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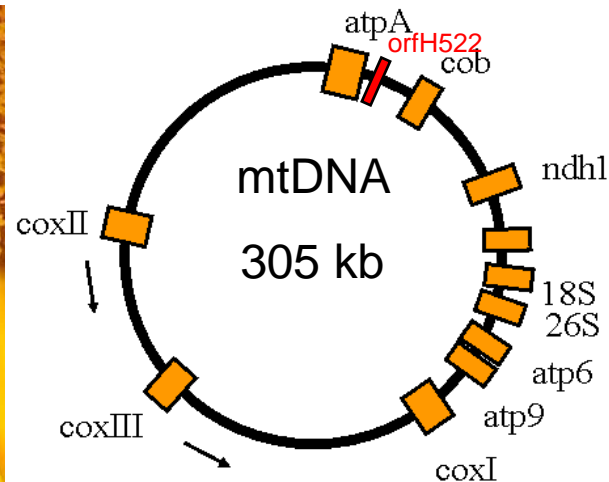


Traditio et Innovatio



# Association studies and marker development for the fertility restorer gene *Rf1* in sunflower

Renate Horn, Aleksandra Radanović, Lena Fuhrmann, Yves Sprycha, Sonia Hamrit,  
Milan Jocković, Dragana Miladinović and Constantin Jansen



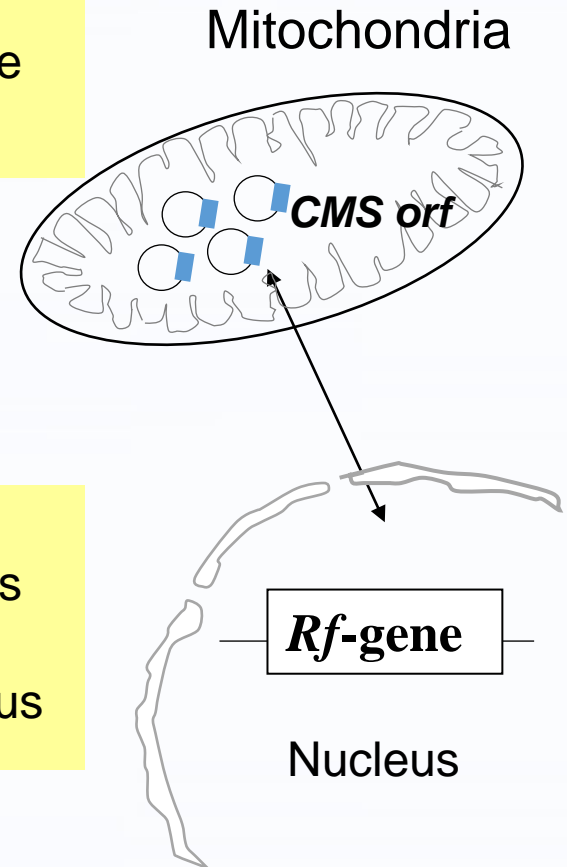
# Cytoplasmic male sterility (CMS) and fertility restoration



- no functional pollen
- maternal inherited trait
- based on changes in the mitochondrial DNA

