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**Molecular studies of plant
genetic resources:
theoretical and applied
aspects**

Irina Anisimova

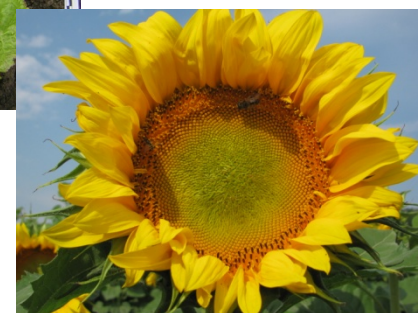


- **Molecular studies of collections of plant genetic resources in the Department of Genetics (VIR)**
- **Molecular genetic studies of the CMS-*Rf* genetic system in sunflower**



Plant objects:

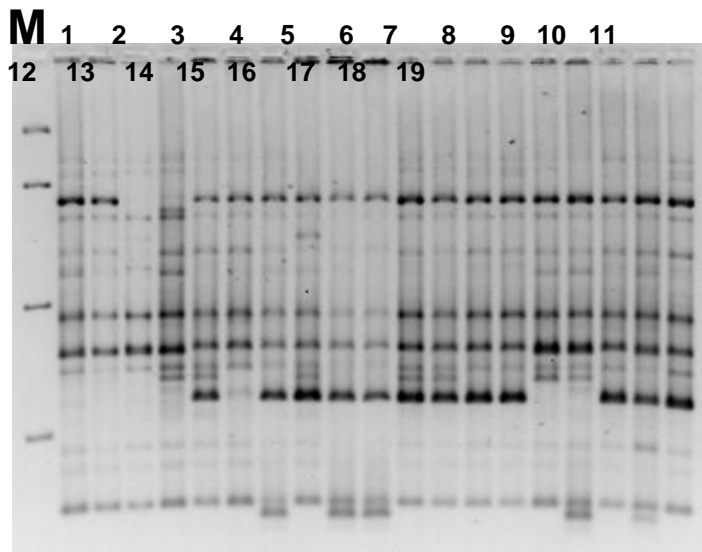
- Cereals (barley, sorghum)
- Oil seed plants (sunflower)
- Vegetable crops (lettuce)



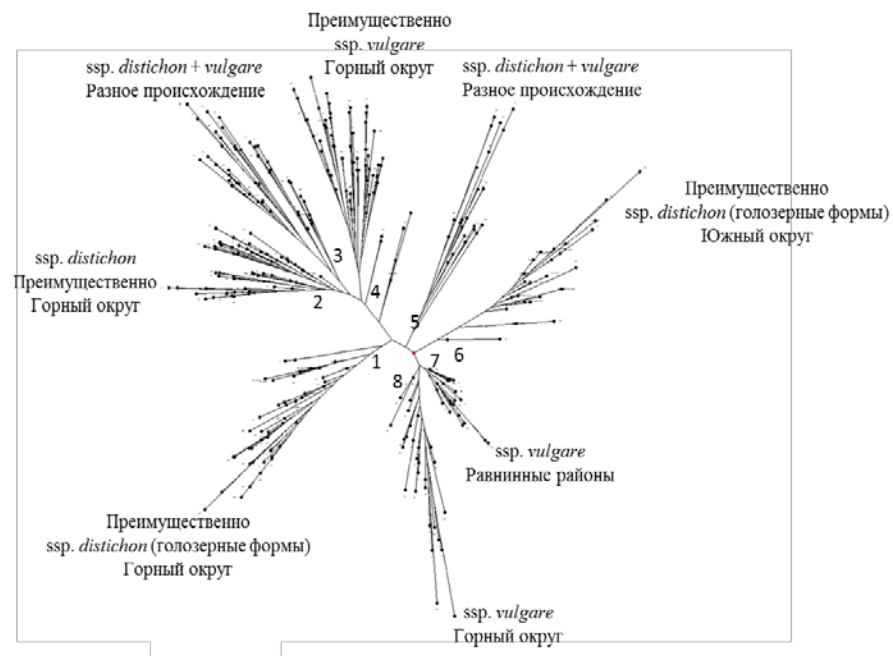


- Genotyping
- Structuring of genetic diversity (seed proteins, DNA markers)
- Molecular screening for identification of agronomically valuable genotypes (PCR markers)
- Identification of mutations and search for new alleles of the genes controlling agronomically valuable characters and traits (earliness, disease resistance, pollen fertility restoration)

Genotyping of barley landraces from Dagestan



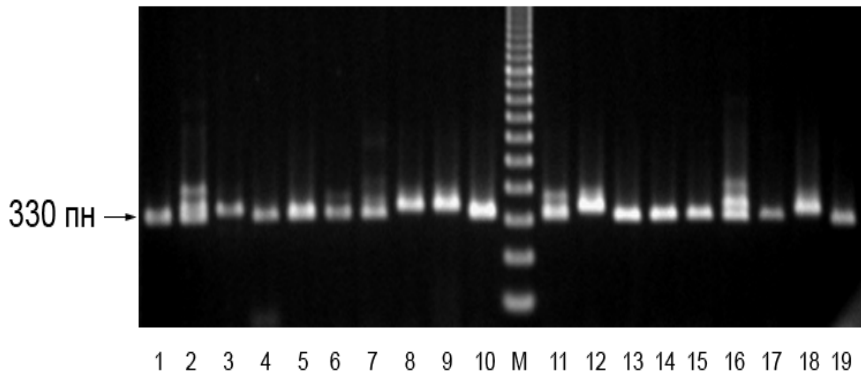
ISSR15



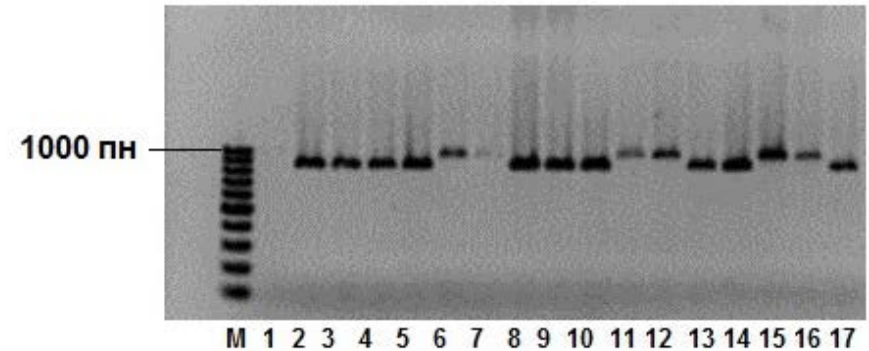
Cluster analysis of 265 barley accession from Dagestan

Абдуллаев Р.А. и др. Генетическое разнообразие местных форм ячменя из Дагестана // Доклады РАСХН, 2014. №5.

