Vavilov Institute of Plant Industry (VIR)

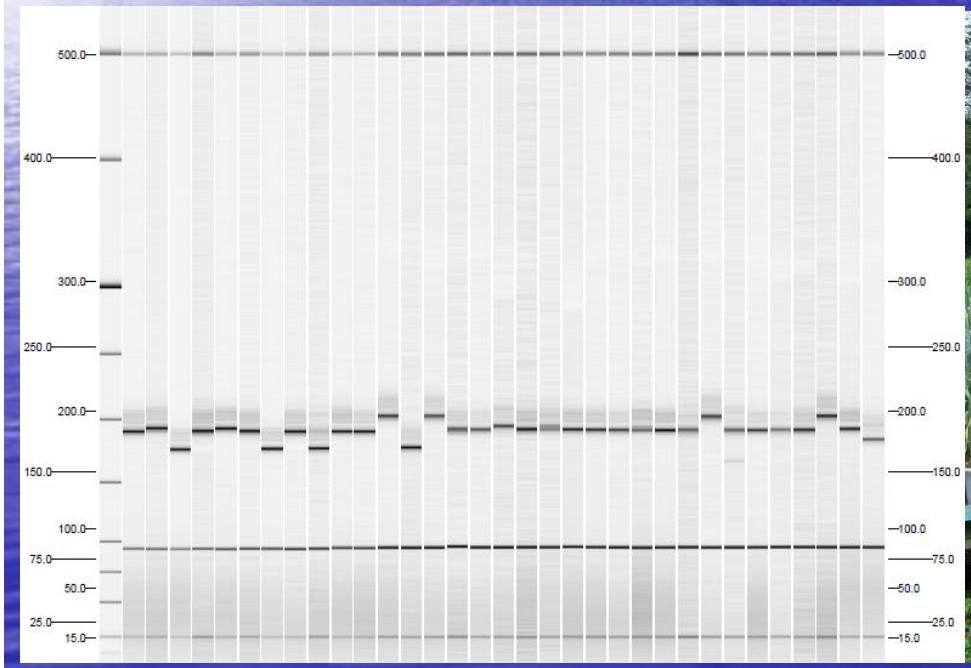
Molecular Marker Laboratory

Ongoing projects:

- SSR-profiling of Russian wheat and barley varieties for the Distinctness, Uniformity and Stability (DUS) tests to provide Plant Breeder's Rights (PBR) Ministry of education and Science, №16.M04.12.0008
- Assessment of the VIR germplasm collection with functional molecular markers

- **SSR-profiling of 310 wheat and 180 barley varieties for the Distinctness, Uniformity and Stability (DUS) tests to provide Plant Breeder's Rights**

- **Assessment of Vrn/Ppd allele combinations for the cultivars to predict their vernalization & photoperiod requirement**

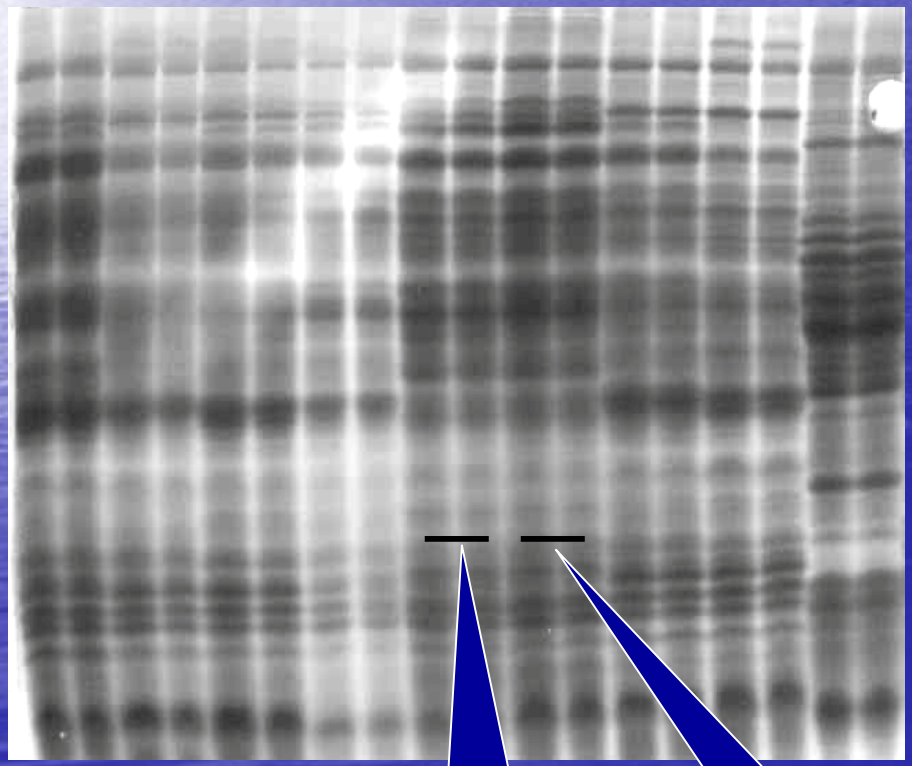


Государственный контракт: **№16.M04.12.0008**

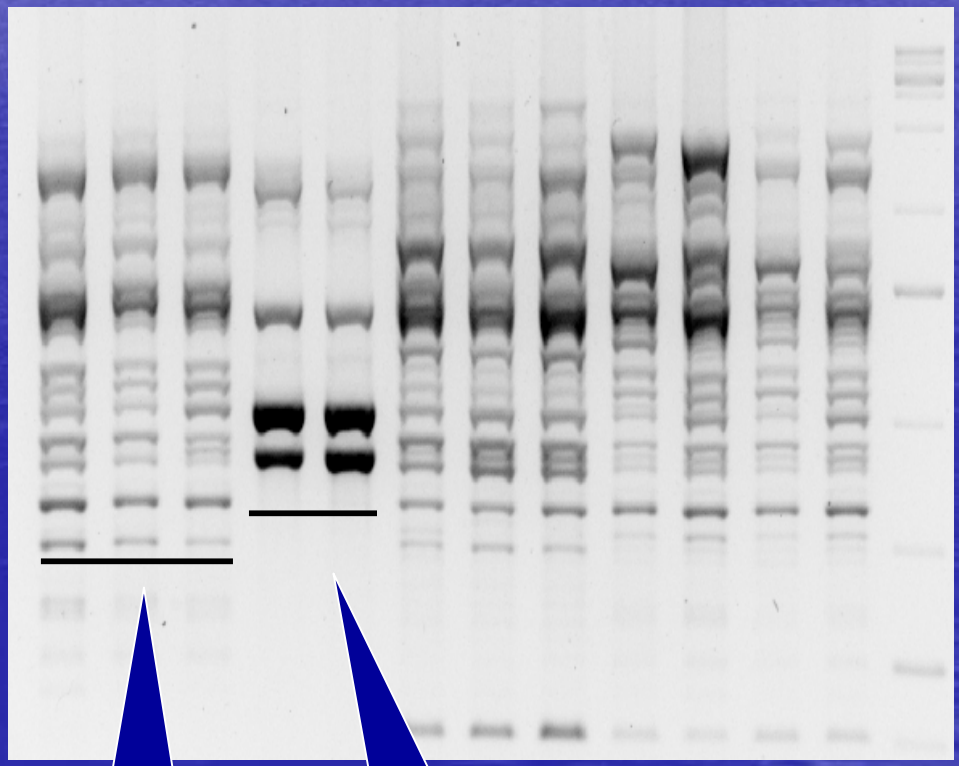
Why SSR-profiling for the DUS test?

Example: Identification of *Lupinus angustifolius* L. cultivars by storage protein electrophoresis and ISSR markers

Storage protein electrophoresis



ISSR (Inter Simple Sequence Repeat) HB12



UPOV

UPOV/INF/17/1
ORIGINAL: English
DATE: October 21, 2010

INTERNATIONAL UNION FOR THE PROTECTION OF NEW VARIETIES OF PLANTS

GENEVA

GUIDELINES FOR DNA-PROFILING: MOLECULAR MARKER SELECTION AND DATABASE CONSTRUCTION ("BMT GUIDELINES")

adopted by the Council
at its forty-fourth ordinary session
on October 21, 2010

A Microsatellite Map of Wheat

Roder et al., 2008
Genetics 149: 2007–2023 (August 1998)

Song et al., 2005
Theor. Appl. Genet 110:550-560

2.2.1.3 For effective microsatellite analysis, **selecting high quality markers is essential**. This includes a consideration of *inter alia*:

(a) the degree of "stuttering" (production of a series of one or more bands, differing by 1 repeat unit in size);

(b) (n+1) peaks; Taq-polymerase often adds 1 bp to the end of a fragment. This can be prevented by using "pigtailed" primers;

(c) the size of the amplification product;

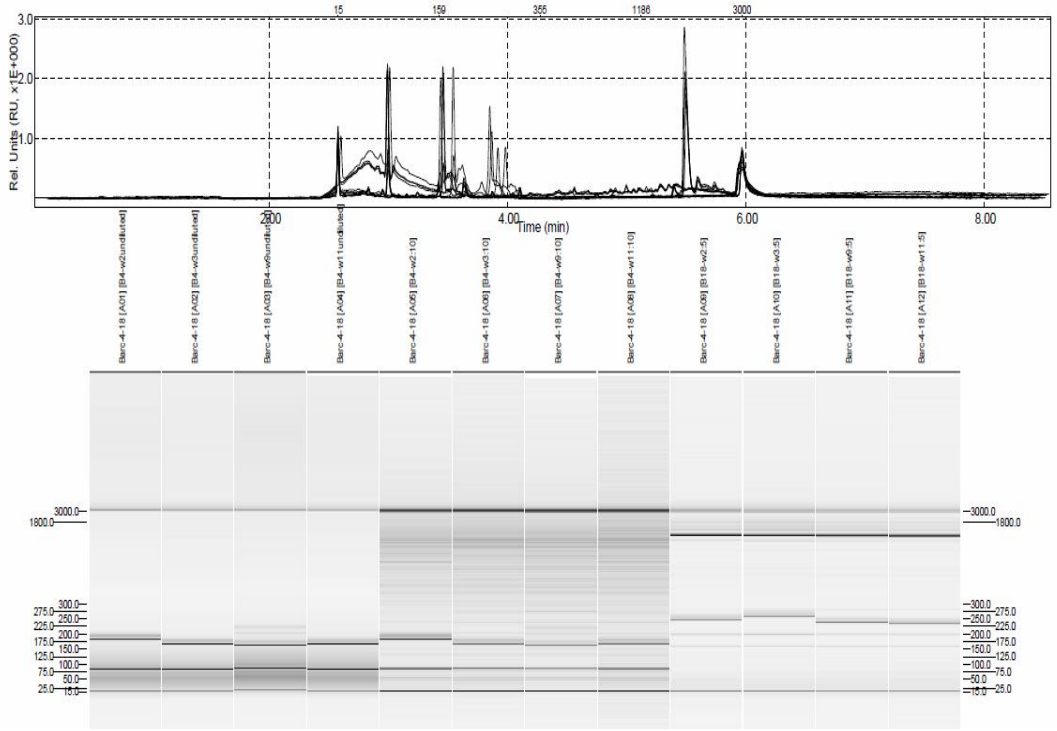
(d) effective separation between the various alleles in suitable detection systems;

(e) reliable and reproducible scoring of the alleles in different detection systems;

(f) the level of polymorphism between varieties (note that this requires analysis of a significant number of varieties);

(g) avoidance of linkage

«...The QIAxcel system —for effortless DNA fragment analysis...»
(www.qiagen.com)



The QIAxcel system provides:

Safety and convenience with ready-to-use gel cartridges

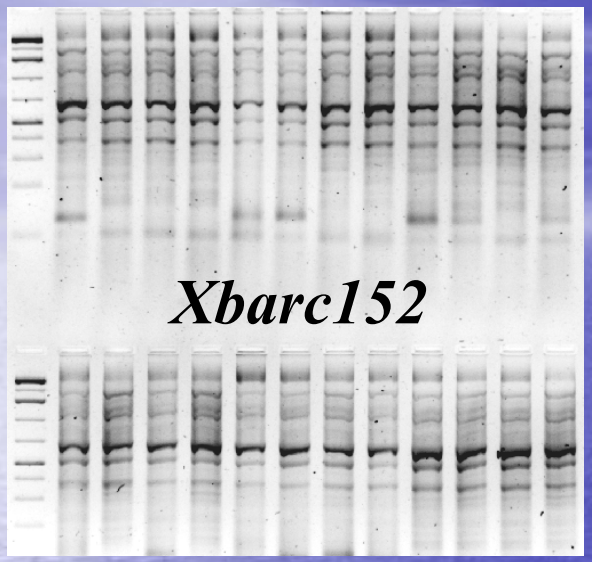
Analysis of up to 96 samples without manual intervention

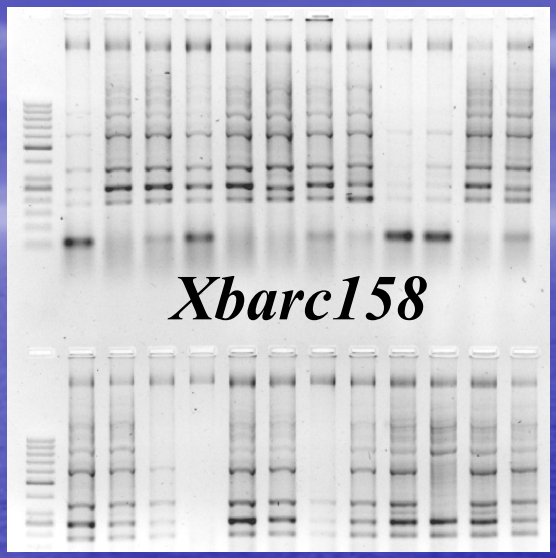
Confident data interpretation with a resolution down to 3–5 bp

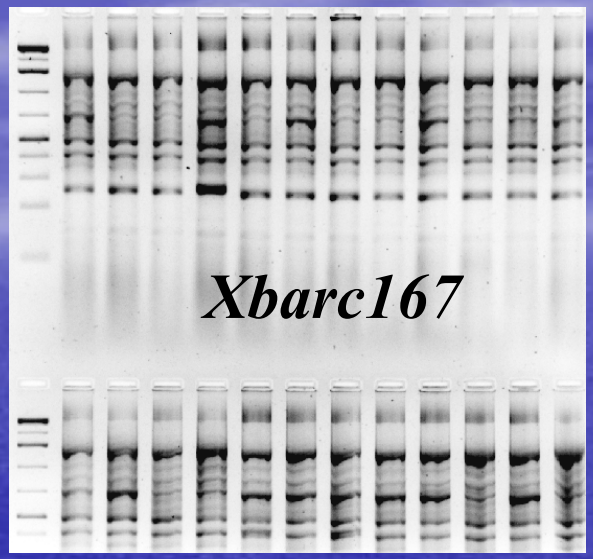
Standardized processing with digital data collection

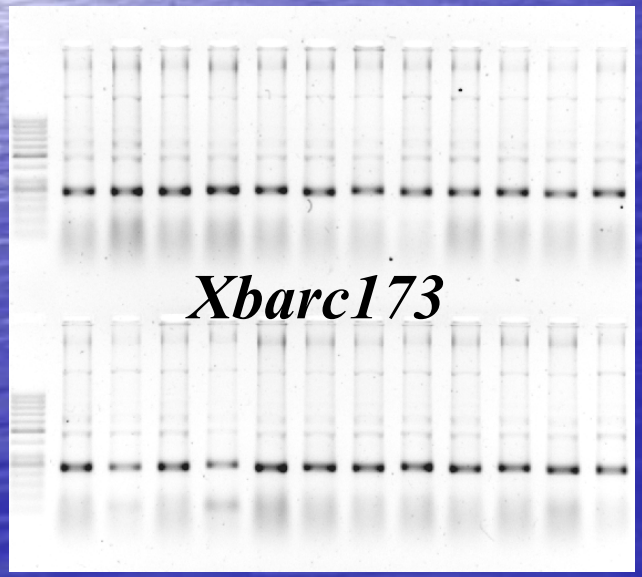
Does not need fluorescent labeling of primers!