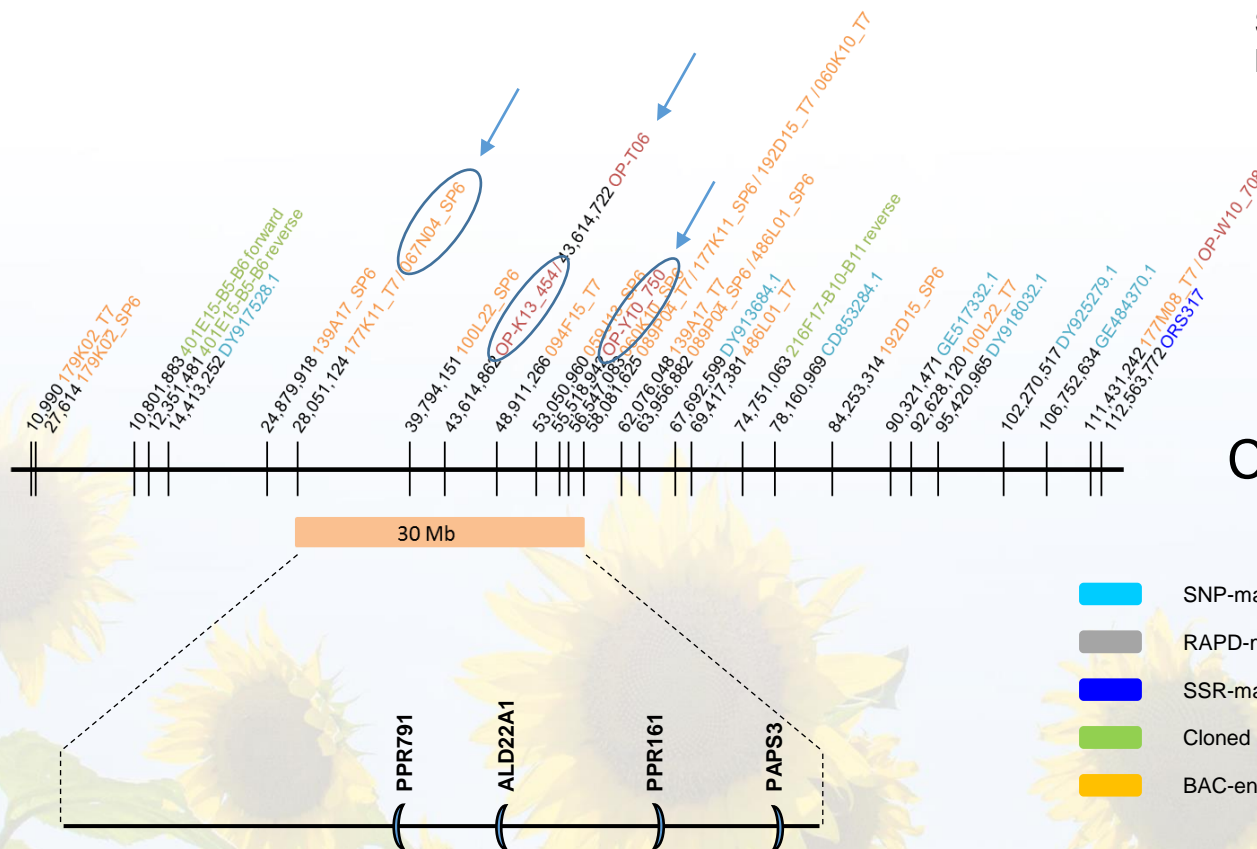


- restoration of fertility by dominant nuclear gene/s
- interactions between mitochondria and nucleus