Molecular screening and identification of carriers of valuable genes



Идентификация гена *Dm3*, определяющего устойчивость *Lactuca L.* к *Bremia lactucae* Regel с использованием SCAR – маркера V12 (Paran , Michelmore , 1991)



Идентификация гена *Radv*, определяющего устойчивость *Helianthus annuus L.* к *Puccinia helianthii* Schwein. С использованием SCAR –маркера SC004 (Lawson et al. 1998)

Identification of a new allele of the *eam8* gene in a barley accession from Dagestan



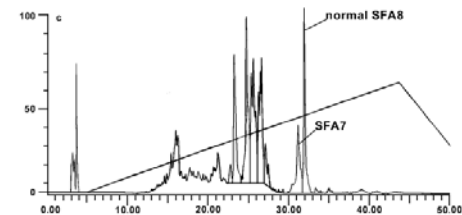
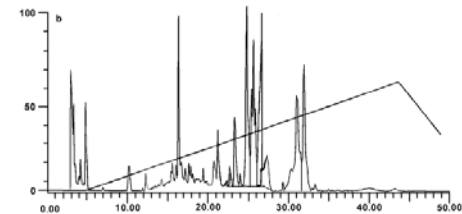
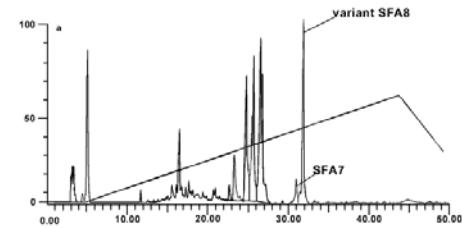
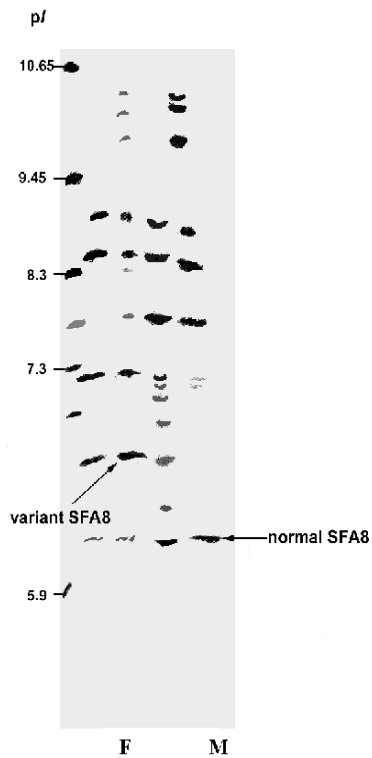
10 hrs photoperiod,
Low daily 10°C) and
high night (20°C)
temperatures

Mutation *eam8* in barley accession K-14891 from Dagestan

Identification of a new allele of the *eam8* gene in a barley accession from Dagestan

<i>Bonus_AEZ53982.1</i>	85	IGGIDR	PLFPSFCVPS	NEPVLPQHI	NTNSSGHATS	GRLS	124
<i>k-17429</i>	85	124
<i>k-16377</i>	85	124
<i>Bonus_mat-a.11</i>	85	124
<i>k-14891</i>	85	124
<i>Bonus_AEZ53982.1</i>	125	TQLKSK	DAYAAGSTAE	CTSSHGRDNN	AKNSSGNKLT	NDDD	164
<i>k-17429</i>	125	164
<i>k-16377</i>	125	164
<i>Bonus_mat-a.11</i>	125	164
<i>k-14891</i>	125	164
<i>Bonus_AEZ53982.1</i>	165	FTVPSV	FCSGVRPRSN	HEEARIQENS	THLPATSPYK	SGPT	204
<i>k-17429</i>	165	204
<i>k-16377</i>	165	G...	204
<i>Bonus_mat-a.11</i>	165ECVLVLT	MRKRGSKRIP	HTYQLQVHIR	V.LR	204
<i>k-14891</i>	165	LRFLPS	SAPECVLVLT	MRKRGSKRIP	HTYQLQVHIR	V.LR	204
<i>Bonus_AEZ53982.1</i>	205	VSKPTA	KFP				213
<i>k-17429</i>	205				213
<i>k-16377</i>	205				213
<i>Bonus_mat-a.11</i>	205	CPNQLQ	N..				213
<i>k-14891</i>	205	CPNQLQ	N..				213

Analysis of nucleotide polymorphism in the *SFA8* gene of sunflower



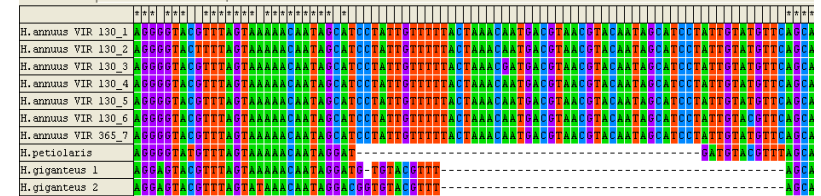
Nucleotide and amino acid sequences of SFA8

