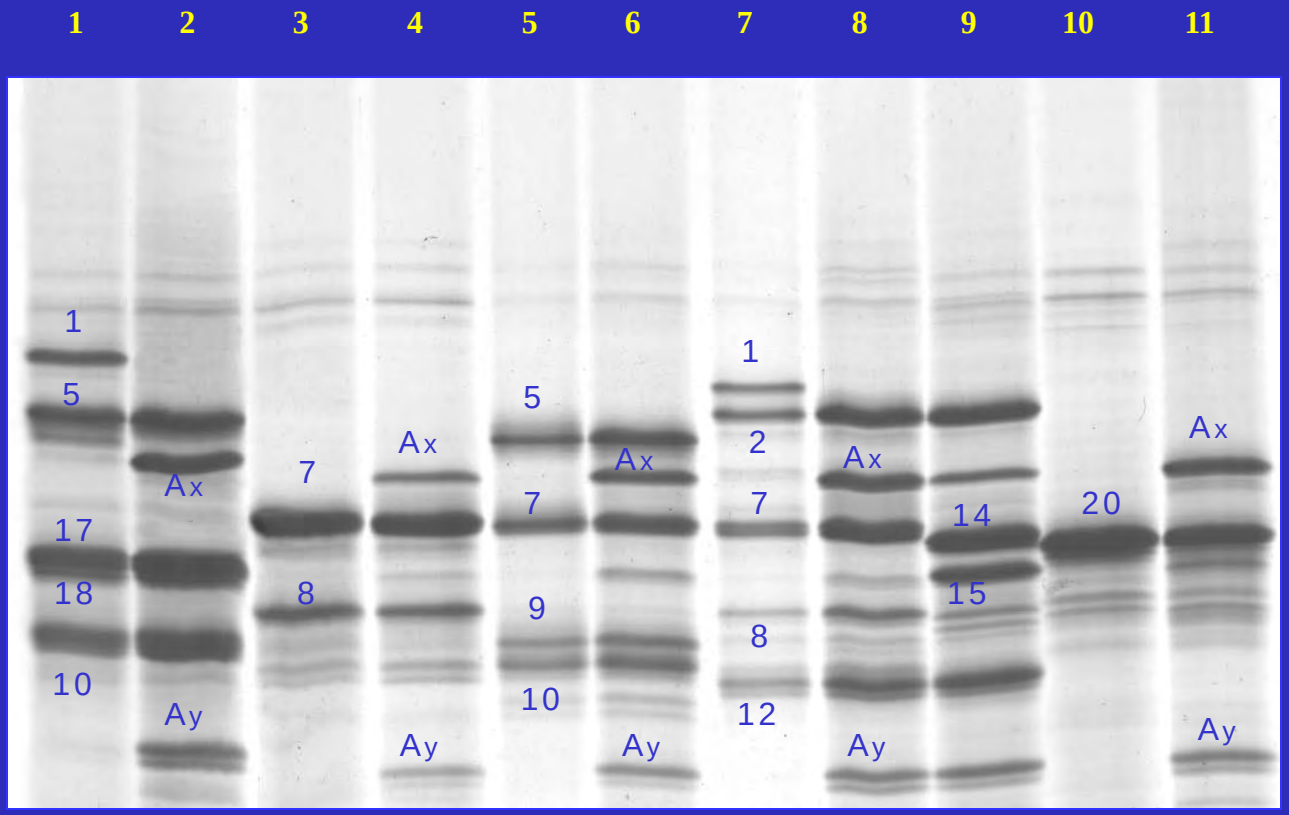
Mobility on SDS-PAGE



Enlarging diversity for glutenin subunits



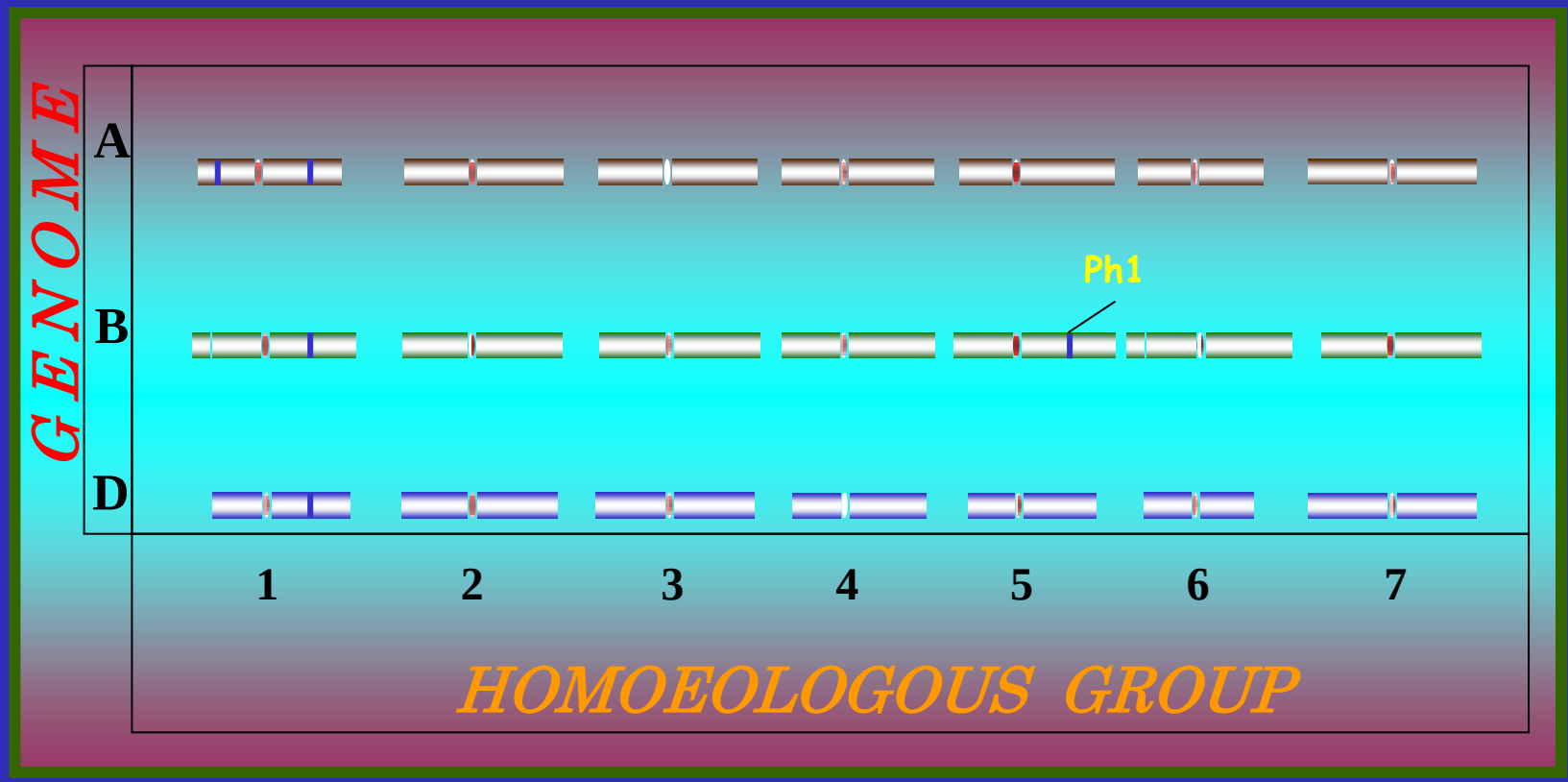
Bread-making improvement through increase of number of glutenin subunits in durum and bread wheat



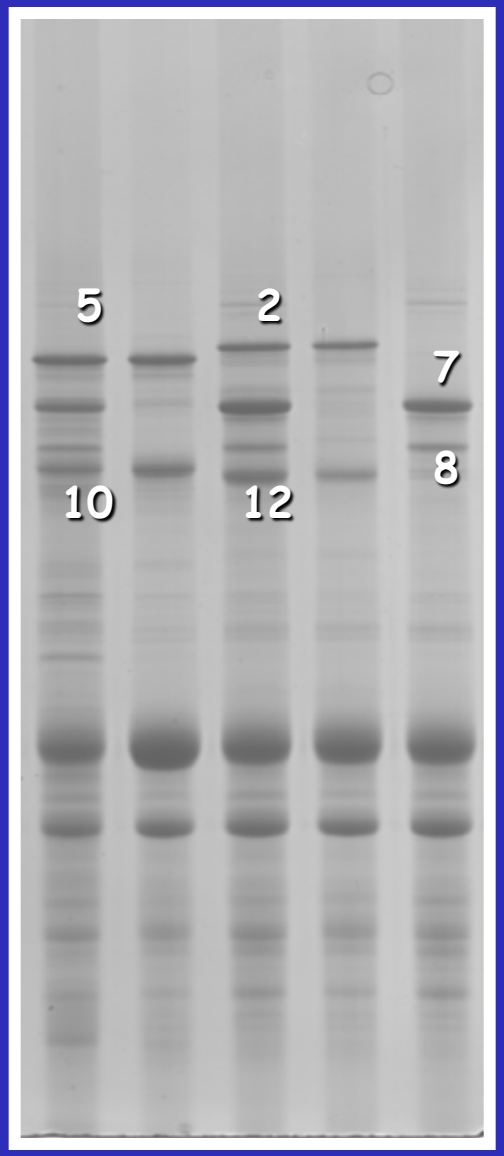
Durum wheat with bread-making potential

Chromosome engineering

Transferring of segment of alien chromosomes carrying particular desired genes to wheat chromosomes (Sears, E.R. 1972)