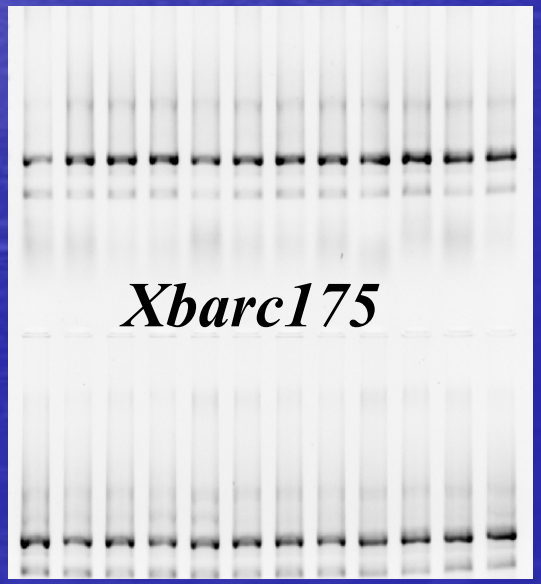
Searching for SSR-marker with no PCR problem

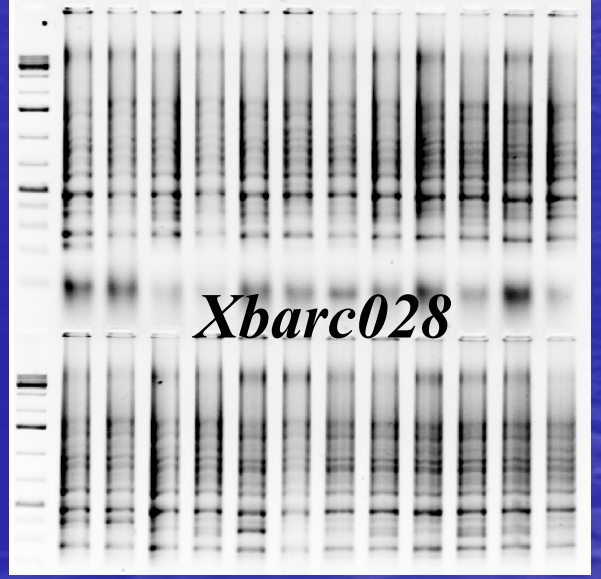




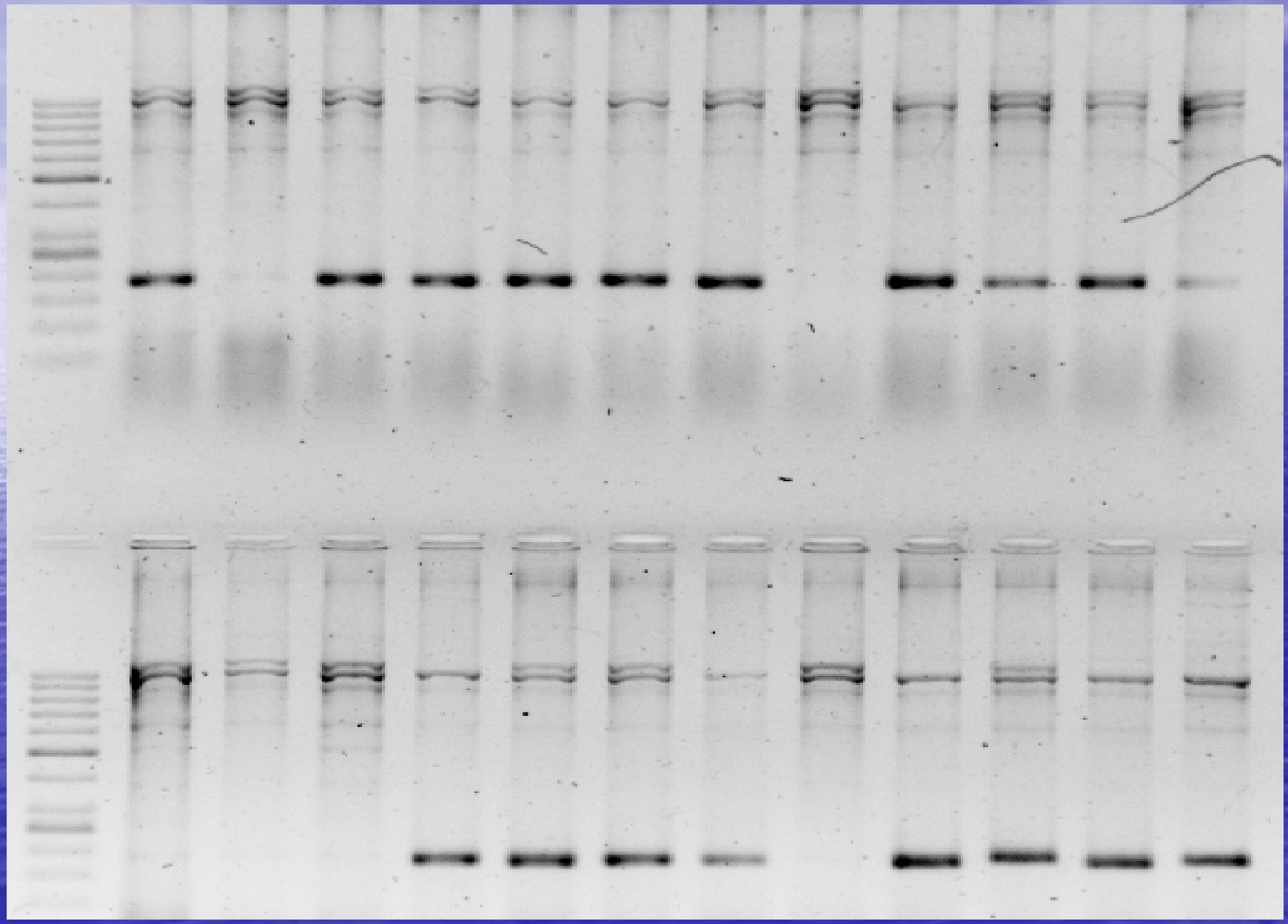




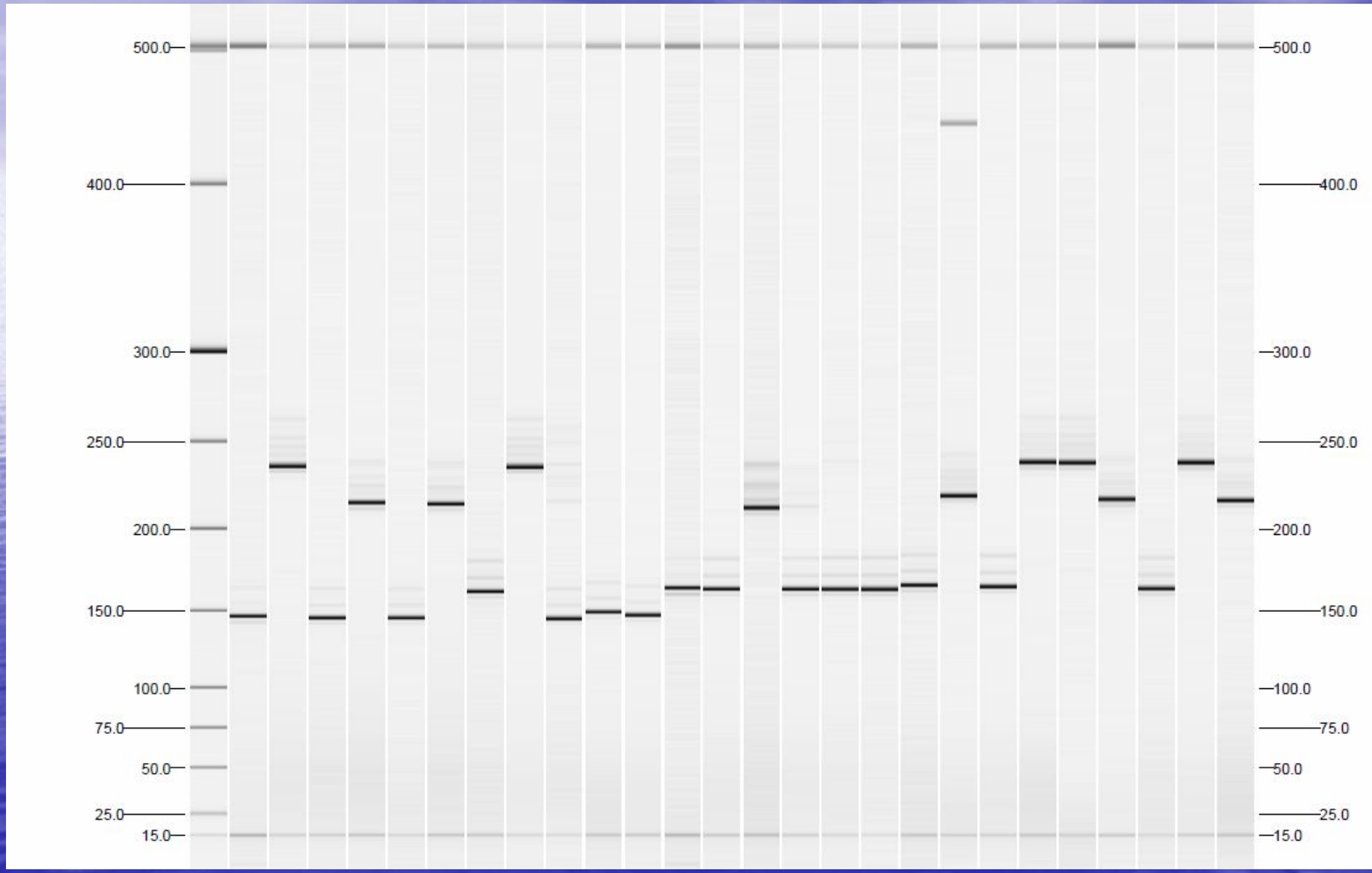




Searching for SSR-marker without null alleles

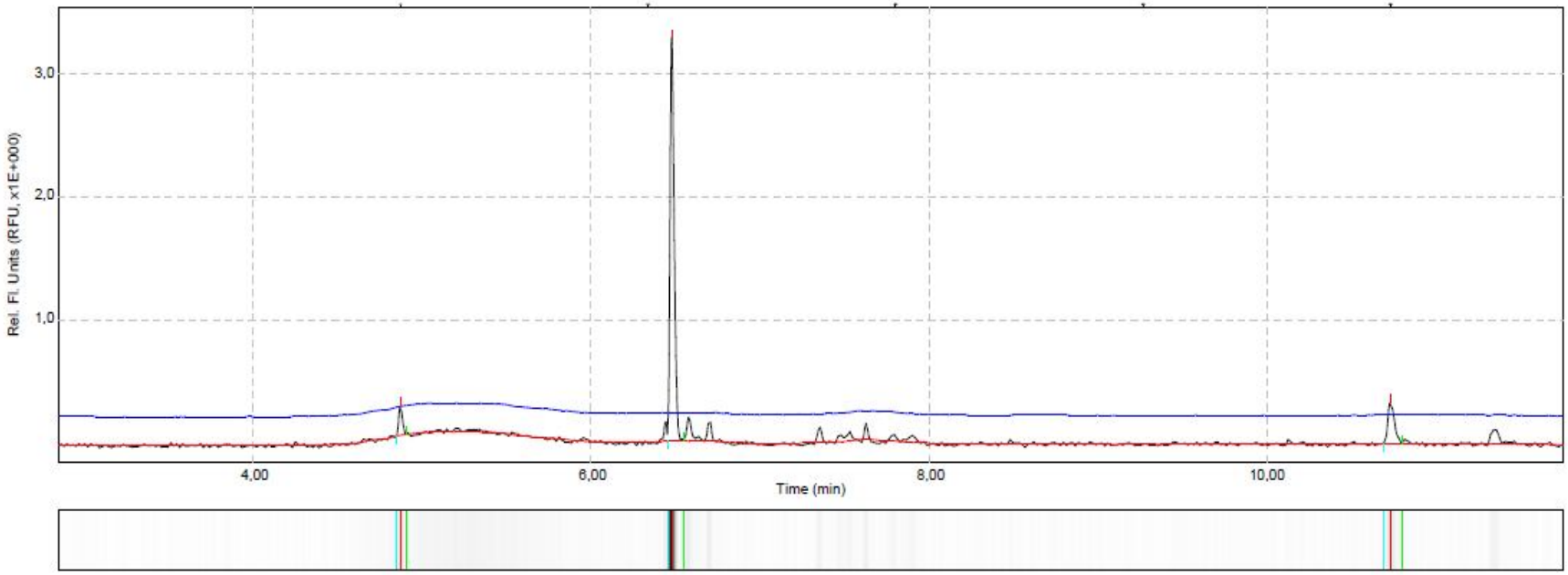


Searching for highly polymorphic SSR-markers



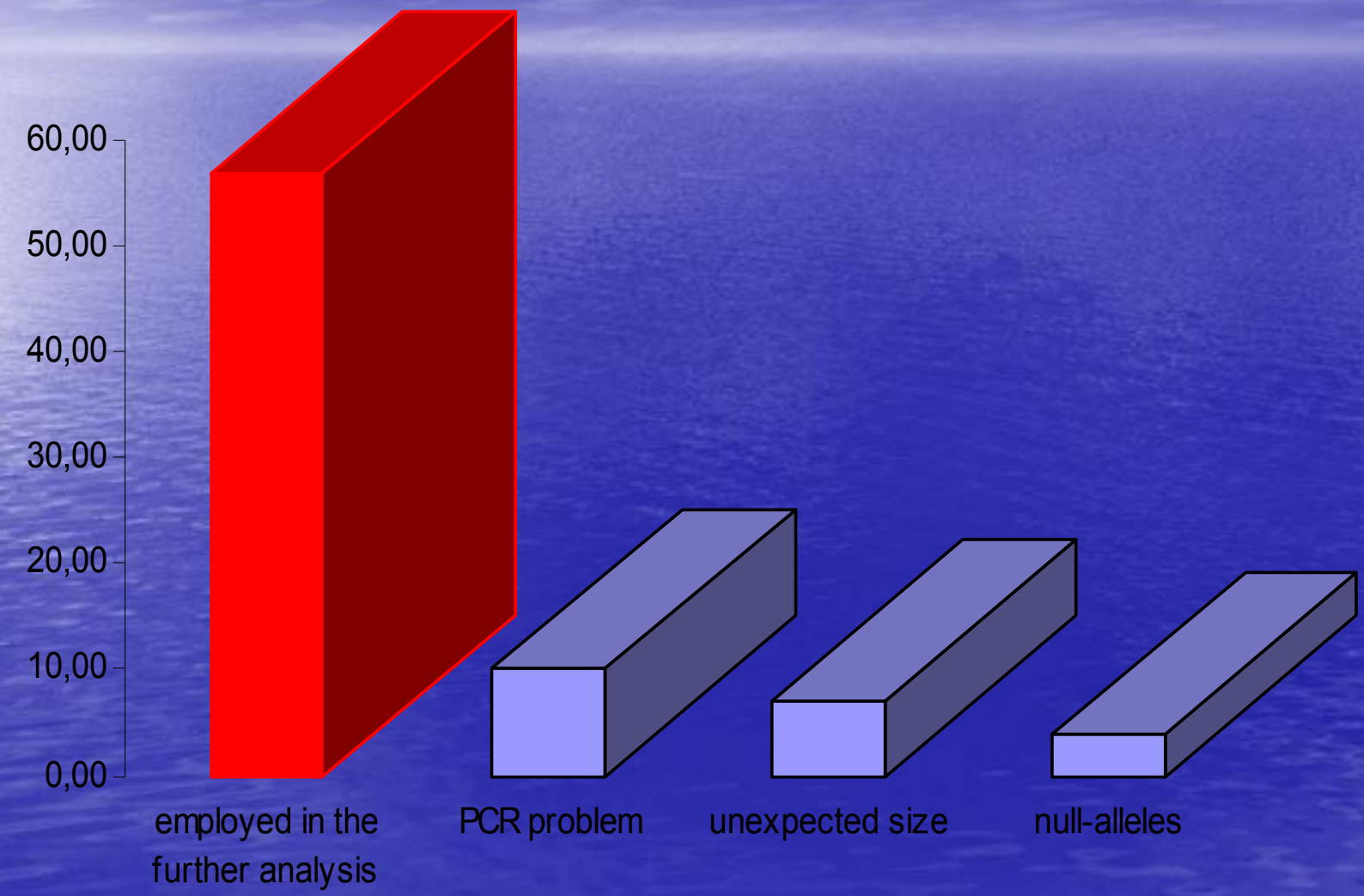
Perfect SSR-marker should be easy to score

Filename: G:\QIAxcelBioCalculator\2012-GK-wheat\040712-barc77\pl1-barc77-run1-A-11.hda 07/04/12
User ID:
Method: C:\Program Files\QIAxcel BioCalculator\Methods\0M700.mtd (Injection time: 10,0 sec)
PlateID:
Cartridge: C120314074 (Cal) (34 runs left)
Sample Info: pl1-barc77-89

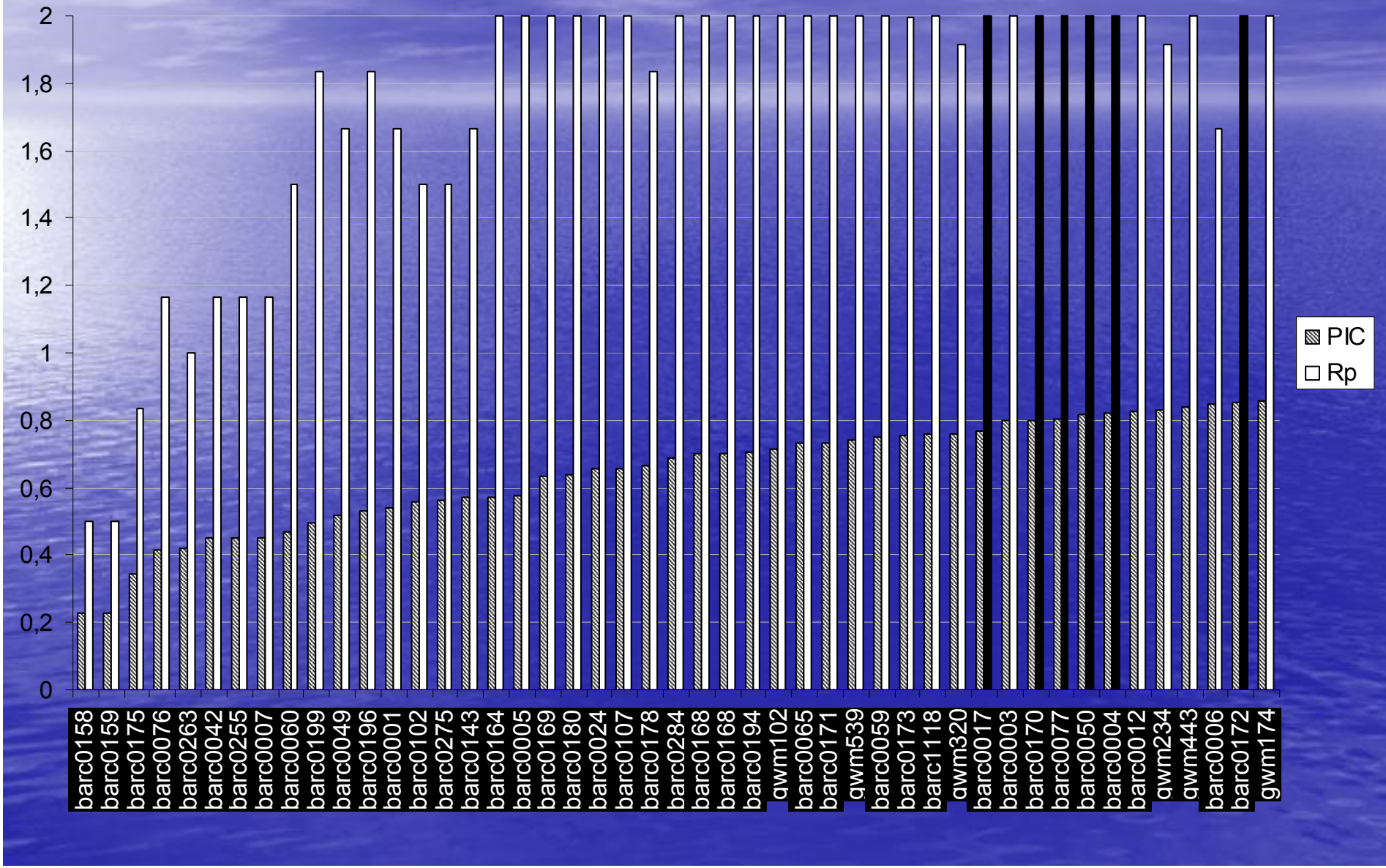


#	name	time	reltime	size (bp)	conc (ng/ul)
1		4,873	0,0000	0,0	0,00
2		6,478	0,2739	144,5	0,00
3		10,733	1,0000	0,0	0,00
Tot				144,5	0,00

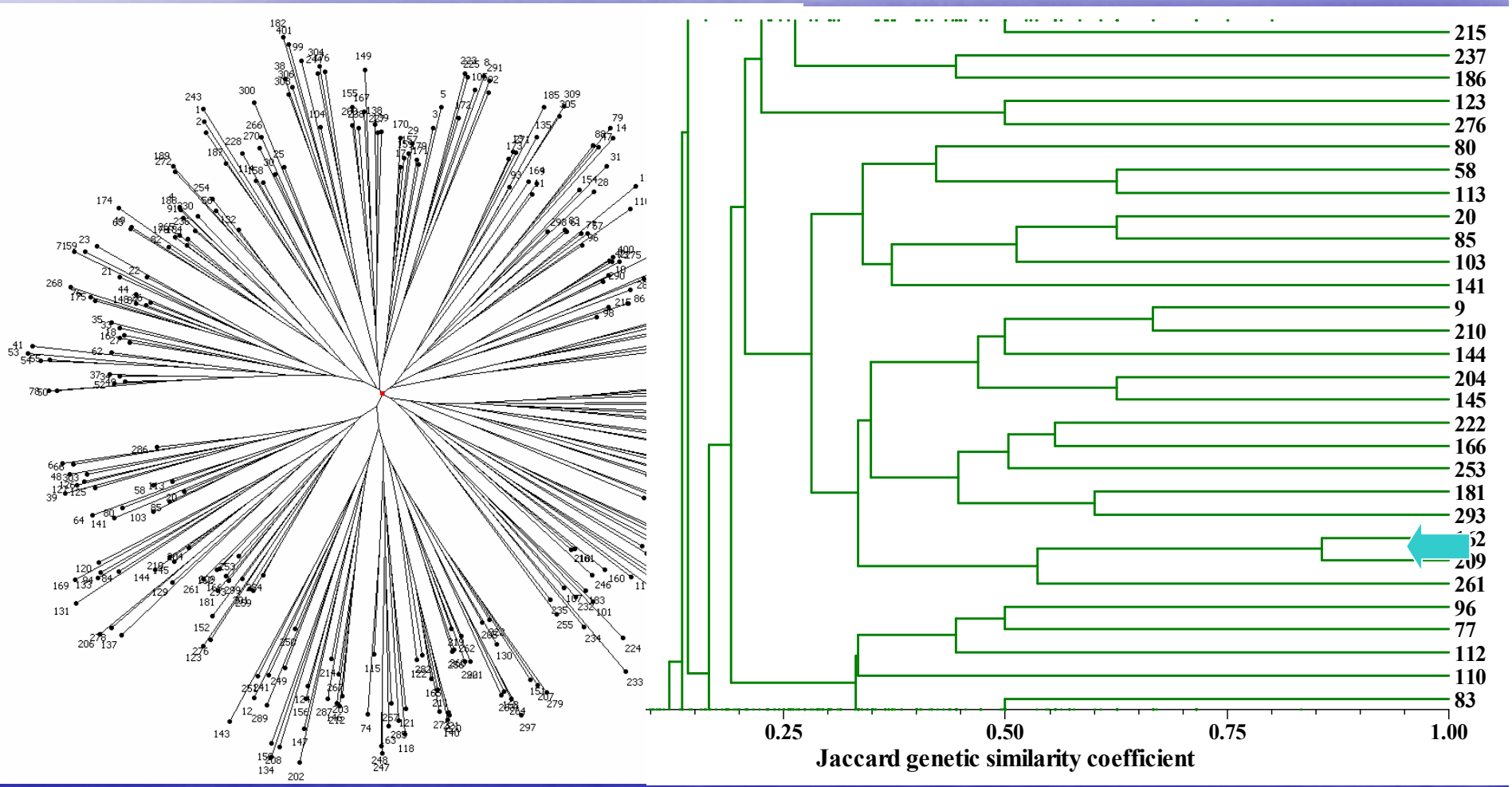
21 SSR-markers were out sorted due to the technical problems
57 SSR-markers were further proceeded



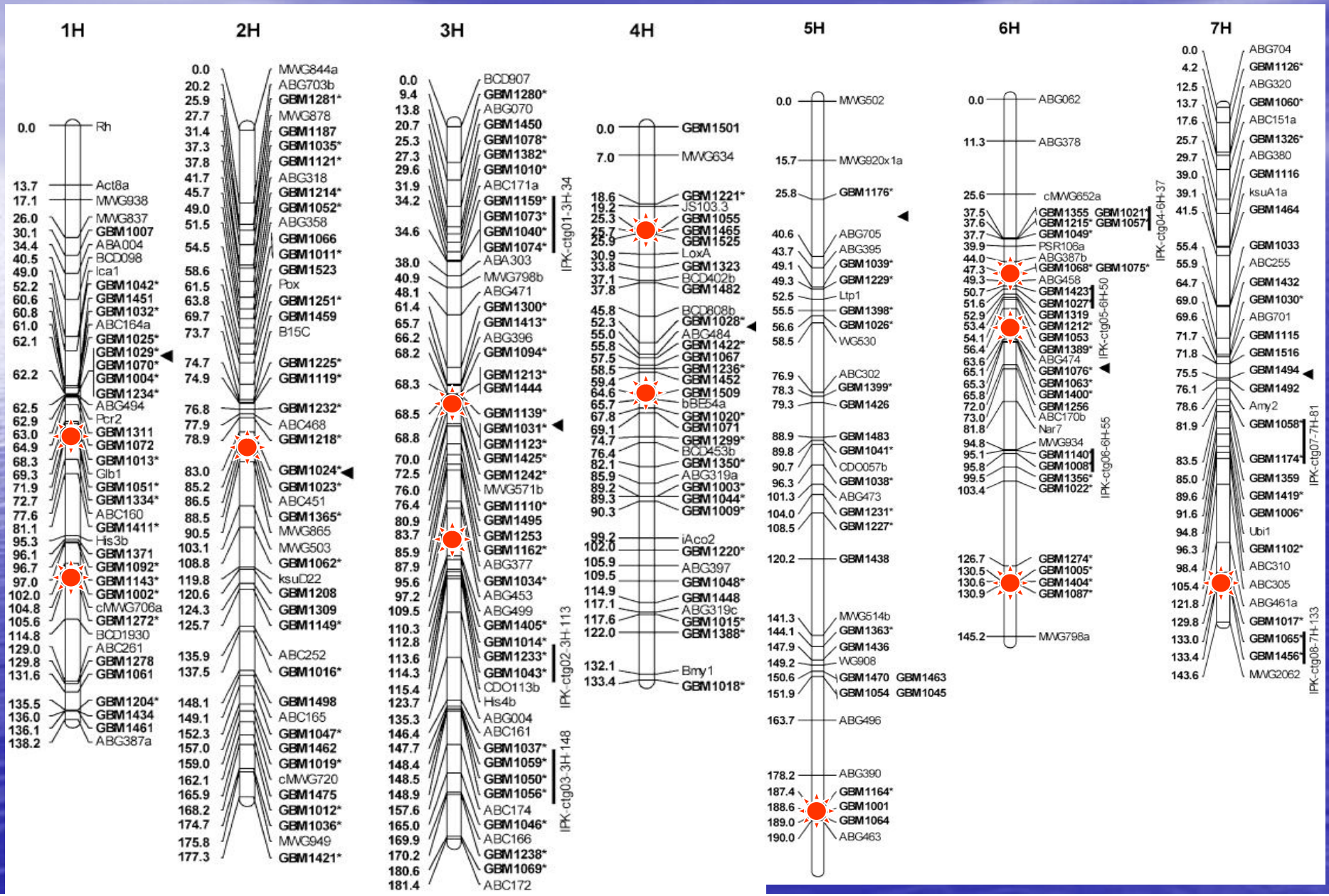
Six selected SSR-markers generated 76 polymorphic alleles among 310 Russian wheat cultivars