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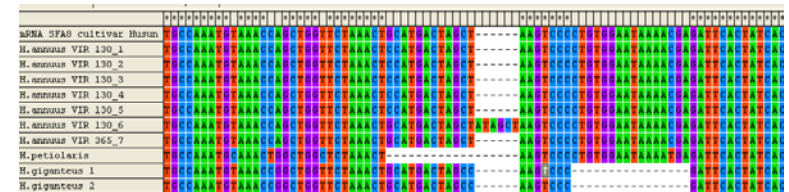
1   M A R F S I V F A A A G V L L L V A
1   tctacaATGGCAAGGTTTTCGATCGTGTTCGACGACGAGGACTCTCTCTCGTAGCC
19  M A A V S E A S T T T I I T T I E E N
61  ATGGCGGCAGTTTCTGAGGCTCCACCAACAACATCATCACCACCATCATAGAGGAGAAC
39  P Y G R G R T E S G C Y Q Q M E E A E T
121 CCCTATGGCAGAGGTAGAACTGAATCCGGATGCTATCAGCAGATGGAGGAGCGGAGAGC
59  L N H C G M Y L M K N L
181 CTCAACCACTGCGGGATGTACCTCATGAAAANTTAGgtagccctgattttttttttggg
241  ggaggccagacataccccctstataagaattcagagggtagcgtaacbaatttttagtaggggt
301  acylltagtaaaaaaataagcaccctattgttttactaaacaatgaagttaacgtacaat
361  agcatcctattgtatgttcagcaacaacccttataaaaaaaaaacaatcacacacacgcat
421  catttagtttgctacatttactactaactcaactcaatgattgttgtagtttaaaagtgtc
71   G E R R Q V S P
481  tgaatttatatggcctgtggtgattttttctgatacggagaaaggaggcaagttagtc
79   R M R E E D H K Q L C C M Q L K N L D E
541 CCAGAATGAGAGAAGAGGATCACAAGCAACTTTGCTGCACTGAAGAACCTAGATG
99   K C M C P A I M M L L N E P M W I R M R
601 AGAAGTGCATGTGTCGGCAATCATGATGATGTTGAACGAAOCCAATGGATAGGATGC
119  D Q V M S M A H N L P I E C N L M S Q P
661 GAGATCAAGTGTAGCATGGCTCATAAOCCTGCCATAGAATGCAATCTCATGTCCCAAC
139  C Q M
721 CATGCCAATGtaaaaccagctggttctaactccatgactgtaagtccccctgtggaat
781 aaaaagagattcactatcacatcgtcgtgtgtgtgtttcaatgtaatacgtccatcg
841 tgtgtctcgttta
    
