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ORIGINAL: English
DATE: May 26, 2010

INTERNATIONAL UNION FOR THE PROTECTION OF NEW VARIETIES OF PLANTS
GENEVA

WORKING GROUP ON BIOCHEMICAL AND MOLECULAR TECHNIQUES AND DNA PROFILING IN PARTICULAR

Twelfth Session
Ottawa, Canada, May 11 to 13, 2010

ADDENDUM

AN OVERVIEW OF DNA-BASED VARIETY IDENTIFICATION AT THE CANADIAN GRAIN COMMISSION

Document prepared by an expert from Canada

Q U A L I T Y I N N O V A T I O N S E R V I C E

Canadian Grain Commission | Commission canadienne des grains

Canada

DNA-based Variety Identification at the Canadian Grain Commission

Daniel Perry
Grain Research Laboratory

Canadian Grain Commission

The CGC:

- Is the regulator of Canada's grain handling industry
- Is the official certifier of Canadian grain
- Conducts research into grain quality

The Grain Research Laboratory

The GRL:

- Conducts research to understand grain quality and grain safety factors
- Develops methods and tests for measuring and evaluating grain quality and grain safety
- Monitors grain in Canada's grain handling system for quality and safety

Variety Identification in the GRL

Wheat

- Marketed by class – designated varieties
- DNA methods to complement protein-based variety monitoring of hexaploid wheat
- DNA methods to monitor variety composition of durum shipments

Barley

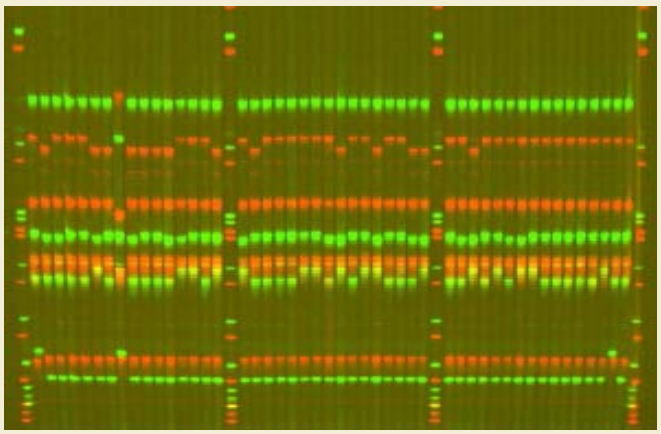
- Certify varietal purity of malting barley cargos

Microsatellite methods

- Multiplexed marker sets
  - published sources
  - tailed primers
  - Li-Cor DNA analyzers
- Single kernel analyses
  - 96-well plate format DNA extraction

