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Metagenomics of Distant Hybrids in the Genus *Ribes* (Grossulariaceae) †

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Abstract: Currant-gooseberry intersectional hybrids were created artificially using polyploidy method. By morphological, palynomorphological, karyological and other characteristics, currant-gooseberry hybrids are contrasting. We performed a comparative metagenomic analysis of three distant hybrids of known origin and their parental forms by locus-specific NGS sequencing on the Illumina MiSeq platform. The ribotypes of 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 hybrids of unknown origin.

Keywords: distant hybrids; intragenomic polymorphism; 35S rRNA; NGS

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1. Introduction

Just as in traditional metagenomics, the species composition of environmental samples is determined according to DNA marker regions, so a comparative analysis of intragenomic polymorphism of plants based on the same methods shows the presence of a large number of haplotypes of various origins and allows to study, with some limitations and accuracy, the history of hybridization processes. In this work, we will illustrate how ITS1 5.8S rRNA marker region changes in distant hybrids after a small number of hybridization steps.

The genus *Ribes* L. consists of several subgenera and approximately 150 species [1,2]. Some authors divide the genus into two genera—currants and gooseberries [3–5]. Currant-gooseberry hybrids were created artificially [6,7]. By morphological, palynomorphological, karyological and other characteristics, currant-gooseberry hybrids are contrasting [8,9]. Tetraploid hybrids have pollen grains with an intermediate type of pollen [10,11]. The pollen of the triploid DCGL is not typical for the family; it is ugly and has a warty exine structure [12]. We selected palynomorphologically contrasting hybrids distinguishable from the parental species (Josta, Kroma, Dlinnokistnaya CGL) and their parental forms: *R. niveum* Lindl., *R. reclinatum* L., *R. divaricatum* Douglas and *R. nigrum* L. ssp. *europeum*.

2. Materials and Methods

Plant material from the garden collection of Komarov Botanical Institute of the Russian Academy of Sciences and collection of black currant and gooseberry NPB "Pushkin and Pavlovsk laboratory of VIR" (vouchers VIR), St. Petersburg, Russia have been studied.

Kroma (VIR k-32609). Allotetraploid (4n = 32). It was bred in Sweden, at the agricultural station in Alnarp, by crossing hybrid forms (*R. nigrum* × *Grossularia*) × (*R. nigrum* × *G. nivea*). It is of interest for breeding as a source of high resistance to fungal diseases and gall mites, a high content of pectin substances in fruits and ascorbic acid in leaves [11,13].

Jošta (VIR k-34031). Allotetraploid (4n = 32). It was created in the 70s of the last century at the Max Planck Institute, initial forms: (*R. nigrum* × *G. reclinata*) × (*R. nigrum* × *G. divaricata*). The variety is of interest for breeding as a source of high resistance to American powdery mildew, anthracnose and gall mites, a high content of pectin substances in fruits (1.12%), ascorbic acid and P-active substances in leaves [11,13].

Dlinnokistnaya CGL (VIR k–14550). Triploid (3n = 24). Bred in Michurin FNC, initial forms: Kyzyrgan (*R. altissimum* Turcz.) × Eighth Davison (*R. nigrum* L. ssp. *europeum*) [14]. *R. altissimum* is a natural far hybrid of red and black currant [5].

Intragenomic polymorphism was studied by locus-specific NGS sequencing on the Illumina MiSeq using total DNA samples [15] and primers ITS1P [16], ITS2 [17]. Amplification was carried out according to the protocol: initial denaturation 98 °C for 5 min; 30 cycles (98 °C 5 s, 56 °C 5 s, 72 °C 15 s); final synthesis 72 °C 1 min; storage 12 °C.

Data processing was performed using FastQC (Babraham Bioinformatics), Trimmomatic [18], Fastq-join [19], Vsearch [20], SplitsTree [21] and TCSBU [22]. ZOTU filtering was performed manually using Mega 7 [23] and BLAST NCBI.

3. Results

3.1. Pseudogenes

It is well known that almost all gooseberries and currants are hybrids to one degree or another, and in hybrids some ribotypes are eliminated. The studied samples contain ribotypes with extended deletions, possibly affecting splicing, since it is believed that splicing is associated with the secondary structure of the transcript. There are many pseudogenes in parental forms, but most of them are not preserved in hybrids and cannot be used for comparative analysis of ribotypes (Table 1, Figure 1).

Table 1. Highly homologous currant ribotypes (pseudogenes) with long deletions.

Deletion	Length (bp)	Start	End	Number of 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	**16**	**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**

