

Reviewer assessment

Author of thesis: Denisa Kubíková

Title: Cytonuclear interactions in grass autopolyploids and allopolyploids

Type of thesis: Bachelor

Evaluation criteria		Grades						
		A	B	C	D	E	F	Non-evaluable
1	Scope of thesis, chapters proportion		x					
2	Review quality (i.e. quality and accuracy, number of references used)		x					
3	Objectives achievement		x					
4	Accuracy and completeness of figures and tables legends (i.e. understandability, consistency, abbreviation explanation, correct using of units)			x				
5	Accuracy of references using (i.e. absence of references quoted in text and list of references, formal stylistic consistency)			x				
6	Accuracy of summary in Czech and English		x					
7	Graphic quality of text and figures			x				
8	Language and stylistic quality, using of valid/ standard terminology and nomenclature			x				
9	Choice of appropriate experimental methods	x						
10	Comprehensibility and conciseness of used methods description		x					
11	Quality of experimental data processing			x				
12	Results interpretation			x				
13	Discussion (results summary and its implementation in the context of current research/knowledge)		x					

Final Grade	C
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Conclusion: thesis is recommended to defence

Olomouc 23 May 2022

Signature: Doc. Ing. Petr Smýkal, Ph.D.

The bachelor thesis of Denisa Kubíková entitled: ***Cytonuclear interactions in grass autopolyploids and allopolyploids***, focuses on the study of polyploidy in plants.

The aim of this bachelor thesis was to evaluate intracellular changes following polyploidization and to count copy numbers of genes involved in cytonuclear interactions in auto- and allopolyploids.

In the theoretical part on 17 pages, the introduction of polyploidy and its mechanism of establishment in plants is provided. There are sections devoted to auto- and allo-polyploidy, however, they could be better grouped, in order to avoid extensive subsections into rather short paragraphs. It is followed by a description of the advantage and disadvantages of polyploidy, with a focus on heterosis and biomass in relation to studied grass plants.

The emphasis is given to the little-studied phenomenon of disruption of stoichiometry between the nuclear-encoded and organelle-encoded genes involved in cytonuclear interactions.

The practical part of the thesis deals with: a) microscopic analysis of diploid and polyploidy samples of studied plant material, grasses (namely: *Lolium multiflorum* and *Festuca pratensis*) and measurement of the cell size and counting the number of chloroplasts, and b) determination of the absolute gene copy number for the selected set of genes by digital PCR method. Quite a good number of samples were used for cell size measurement. The data were processed and presented graphically in violin plots, however, the table with specific values would be useful.

Moreover, the proper statistical analysis and comparison between samples should be done, in order to evaluate the significance of differences. It is not sufficient to say that for example they differ in 25.6% in cell volume etc.

There could be a better description of studied plant material, e.g. chromosomal numbers, and genome size. Similarly, some equipment should be described more precisely, providing type etc. (for example confocal microscope, droplet generator).

I value extent of experimental work (122 evaluated plants with about 60 cells per variety used for chloroplast counting), optimization of droplet digital PCR used for copy number estimation.

Some heading, terminology could be better used, for example: 4.2.2.3 Condition optimization (of what?) (p. 25). ddPCR is first explained as a term in full on page 28 (Droplet digital PCR). Description of figures should be self-explanatory including a description of all samples. Currently, the reader has to go back and forward to search for a sample identity. Figure 14 is

out of focus and the description of not comprehensive – it likely shows the temperature gradient of PCR. The size of the MW marker is not provided.

The discussion part is relatively short but provides a critical reflection of the author's work to published data.

In the abstract and conclusion, there is a statement related to differences between freshly established polyploids and subsequent generations. How these were assessed, is neither provided in the material nor mentioned in the results.

Questions:

- It is mentioned that polyploidy cells/tissues can be found in an otherwise diploid organism, this is cited for animals (p. 3), what about the occurrence of this phenomenon in plants?
- Is it correct to say that in crops, autopolyploidy is frequent? In the case of bananas or potato. Are those indeed autopolyploids? What about allopolyploidy in crops?
- What is the reported frequency of unreduced gametes, truly only up to 2% (p.5)?
- How old were sampled plants? It is stated that young leaves were used for measurement, however cell size might differ during the development (also during vegetative vs generative stage).
- How the volume of chloroplasts was measured? These are irregular, rod-like structures.
- How the heterosis was measured and quantified? First mentioned in results on page 30.