# Physical localization of the *Rf1* gene (proximal region)

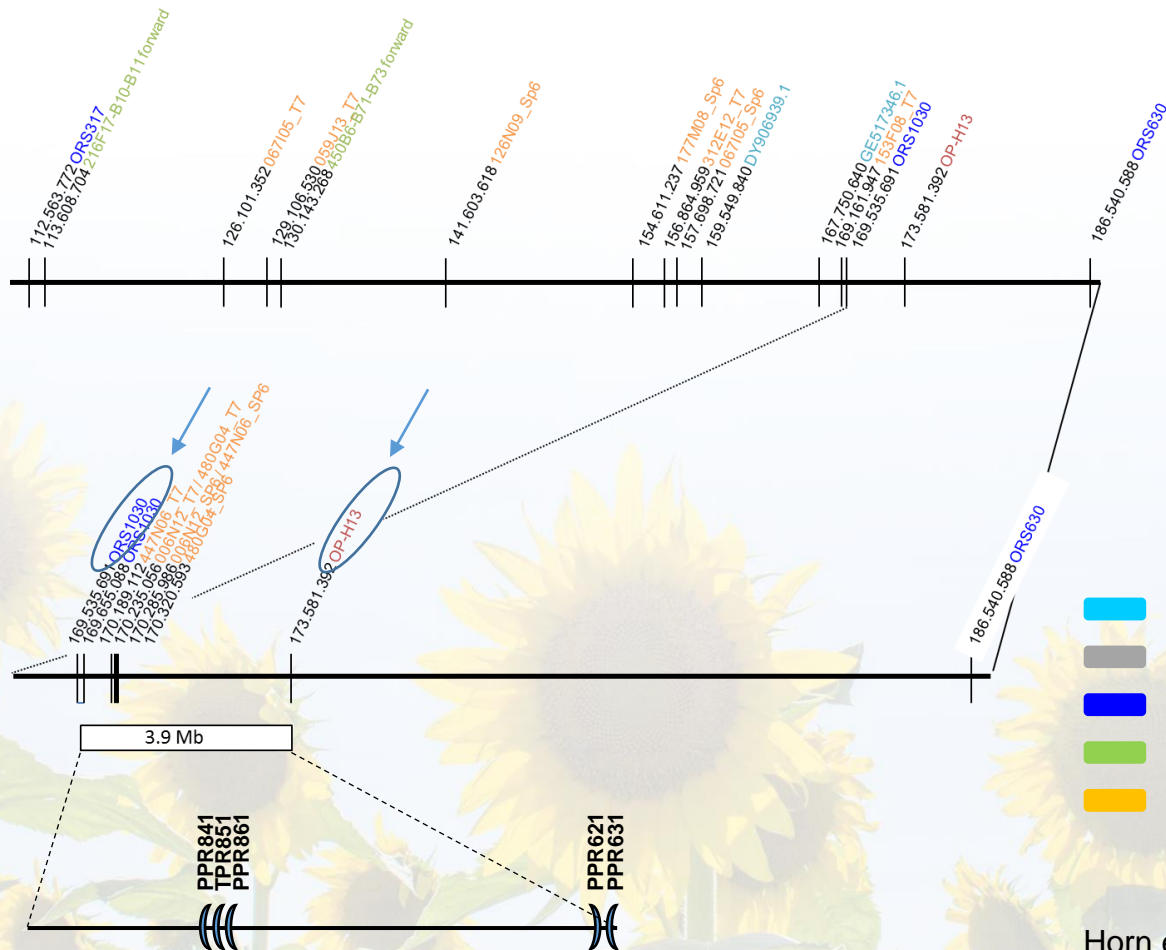
Sunflower genome sequence  
HanXRQ (Badouin et al. 2017)