```

Polymorphism within exon and intron of SFA8

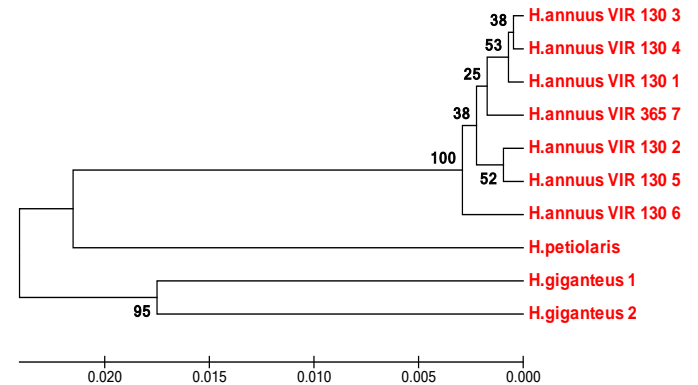
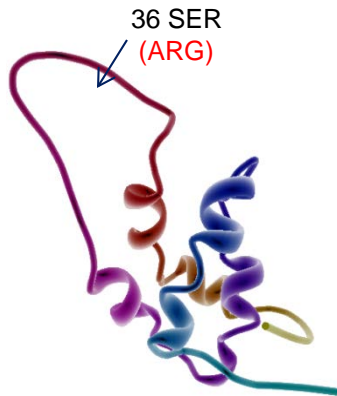
A

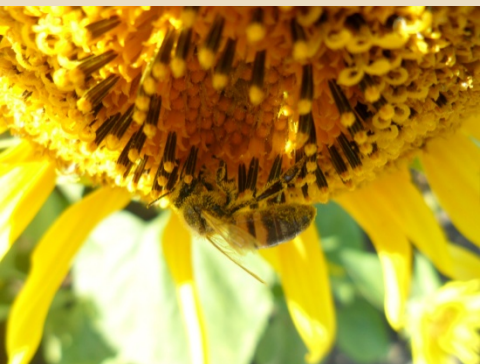


B



Quarternary structure of SFA8 protein

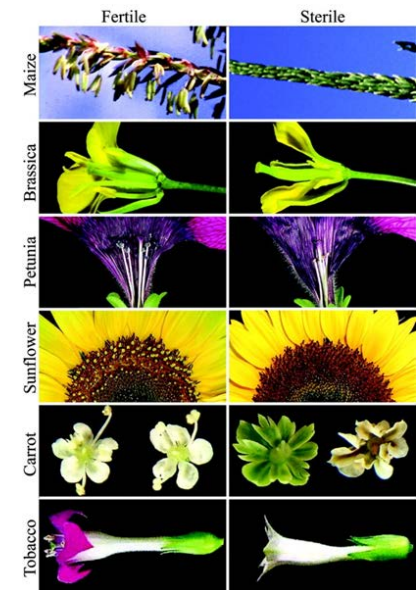




**SUNFLOWER COLLECTION IN THE
STUDIES OF GENETIC MECHANISMS OF
POLLENFERTILITY RESTORATION**

CMS-Rf genetic system

- A basis for hybrid seed production
- A model for studying mechanisms of interaction between nuclear and organelle genomes



ФЕНОТИПЫ ЦМС
(Hanson & Bentolila,
2004)

CMS-*Rf* genetic system in sunflower

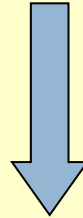
- 70 CMS sources; CMS PET1 is widely used
- The character of fertility restoration is poorly studied; genetic control is not well known
- 1-5 *Rf* genes is reported

Heterotic hybrid production



Материнская стерильная линия на основе ЦМС РЕТ1

x



*Отцовская линия
восстановитель
фертильности пыльцы,
несущая ген Rf1*



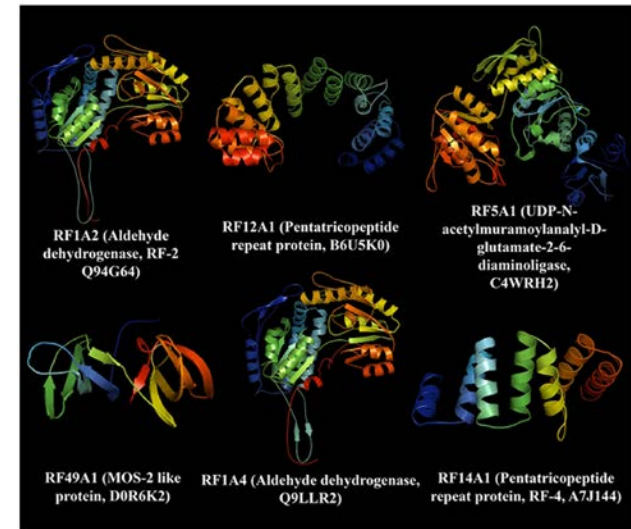
Для создания гетерозисного гибрида необходимо стерильную линию ЦМС скрестить с линией восстановителем фертильности пыльцы



Гибриды F1 проявляют эффект гетерозиса по урожайности. Их выровненность по высоте растений, срокам цветения и созреванию снижает степень поражения болезнями и потери урожая при уборке

The nature of sunflower *Rf* genes is unknown

- The majority of known *Rf* genes in plants encode PPR-proteins (PPR - pentatricopeptide repeats of 35 amino acids)
- Separate gene family
 - RFL-PPR* (*Restorer-of-Fertility-Like-PPR*) (Fujii et al., 2011)
- The main features of *RFL-PPR* genes :
 - ✓ Cluster organization
 - ✓ High homology within cluster
 - ✓ High rate of divergence in PPR motives
 - ✓ The products of *RFL-PPR* genes interact with products of aberrant mitochondrial genes associated with CMS



Proteins encoding by *Rf* genes
(Kotchoni et al. 2010)

Rf genes and their products

Вид	Тип ЦМС	Ген <i>Rf</i>	Белок	Функция белка	Ссылки
<i>Zea mays</i>	T	<i>Rf1</i>	Не идентифицирован	Процессинг и пост-транскрипционное снижение уровня белка T-URF13	Dewey et al., 1987 Wise et al., 1999
<i>Oryza sativa</i>	BT	<i>Rf1</i>	PPR	Процессинг мРНК	Wang et al., 2006
	HL	<i>Rf5</i>	PPR	Процессинг транскрипта <i>atp6-orf79</i>	Hu et al., 2012
<i>Sorghum bicolor</i>	A1	<i>Rf1</i>	PPR13	Неизвестна	Klein et al., 2005
	A1	<i>Rf2</i>	PPR	Процесинг мРНК	Jordan et al., 2010
<i>Brassica napus</i>	Ogura	<i>Rfo</i>	PPR	Пост-транскрипционная регуляция	Brown et al., 2003
<i>Petunia × hybrida</i>	pcf	<i>Rf</i>	PPR592	Деградация мРНК и снижение уровня белка PCF	Bentolila et al., 2002
<i>Helianthus annuus</i>	PET1	<i>Rf1</i>	????	????	

Materials



**130 lines of sunflower genetic collection
with different ability for suppression of
CMS phenotype**

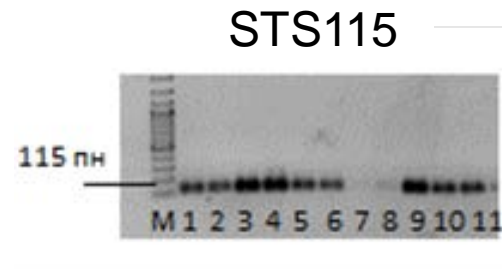
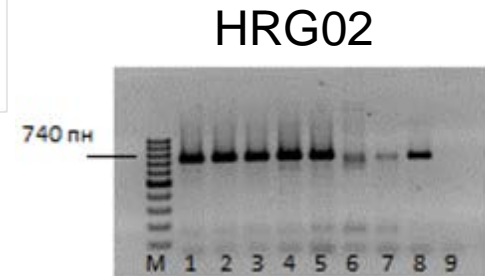
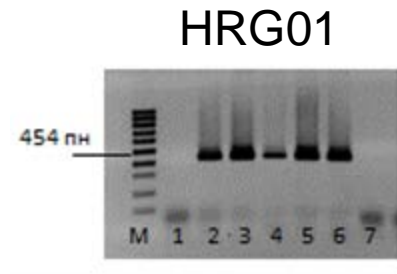
Aims of the study



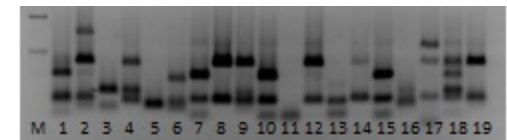
- Clarification of peculiarities of structural and functional diversity of the genes controlling pollen fertility restoration
- Estimation of genetic diversity of sunflower genetic collection

Molecular marking of lines

- 8 PCR markers (STS, SCAR, SSR, CAPS), tightly linked to the *Rf1* locus
- Mitochondrial STS markers *orfH522* и *atp9*
- CAPS markers of *RFL-PPR* genes

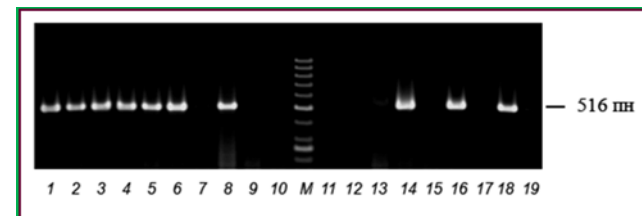


CAPS/H13/*Hinfl*



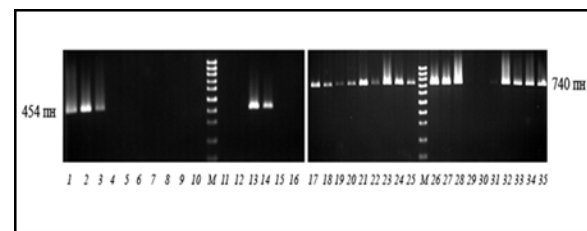
Molecular markering of lines

С помощью митохондриального маркера *orfH522*, ассоциированного с ЦМС РЕТ1, у 107 линий идентифицирована стерильная (РЕТ1 или иного типа) цитоплазма



Линия	Наличие маркера		Тип цитоплазмы (S – стерильная, F – фертильная)
	HRG01	HRG02	
ВИР109А (ЦМС)	-	-	S
ВИР558	+	+	S
ВИР792	+	+	S
ВИР793	+	+	S
ВИР581	+	+	S
ВИР681	+	+	S
ВИР653	+	+	F
ВИР740	+	+	F

С помощью молекулярных маркеров подтверждено наличие гена *Rf1* у большинства линий, восстанавливающих фертильность в скрещиваниях с линиями ЦМС (РЕТ1)



Polymorphism of the SSR loci linked to the *Rf1* gene



Locus	Alleles	Number of lines
ORS511	Нулевой	9
	158-162	71
	210-214	3 (ВИР343, ВИР381, ВИР210)
	198	1 (ВИР130Б)
	244	1 (ВИР370)
ORS224	Нулевой	39
	148-153	43
	105	1 (ВИР210)
	124	1 (ВИР376)
ORS799	Нулевой	34
	165-170	47

Nucleotide polymorphism of mitochondrial locus *atp9*