Microsatellite methods

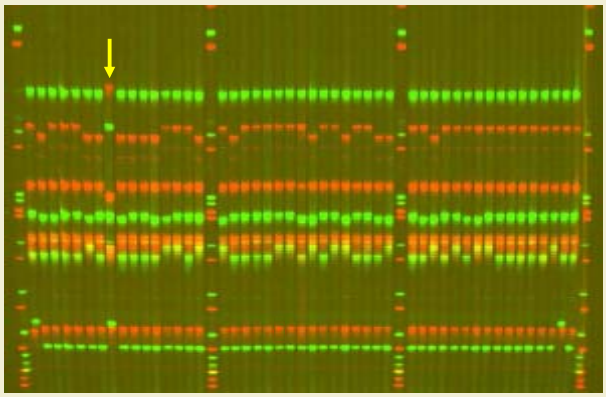
Malting barley varietal purity



700 Channel 800 Channel

Microsatellite methods

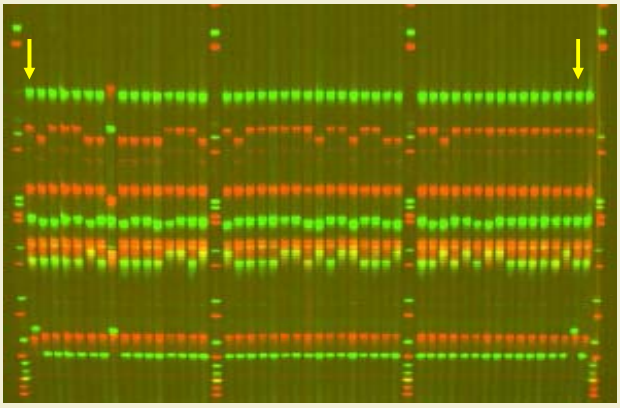
Malting barley varietal purity



700 Channel ■ 800 Channel ■

Microsatellite methods

Malting barley varietal purity



700 Channel ■ 800 Channel ■

Microsatellite methods

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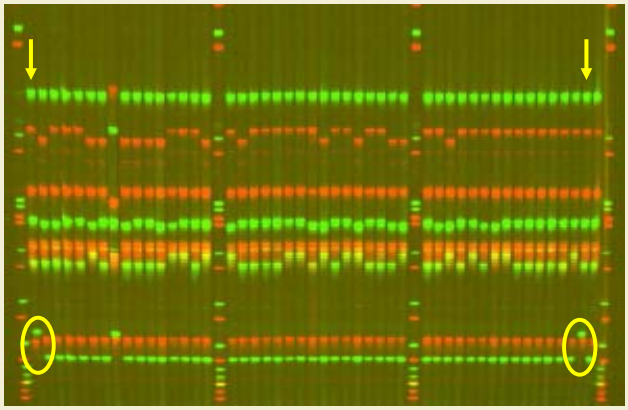


700 Channel 800 Channel

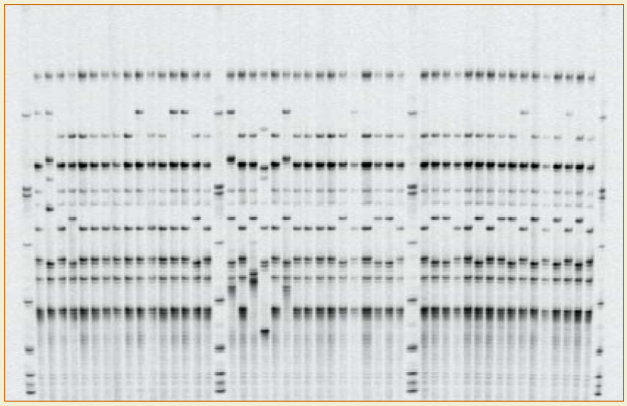
Microsatellite methods

Challenges for barley variety ID

Variety	Pedigree
'CDC PolarStar'	OIU003/CDC Kendall (bx)
'Norman'	Selection from CDC Kendall
'Merit 16'	Merit//Merit/2B95-8129
'Merit 57'	Merit//Merit/2B94-5744