Chromosome 13

Horn et al. 2019 IJMS 2019, 20:1260

# Physical localization of the *Rf1* gene (distal region)

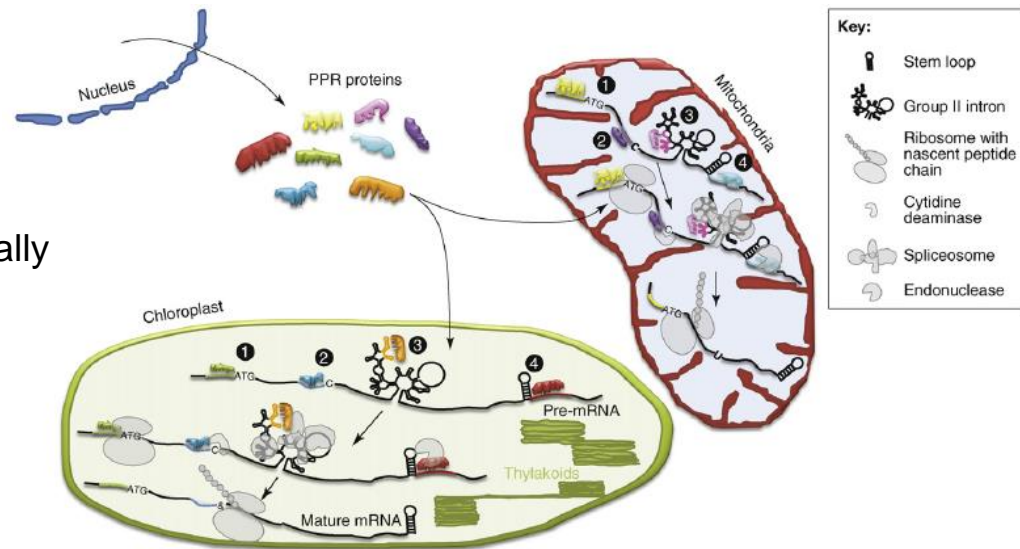


Chromosome 13

Horn et al. 2019 IJMS 2019, 20:1260

# Plant PPR proteins

- P** only canonical 35 aa PPR motifs
- PLS** characteristic triplets: **P**, **L** (long generally 36 aa), **S** (short, generally 31 aa)
- E** with E domain in C-terminus
- DYW** plus terminal DYW domain



TRENDS in Plant Science



## Involved in organellar processes:

1. translation
2. RNA editing
3. Splicing
4. RNA stability

## Additionally:

probable aldehyde dehydrogenase 22A1  
poly(A) polymerase 3 (PAPS3)  
tetratricopeptide-like repeat helical domain

TRENDS in Plant Science

Schmitz-Linneweber & Small (2008) Trends Plant Science 13: 663-670

# Association panel for fertility restoration



Field trial – Bandow near Schwaan, Germany

Maintainer	Restorer
54-3	GN 0778
Armavirsky 3497	RHA 282
Arrowhead	UGA-SAM1-010
CM104	UGA-SAM1-012
CM259	UGA-SAM1-024
CM275	UGA-SAM1-041
CM526	UGA-SAM1-055
CM63	UGA-SAM1-057
Krasnodarets	UGA-SAM1-100
No. 2	UGA-SAM1-101
ROMSUN V3355	UGA-SAM1-118
UGA-SAM1-053	UGA-SAM1-119
UGA-SAM1-082	UGA-SAM1-121
UGA-SAM1-109	UGA-SAM1-136
UGA-SAM1-110	UGA-SAM1-149
UGA-SAM1-140	UGA-SAM1-161
UGA-SAM1-153	UGA-SAM1-165
UGA-SAM1-155	UGA-SAM1-169
UGA-SAM1-156	UGA-SAM1-181
UGA-SAM1-171	UGA-SAM1-186
UGA-SAM1-183	UGA-SAM1-191
UGA-SAM1-185	UGA-SAM1-195
UGA-SAM1-219	UGA-SAM1-198
UGA-SAM1-224	UGA-SAM1-199
UGA-SAM1-264	UGA-SAM1-204
UGA-SAM1-273	UGA-SAM1-207
D-75-10	UGA-SAM1-218
	UGA-SAM1-242
	UGA-SAM1-243
	UGA-SAM1-276
	UGA-SAM1-278
	UGA-SAM1-288



