IECPS 2021
The 2nd International Electronic Conference on Plant Science
01–15 DECEMBER 2021 | ONLINE
10th Anniversary 2012 – 2021
Chaired by DR. ADRIANO SOFO
plants
MDPI

Metagenomics of distant hybrids in the genus Ribes (Grossulariaceae)

Eduard Machs [1,*], Olga Gavrilova [1], and Olga Tikhonova [2]

[1] Komarov Botanical Institute of the Russian Academy of Sciences, 197376, Russia, St.Petersburg, ul. prof. Popova, dom 2;
[2] Vavilov All-Russian Institute of Plant Genetic Resources, 190000, Russia, St.Petersburg, ul. Bolshaya Morskaya, 42.
* Corresponding author: emachs@binran.ru

Abstract: Metagenomics of distant hybrids in the genus Ribes (Grossulariaceae)

The genus Ribes consists of several subgenera and approximately 150 species. Some authors divide the genus into two genera - currants and gooseberries. Within the genus, the level of hybridization is higher into gooseberries than in intersectional currants, currant-gooseberry and intersectional currants hybrids were created artificially using polyploidy method. By morphological, palynomorphological, karyological and other characteristics, currant-gooseberry hybrids are contrasting. Tetraploid currant-gooseberry hybrids Josta and Kroma have pollen grains with an intermediate morphological type, and pollen of the triploid Dlinnokistnaya CGL is not typical for the family. We performed a comparative metagenomic analysis of these three hybrids of known origin and their parental forms (*R. niveum*, *R. reclinatum*, *R. divaricatum* and *R. nigrum*) in order to assess changes in their genomes for the ITS1 of 35S rRNA gene. Intragenomic polymorphism was studied by locus-specific NGS sequencing on the Illumina MiSeq platform using primers ITS-1P and ITS-2. It was shown that the ribotypes of the hybrids correspond to those of the parental forms. Ribotypes of unknown origin, highly homologous to other currants, were found. It has been shown that most of the pseudogenes are not conserved in hybrids. Comparative plant metagenomics is an informative method for studying hybridization and hybrids of unknown origin.

Keywords: distant hybrids; intragenomic polymorphism; 35S rRNA

Distant hybrids

Kroma, к-32609. 4n=32. Sweden (Alnarpe).
(*R. nigrum* × *Grossularia*) × (*R. nigrum* × *G. nivea*).

Jošta, к-34031. 4n=32, Germany, Max Planck Institute.
(*R. nigrum* × *G. reclinata*) × (*R. nigrum* × *G. divaricata*).

Dlinnokistnaya CGL, к-14550. 3n=24. Russia, Michurin institute.
Kyzirgan (*R. altissimum*) × Davison's eighth form (*R. nigrum*).