Introgression of the *Glu-D1* alleles into the durum wheat cultivar Svevo

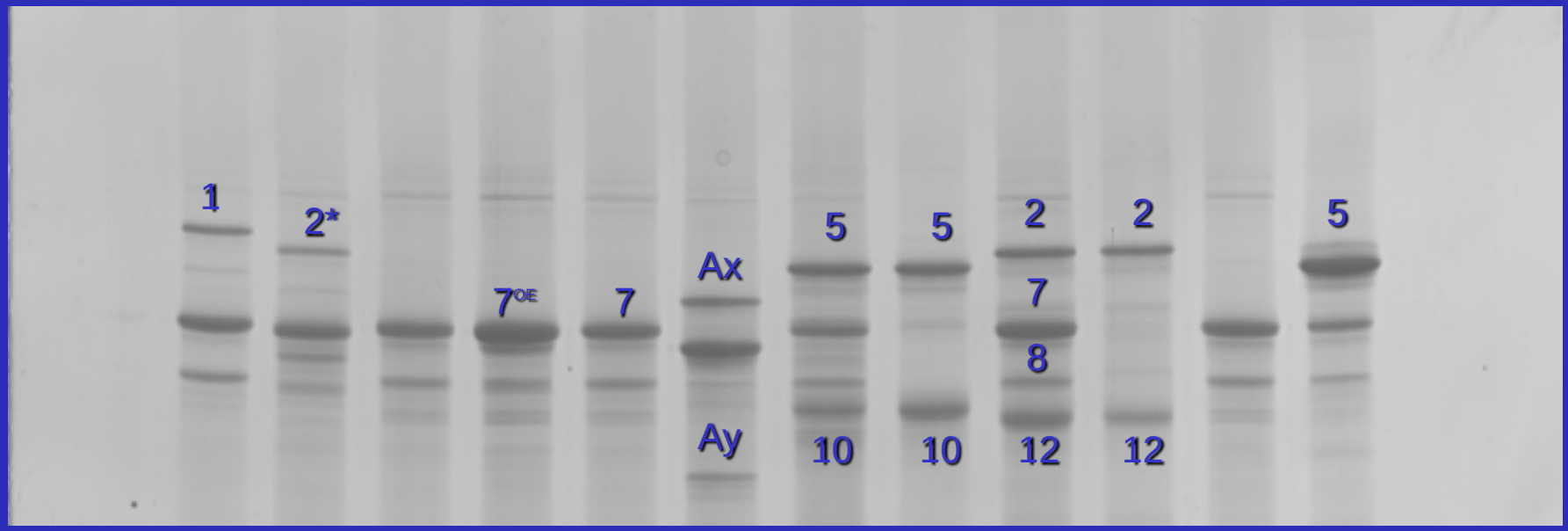


Effect of the alleles 2+12 and 5+10 on quality characteristics of durum wheat

	Genotypes	Protein content	Gluten Index	Alveograph parameters P	L	G	W	P/L	%UPP
VT	Svevo	18,3	54,0	78,0	84,0	20,4	165,0	0,9	44,1
	Svevo 2 + 12	16,3	99,0	102,0	119,0	24,3	370,0	0,9	55,8
	Svevo 5 + 10	15,9	100,0	180,0	69,0	18,5	500,0	2,6	63,9
BO	Svevo	17,4	58,0	62,0	93,0	21,5	120,0	0,7	41,0
	Svevo 2 + 12	18,3	100,0	97,0	134,0	25,8	320,0	0,7	53,0
	Svevo 5 + 10	17,5	100,0	169,0	72,0	18,9	480,0	2,4	62,0
FG	Svevo	13,7	74,0	75,0	83,0	20,3	165,0	0,9	42,6
	Svevo 2 + 12	12,9	99,0	88,0	88,0	20,9	220,0	1,0	52,0
	Svevo 5 + 10	12,8	97,0	147,0	38,0	13,7	240,0	3,9	61,0

Major difference between the pairs 2+12 and 5+10 the extra Cys residue of subunit 5 compared to subunit 2

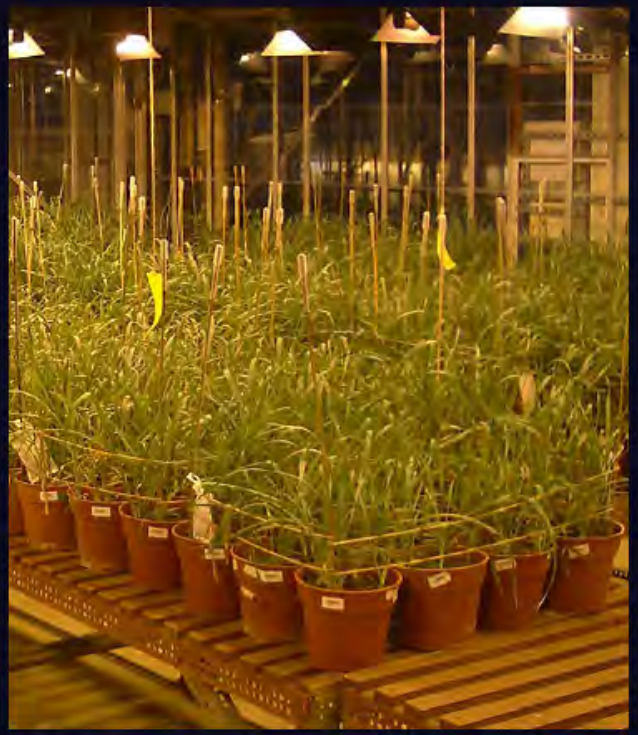
Enlarging diversity for glutenin subunits in durum wheat



Mutagenesis

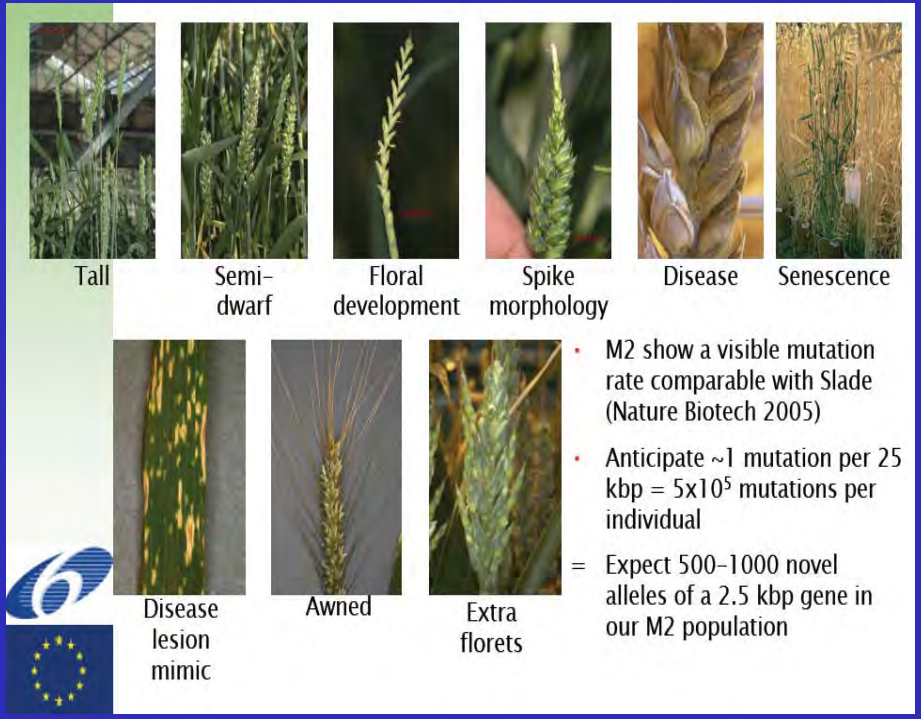
Production of mutagenised bread wheat lines (cv Cadenza, Rothamsted)

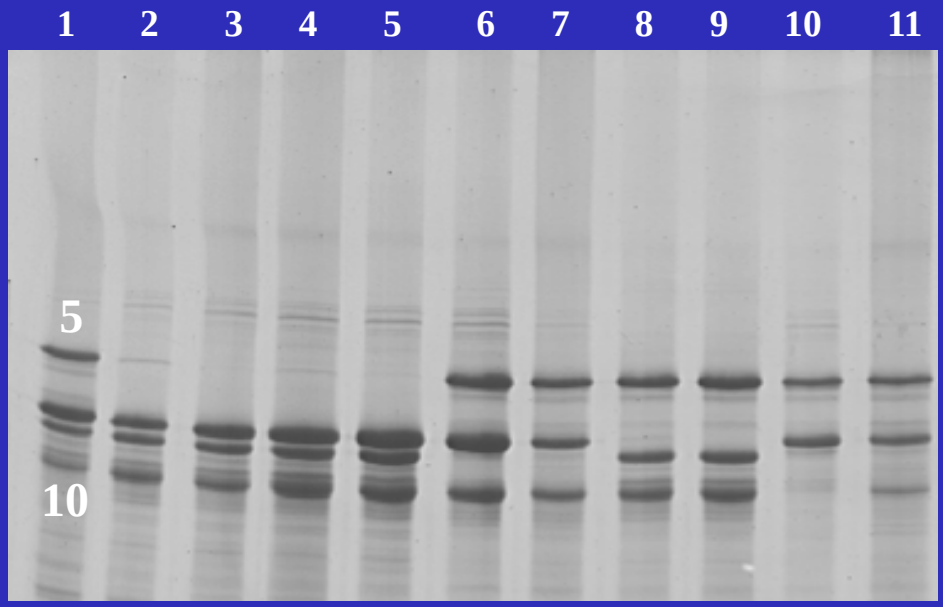
1. Assessed lethal dose of EMS (test 0.3%, 0.6%, 1.2%, 2.4%).
2. Used 0.6% + 0.9% (~30% non-germinating seeds) on ~8000 dry seeds 16h.
3. Sowed seeds and grew to maturity. Collected 1 M_1 ear per plant.
4. Sowed 1 M_2 seed from each ear
5. Harvested young leaf tissue for genomic DNA
6. Estimated DNA concentrations & archived.
7. Harvested M3 seed & archived.