27 maintainer lines, 32 restorer lines

U.S. National Plant Germplasm System

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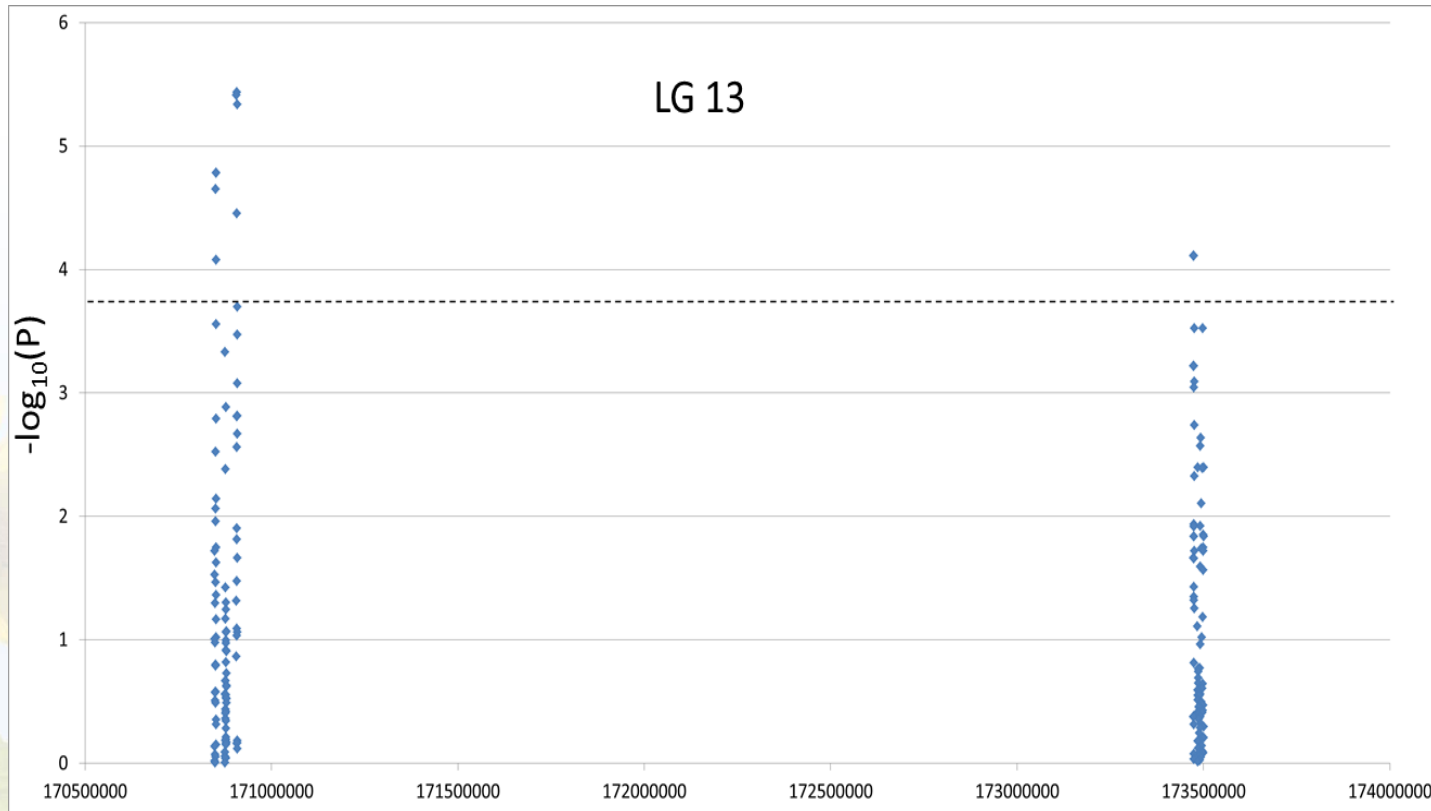
Agriculture and Agri-Food Canada

Plant Gene Resources of Canada: GRIN-CA Information

# SNP-analyses within potential candidate genes

	Gene ID	Short Name	Total Variants	Whole genome area (including 2000 bp upstream and 500 bp downstream of the gene)		Exon	
				No. SNPs	No. INDELS	No. SNPs	No. INDELS
30 Mb	HanXRQChr13g0392791	PPR791	7	-	7	-	-
	HanXRQChr13g0393411	ALD22A1	9	-	9	-	6
	HanXRQChr13g0394161	PPR161	-	-	-	-	-
	HanXRQChr13g0394751	PAPS3	7	4	3	2	2
3.9 Mb	HanXRQChr13g0418841	PPR841	46	36	10	22	3
	HanXRQChr13g0418851	TPR851	56	45	11	24	4
	HanXRQChr13g0418861	PPR861	30	24	6	1	-
	HanXRQChr13g0419621	PPR621	30	26	4	22	1
	HanXRQChr13g0419631	PPR631	92	75	17	7	-