Parental forms

***Ribes niveum*, k-28706**

***Ribes reclinatum*, k-8196**

***Ribes divaricatum*, k-8187**

***Ribes nigrum* ssp. europeum**

***Ribes nigrum* Davison's eighth form.**

***Ribes altissimum* Turcz. Lost.**

IECPS 2021


R. niveum


R. divaricatum


R. nigrum ssp. europeum


Kroma


Jošta


Dlinnokistnaya CGL

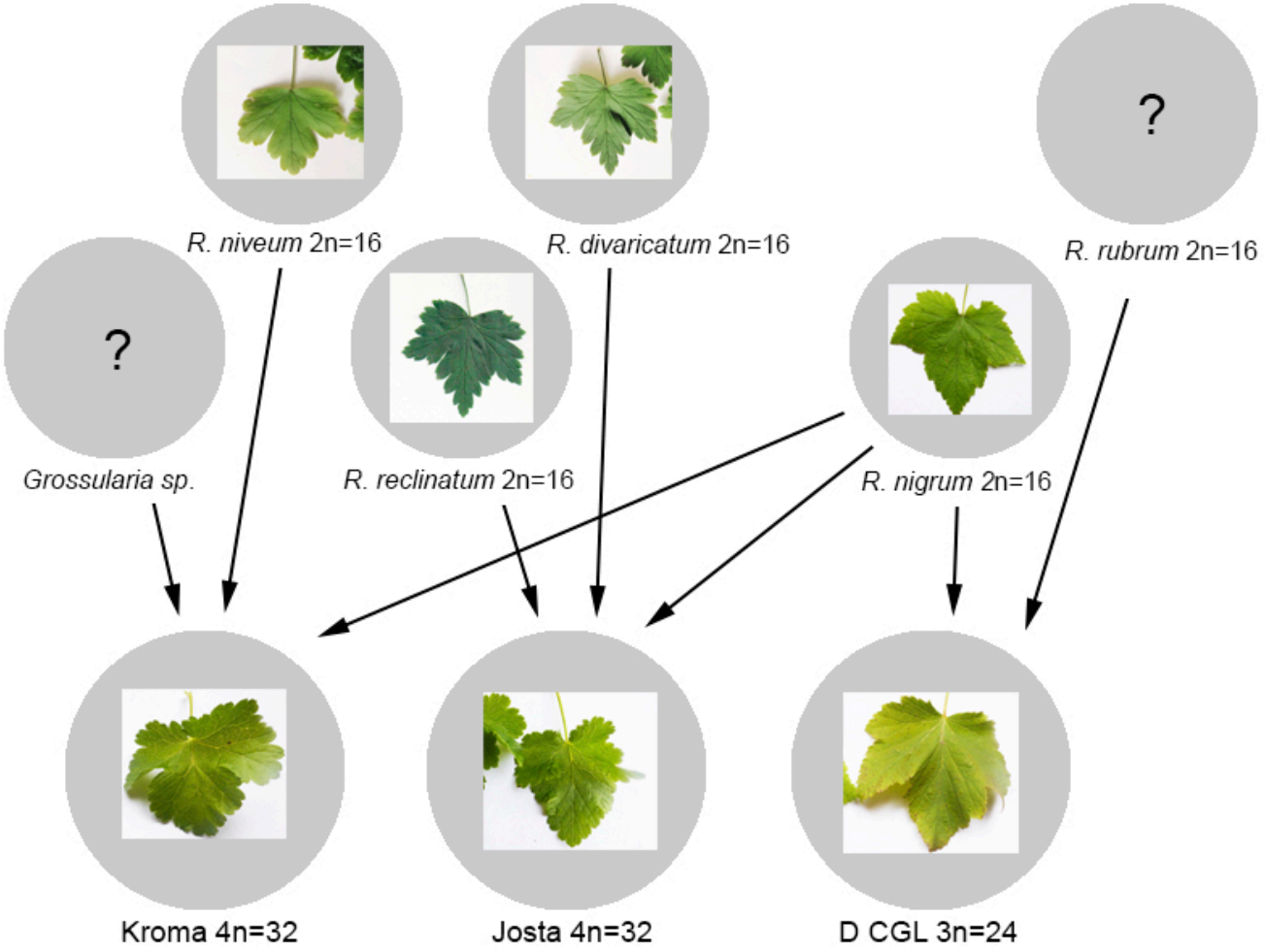
IECPS 2021

Typical morphological and biological characteristics of hybrids and their parental forms