Microsatellite methods

Durum wheat monitoring

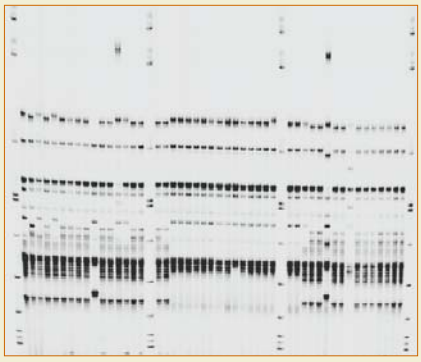


700 or 800 Channel

Theor Appl Genet (2004) 109:55-61

Microsatellite methods

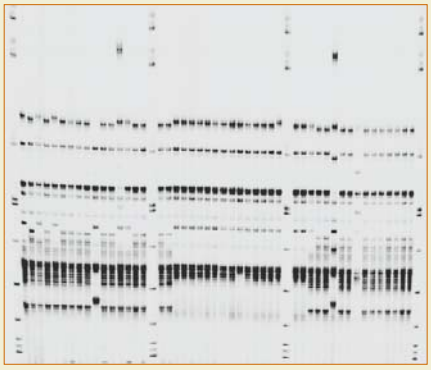
Hexaploid wheat analysis



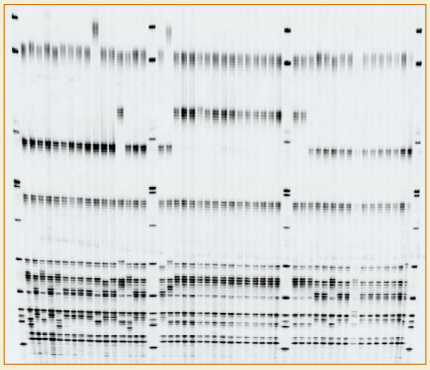
Durum set – 700 Channel

Microsatellite methods

Hexaploid wheat analysis



Durum set – 700 Channel



WOOC set – 800 Channel

SNP Research

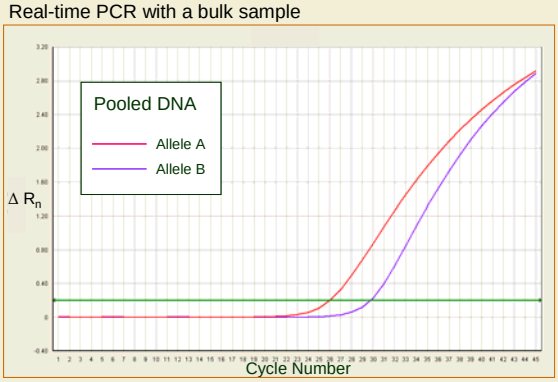
SNP-based markers

- Less user intervention
- Higher throughput
- Quantitative analyses

SNP Research

Ground sample analysis – barley

Real-time PCR with a bulk sample



SNP Research

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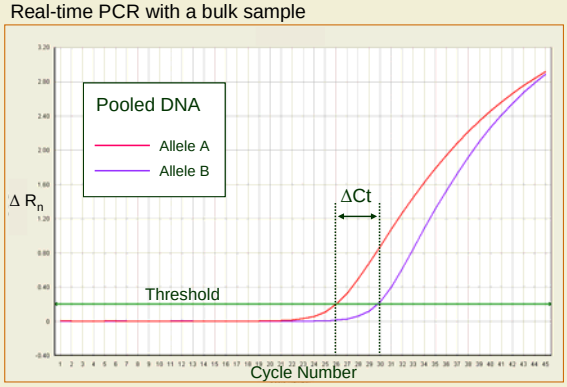
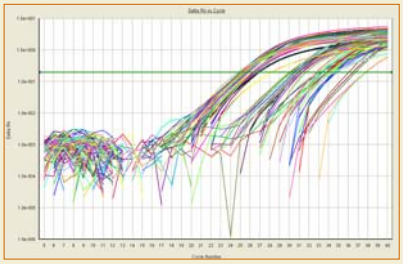


SNP Research

Ground sample analysis – barley

16 markers x 2 alleles x 3 reps = 96 wells



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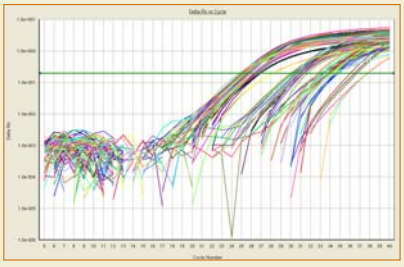

MARKER	Frequency
A******5	0.7
R***2	1.6
M****1	100.0
A****4	2.6
M*****2	0.4
A***8	99.7
A****6	0.4
M*****9	100.0
A****2	97.8
A****1	99.3
B*****8	1.3
A**3	98.5
N**1	0.5
A***5	0.0
M*****1	0.3

SNP Research

Ground sample analysis – barley

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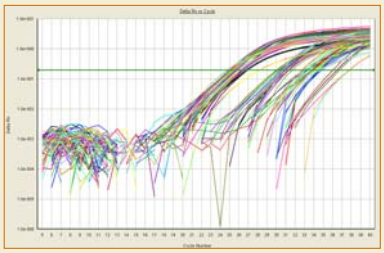

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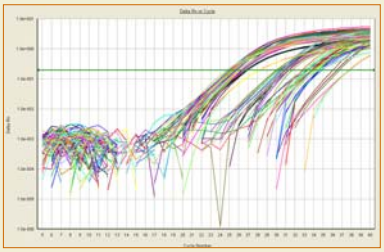
A****2	97.8
A****1	99.3
B*****8	1.3
A**3	98.5
N**1	0.5
A***5	0.0
M*****1	0.3

ESTIMATED SAMPLE COMPOSITION	
AC Metcalfe	1.48 (0.05)
CDC Copeland	98.06 (0.05)
Harrington	0.46 (0.02)