```

      10      20      30      40      50      60      70      80      90
X51895.1  1  GGTGCAAAAT CAATAGGGGC CCGAGCTGCT ACAATTCGTT CAGCGGGAGC TGCTATCGGT ATTGGAAACG TCCTTAGTTC CTCGATTCAT
Zelenka  1  .....
VIR763   1  .....
VIR743   1  .....
VIR449   1  .....
VIR377   1  .....
H.petiolaris 1  .....
H.mollis  1  .....
H.agrophyllus 1  .....

      100     110     120     130     140     150     160     170     180
X51895.1  91  TCCGTGGCTC GGAATCCATC ATTGGCTAAA CAATCATTTG GTTATGCCAT TTTGGGCTTT GCTCTAACCG AAGCTATTGC ATCGTTTGCC
Zelenka   91  .....
VIR763   91  .....
VIR743   91  .....
VIR449   91  .....
VIR377   91  .....
H.petiolaris 91  .....
H.mollis  91  .....
H.agrophyllus 91  .....

      190     200     210     220     230     240     250
X51895.1  181 CCAATGATGG CCTTCTGAT CTCATCCGTT ATTCGATCA AAGAATCAAA GAAAGAAGGT TGAAGT --- 246
Zelenka   181 .....A T.C.GATCA. .GA.TCA..G A..GA.G.T. GA.GT.TCAT AA 252
VIR763    181 .....A T.C.GATCA. .GA.TCA..G AT.GA.G.T. GA.GT.TCAT AA 252
VIR743    181 .....A T.C.GATCA. .GA.TCA..G A..GA.G.T. GA.GT.TCAT AA 252
VIR449    181 .....A T.C.GATCA. .GA.TCA..G A..GA.G.T. GA.GT.TCAT AA 252
VIR377    181 .....A T.C.GATCA. .GA.TCA..G A..GA.G.T. GA.GT.TCAT AA 252
H.petiolaris 181 .....A T.C.GATCA. .GA.TCA..G A..GA.G.T. GA.GT.TCAT AA 252
H.mollis   181 .....A T.C.GATCA. .GA.TCA..G A..GA.G.T. GA.GT.TCAT AA 252
H.agrophyllus 181 .....A T.C.GATCA. .GA.TCA..G A..GA.G.T. GA.GT.TCAT AA 252

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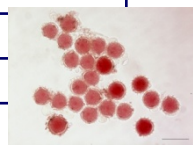
Выравнивание фрагмента смысловой последовательности гена *atp9* у фертильных линий ВИР763, ВИР449, ВИР218, ВИР377, староместного сорта Зеленка, диких видов *H.petiolaris*, *H.agrophyllus*, *H.agrophyllus*; X51895.1- последовательность линии HA89 (Recipon, 1990)

Hybridological analysis

- 15 F1 hybrid combinations of crosses between CMS PET1 and fertile lines were grown in different regions of Russia: manifestation of the pollen fertility restoration trait is independent on climatic conditions.
- Different segregation pattern in the F2 depending on cross combinations : monogenic or digenic

The results of test crosses between CMS PET1

№ п/п	Maternal line (CMS PET1)	Paternal line		Fertility of F1
		name	Cytoplasmon type	
1	ВИР116А	ВИР160	ЦМС Х или фертильный	S
2	то же	ВИР195	ЦМС РЕТ1	F
3	то же	ВИР210	ЦМС РЕТ1 или Х	F
4	то же	ВИР211	ЦМС Х или фертильный	S
5	то же	ВИР364	ЦМС РЕТ1	F
6	то же	ВИР365	ЦМС РЕТ1	F
7	то же	ВИР366	ЦМС Х или фертильный	S
8	то же	ВИР371	фертильный	S
9	то же	ВИР387	ЦМС Х или фертильный	S
10	то же	ВИР558	ЦМС РЕТ1	F
11	то же	ВИР729	ЦМС РЕТ1	F
12	то же	ВИР740	ЦМС Х или фертильный	F
13	то же	RIL80	ЦМС РЕТ1	F
14	то же	RIL130	ЦМС РЕТ1	F
15	ВИР109А	ВИР364	ЦМС РЕТ1	F
16	то же	ВИР558	ЦМС РЕТ1	F