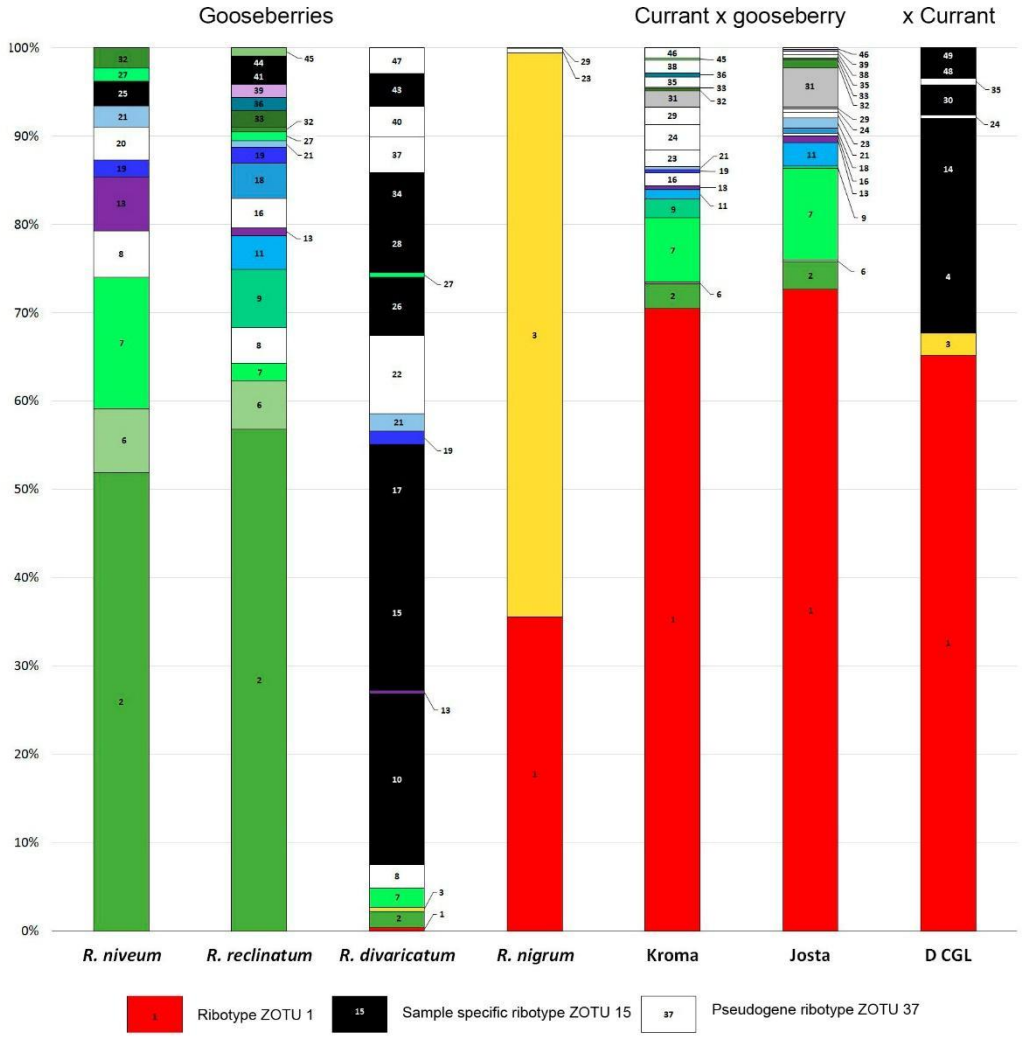

Figure 1. Ribotypes of distant hybrids and parental forms. Numbers and colors indicate comparable ZOTU (Zero-radius Operational Taxonomic Unit). Incomparable sample specific ribotypes are indicated as black, pseudogenes as white.

3.2. Ribotypes

The results of metagenomic analysis of 50 most frequent ribotypes are presented on Figure 1. Zotu1 of the parental form *R. nigrum* was found in all three hybrids in an amount of more than 60% and in a small amount in *R. divaricatum*. The second ribotype of *R. nigrum* (Zotu3) was found in a small amount only in D CGL and *R. divaricatum*. These two ribotypes differ in only one nucleotide substitution C/A. However, their inheritance is asymmetrical, and Josta and Kroma do not have Zotu3. Thus, only two ribotypes of *R. nigrum* were found in Kroma and Josta: Zotu1 and the pseudogene Zotu29.

The other ribotypes of currant-gooseberry hybrids came from gooseberries. Concerning *R. divaricatum*, the ribotypes Zotu13, Zotu19, and Zotu21 found in hybrids are also found in other gooseberries, so it is impossible to reliably trace their inheritance. In addition, *R. divaricatum* differs from other gooseberries by the presence of a number of specific ribotypes (Zotu10, 15, 17, 26, 28, 34, 43), a small number of *R. nigrum* ribotypes (Zotu1, 3), and a number of pseudogenes (Zotu8, 22, 37, 40, 47). This indicates that *R. divaricatum* may be a natural far hybrid, having in its history the parental form *R. nigrum*. The ribotypes of *R. niveum* and *R. reclinatum* (Zotu2, 6, 7, 9, 11, and others) are well traced in the Kroma and Josta. In D CGL, no gooseberry ribotypes were found.

4. Discussion

More than a half of ribotypes-pseudogenes were found for all seven samples (282 Zotu), and in most cases multiple deletions were observed. For example, Table 1 lists the deletions for the first 50 Zotu. Only Zotu38 has a unique deletion, other deletions are characteristic of several ribotypes. Probably, pseudogenes are not inherited, since in most cases they are characteristic of the sample. Therefore, they cannot be used for comparative analysis of hybrids. It is possible that the elimination of ribotypes in hybrids occurs at a high rate through multiple mutations. Note that long deletions are the only trait indicating that the ribotype is a pseudogene. This cannot be said about ribotypes with many nucleotide substitutions, because even if they are not singletons, their frequency is low, and the variety of substitutions is very high, which significantly reduces homology.

We believe that the first 50 Zotu are sufficient for a comparative metagenomic analysis of distant hybrids and hybrids of unknown origin. For example, in case of D CGL, it can be seen that there are a large number of specific ribotypes, probably corresponding to the lost parental forms from red and black currants, since they are highly homologous to *R. janczewskii* Pojark., *R. himalense* Royle ex Decne, *R. petraeum* Wulfen, *R. triste* Pall., *R mandshuricum* Kom., *R. palczewskii* Pojark.. In addition, *R. reclinatum* and Kroma probably contain ribotypes of unknown origin, highly homologous (85%) to *R. andicola* Jancz. Since there is no evidence that the species *R. andicola* could be the parental form of the Kroma, it can be assumed that the ribotypes of this group could have been contained in the earlier parental forms of gooseberries. We believe that a comparative metagenomic analysis of hybrids is quite informative, since it does not contradict the history of hybrids of known origin

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Conflicts of Interest: The authors declare no conflict of interest.

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