12x8 array of Cadenza M_2 lines

Mutations in the high-molecular weight glutenin subunit genes





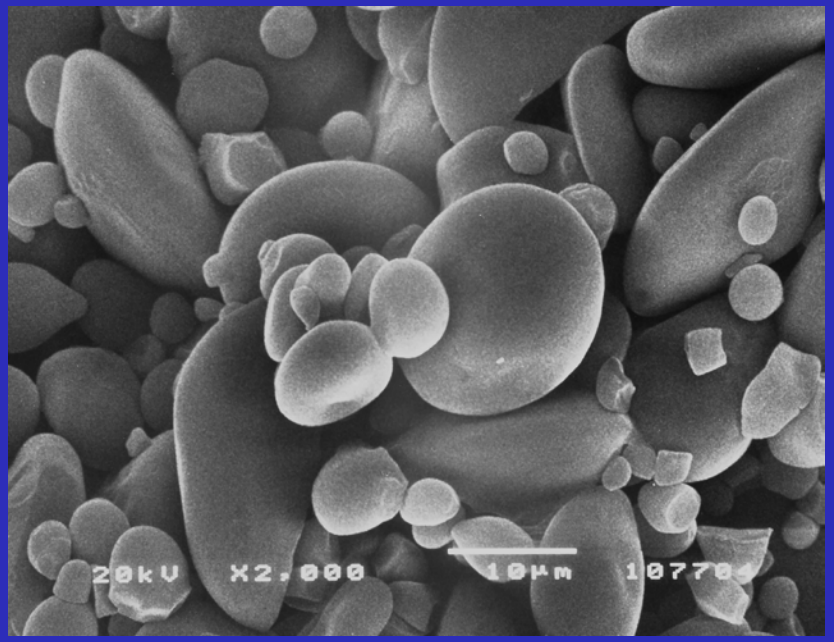

```
Bx14  750 GGGCAACAAGGTCAGCAGCCAGGACAAGGGCAACGACCATGACAAGGACAACAAGGGTACTACCCAA
          |||||||||||||||||||||||||||||||||||||||||||| ||||||||||||||||||||||||
Bx7       GGGCAACAAGGTCAGCAGCCAGGACAAGGGCAACGACCAGGACAAGGACAACAAGGGTACTACCCAA


Bx14  250 GQQGQQPGQGQRP-QGQQGYYP
          ||||||||||||||||||||||
Bx7       GQQGQQPGQGQRPGQGQQGYYP
```

Starch

Major component of the human diet. In particular, starch-based foods contribute approximately one-third of the total weight of our dietary intake, mainly from foods such as bread, pasta, potatoes and rice.

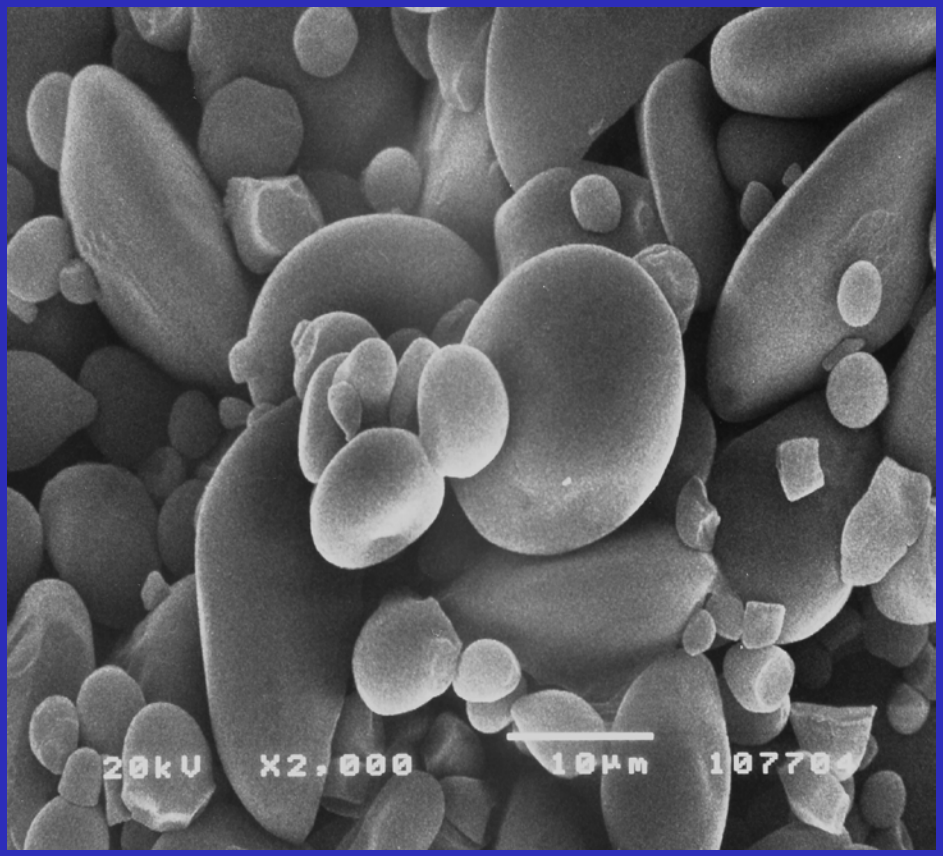
Starch



Starch content in wheat ranges from 65% to 75%

Contains two types of polymers:
Amylose (25%)
Amylopectin 75%)

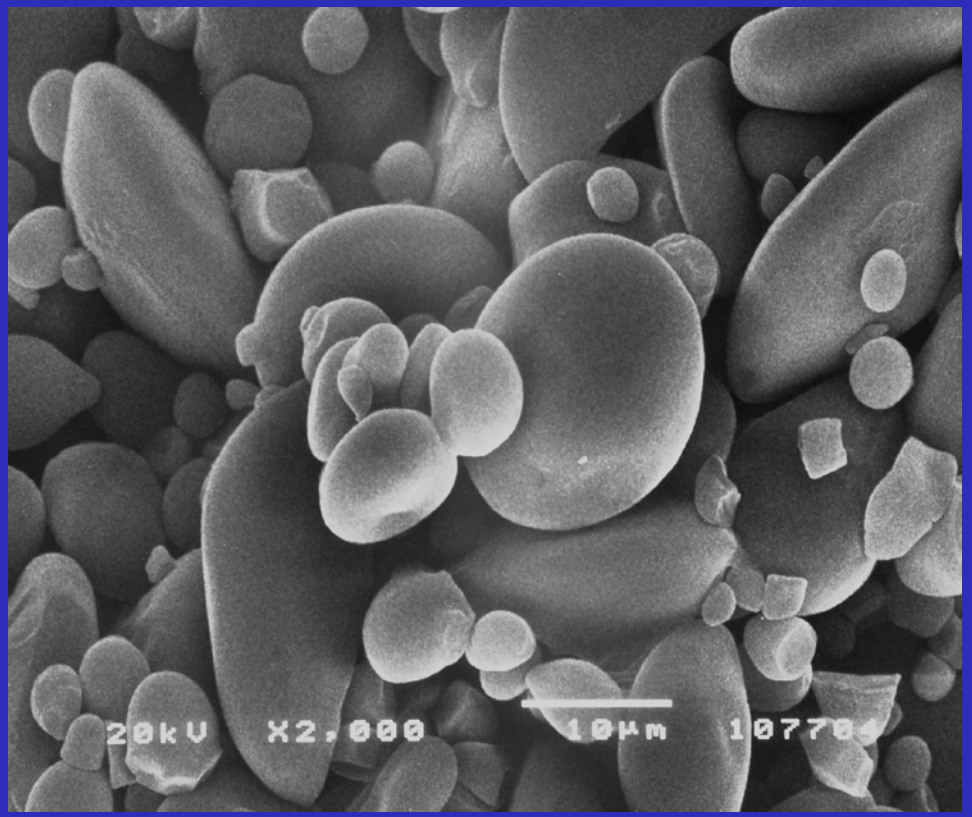
Low Amylose Starch



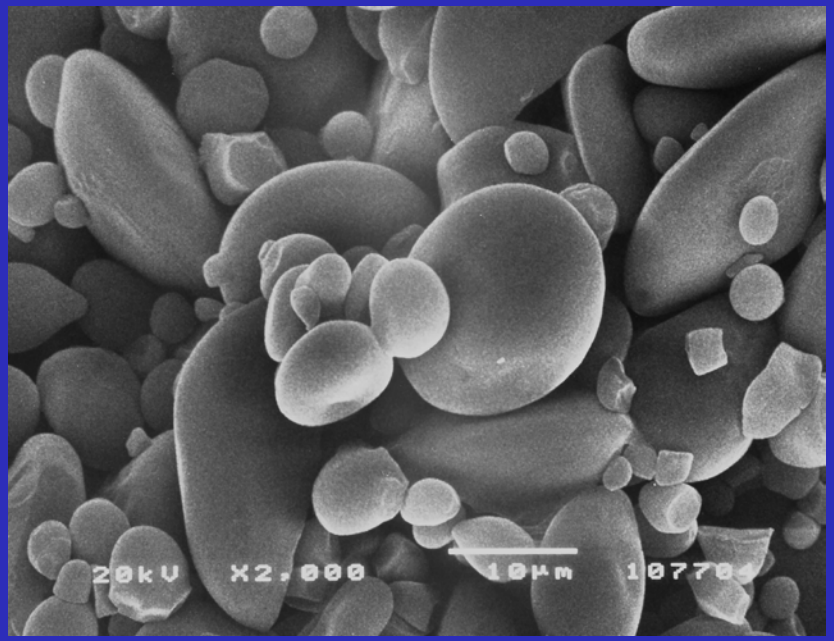
- ✓ Food industry
  - Frozen products
  - Fat replacement
  - Extruded products
  - Shelf life
- ✓ Paper industry
- ✓ Adhesive industry
- ✓ Feed industry