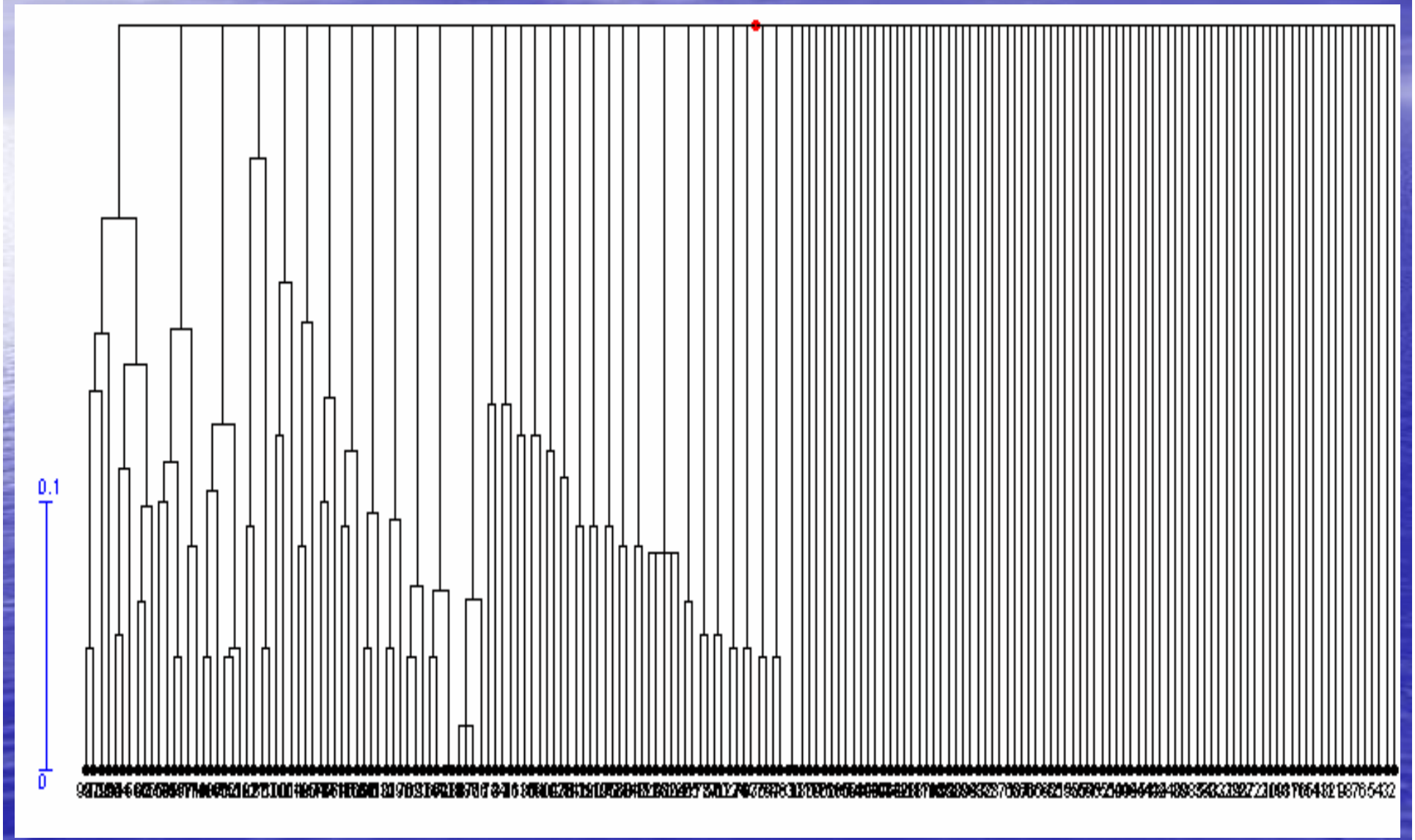
Each of 310 Russian wheat cultivars can be identified by the unique SSR-allele combination of the 6 SSR loci



Pilot experiment: 12 preliminary selected SSR-markers were able to discriminate 180 barley varieties



Pilot experiment: Each of 180 Russian barley varieties from VIR collection can be recognized by the unique SSR-allele combination



The catalogue of 310 Russian wheat cultivars assayed by functional and SSR markers

						Ppd Vrn alleles				SSR genotyping					
Тип развития	№ каталога ВИР	Сорт	Ботаническая разновидность	Происхождение	Репродукция	Ppd-D1	Vrn-B1	Vrn-D1	Vrn-B3	Barc 1	Barc 2	Barc 3	Barc 4	Barc 5	Barc 6
оз.	63002	Тау	erythrospermum	Россия, Владимирская обл.	Ориг 2005	R	R	R	R	287	119	231 237	178	241	188
оз.	64491	Авеста	lutescens	Россия, Ростовская обл.	Ориг 2005	D	R	R	R	281	119 131	231 237	178	151 166	188
оз.	64492	Агра	lutescens	Россия, Ростовская обл.	Ориг 2005	D	R	R	R	281	116	234 237	172	151 220	185
оз.	64914	Айвина	lutescens	Россия, Краснодарский край	Ориг 2008	D	R	R	R	281	119	234	175	241	185
оз.	65077	Ариадна	lutescens	Россия, Белгородская обл.	Ориг 2009	D	R	R	R	278	131	237	166	151 220	185

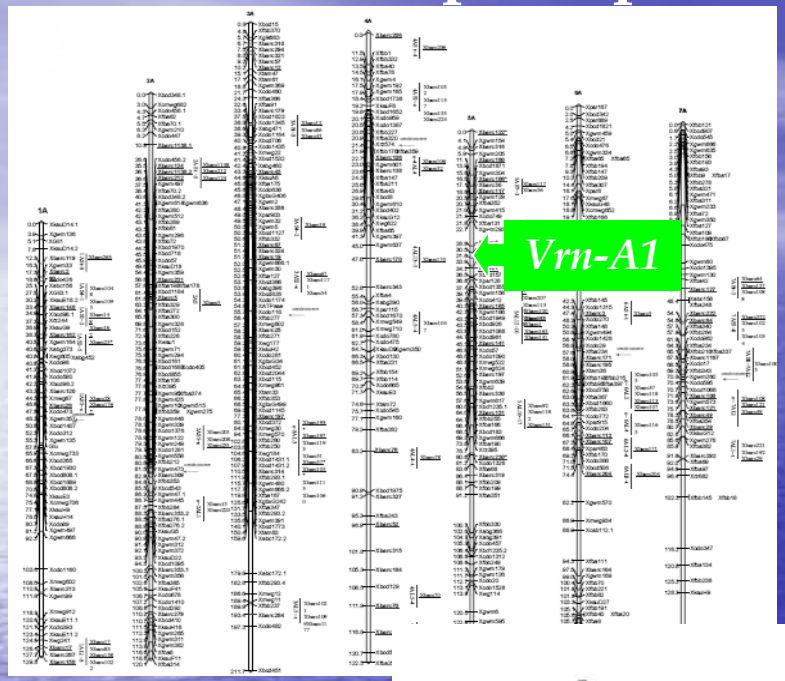
Heading date of wheat and barley cultivars

25% - *Ppd*
Photoperiod response

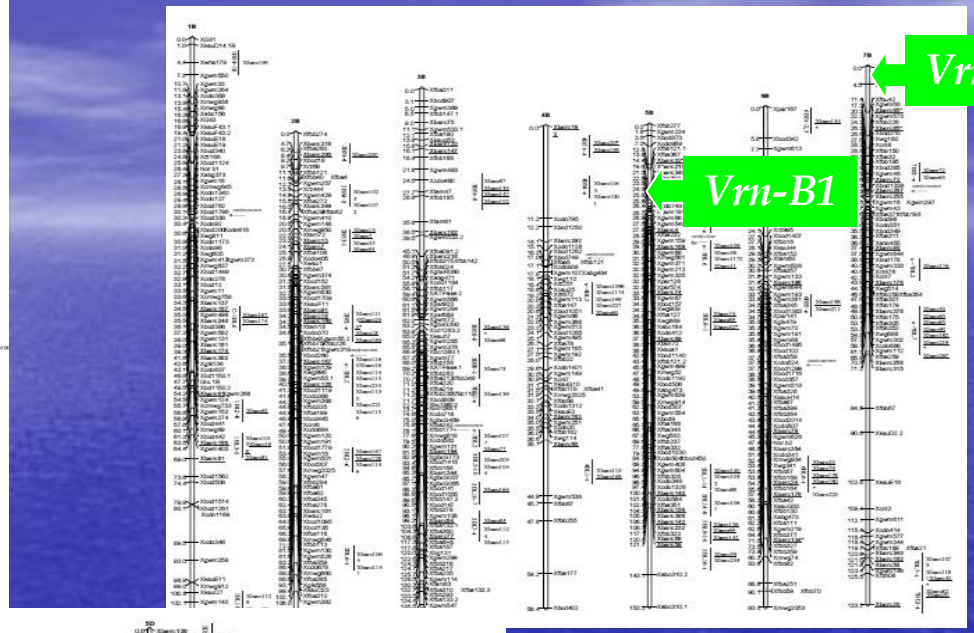
70% - *Vrn*
Vernalisation response

5% - *eps*
Earliness per se

PCR assay available for vernalisation response (*Vrn*) and photoperiod sensitivity (*Ppd*) genes