SNP Research

Ground sample analysis – barley

16 markers x 2 alleles x 3 reps = 96 wells



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ESTIMATED SAMPLE COMPOSITION	
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Harrington	0.46 (0.02)

SNP Research

Ground sample analysis – blind samples

Sample		AC Metcalfe	CDC Copeland	CDC Kendall	Harrington	Stein	Legacy
A	Actual	**92**	4	2	2		
	Estimated						
B	Actual	2		94		2	2
	Estimated						
C	Actual	2	**96**		2		
	Estimated						
D	Actual	**94**	1	2	1	2	
	Estimated						
E	Actual	2	**94**	2	2		
	Estimated						
F	Actual	2	2	2	**94**		
	Estimated						

SNP Research

Ground sample analysis – blind samples

Sample		AC Metcalfe	CDC Copeland	CDC Kendall	Harrington	Stein	Legacy	CDC Dolly
A	Actual	**92**	4	2	2			
	Estimated	**92.6**	4.1	1.9	1.4			
B	Actual	2		94		2	2	–
	Estimated	2.5		94.9		1.2	0.9	0.5
C	Actual	2	**96**		2			
	Estimated	2.3	**96.4**		1.3			
D	Actual	**94**	1	2	1	2		
	Estimated	**94.7**	0.9	2.0	1.2	1.3		
E	Actual	2	**94**	2	2			
	Estimated	2.3	**94.8**	1.8	1.2			
F	Actual	2	2	2	**94**			
	Estimated	2.4	1.6	2.3	**93.8**			

SNP Research

Ground sample analysis – blind samples

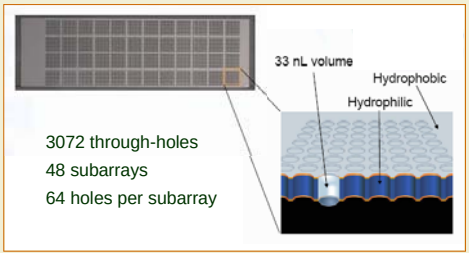
Sample		AC Metcalfe	CDC Copeland	CDC Kendall	Harrington	Stein	Legacy	CDC Dolly
A	Actual	**92**	4	2	2			
	Estimated	**92.6**	4.1	1.9	1.4			
B	Actual	2		94		2	2	–
	Estimated	2.5		94.9		1.2	0.9	0.5
C	Actual	2	**96**		2			
	Estimated	2.3	**96.4**		1.3			
D	Actual	**94**	1	2	1	2		
	Estimated	**94.7**	0.9	2.0	1.2	1.3		
E	Actual	2	**94**	2	2			
	Estimated	2.3	**94.8**	1.8	1.2			
F	Actual	2	2	2	**94**			
	Estimated	2.4	1.6	2.3	**93.8**			