Segregation patterns in the F2

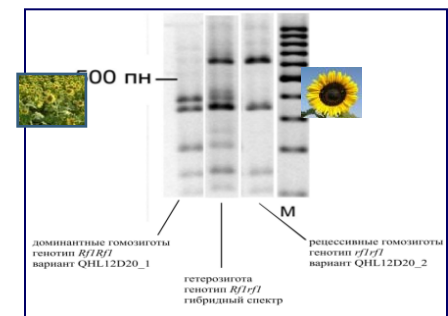
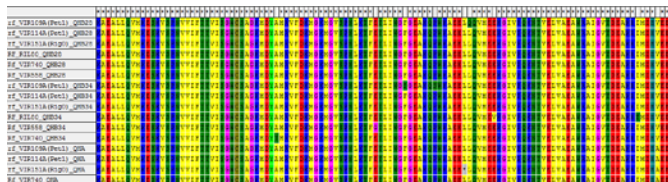
№ п/п	Hybrid combination	Number of plants	Segregation		Theoretically expected ratio	χ^2
			F	S		
1	ВИР109 × ВИР558	133	109	24	3 : 1	3,43
2	ВИР116 × RIL130	59	43	16	3 : 1	0,14
3	ВИР116 × ВИР365	93	77	16	3 : 1	3,01
4	ВИР116 × ВИР365	34	26	8	3 : 1	0,07
5	ВИР116 × ВИР210	43	26	17	9 : 7	0,31



Фенотипы растений расщепляющейся гибридной популяции F2 (ВИР116 x ВИР210)

Verification of the hypothesis on belonging sunflower *Rf1* genes to the *RFL-PPR* family

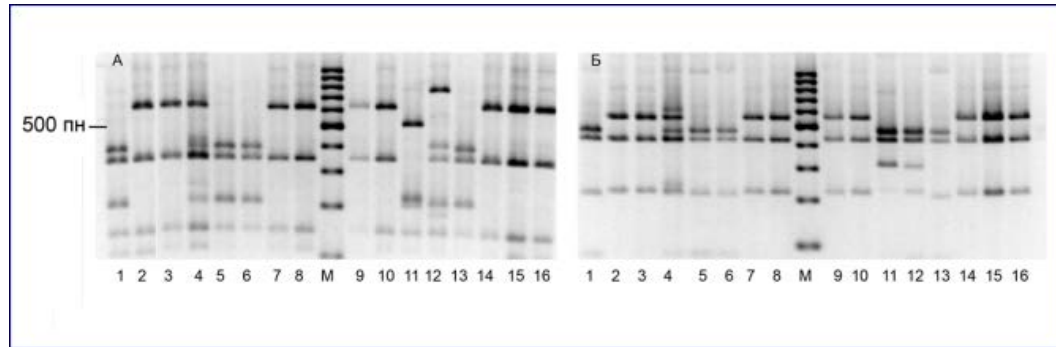
- Sequencing of the EST fragments (<http://www.cgp.edu/>) homologous to the known *Rf* genes of other plants
- Comparative analysis of the polymorphism revealed between the genotypes with different functional state of the *Rf* genes; identification of SNPs; developing markers of *RFL-PPR* genes
- Analysis of joint inheritance of the *RFL-PPR* genes variant and common molecular markers linked to the *Rf1* locus



Verification of the hypothesis on belonging sunflower *Rf1* genes to the *RFL-PPR* family

- Nucleotide polymorphism of 10 PPR fragments was investigated
- The co-dominant CAPS markers were developed and their occurrence in the lines of genetic collection was studied
- For the fragments **QHL12D20** и **QHB20M13** an association with the functional state of *Rf* genes was demonstrated

Group of lines (cytoplasmon type)	Number of line with marker variants L12D20/ <i>HaeIII</i>		Number of lines with markers variants B20M13/ <i>RsaI</i>	
	1	1.2	1	2
Autofertile (S PET1)	27	1.17	43	25
Autofertile (F or S(X))	5	1.3	5	3
CMS lines and fertile analogs	0	1.4	0	4