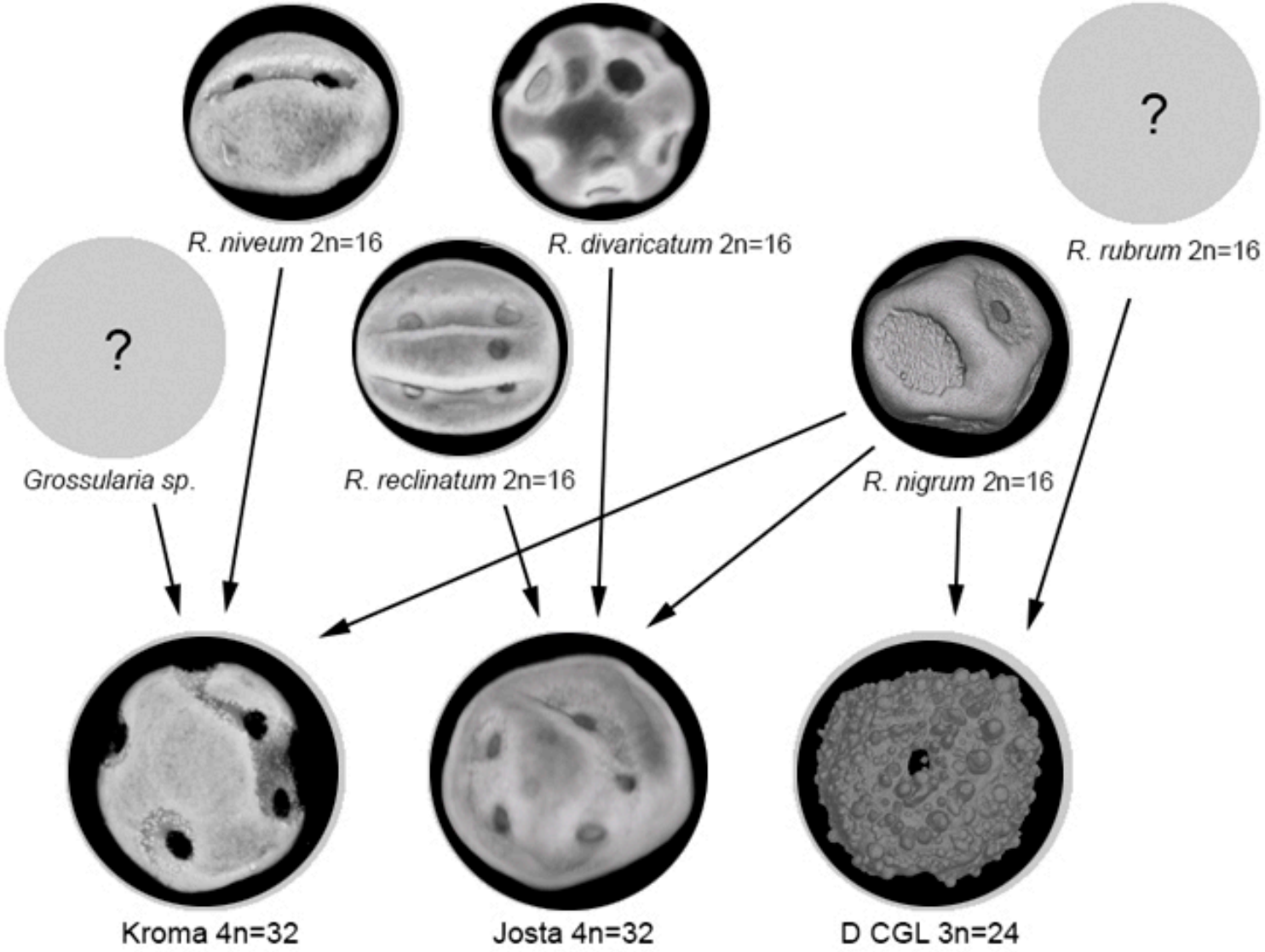
Characters	*R. reclinatum*	*R. divaricatum*	*R. niveum*	Josta	Kroma	*R. nigrum*	*R. altissimum*	*R. rubrum*	D CGL
Pollen type	Gooseberry			Intermediate		Currant			Atypical
Brush type Leaf type Flower type Raceme Fruit spur Calyx	Gooseberry					Black currant	Red currant		
Raceme length	Short					Polymorphic			Long
Flowers	1 - 3			2 - 4		3 - 15	20 - 40		11 - 16
Throns	+			-					
Fruits	Medium	Small		Big		Small			
Resistance (powdery mildew)	+					Polymorphic	-		+
Resistance (antracnose)	-							Polymorphic	+
Resistance (gall mite)	-			+		-			+