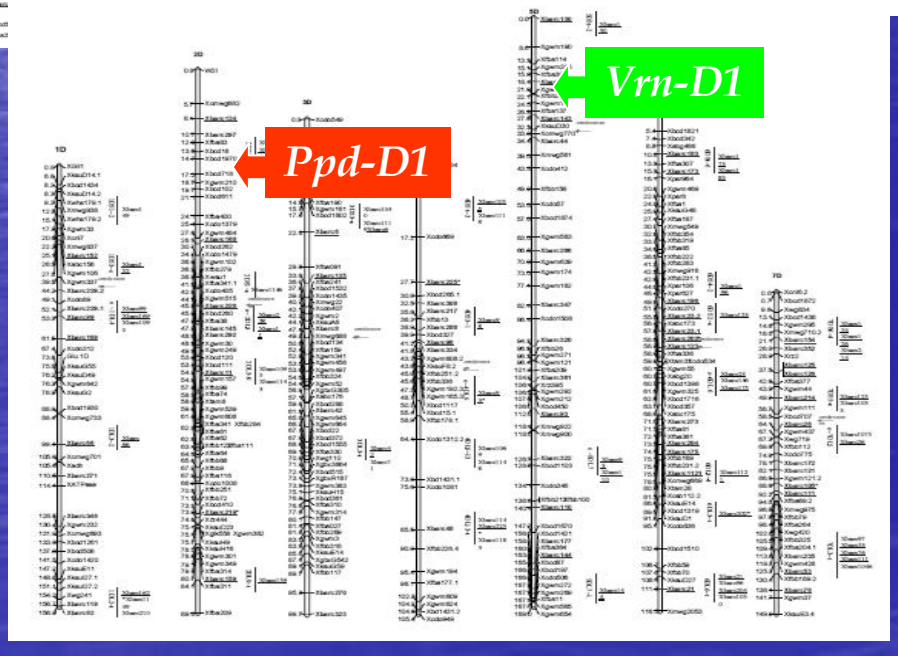


Vrn-A1



Vrn-B3

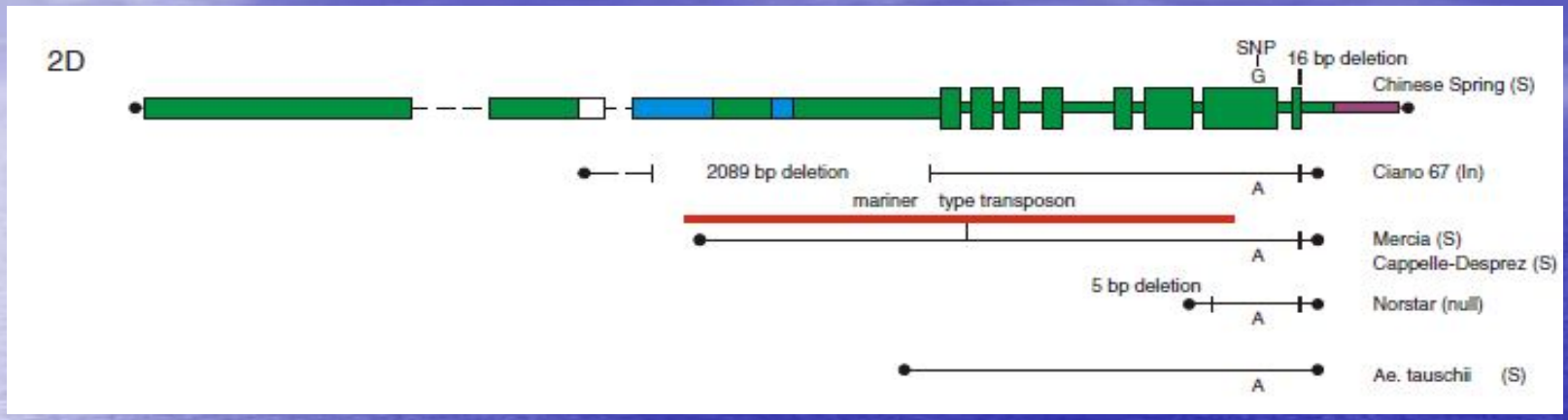
Vrn-B1



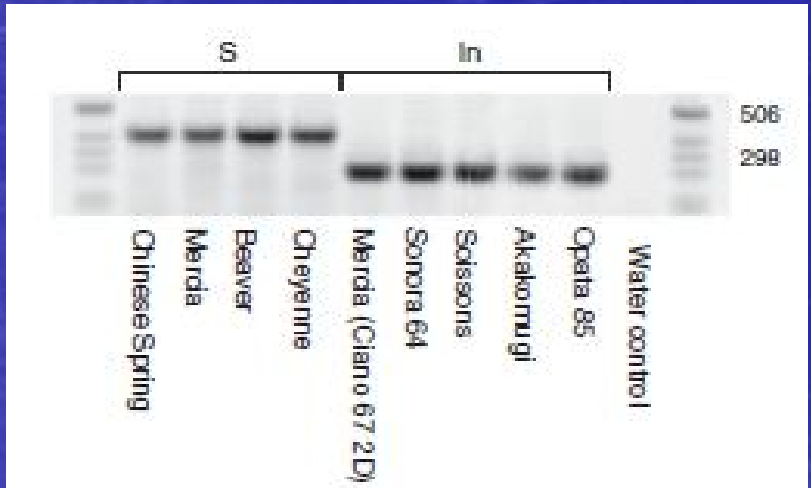
Vrn-D1

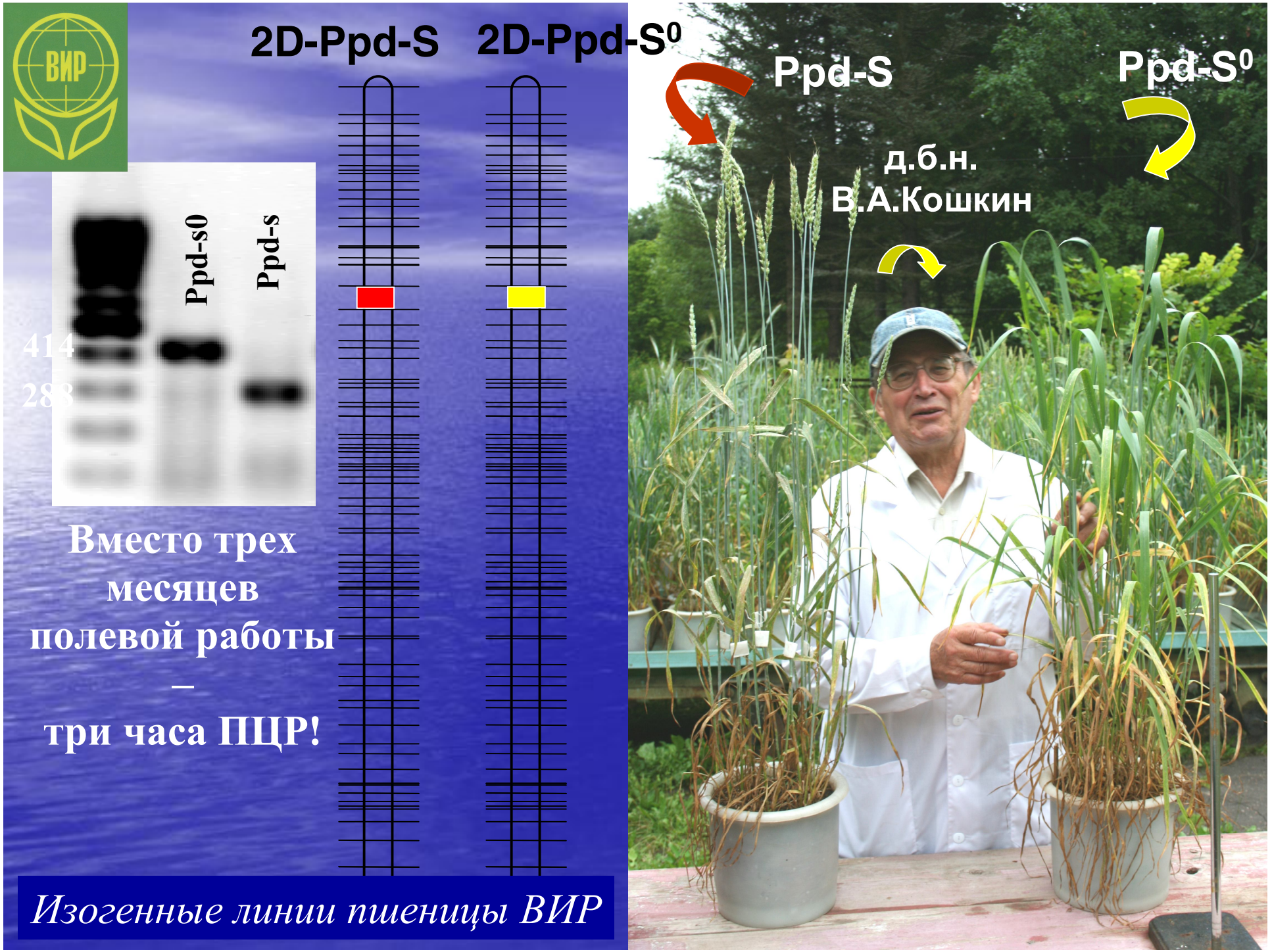
Ppd-D1

Photoperiod insensitive allele of *Ppd-D1* has been identified on chromosome 2D in wheat (*Beales et al., 2007*)



The insensitive allele can be detected by allele-specific PCR-marker

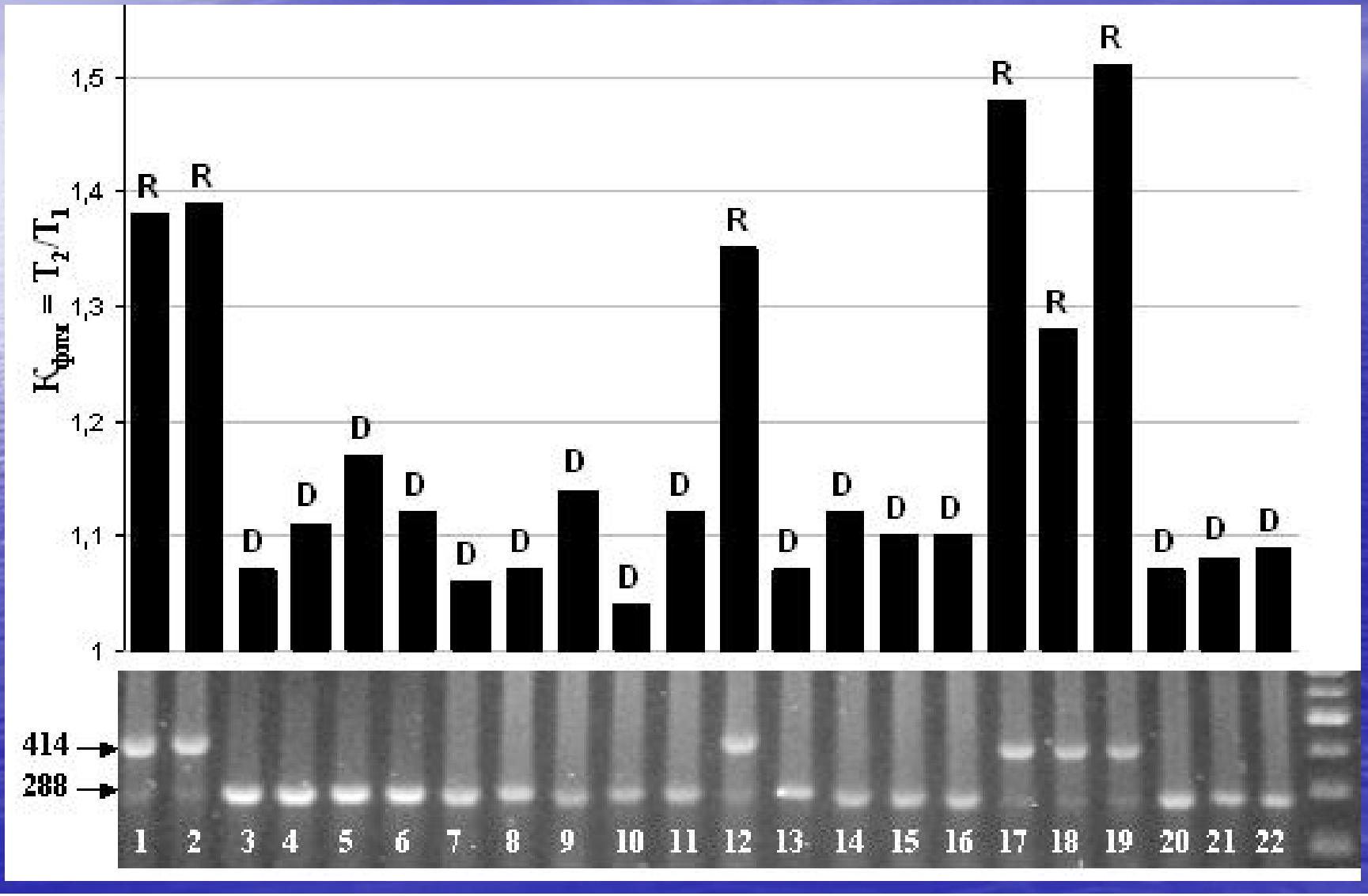



2D-Ppd-S
2D-Ppd-S⁰
Ppd-s0
Ppd-s
414
288
Вместо трех
месяцев
полевой работы
–
три часа ПЦР!
Ppd-S
Ppd-S⁰
д.б.н.
В.А.Кошкин
Изогенные линии пшеницы ВИР

Photoperiod sensitivity and molecular marking of Ppd and Vrn genes for wheat breeding lines of Krasnodar region

№ по п/п	Название	Происхождение	Вариант	Всходы-колошение, сут		$T_1 - T_2$	$К_{ФПЧ}$
				Длинный день T_1	Короткий день T_2		
1	Ласточка озимая	Краснодарский НИИСХ	к	61,1±1,58	94,0±3,29	32,9	1,54
			я	39,5±0,27	54,6±0,34	15,1	1,38
2	Ласточка яровая	«	к	56,9±1,38	93,3±4,22	36,4	1,64
			я	39,9±0,47	55,4±0,34	15,5	1,39
3	Афина озимая	«	к	59,2±0,81	71,3±1,14	12,1	1,20
			я	**42,5±0,27**	**45,5±0,17**	**3,0**	**1,07**
4	Афина яровая	«	к	61,2±1,14	71,1±0,71	9,9	1,16
			я	**42,6±0,42**	**47,1±0,35**	**4,5**	**1,11**
5	Паллада озимая	«	к	59,6±0,54	69,0±0,42	9,4	1,16
			я	**42,7±0,15**	**49,9±0,73**	**7,2**	**1,17**
6	Паллада яровая	«	к	61,3±0,45	70,9±0,23	9,6	1,16
			я	**42,0±0,26**	**47,0±0,29**	**5,0**	**1,12**
7	Л 1120я1633-7	«	к	54,8±0,76	74,1±1,04	19,3	1,35
			я	**44,1±0,79**	**46,8±0,16**	**2,7**	**1,06**
8	Л 1120я16к7	«	к	66,2±0,65	76,7±0,65	10,5	1,16
			я	**43,5±0,56**	**46,7±0,42**	**3,2**	**1,07**
9	Л 1120я16к27	«	к	65,1±0,59	74,2±1,04	9,1	1,14
			я	41,9±0,48	47,9±0,62	6,0	1,14
10	Л 1120я16к12	«	к	кущение*	кущение*	-	-

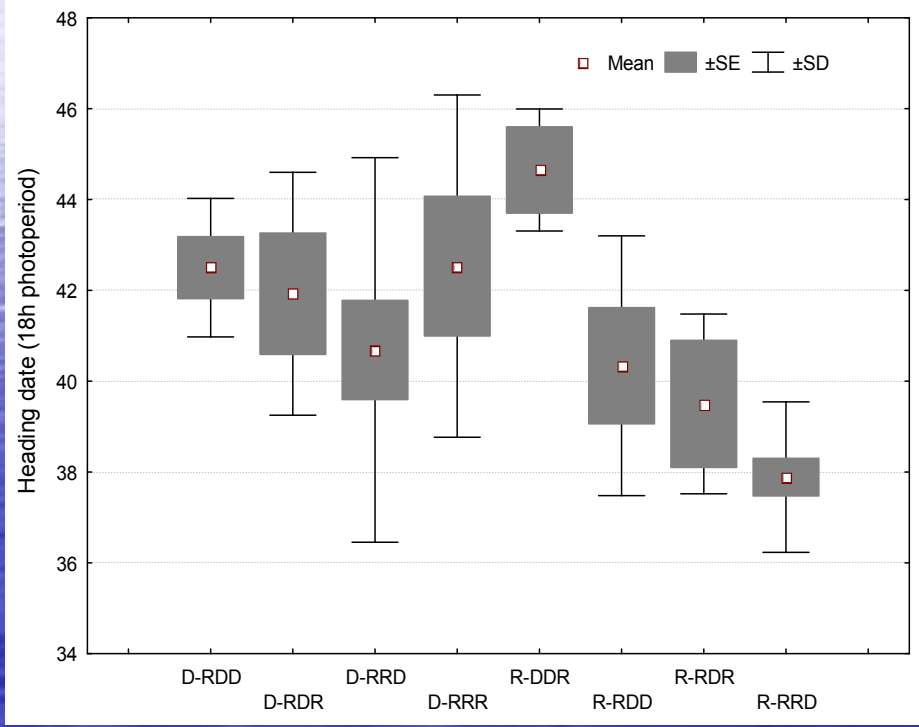
Photoperiod sensitivity and molecular marking of Ppd and Vrn genes for wheat breeding lines of Krasnodar region



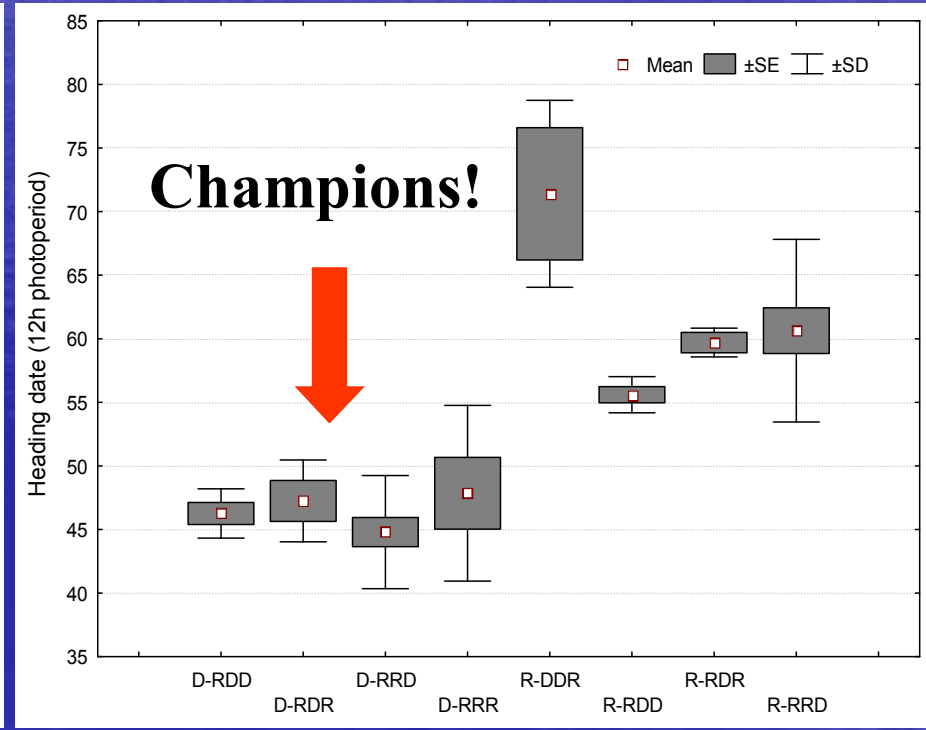
We compared heading date of 57 wheat cultivars carrying different allele combinations of Vrn и Ppd:

D-RDD means dominant/recessive alleles for (*Ppd-D1*)-(*vrn-A1*)-(*Vrn-B1*)-(*Vrn-D1*)

Day length 18h (St.Petersburg)



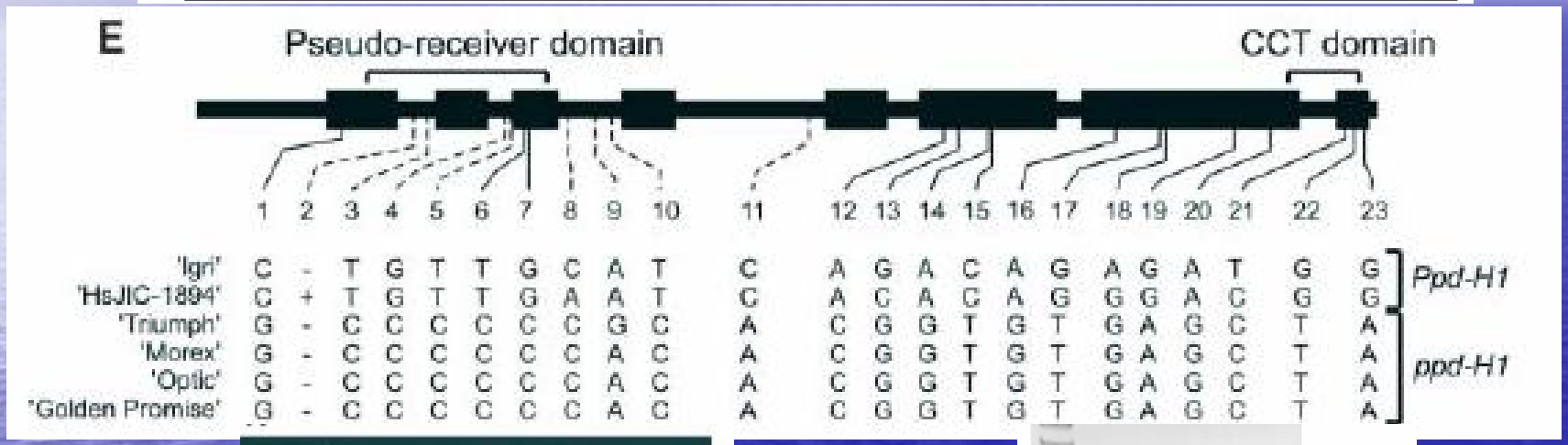
Day length 18h (Krasnodar)

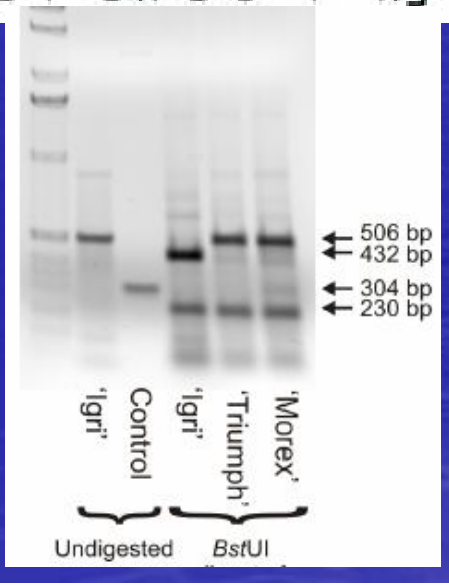


Assessment of barley germplasm collection with functional molecular markers

Science
AAAS

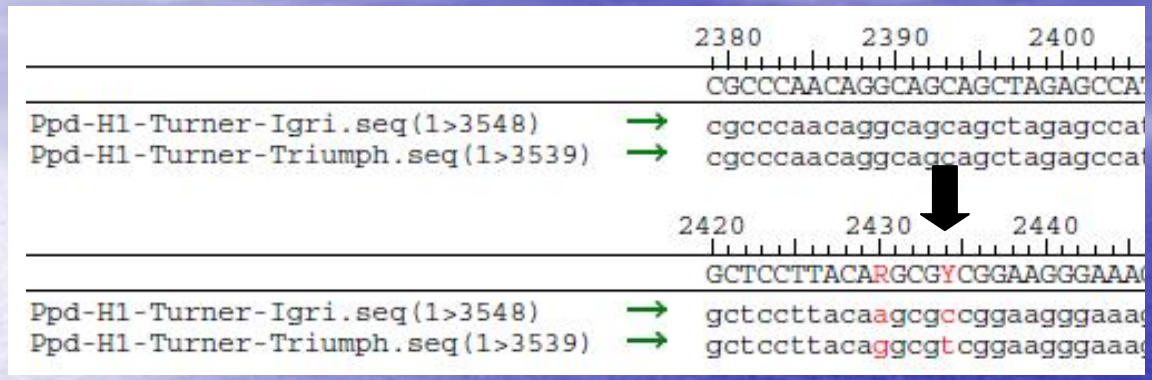
The Pseudo-Response Regulator Ppd-H1 Provides Adaptation to Photoperiod in Barley
Adrian Turner, *et al.*
Science **310**, 1031 (2005);
DOI: 10.1126/science.1117619

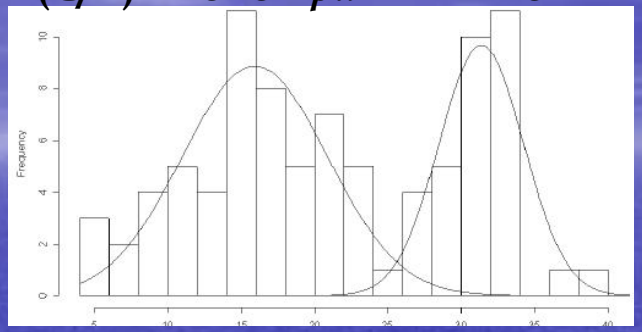




CAPS-marker to identify Ppd-H1 alleles in barley germplasm

Jones et al. (2008) идентифицировали «ключевой» SNP (C/T) в гене *Ppd-H1* ячменя

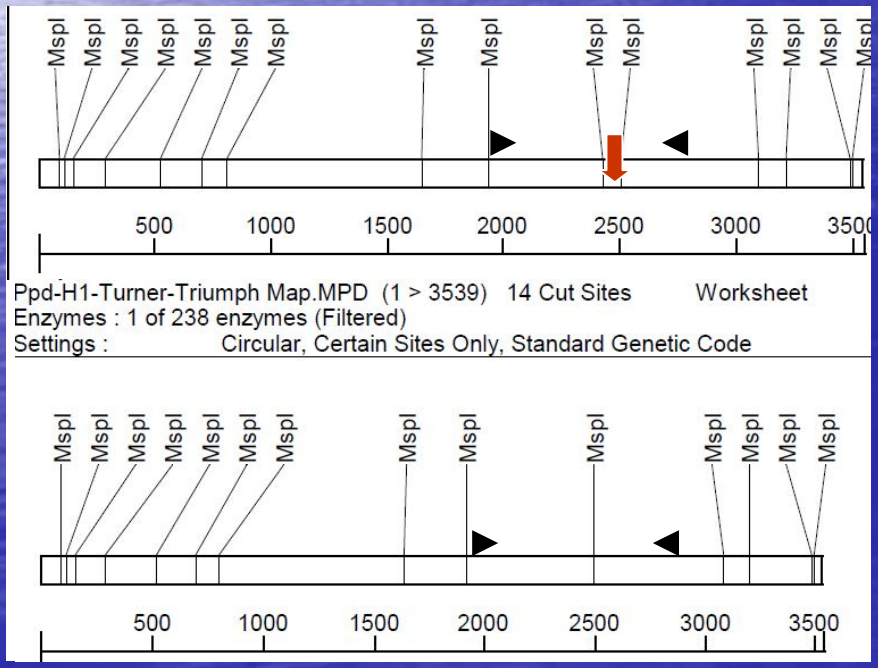




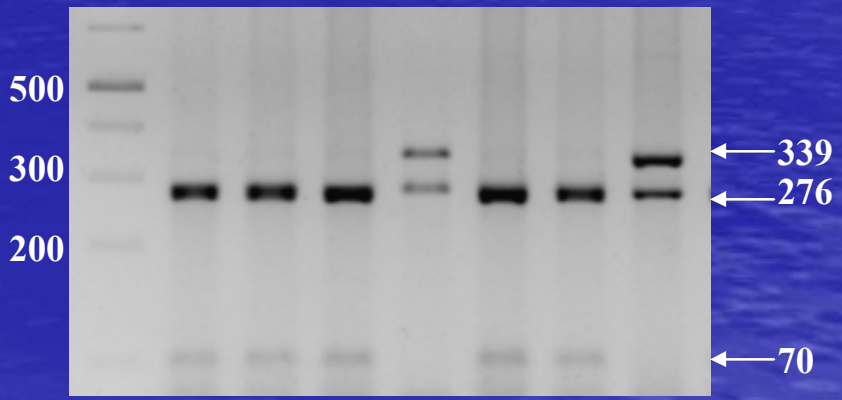
всходы-колошение

«C» Ppd-H1 **«T» ppd-H1**

SNP (C/T) является «мишенью» для рестриктазы MspI

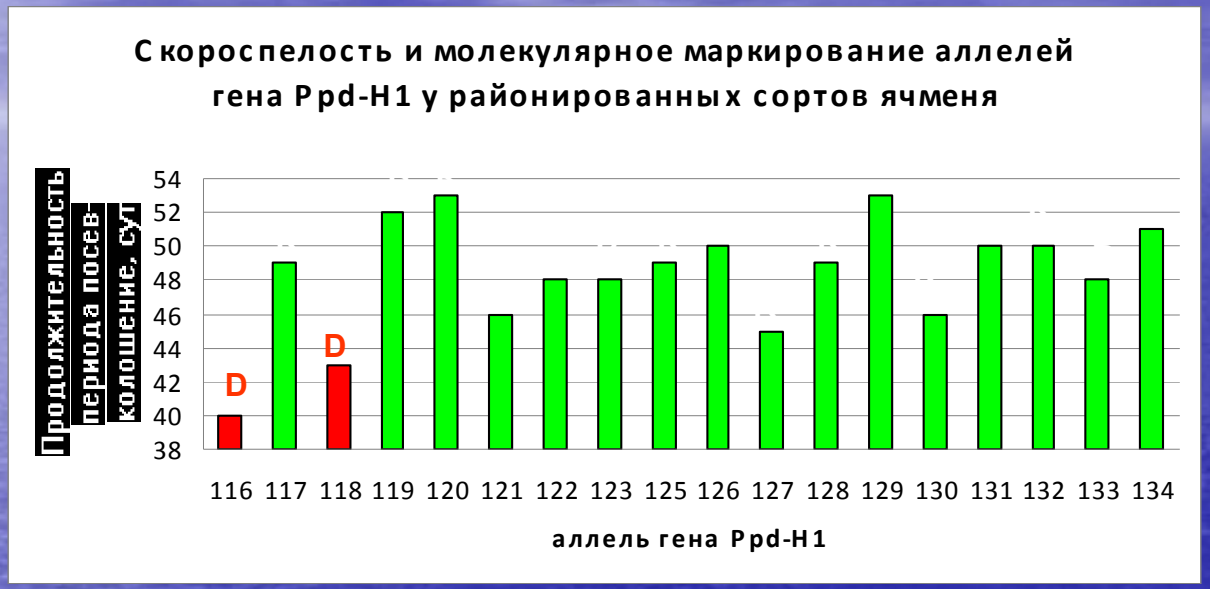


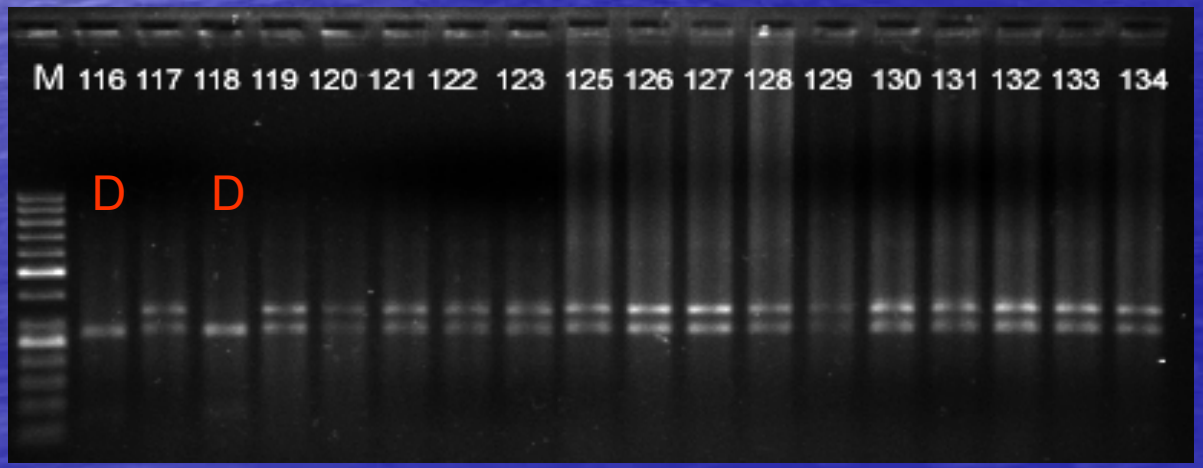
The CAPS-marker easily distinguished alleles Ppd-H1 и ppd-H1 in Russian barley germplasm