**Frequencis of nonsynonimous SNPs within
sunflower and sorghum *RFL-PPR* genes:**

> 4%

**Frequencis of nonsynonimous SNPs within the
gene for sunflower 11S globulin:**

~ 1%i

Polymorphism of EST QHL12D20

```

QHL12D20.yy.abl.cF081137 1  CGGGTAGAG ACCCGGAAA AGCACATT-C GTTATTCGAA GATATGAT-T GATGAAAGTT 58
VIR 109 (cF1eF1) 1  ----GTAGG ACCCGGAAA AGCACATCGC GTTATTCGAA GATATGAT-C GAGCAAGGTC 55
VIR 740 (R1E1F1) 1  ----GTAGG ACCCGGAAA AGCACATT-C GTTATTCGAA GATATGATC GATGAAAGTT 55
                                     ----->
QHL12D20.yy.abl.cF081137 59  GTATTGAAA CCAG-6AATC CTACACTGCT CTCTCTTCGC CTACAGATC- GAGTGGGCT 116
VIR 109 (cF1eF1) 56  GTATTGAAA CCAGAAAGATC CTACACTGCT CTCTCTTCGC CTACAGATCC GAGTGGGCT 115
VIR 740 (R1E1F1) 56  GTATTGAAA CCAG-6AATC CTACACTGCT CTCTCTTCGC CTACAGATC- GAGTGGGCT 116
                                     ----->
QHL12D20.yy.abl.cF081137 117  GTTTAGAAA GCOTTITCAA TACTGACAGA AATGAGAAC ACTCTCAACT GCCACCTGAA 173
VIR 109 (cF1eF1) 116  GTTTAGAAA GCOTTITCAA TACTGACAGA AATGAGAAC ACTCTCAACT GCCACCTGAA 175
VIR 740 (R1E1F1) 116  GTTTAGAAA GCOTTITCAA TACTGACAGA AATGAGAAC ACTCTCAACT GCCACCTGAA 173
                                     ----->
QHL12D20.yy.abl.cF081137 177  TTTTACAGC TATTCARCTC TATAAAGAC ATGCTCTCAT TTTCANAGT TTAGAGAAAT 236
VIR 109 (cF1eF1) 176  TTTTACAGC TATTCARCTC TATAAAGAC ATGCTCTCAT TTTCANAGT TTAGAGAAAT 235
VIR 740 (R1E1F1) 174  TTTTACAGC TATTCARCTC TATAAAGAC ATGCTCTCAT TTTCANAGT TTAGAGAAAC 233
                                     ----->
QHL12D20.yy.abl.cF081137 237  GC-AATCTTT GCTTTCGGAG ATGGCCCTCA CAGGAAATCA A-GCCCAATA CGGTACTTA 293
VIR 109 (cF1eF1) 236  CG-AATCTTT GCTTTCGGAG -ATGGCCCTCA CAGGAAATCA ATGCCCAATA CGGTACTTA 293
VIR 740 (R1E1F1) 234  TGCATCTTT GCTTTCGGAG TATGGCCCTCA CAGGAAATCA A-GCCCAATA CGGTACTTA 292
                                     ----->
QHL12D20.yy.abl.cF081137 294  TAAT-ACCCT TATGATGCA TATGGGAAA CAAAA----- 328
VIR 109 (cF1eF1) 294  TAAT-ACCCT TATGATGCA TATGGGAAA CAAAAAggta acttttccac tegtatat 352
VIR 740 (R1E1F1) 293  TAAT-ACCCT TATGATGCA TATGGGAAA CAAAAAggta acttttccac tegtatat 351
                                     ----->
QHL12D20.yy.abl.cF081137 328  ----- 328
VIR 109 (cF1eF1) 323  acttttccac ttttagagat gtggatgat ggaagtaag gttgagtt tatatttt 404
VIR 740 (R1E1F1) 322  atatatat ttttagagat gtggatgat ggaagtaag gttgagtt tatatttt 411
                                     ----->
QHL12D20.yy.abl.cF081137 405  ----- 405
VIR 109 (cF1eF1) 412  actttttaa tttttaccac aaaaatag agtaaatag catttngtc atgagtttg 463
VIR 740 (R1E1F1) 412  actttttt-----accac aaaaatag agtaaatag catttngtc atgagtttg 463
                                     ----->
QHL12D20.yy.abl.cF081137 328  ----- 328
VIR 109 (cF1eF1) 465  actggtttg cgttttgt ccaaaattt gtttttgc atcgtatc aaaaagttg 524
VIR 740 (R1E1F1) 464  gcaagtttg cgttttgt ccaaaattt gtttttgc atcgtatc aaaaagttg 523
                                     ----->
QHL12D20.yy.abl.cF081137 328  ----- 328
VIR 109 (cF1eF1) 523  aaatcttgc attttaccac ggtttgtaa ctccatcgt tttctcgt taagtccgg 584
VIR 740 (R1E1F1) 524  aaatcttgc attttaccac ggtttgtaa ctccatcgt tttctcgt taagtccgg 583
                                     ----->
QHL12D20.yy.abl.cF081137 328  ----- 328
VIR 599 (cF1eF1) 583  gtttttgt cttttctgt aacttaagc gcaatctg tttttgata ctgtacatt 643
VIR 740 (R1E1F1) 584  gtttttgt cttttctgt aacttaagc gcaatctg tttttgata ctgtacatt 643
                                     ----->
QHL12D20.yy.abl.cF081137 328  ----- 328
VIR 109 (cF1eF1) 644  atggaatg ctgttaccac aagtgaaaa gacagatg cttttaag taacaaaa 702
VIR 740 (R1E1F1) 644  atggaatg ctgttaccac aagtgaaaa gacagatg cttttaag tgcacaaa 703
                                     ----->
QHL12D20.yy.abl.cF081137 328  ----- 328
VIR 704  gacaaaaa ccccttctt aactgtaga aaaaatgag gattgaaca gggatgaa 763
VIR 109 (cF1eF1) 702  -----ta ccccttctt aactgtaga aaaaatgag gattgaaca gggatgaa 754
VIR 740 (R1E1F1) 702  -----ta ccccttctt aactgtaga aaaaatgag gattgaaca gggatgaa 754
                                     ----->
QHL12D20.yy.abl.cF081137 328  ----- 328
VIR 109 (cF1eF1) 764  aatgacaag tttgacact tttgatac gatgogaaa acaaaatt tgcogaaa 823
VIR 740 (R1E1F1) 755  aatgacaag tttgacact tttgatac gatgogaaa acaaaatt tgcogaaa 814
                                     ----->
QHL12D20.yy.abl.cF081137 328  ----- 328
VIR 109 (cF1eF1) 824  ttgaaaact gggcaaaact gggagagga aatggatatt tacttttaa ataatatg 863
VIR 740 (R1E1F1) 813  ttgaaaact gggcaaaact gggagagga aatggatatt tacttttaa ataatatg 874
                                     ----->
QHL12D20.yy.abl.cF081137 328  ----- 328
VIR 109 (cF1eF1) 864  gaagttgaa ctaatgacc atgacaaac gcatgtgta actacaaat attgtctg 942
VIR 740 (R1E1F1) 875  gaagttgaa ctaatgacc atgacaaac gcatgtgta actacaaat attgtctc 934
                                     ----->
QHL12D20.yy.abl.cF081137 328  ----- 328
VIR 109 (cF1eF1) 943  gttattaac tttgtcaaa GATTITGGA CATGAAATCA ACACTGTGG AAGTCTCCG 1002
VIR 740 (R1E1F1) 935  gttattaac tttgtcaaa GATTITGGA CATGAAATCA ACACTGTGG AAGTCTCCG 994
                                     ----->
QHL12D20.yy.abl.cF081137 1002  ACAAGGAAA TGCAGCCCG ATATATGAC CATGARTCA ACCTTAAAG CTTTGTGTG 428
VIR 109 (cF1eF1) 1002  ACAAGGAAA TGCAGCCCG ATATATGAC CATGARTCA ACCTTAAAG CTTTGTGTG 1062
VIR 740 (R1E1F1) 995  ACAAGGAAA TGCAGCCCG ATATATGAC CATGARTCA ACCTTAAAG CTTTGTGTG 1054
                                     ----->
QHL12D20.yy.abl.cF081137 429  CAGTGGGCA ATAGAACAA TGGAAAATG CTCAGAGAG TTCTTAATG CCGAARTCA 488
VIR 109 (cF1eF1) 1053  CAGTGGGCA ATAGAACAA TGGAAAATG CTCAGAGAG TTCTTAATG CCGAARTCA 1122
VIR 740 (R1E1F1) 1055  CAGTGGGCA ATAGAACAA TGGAAAATG CTCAGAGAG TTCTTAATG CCGAARTCA 1114
                                     ----->
QHL12D20.yy.abl.cF081137 489  ACCTAAGTGT AAGACTCTT ATATCTCTT GGATTCATC GGGAAAACC GAACTAAA 548
VIR 109 (cF1eF1) 1123  ACCTAAGTGT AAGACTCTT ATATCTCTT GGATTCATC GGGAAAACC GAACT--- 1178
VIR 740 (R1E1F1) 1115  ACCTAAGTGT AAGACTCTT ATATCTCTT GGATTCATC GGGAAAACC GAACT--- 1170
                                     ----->
QHL12D20.yy.abl.cF081137 549  AAGATAGCT GCTGTATGG A 549
VIR 109 (cF1eF1) 1178  ----- 1178
VIR 740 (R1E1F1) 1170  ----- 1170
    
```