SNP Research

OpenArray for wheat variety ID

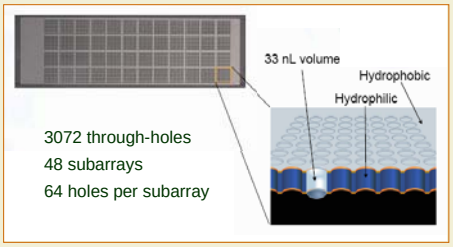
SNP Research

OpenArray for wheat variety ID

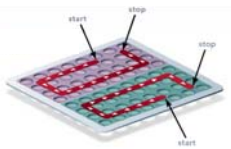


SNP Research

OpenArray for wheat variety ID

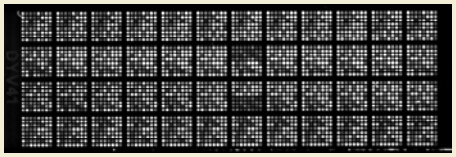


2 samples (kernels) per subarray
96 kernels per plate
32 SNPs per kernel

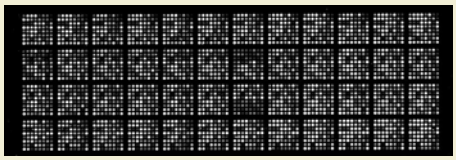


SNP Research

OpenArray – imaging