High Amylose Starch

- Hampers the penetration of cooking oils
- Increases pasta firmness
- Increases resistant starch
- Uses as gelling agent
- Photografic films



Starch & Health



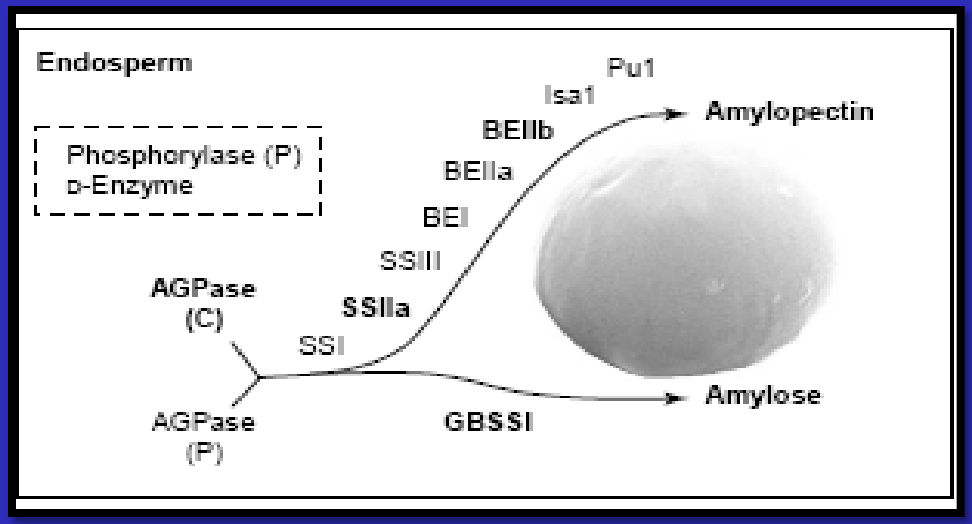
High amylose starches are also classified as resistant starches because they escape digestion passing through the small intestine into the large bowel.

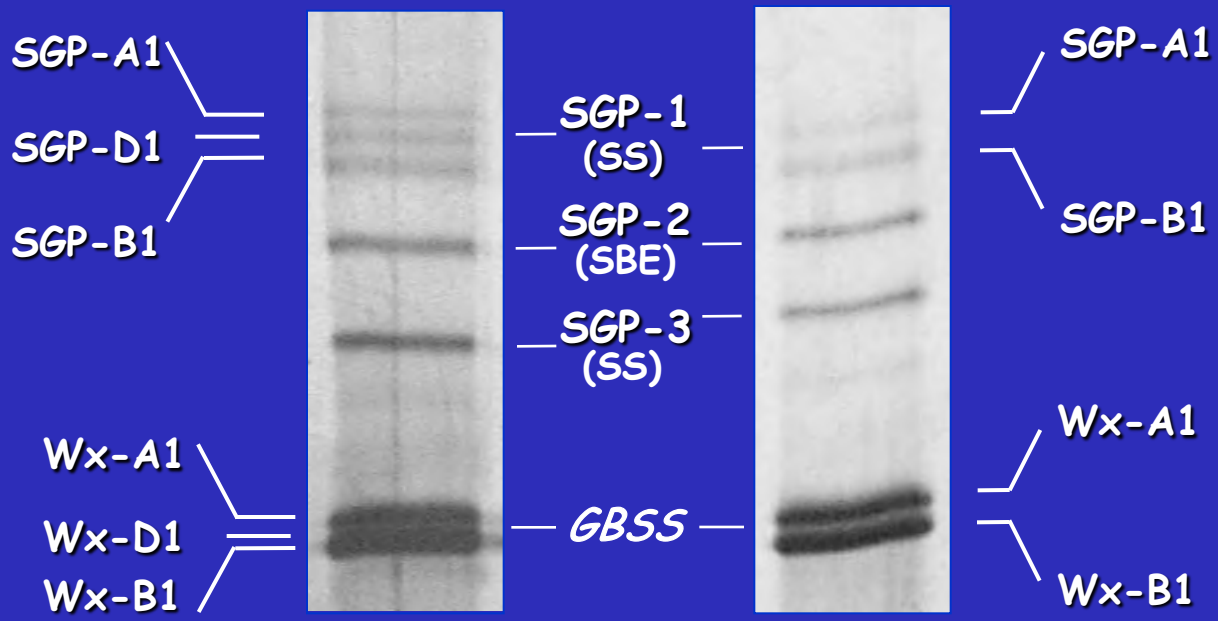
Starch & health

Physiological effects of Resistant Starch

- Improved glycaemic and insulinaemic responses (diabetes)
- Improved bowel health (colorectal cancer)
- Improved blood lipid profile (cardiovascular disease)
- Prebiotic and culture protagonist (colonic health)
- Increased micronutrient absorption (osteoporosis)
- Increased satiety and reduced energy intake (obesity)

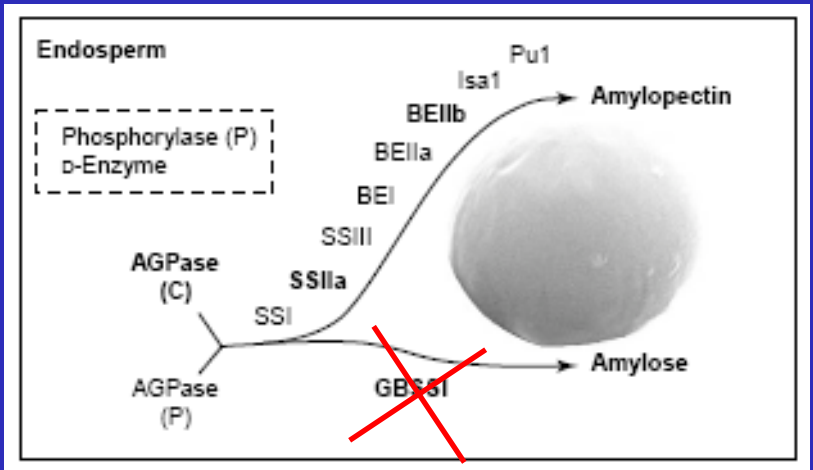
Enzymes involved in amylose and amylopectin synthesis





Production of wheat with altered amylose/amylopectin ratio

LOW AMYLOSE STARCH



Production of near isogenic lines for waxy proteins in durum wheat

Svevo (wt) × Kanto 107 ($Wx\text{-}A1^{-}/B1^{-}$)

F_2 Genotype ($WxA1^{-}/B1^{-}$) × Svevo (wt)

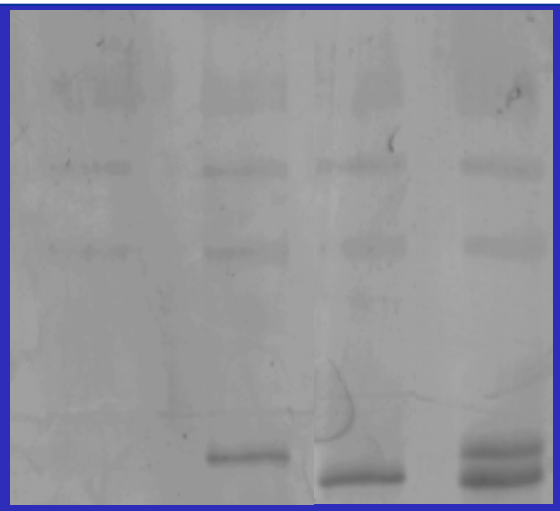
BC_1F_1

BC_6F_1

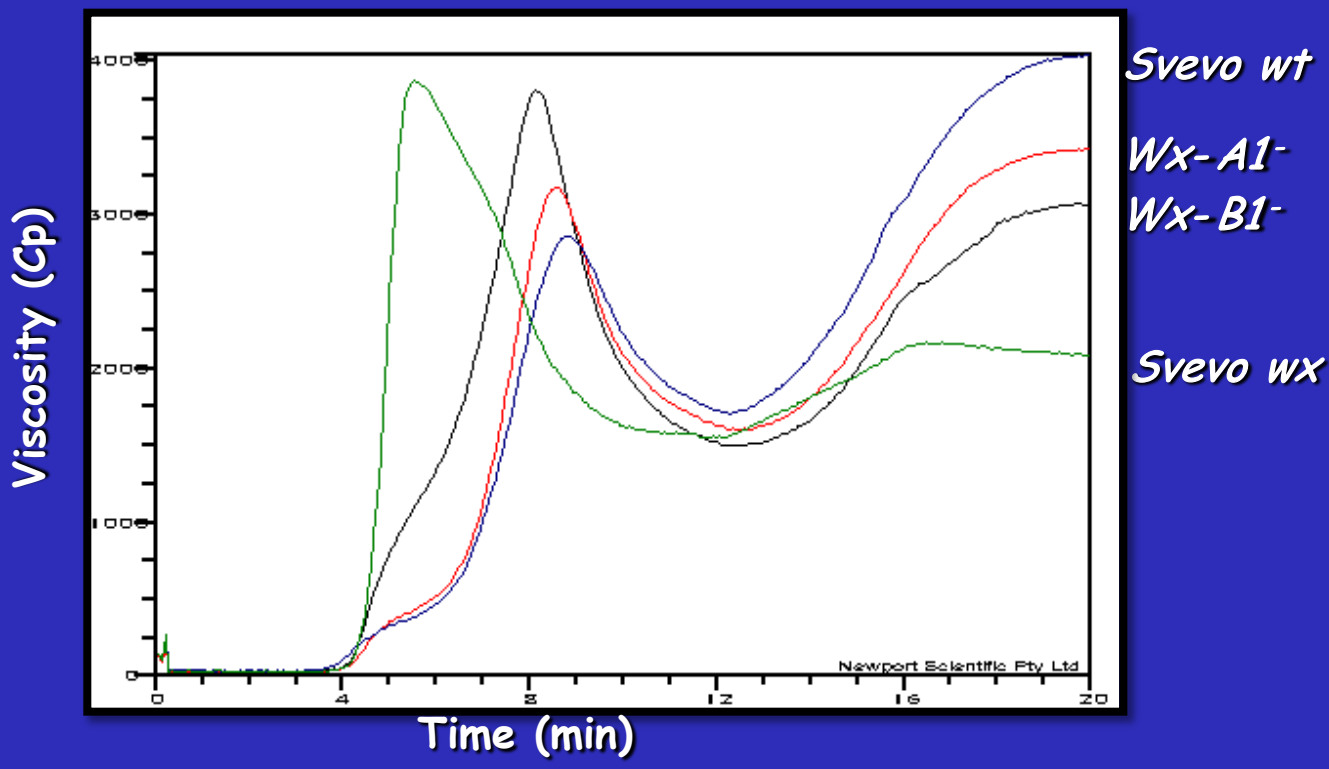
BC_6F_2 Genotype: $Wx\text{-}A1^{-}$;$-B1^{-}$; $Wx\text{-}A1^{-}/B1^{-}$