- 3 PPR motives

- Contains intron 630 bp the sequence of which represents complementary palindrom

- Intron of QHL12D20 is homologous to the intron of the AHBP-1B gene (stress adaptation, high homology to the bZIP transcription factor of arabidopsis)

Nucleotide sequences of the
QHL12D20 of 9 lines have been
deposited to the GenBank
(NCBI) data base (accessions
numbers KJ450920-KJ450928)
(<http://www.ncbi.nlm.nih.gov/nucore>)

Helianthus annuus inbred line VIR151A RIG0 pentatricopeptide repeat-containing protein (RFL-PPR) gene, partial cds

GenBank: KJ450920.1

LOCUS KJ450920 1174 bp DNA linear PLN 12-NOV-2014

DEFINITION Helianthus annuus inbred line VIR151A RIG0 pentatricopeptide repeat-containing protein (RFL-PPR) gene, partial cds.

ACCESSION KJ450920

VERSION KJ450920.1 GI:618765566

KEYWORDS .

SOURCE Helianthus annuus (common sunflower)

ORGANISM [Helianthus annuus](#)

Eukaryota; Viridiplantae; Streptophyta; Embryophyta; Tracheophyta; Spermatophyta; Magnoliophyta; eudicotyledons; Gunneridae; Pentapetalae; asterids; campanulids; Asterales; Asteraceae; Asteroideae; Heliantheae alliance; Heliantheae; Helianthus.

REFERENCE 1 (bases 1 to 1174)

AUTHORS Anisimova, I.N., Alpatieva, N.V., Rozhkova, V.T., Kuznetsova, E.B., Pinaev, A.G. and Gavrilova, V.A.

TITLE Polymorphism among RFL_PPR homologs in sunflower (*Helianthus annuus* L.) lines with varying ability for the suppression of the cytoplasmic male sterility phenotype

JOURNAL Russ. J. Genet. 50 (7), 712-721 (2014)

REFERENCE 2 (bases 1 to 1174)

AUTHORS Anisimova, I.N., Alpatieva, N.V., Rozhkova, V.T., Kuznetsova, E.B., Pinaev, A.G. and Gavrilova, V.A.

TITLE Direct Submission

JOURNAL Submitted (14-FEB-2014) Department of Genetics, N.I. Vavilov Institute of Plant Industry, Bolshya Morskaya, 42, Saint Petersburg 190000, Russia

/translation="KQPEKAHSLFQDMIDEGCIVNQESYTALLSAYSRSGLFRKAFSI

LDDMRNTPNCHPDVYTYSILIKSCLHFHEFEKVQSLLEMASQGKPNPTVYNTLIDA
YGKAKRFVDMESTLVEMLRQRECKPDVWVTMNSTLRAFGGSGQIETMEKCYEKFLSAGI
QPNVKTFNILLDSYGKTGN"

ORIGIN

F₂ segregation by the alleles of the QHL12D20, HRG02 и STS115

Combination (loci)	F ₂ phenotypes						Theoretical ly expected ratio	χ ²
	A-Bb	A-BB	A-bb	aaBb	aaBB	aabb		
ВІР116 x ВІР558 (L12D20, HRG02)	24	12	3	2	0	7	6:3:3:2:1: 1	14,1 7
ВІР116 x ВІР740 (L12D20, STS115)	21	8	10	8	4	6	6:3:3:2:1: 1	0,89

При P < 0, 005 χ² = 11,07

Acknowledgements



Department of Genetics (VIR)

Department of Genetic Resources of Oil and Fiber Crops (VIR)

Cuban Experiment Station (VIR)

**Research Centre “Genomic Technologies” (All-Russia Institute of
Agricultural Microbiology)**

**The study was supported by Russian Foundation for Basic Research
(project code №12-04-00239)**

Thank you for your attention!