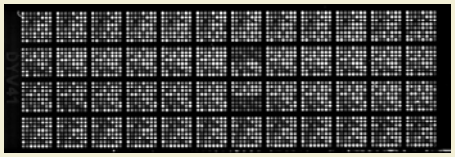
Fam



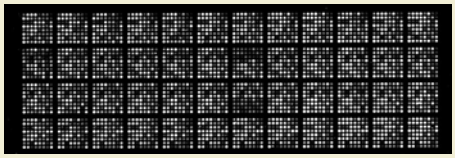
Vic

SNP Research

OpenArray – automated genotyping



Fam



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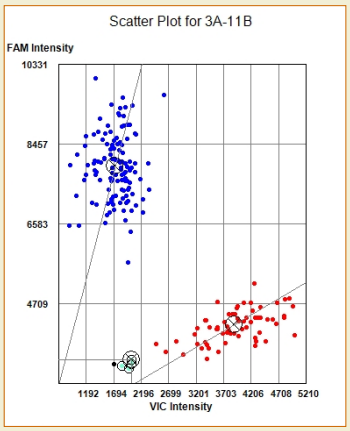
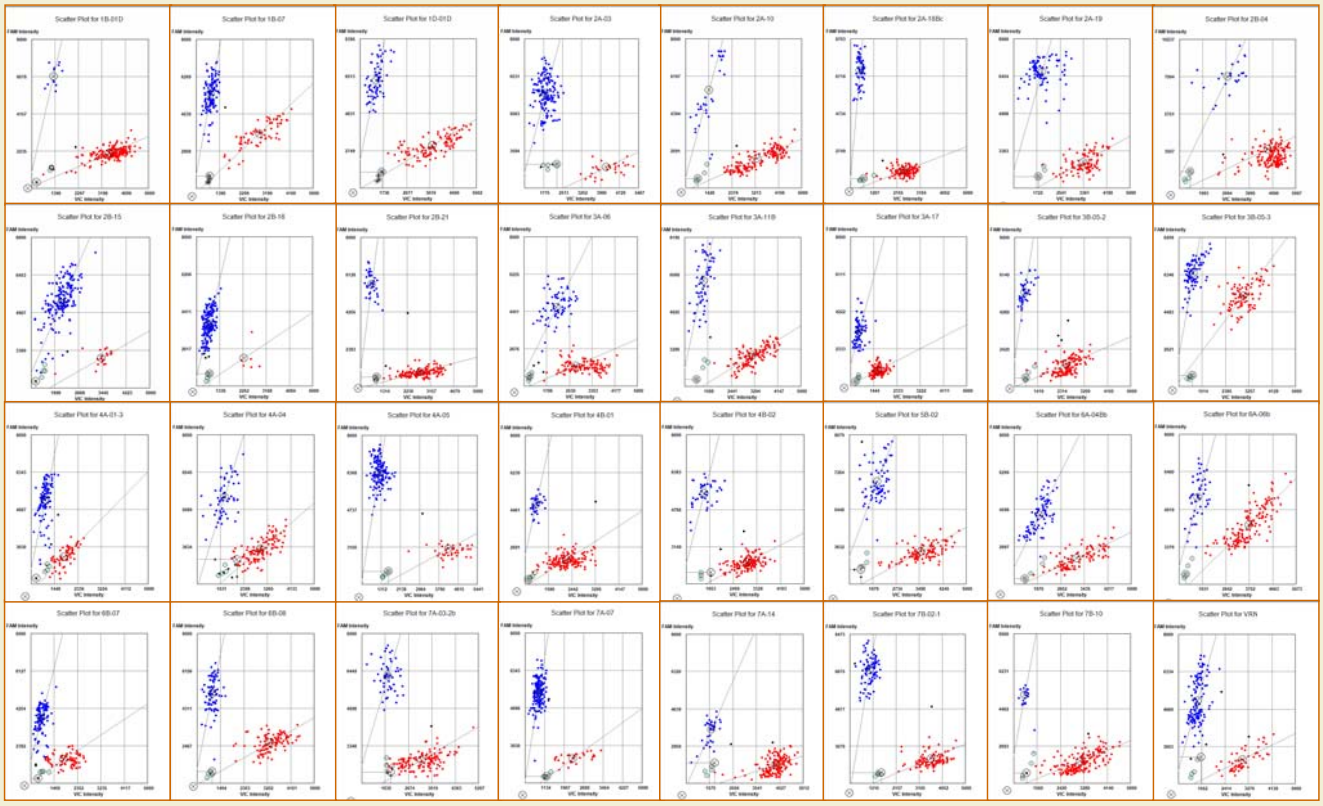
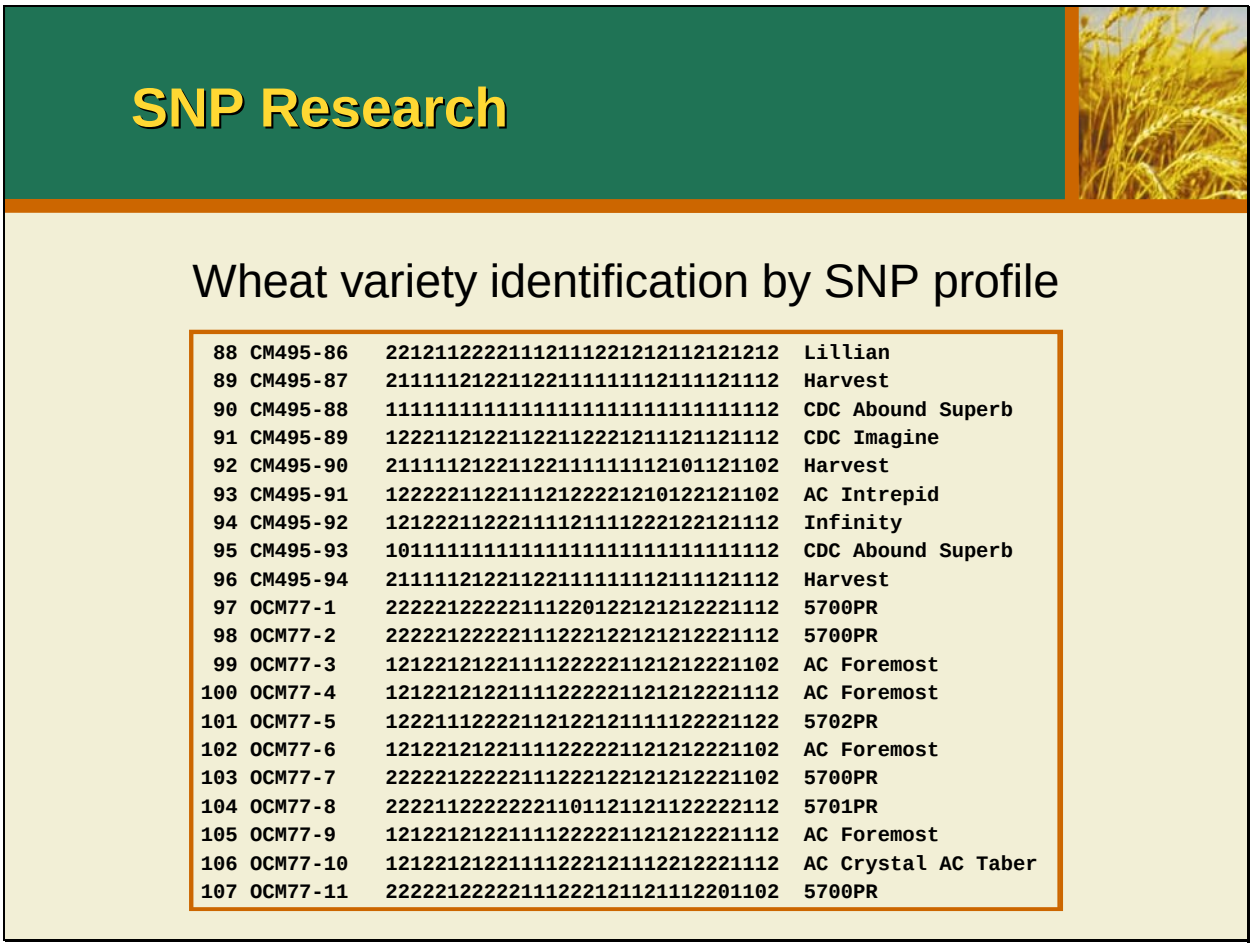


SNP Research





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