# Association studies for the restorer *Rf1* gene



3.9 Mb region

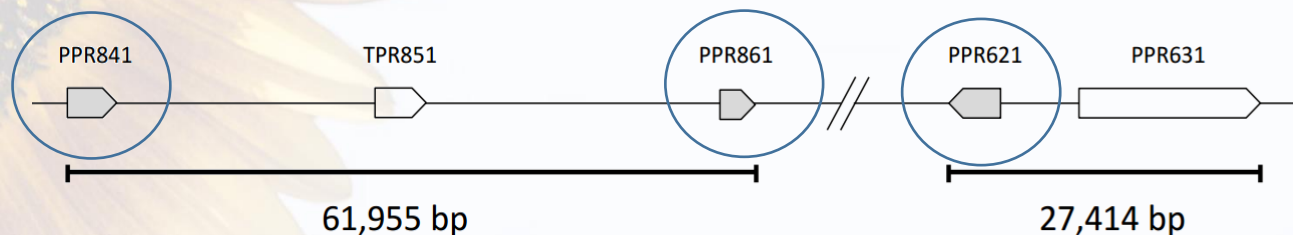
Distribution of all SNPs in the 3.9-Mb-region

Horn et al. 2019  
IJMS 2019, 20:1260



# Haplotypes for restorer and maintainer lines

Genotypes	<b>PPR841.26</b>	PPR841.29	<b>PPR841.38</b>	PPR841.39	PPR861.3	PPR861.9	PPR861.11	<b>PPR861.19</b>	<b>PPR621.5</b>	<b>PPR621.11</b>	Percentage
SNP	T/C	G/A	G/A	C/T	G/A	A/T	C/T	G/C	G/C	C/A	
HanXRQ	TT	GG	GG	CC	GG	AA	CC	GG	GG	CC	
Maintainer	TT	GG	GG	CC	GG	AA	CC	GG	GG	CC	85.2 %
Restorer	CC	GA	AA	CT	AA	TT	CT	CC	CC	AA	56.3 %
Restorer	CC	GA	AA	CY	RA	WT	CT	CC	CC	AA	81.3 %



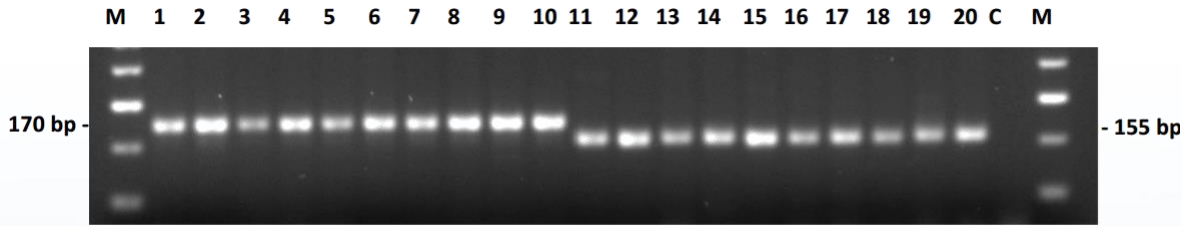
Schematic distribution of the five candidate genes in the 3.9-Mb-region derived from the HanXRQ genome annotation