IECPS 2021

Polyploidy method



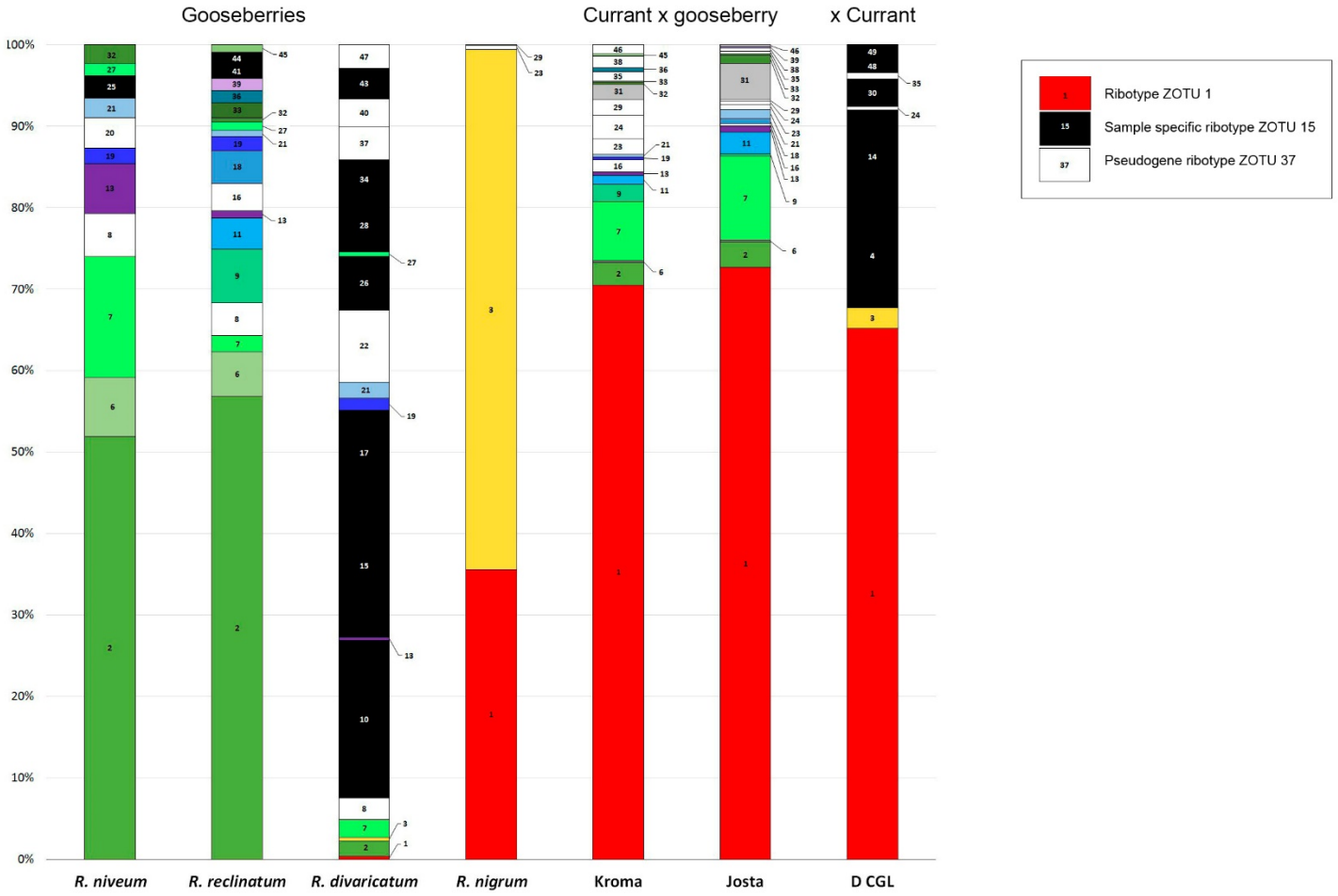
Palynomorphology



NGS Methods

- **DNA extraction:**
  Doyle&Doyle [1]
- **Primers:**
  ITS-1P [2], ITS-2 [3]
- **Thermal Cycler:**
  C-1000 Bio-Rad
- **Phire Hot Start II DNA Polymerase:**
  Thermo Scientific Phire Plant Direct PCR Master Mix (#F-160)
- **Cycling protocol:**
  Initial denaturation: 98°C 5 min.
  30 cycles: denaturation 98 °C 5 s, annealing 56 °C 5 s, extension 72 °C 15 s.
  Final extension: 72 °C 1 min.
- **Sequencing:**
  Illumina MiSeq
- **Data processing:**
  Trimmomatic [4], Fastq-join [5], Vsearch [6], Mega [7], BLAST, Excel.