BC_6F_3

BC_6F_4 Genotype: $Wx\text{-}A1^{-}$;$-B1^{-}$; $Wx\text{-}A1^{-}/B1^{-}$



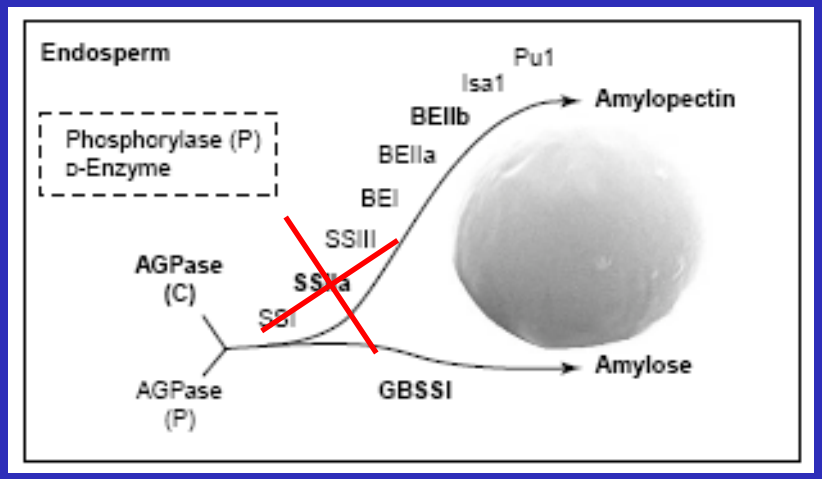
RVA and amylose content of partial and complete waxy durum wheat



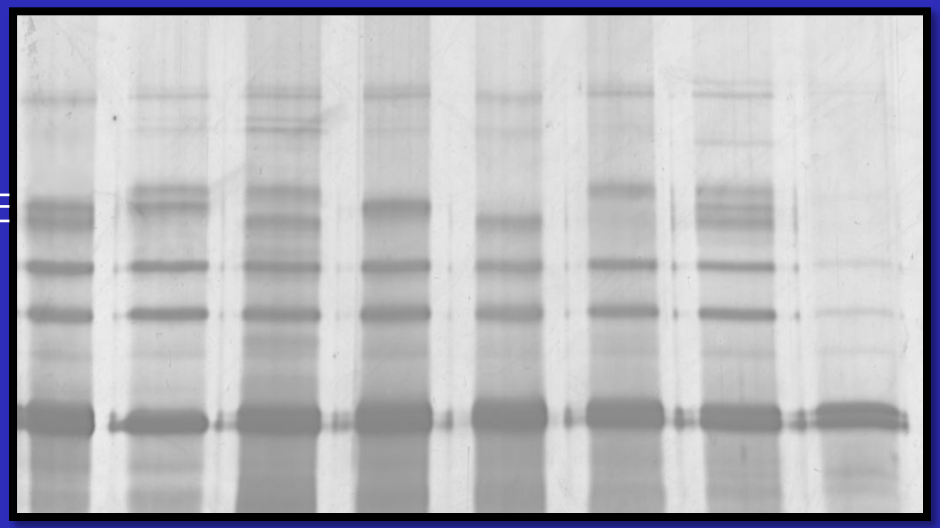
Genotypes	*Wt*	*Wx-A1⁻*	*Wx-B1⁻*	*Wx*
Amylose content (%)	29.7	27.5	21.8	2.5