Horn et al. 2019  
IJMS 2019, 20:1260

# Marker development for the *Rf1* gene

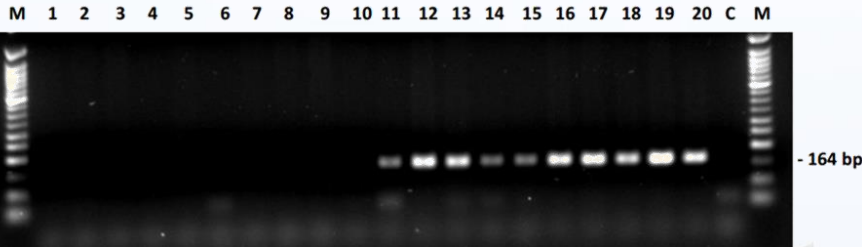
restorer lines

maintainer lines

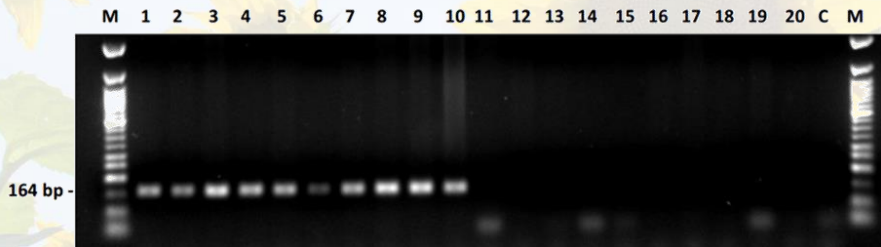


PAMSA Marker - 67N04\_P

G to A mutation in the BAC-end sequence 67N04-B2 BamHI (RHA 325) observed in comparison to HanXRQ