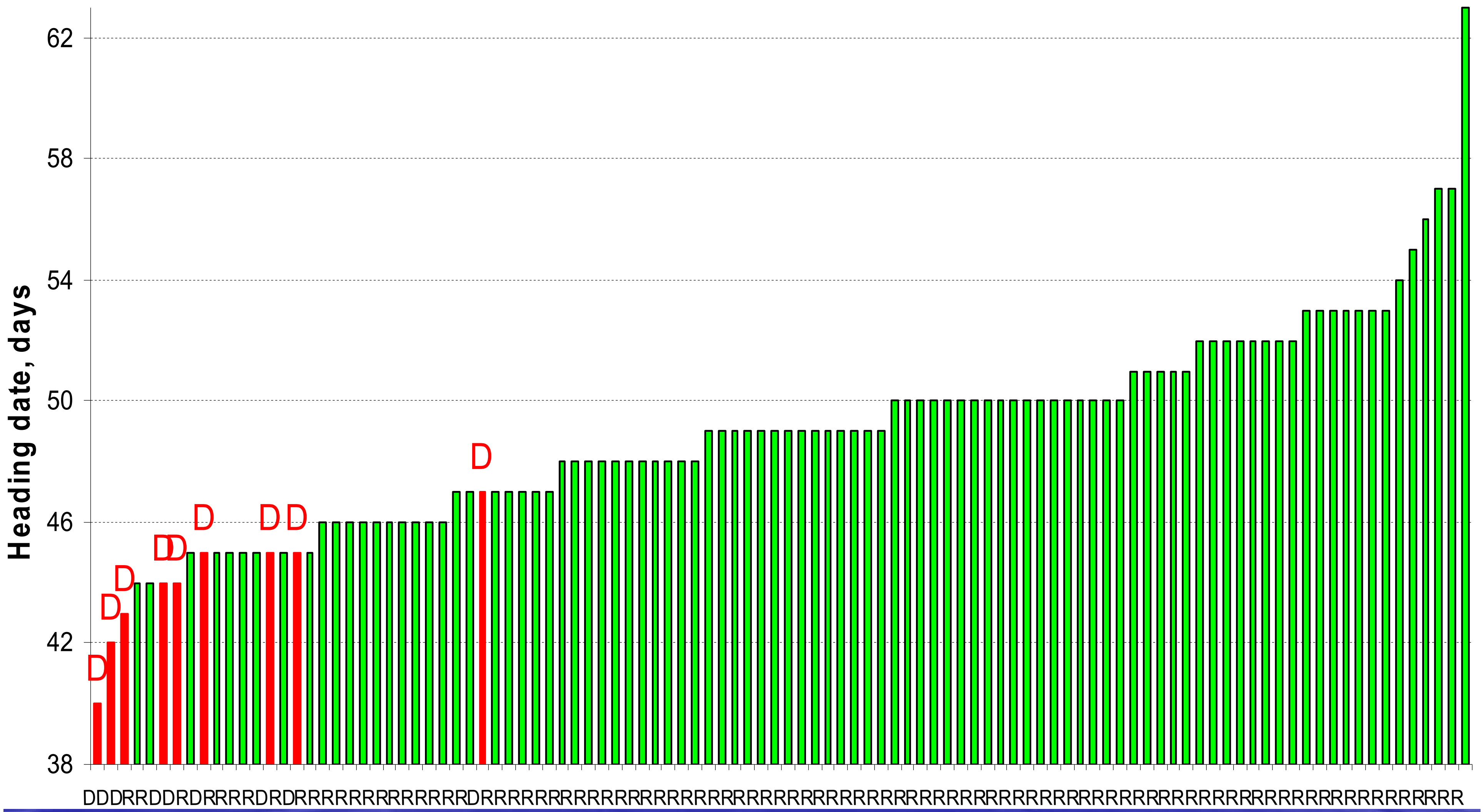
лаборатория мониторинга генетической эрозии

We screened Russian barley varieties with the CAPS marker end estimated heading date

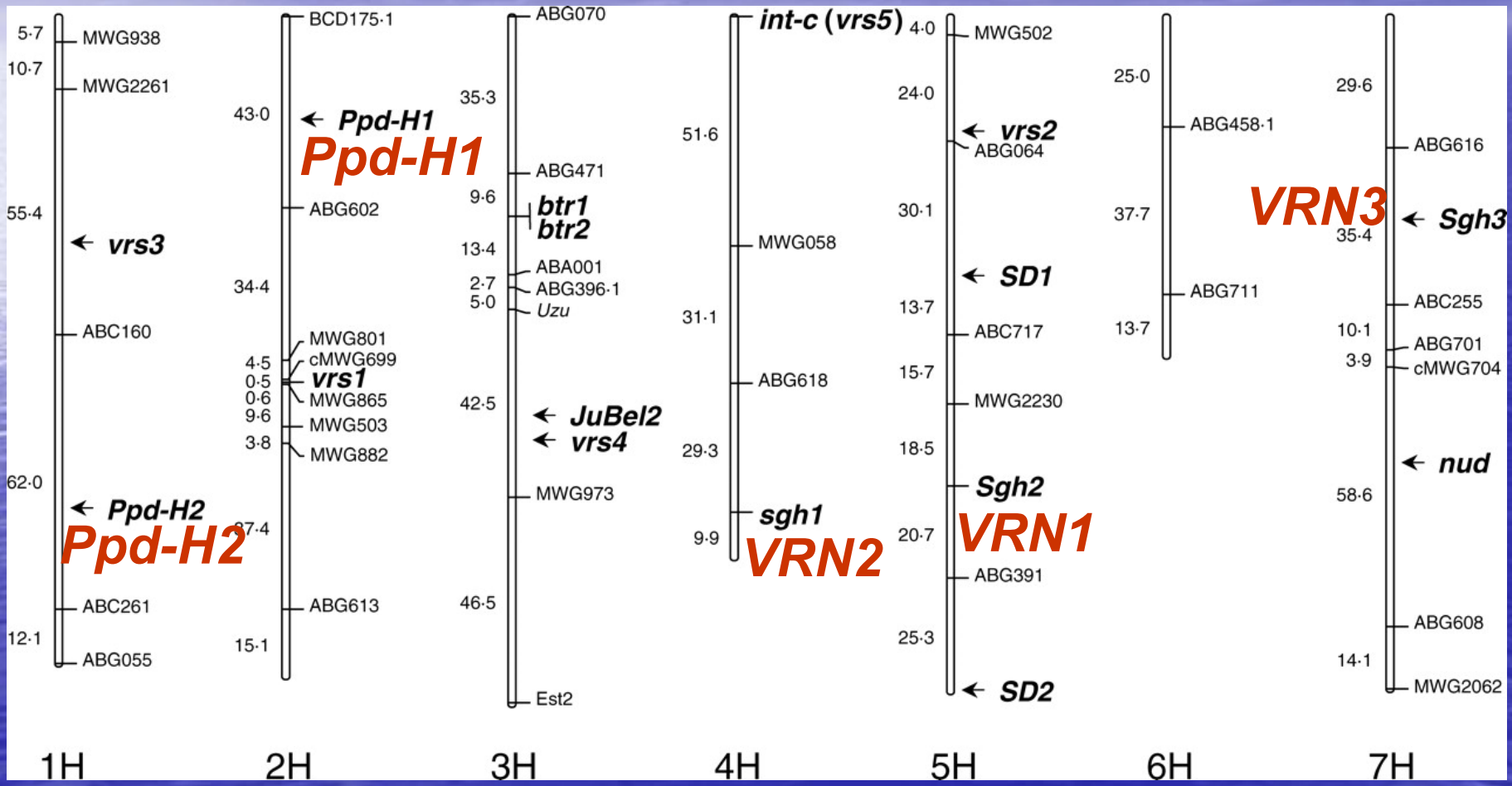




Barley varieties that carry dominant (D) Ppd-H1 allele transit to flowering much faster (St.Petersburg, 2013)

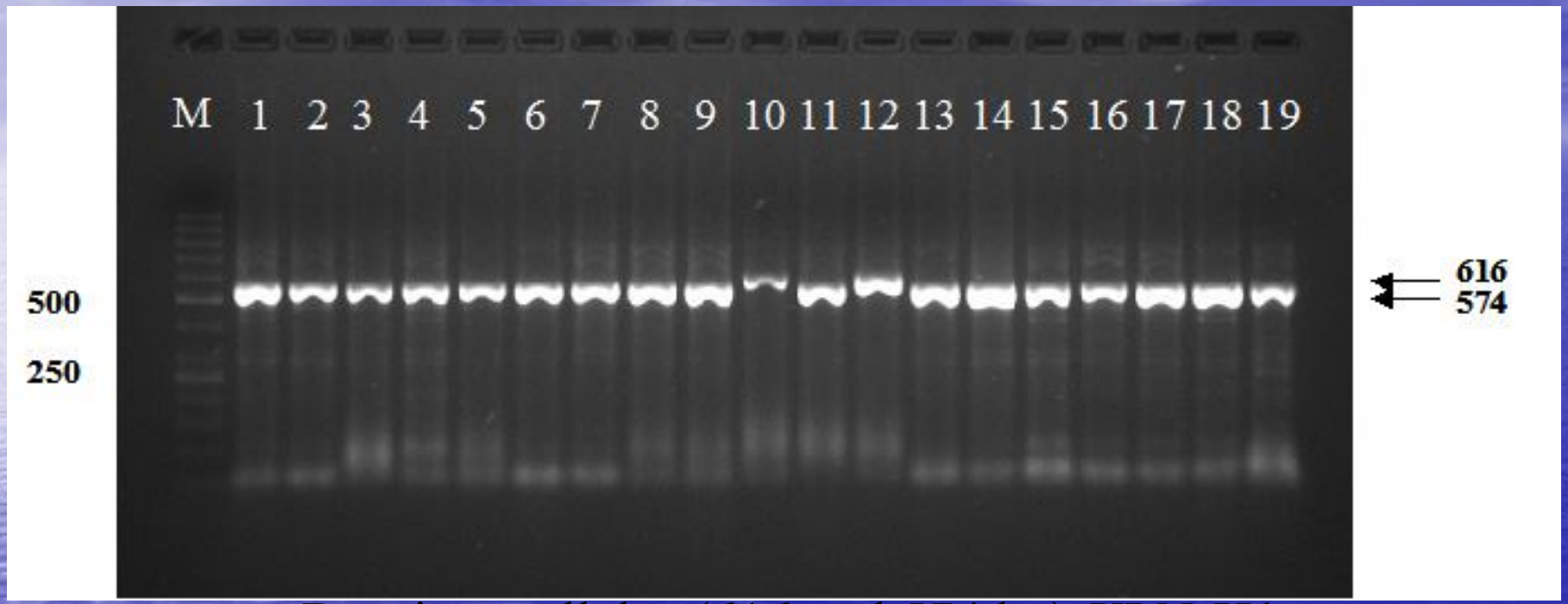


Allele diversity of *Ppd* (photoperiod response) and *VRN* (vernalization response) affect Headind Date in barley



Consensus map of barley domestication-related genes, **Pourkheirandish, M. *et al.* Ann Bot 2007**

Allele-specific PCR to identify *Vrn-H1* alleles in barley varieties propagated in Russia

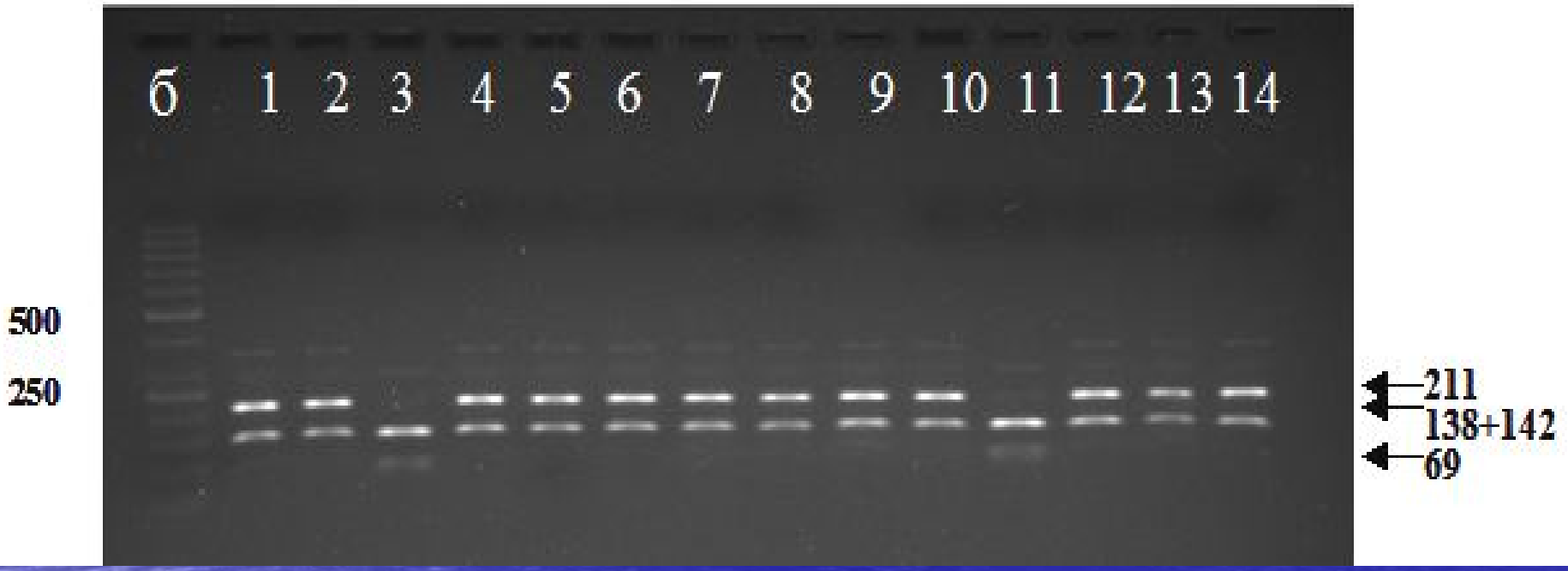


Dominant alleles (616 and 574 bp) *VRN-H1*

Сорта: 1-Прерия, 2- Харьковский 99, 3-Биос-1, 4-Волгарь, 5-Джин, 6-Ача, 7- Соболёк, 8-Skarlett, 9- Сонет, 10- Зерноградец 770, 11-Задонский 8, 12-Рахат, 13-Раушан, 14-Мик-1, 15- Зерноградский, 16-Приазовский 9, 17-Челябинский 99, 18-Безенчукский 2

Zlotina M.M., et al, (2013). Russian Journal of Genetics: Applied Research, Vol. 3, No. 4, pp. 254–264.

CAPS marker to identify *Vrn-H3* alleles in Russian barley varieties



Dominant (138+142+69 bp) and recessive (211+138 bp) alleles *Vrn-H3*
Сорта: 1- Владимир, 2- Велес, 3- Биом, 4-Бином, 5-Ястреб, 6-Т-12, 7-Жозефин, 8- Таловский 9, 9-Марни, 10-Прикумский 47, 11-Щедрый, 12-Родник, 13-Тимерхан, 14-Саншайн.