Production of wheat with altered amylose/amylopectin ratio

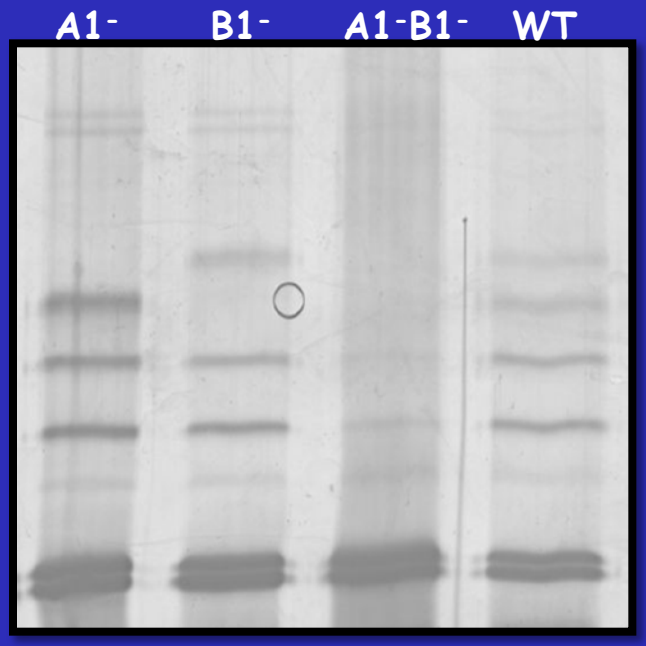
HIGH AMYLOSE STARCH

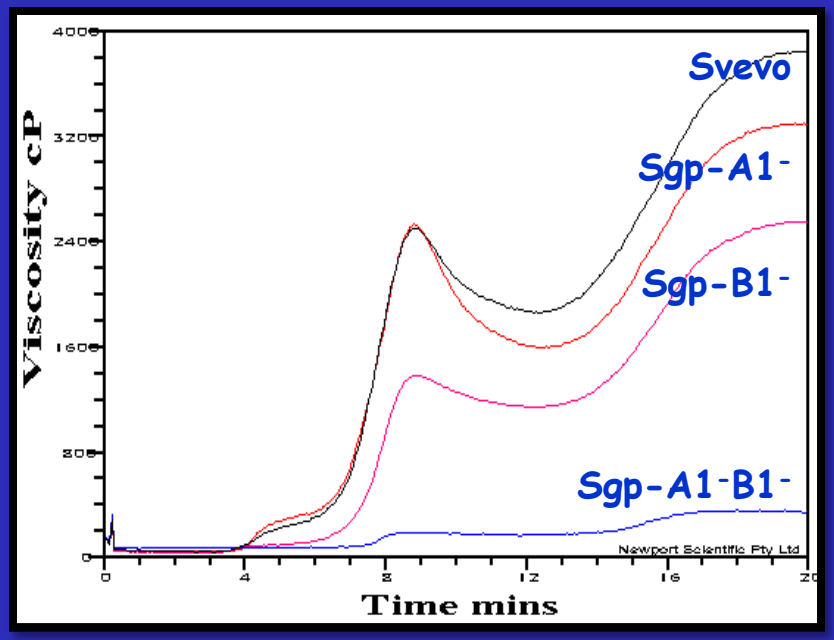


Sgp-A1
Sgp-D1
Sgp-B1



Production of a set of Sgp-1 null lines in durum wheat

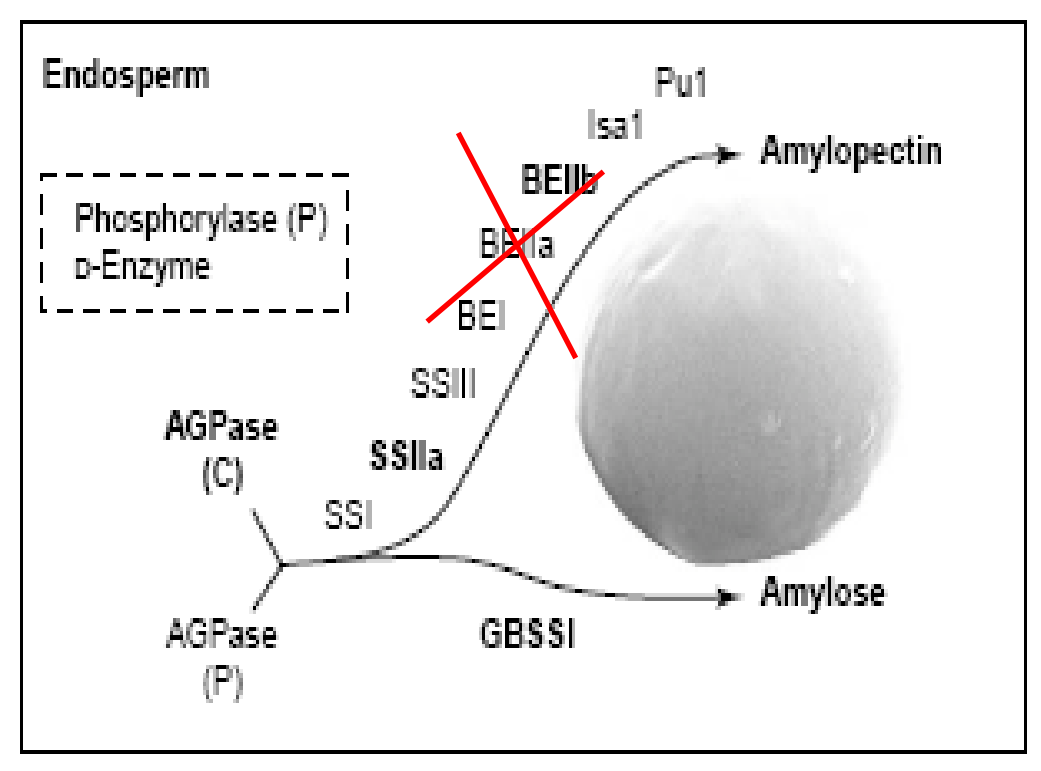




Genotype	Sgp-A1-	Sgp-B1-	Sgp-A1-B1-	Svevo
Amylose Content	24.3	29.1	43.6	23

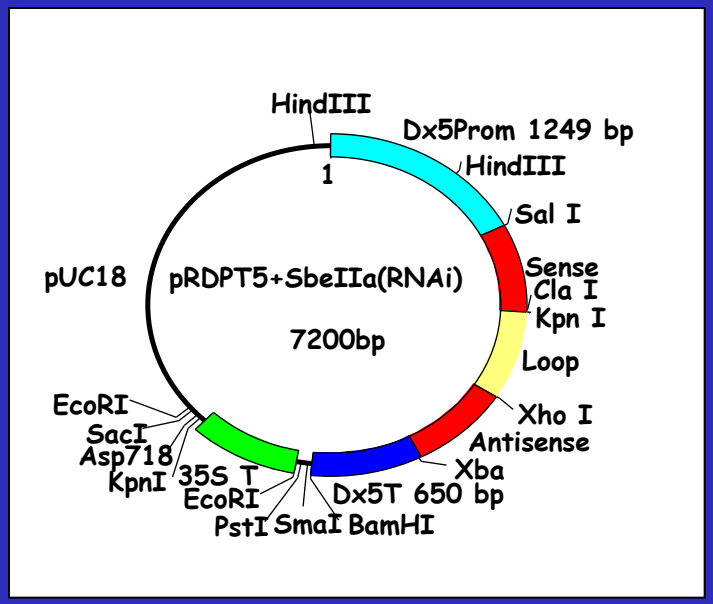
Production of wheat with altered amylose/amylopectin ratio