PPR621.5M - G for maintainers



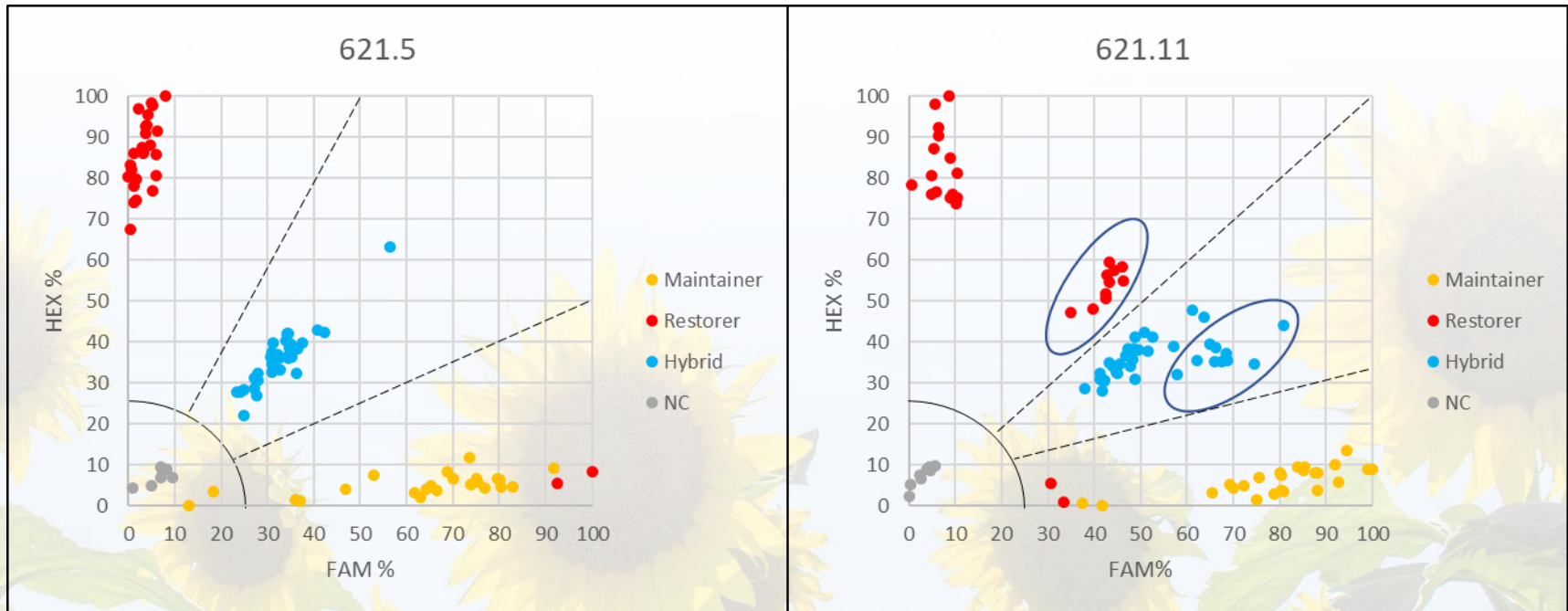
PPR621.5R - C for restorers

Horn et al. 2019  
IJMS 2019, 20:1260

Validation in an association panel consisting of 557 accessions

# KASP marker development for the *Rf1* gene

Candidate gene PPR621



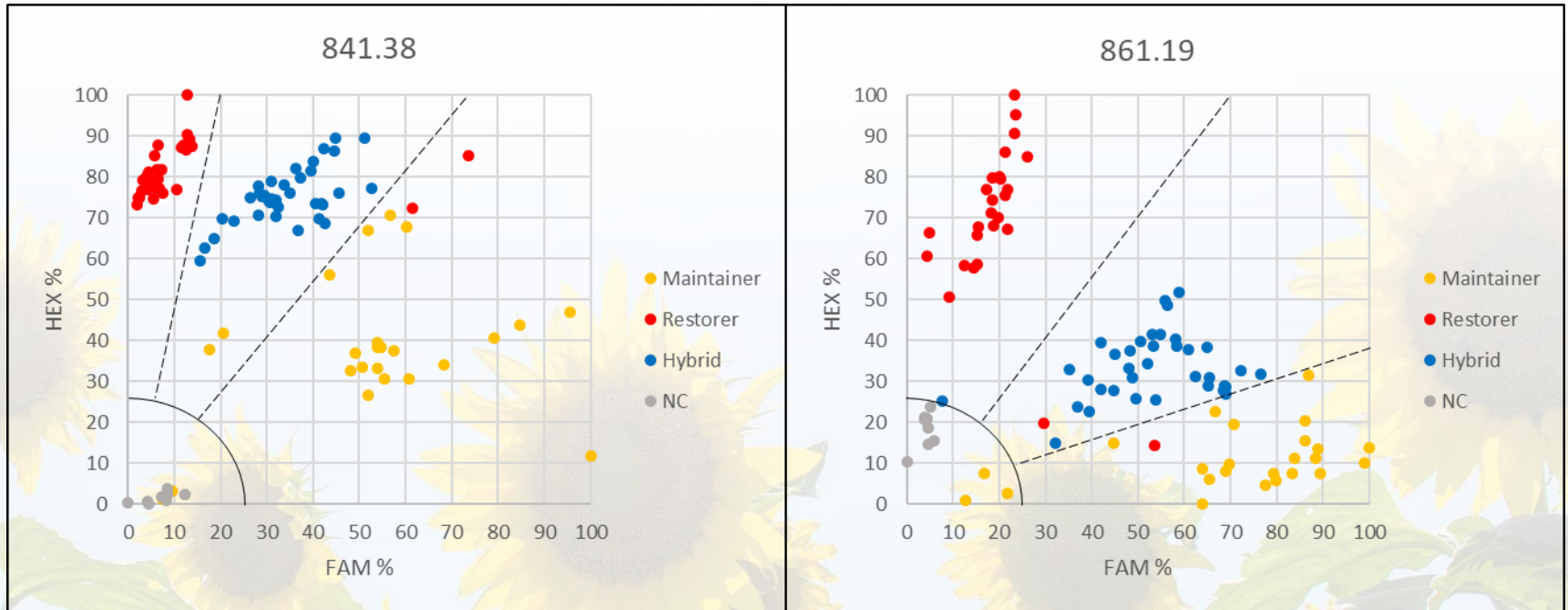
G/C

C/A

Radanovic et al. 2022  
Genes 2022, 13:465

# KASP marker development for the *Rf1* gene

Candidate genes PPR841 and PPR861



G/A

G/C

Radanovic et al. 2022  
Genes 2022, 13:465



## Summary

- Physical localization of the restorer gene *Rf1* in two separate regions of 30 Mb and 3.9 Mb on chromosome 13
- Nine potential candidate genes annotated in these regions
- Association studies in an association panel of 59 lines (27 maintainer lines and 32 restorer lines) via NGS
- SNP analyses revealed 10 SNPs significantly associated with fertility restoration within three potential candidate genes
- Effects of SNPs on the amino acid sequences revealed
- Marker development and analyses in a large association panel
- KASP markers for line development and genetic purity testing

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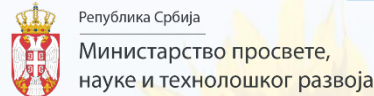
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