Results



IECPS 2021

Pseudogenes with long deletions

Deletion	Length	Start	End	Num ZOTU	ZOTU
1	4	289	292	425	22 29
2	5	45	49	1123	8 27 37 46
3	11	56	66	364	23 35
4	15	153	167	121	38
5	21	99	119	813	16 20 47 50
6	22	33	54	813	16 20 47 50
7	48	72	119	364	23 35
8	57	190	245	354	32 40

- The pseudogene is a highly homologous currant ribotype with a long (4-57 bp) deletion.
- The position and length of the deletions are not random.
- The same deletions are found in many ribotypes.
- Pseudogenes of parental forms were not found in hybrids.
- Probably, pseudogenes are formed during hybridization and are eleminated.
- Possibly, the mechanism of elimination of ribotypes of parental forms is associated with rapid multiple mutations, long deletions, changes in the secondary structure of ITS1, and splicing.

Conclusion

- Distant hybridization leads to mixing of morphological characters and genome rearrangement.
- Both morphological characters and ribotypes are inherited asymmetrically.
- In Kroma and Josta, the morphological characters correspond to gooseberry type, although in terms of ribotypes these hybrids are closer to *R. nigrum*
- The pollen of Kroma and Josta is highly fertile and viable, it has the characteristics of currants and gooseberries.
- The distant hybrid of black and red currants D CGL has sterile and atypical pollen.
- The distribution of ribotypes of parental forms in hybrids is rather complex.
- The ribotypes of the parental forms are retained in hybrids.
- Some ribotypes are eliminated after several hybridization steps.
- The number of different ribotypes-pseudogenes in hybrids is at least 3 times higher as compared with studied parental forms.

References

1. Doyle JJ, Doyle JL. A rapid DNA isolation procedure for small quantities of fresh leaf tissue. Phytochemical Bulletin. 1987;19(1):11-15.
2. Ridgway K. P., Duck J. M., Young J. P. W. Identification of roots from grass swards using PCR-RFLP and FFLP of the plastid trnL (UAA) intron // BMC Ecology. 2003. Vol. 3(8e).
3. White, T. J., T. Bruns, S. Lee, and J. W. Taylor. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. Pp. 315-322 In: PCR Protocols: A Guide to Methods and Applications, eds. Innis, M. A., D. H. Gelfand, J. J. Sninsky, and T. J. White. Academic Press, Inc., New York.
4. Bolger, A. M., Lohse, M., & Usadel, B. (2014). Trimmomatic: A flexible trimmer for Illumina Sequence Data. Bioinformatics, btu170.
5. Aronesty E. (2013). TOBioiJ : "Comparison of Sequencing Utility Programs", DOI:10.2174/1875036201307010001
6. Rognes T, Flouri T, Nichols B, Quince C, Mahé F. (2016) VSEARCH: a versatile open source tool for metagenomics. PeerJ 4:e2584. doi: 10.7717/peerj.2584
7. Sudhir Kumar, Glen Stecher, Michael Li, Christina Knyaz, and Koichiro Tamura (2018) MEGA 7: Molecular Evolutionary Genetics Analysis across computing platforms. Molecular Biology and Evolution 35:1547-1549

Acknowledgments

- The study was carried out using the equipment of the resource center "Genomic Technologies, Proteomics and Cell Biology" of ARRIAM
- This research was funded within the framework of the state assignment of Komarov Botanical in-stitute RAS on topics AAAA-A18-118040290161-3 and No. AAA-A18-118031690084-9 and within the framework of the Agreement with the Ministry of Science and Higher Education of the Russian Federation on the provision of a grant from the federal budget in the form of a subsidy No. 075-15-2021-1056. Komarov Botanical institute RAS..
- Federal Research Center Vavilov All-Russian Institute of Plant Genetic Resources.