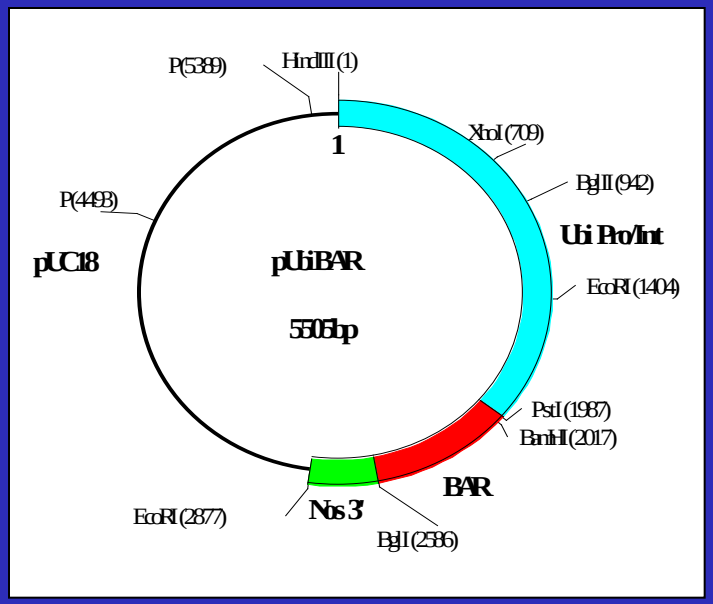
HIGH AMYLOSE STARCH



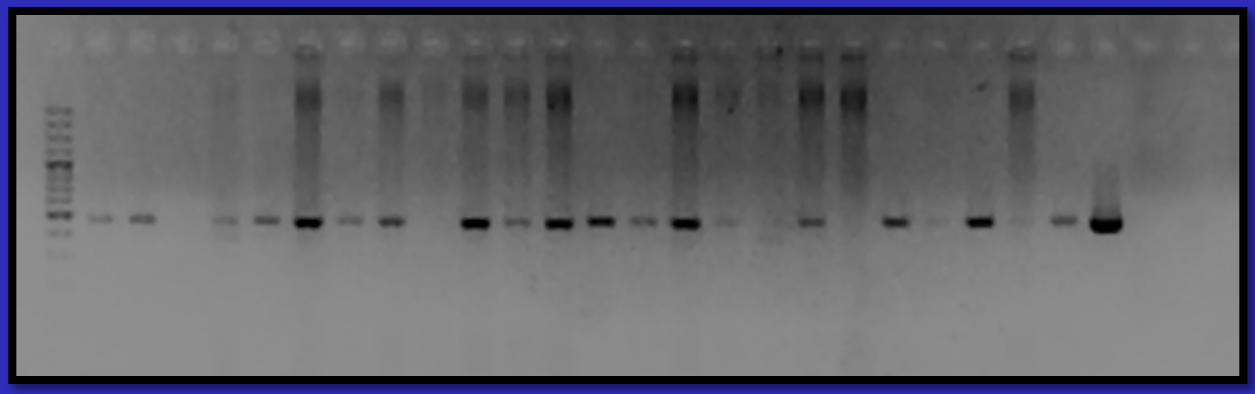
Silencing of *SbeIIa* by RNAi

Biolistic transformation



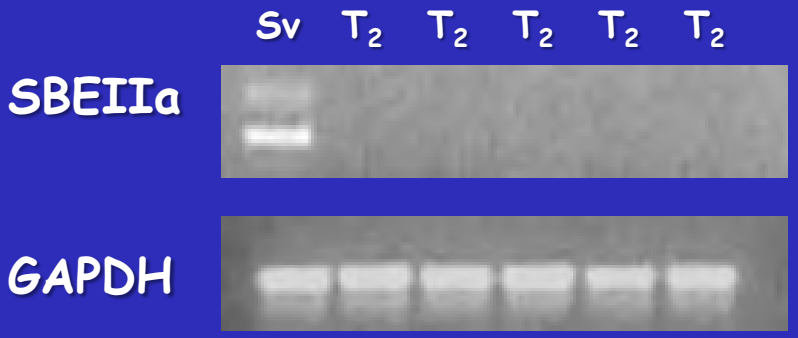


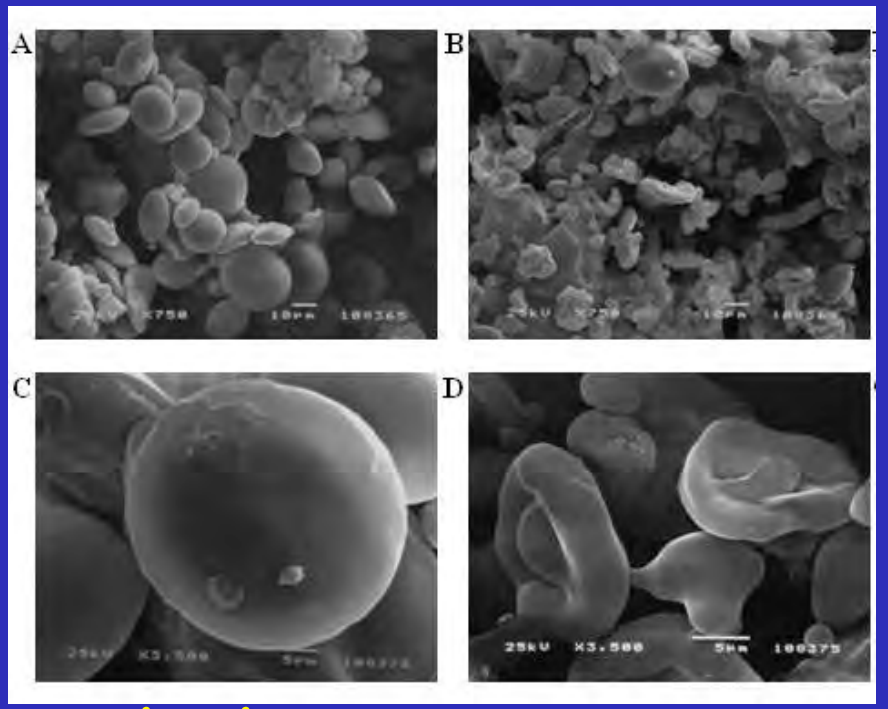
Transformation of 3000 Svevo embryos



- 48 transgenic lines

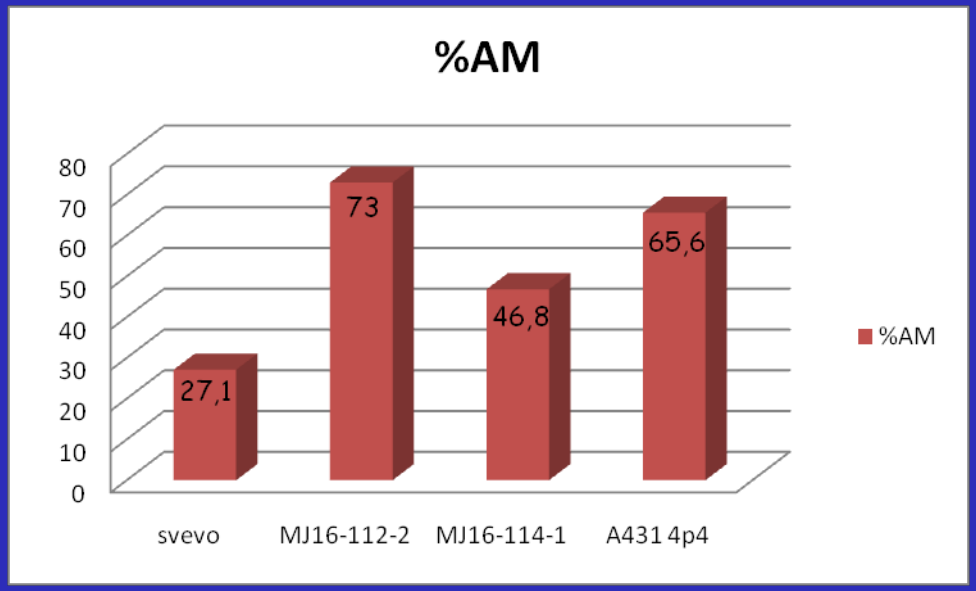
RT-PCR



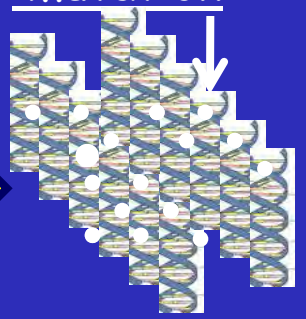


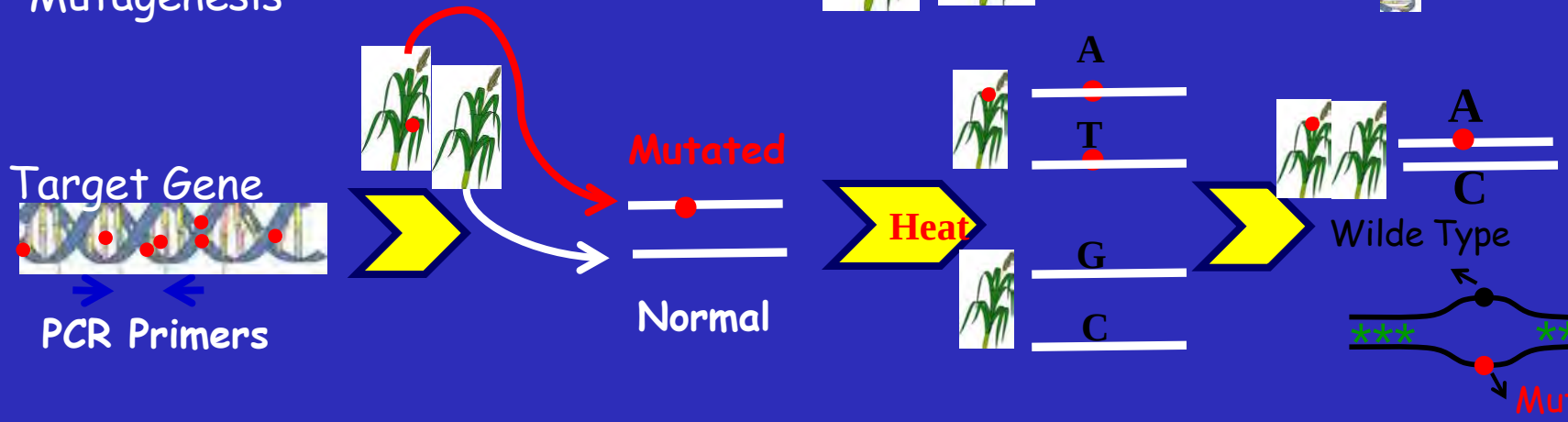
Amylose content

Sample	ABS 620nm	%AM
svevo	0,2915	27,1
MJ16-112-2	0,677	73,0
MJ16-114-1	0,4575	46,8
A431 4p4	0,615	65,6

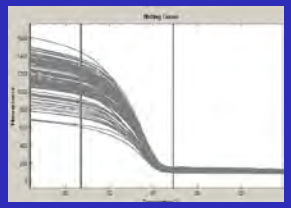


TILLING

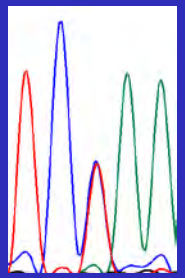




HRM



Detection of mismatch



Sequencing

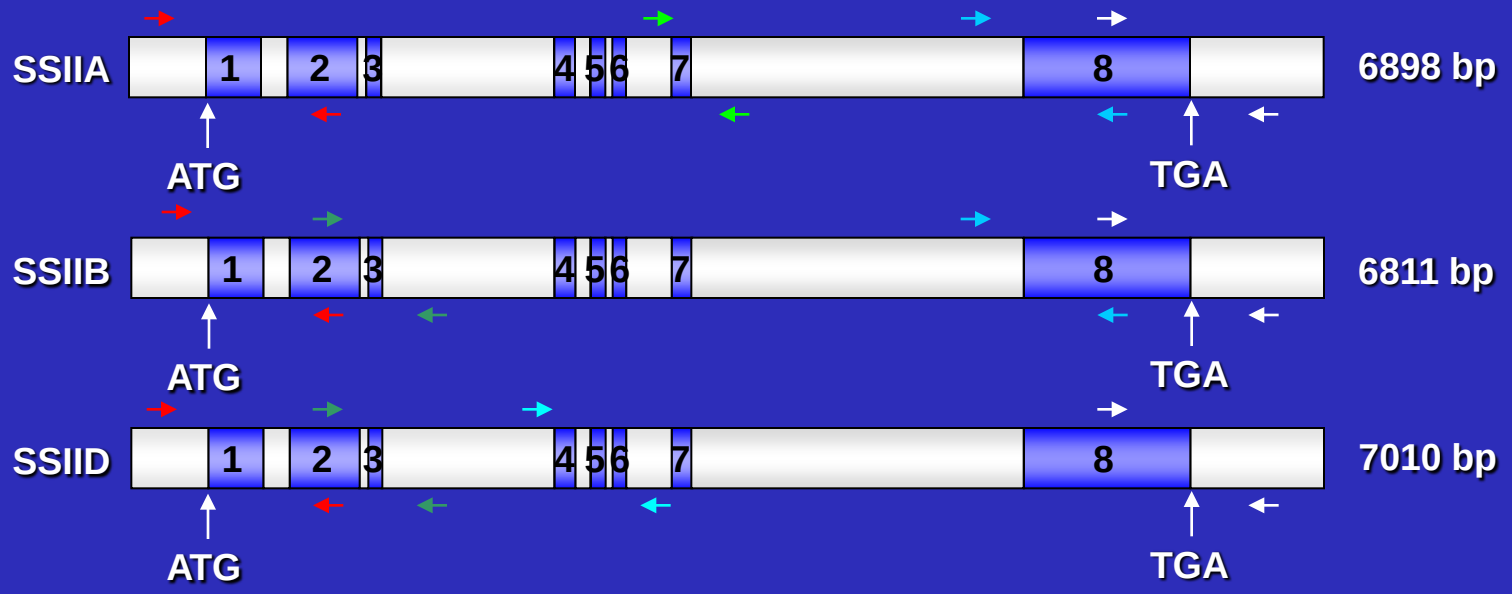
aaatacatagccccctag STOP **Stop codon**

MAQNGAPQ* **Truncated protein**

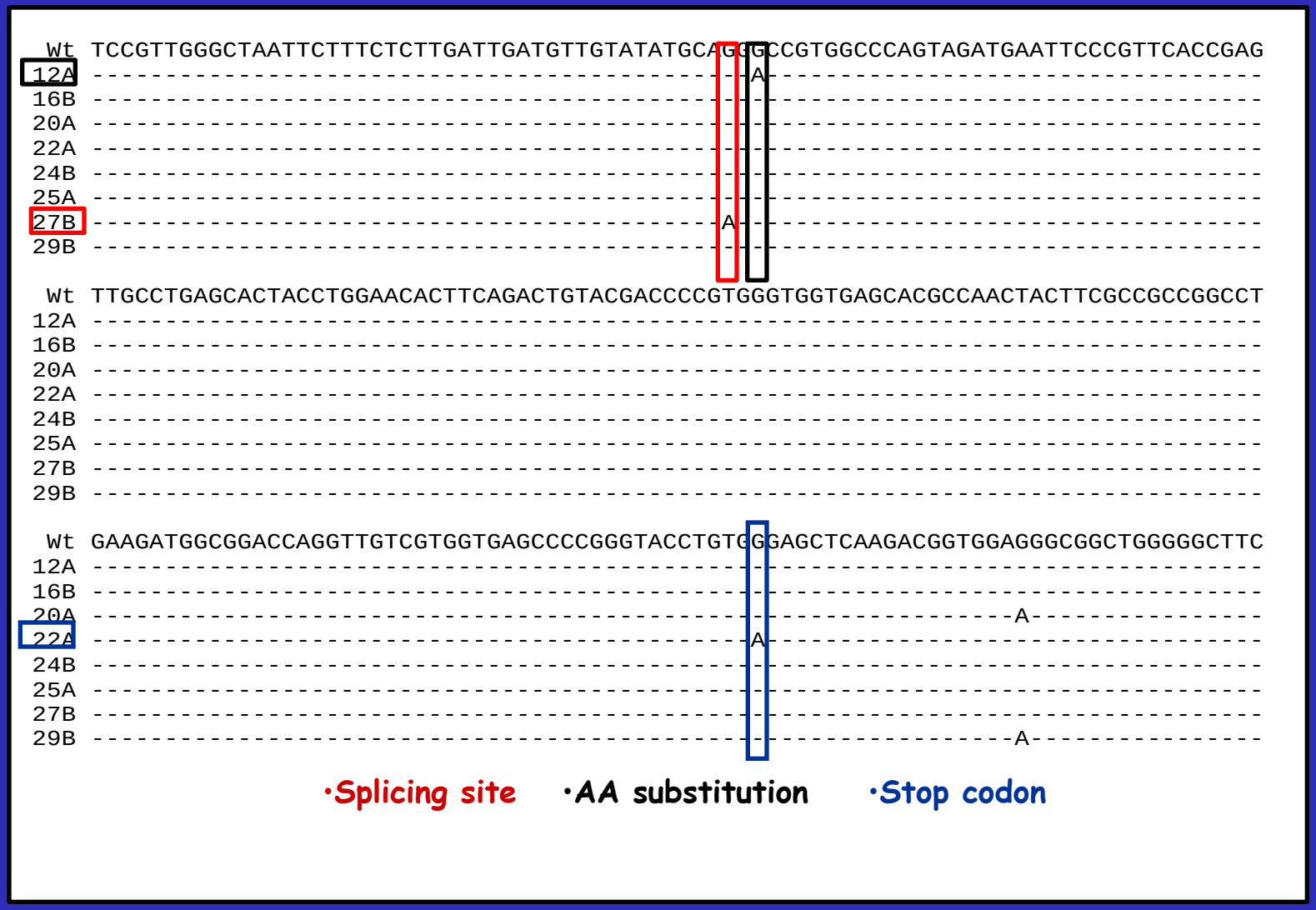
SBEIIa **Loss of Enzyme Functionality!!!**