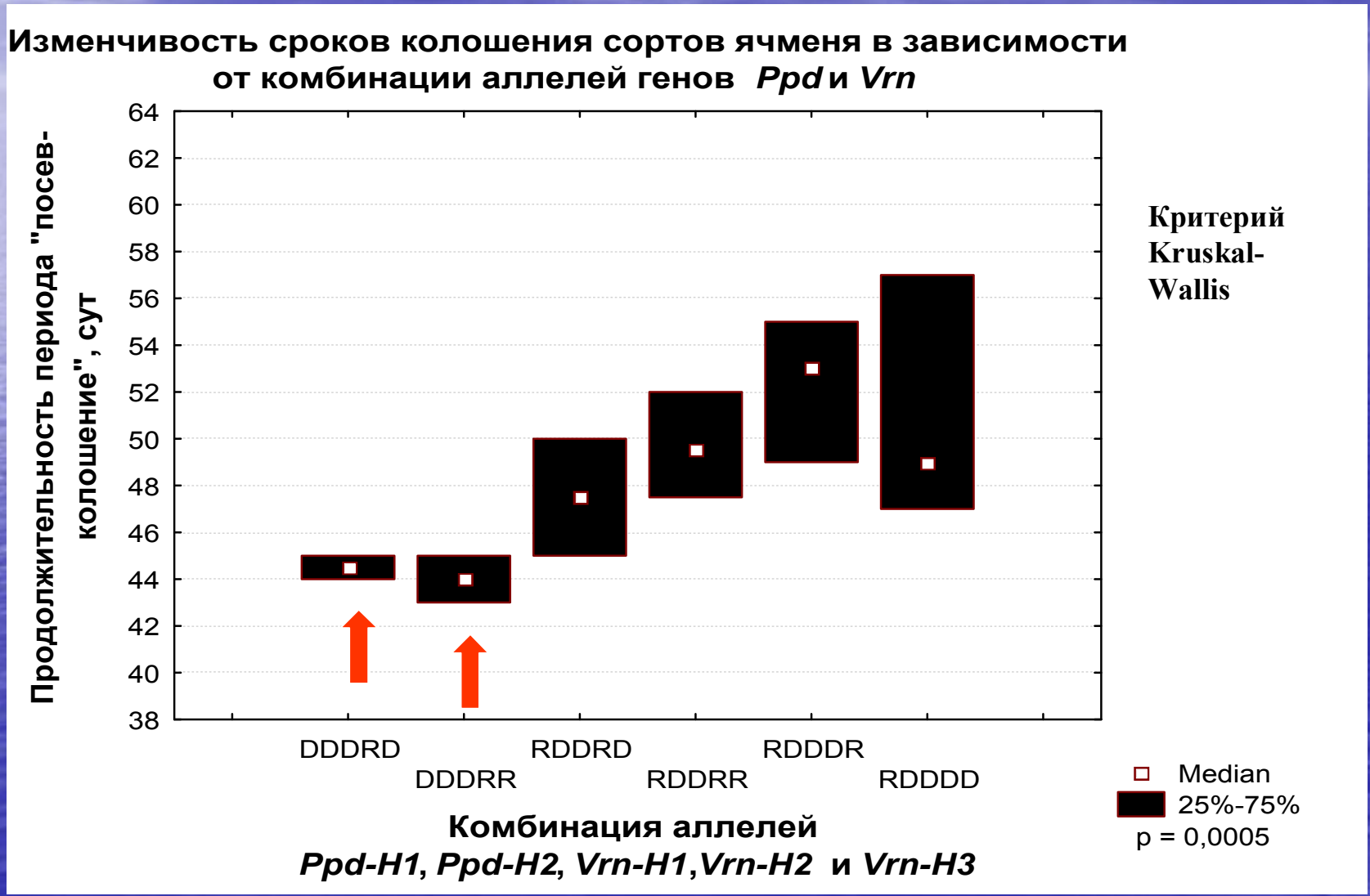
Zlotina M.M., et al, (2013). Russian Journal of Genetics: Applied Research, Vol. 3, No. 4, pp. 254–264.

Allelic combinations of the Ppd-H1, Ppd-H2 and Vrn-H1, Vrn-H2, Vrn-H3 genes have been investigated with allele-specific molecular markers in 91 spring barley cultivars recommended for use in Russia and Belarus

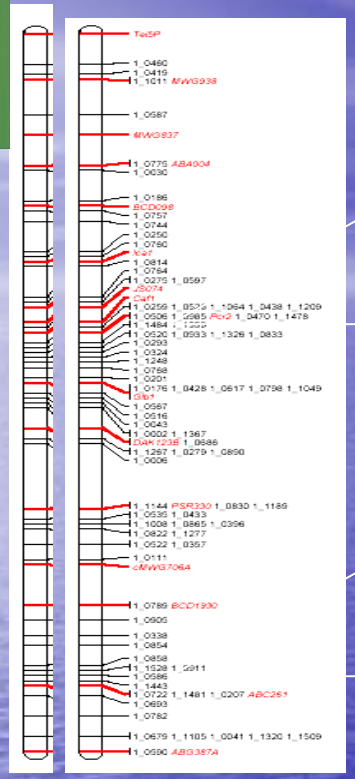
Название сорта, линии	№ по каталогу	Происхождение	Т	*Ppd-H1*	*Ppd-H2*	*Vrn-H1*	*Vrn-H2*	*Vrn-H3*	Обозначение в анализе
Биом	30984	Новосибирская обл.	45	D	D	D	R	D	DD-DRD
Прикумский 47	31045	Ставропольский край	44	D	D	D	R	D	DD-DRD
Мамлюк	28151	Красноярский край	40	D	D	D	R	R	DD-DRR
Баган	29040	Новосибирая обл.	43	D	D	D	R	R	DD-DRR
Безенчукский 2	30799	Самара	45	D	D	D	R	R	DD-DRR
Симон	30898	Кемеровская обл.	47	D	D	D	R	R	DD-DRR
Одесский 22	30969	Украина	44	D	D	D	R	R	DD-DRR
Волгодон	31104	Самарская обл.	45	D	D/R	R	R	R	DD-RRR

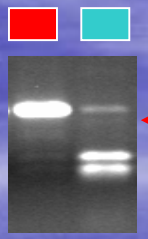
Zlotina M.M., et al, (2013). Russian Journal of Genetics: Applied Research, Vol. 3, No. 4, pp. 254–264.

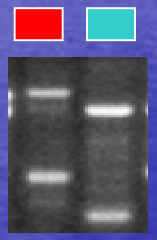
Barley cultivars with the dominant *Ppd* alleles (DD-DRD, DD-DRR), significantly (p=0,0005) faster transit to flowering



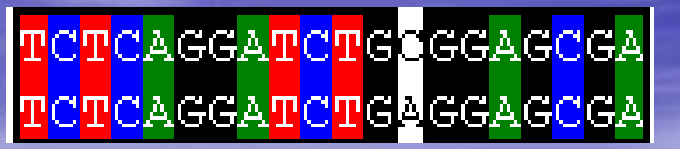
Marker-assisted selection using SSR-markers

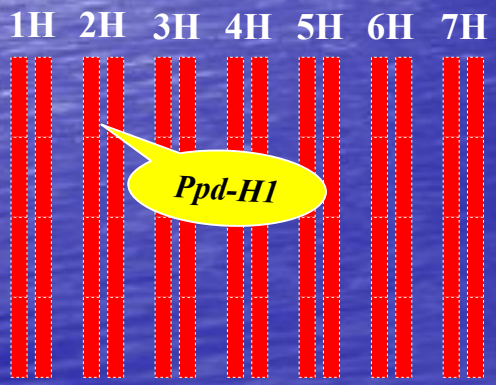






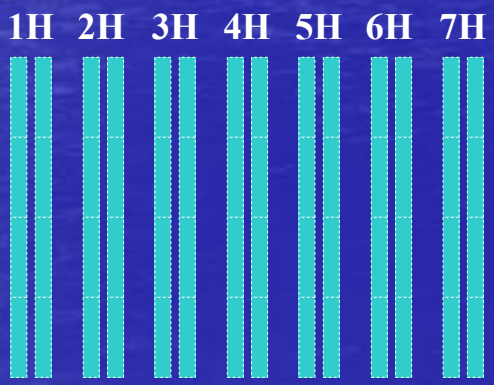
Morex (M)
Steptoe (S)



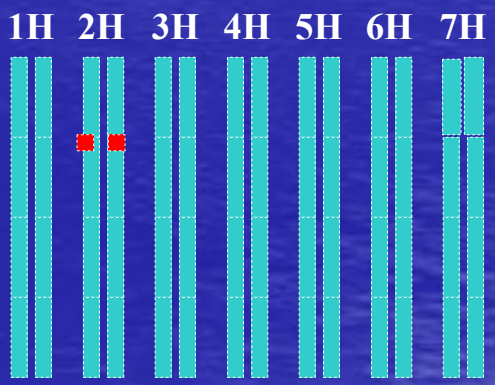


Donor of the insensitive Ppd-D1 allele

X

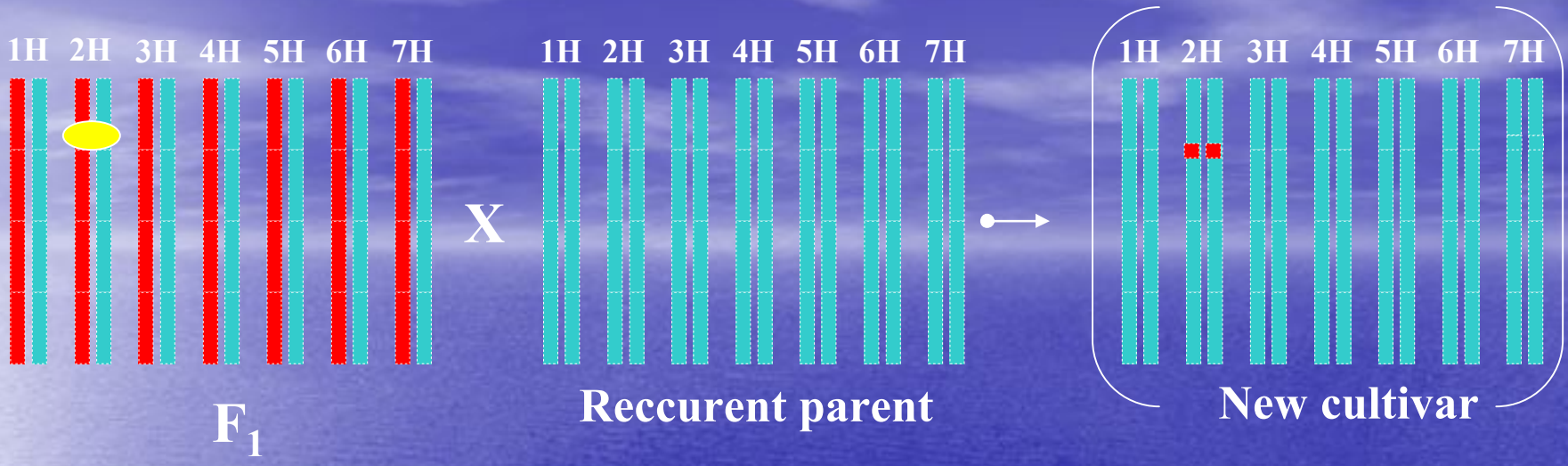


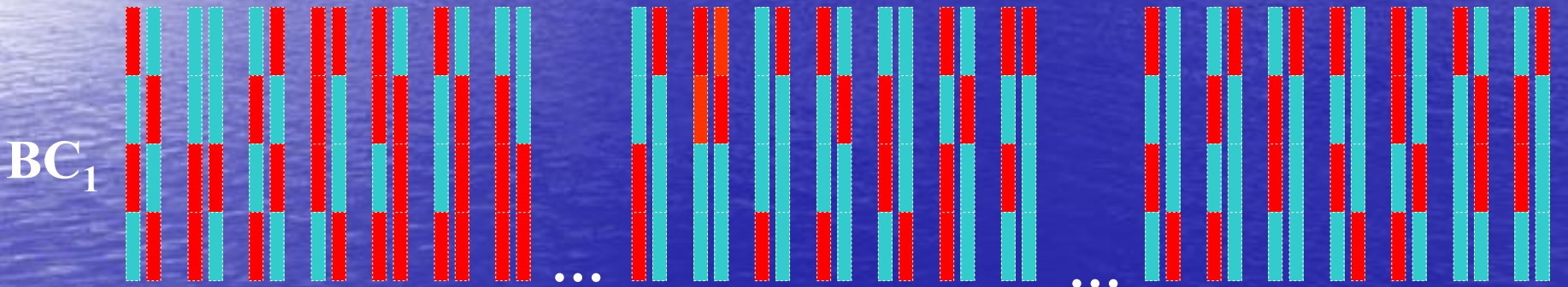
Recurrent parent



New cultivar

Three backcrosses instead of six ?

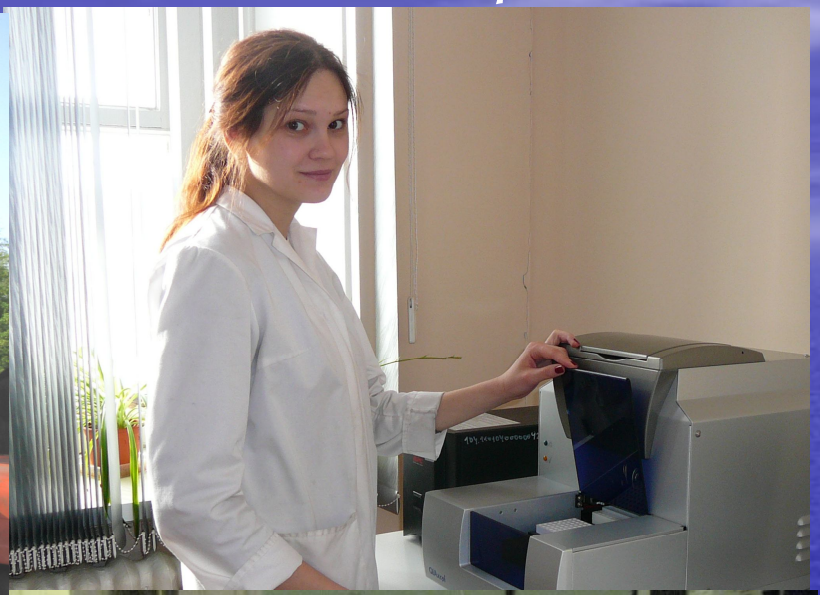




Genetic background recovered in: 37% 66% 62%

Рекомендуем:

X ! ?

Благодарим за внимание!

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