TILLING in wheat

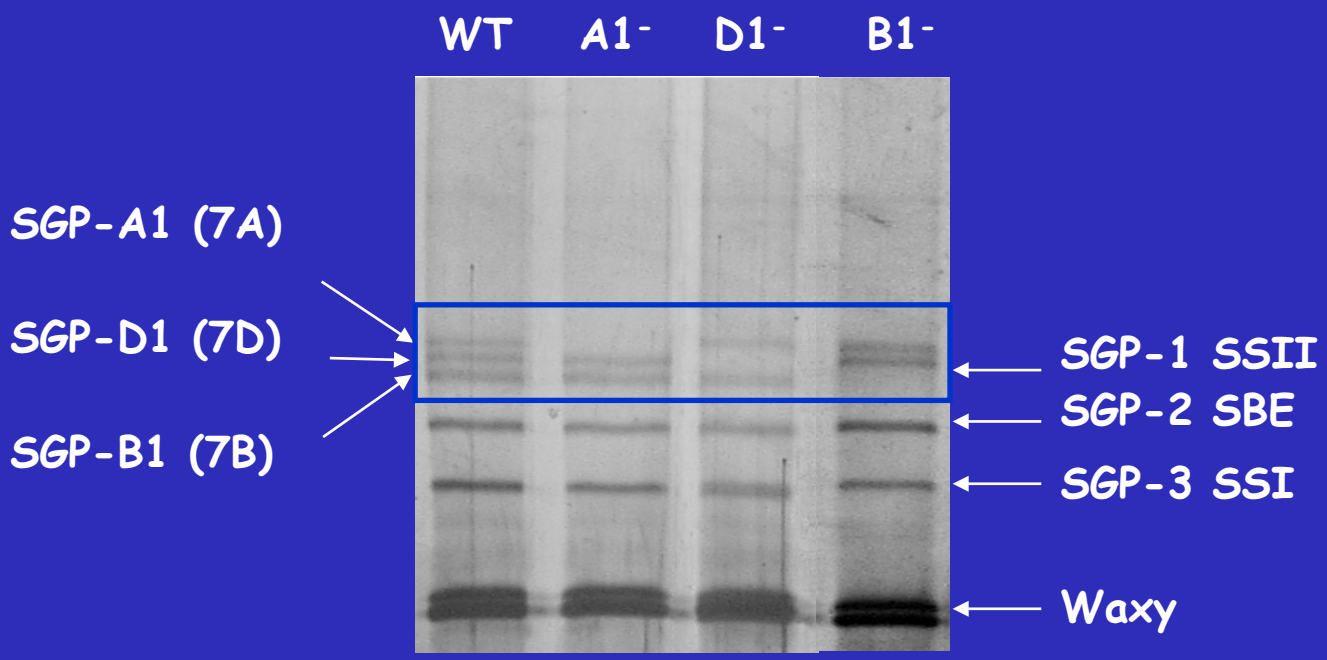
Identification of SSII genome specific primers



SGP-A1 (*SSIIa*) mutants

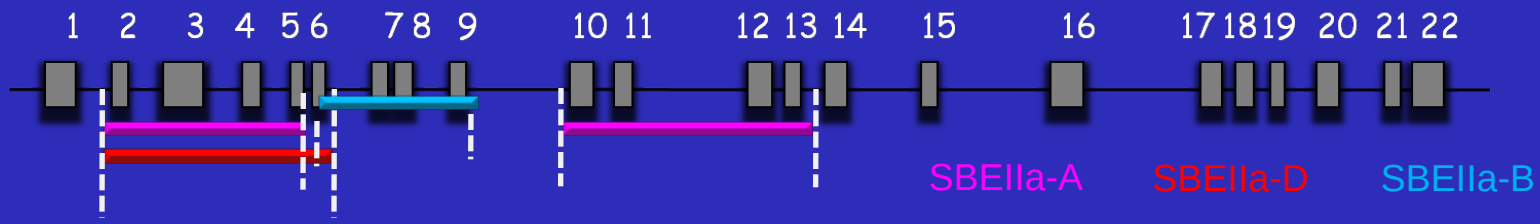


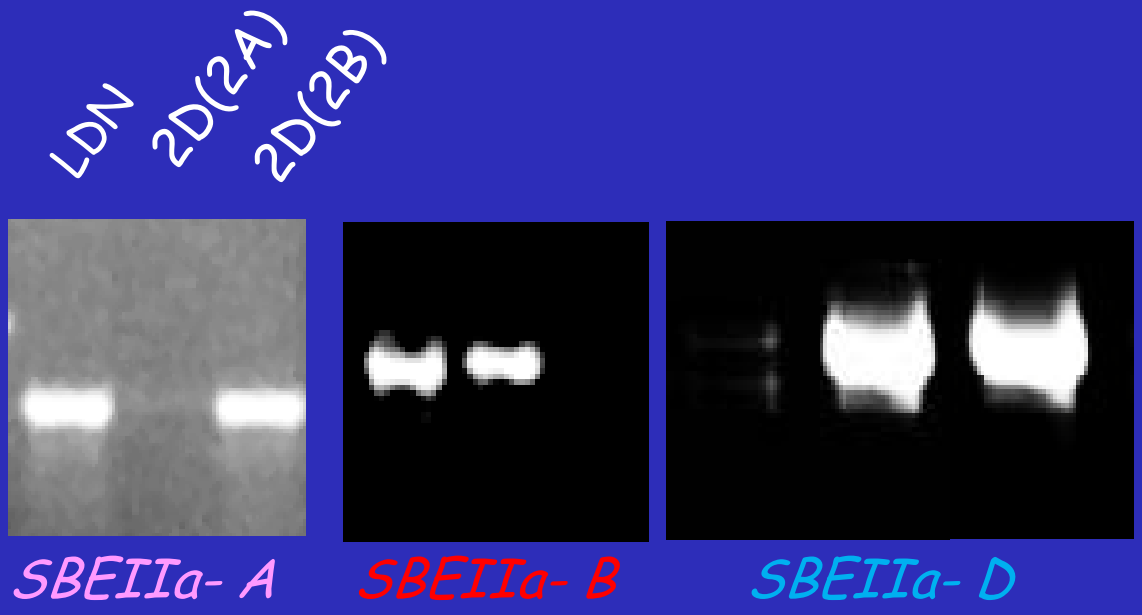
Identification of Sgp-1 null lines



TILLING
Targeting Starch Branching Enzyme IIa

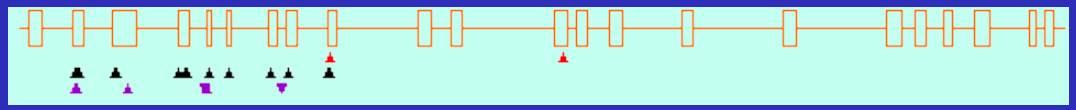
GENE REGIONs TARGETED



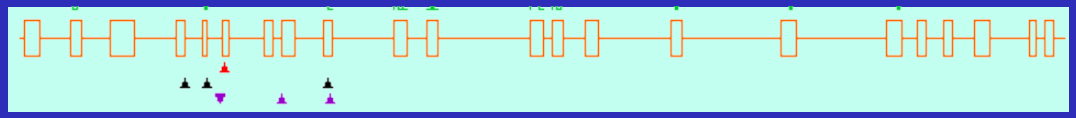


TILLING SbeIIa genes

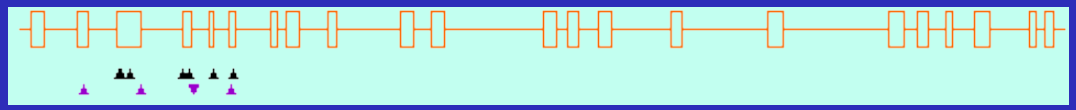
SBEIIa-A



SBEIIa-B



SBEIIa-D



GENES	wSBEIIa-A	wSBEIIa-B	wSBEIIA-D
Subset size	2496	1150	1920
Fragment size	1500-2000	1700	2000
Mutants	30	10	25
Nonsense MUTs	2	1	0

Mutation frequency

1 / 40Kbs

Conclusions

- A range of materials and approaches can be used to manipulate protein and starch composition with the possibility to tailor wheat cultivars with superior technological and nutritional characteristics;

- Particular success has been achieved using classical breeding, in addition mutagenesis is gaining again popularity thanks to the integration with genomic resources;

- The availability of gene sequence information along with informatics tools that maximise the probability of recovering useful mutations will make this approach a strong contributor to genomic studies and crop breeding in the near future;

- These approaches are also most likely to find acceptance by consumers and regulatory bodies.

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