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Faculty
of Science

Jihočeská univerzita
v Českých Budějovicích
University of South Bohemia
in České Budějovice

STATEMENT OF THE BACHELOR THESIS REVIEWER

Name of the student: Katharina Böttinger

Thesis title: Genomic distribution of major families of satellite DNA in *Vicia faba* and *Lathyrus sativus*

Supervisor: RNDr. Jiří Macas, Ph.D.

Reviewer: RNDr. Magda Zrzavá, Ph.D.

Reviewer's affiliation: University of South Bohemia in České Budějovice, Faculty of Science, Branišovská 1760, České Budějovice, 37005

Point scale¹ Points

(1) FORMAL REQUIREMENTS

Extent of the thesis (for bachelor theses min. 18 pages, for masters theses min. 25 pages), balanced length of the thesis parts (recommended length of the theoretical part is max. 1/3 of the total length), logical structure of the thesis	0-3	3
quality of the theoretical part (review) (number and relevancy of the references, recency of the references)	0-3	3
Accuracy in citing of the references (presence of uncited sources, uniform style of the references, use of correct journal titles and abbreviations)	0-3	2
Graphic layout of the text and of the figures/tables	0-3	2
Quality of the annotation	0-3	3
Language and stylistics, complying with the valid terminology	0-3	3
Accuracy and completeness of figures/tables legends (clarity without reading the rest of the text, explanation of the symbols and labeling, indication of the units)	0-3	3
Formal requirements – points in total		19

(2) PRACTICAL REQUIREMENTS

Clarity and fulfillment of the aims	0-3	3
Ability to understand the results, their interpretation, and clarity of the results, discussion, and conclusions	0-3	3
Discussion quality – interpretation of results and their discussion with the literature (absence of discussion with the literature is not acceptable)	0-3	3
Logic in the course of the experimental work	0-3	3
Completeness of the description of the used techniques	0-3	3

¹ Mark as: 0-unsatisfactory, 1-satisfactory, 2-average, 3-excellent.

Experimental difficulty of the thesis, independence in experimental work	0-3	3
Quality of experimental data presentation	0-3	3
The use of up-to-date techniques	0-3	3
Contribution of the thesis to the knowledge in the field and possibility to publish the results (after eventual supplementary experiments)	0-3	3
Formal requirements – points in total		27

POINTS IN TOTAL (MAX/AWARDED)

48

46

The aim of the bachelor thesis was to perform the physical mapping of 14 satellite sequences previously identified in genomes of *Vicia faba* and *Lathyrus sativus* by means of bioinformatics approaches. The satellites were predicted to be either highly abundant in the genome of particular species or possibly associated with centromeric histone CENH3. The work involved several methods, starting with optimization of chromosome preparation, PCR amplification of the satellite sequences, cloning, two methods of probe labeling, and two variants of FISH – simple FISH mapping and FISH mapping combined with immunodetection of CENH3. All but one satellite sequences were successfully mapped on the chromosomes. The satellites associated with CENH3 are indeed located in the centromeres and the FISH combined with CENH3 immunodetection showed co-localization of one satellite with this centromeric histone. I highly appreciate that the results are presented synoptically in diagrams of individual chromosomes, as well as in tables summarizing all data. The text itself is nicely written, it has reasonable length, and it is nearly free of formal errors. The thesis brings new interesting data which can be easily published.

Suggestions and questions, to which the student has to answer during the defense:

Comments

1. In the fig. 1 the centromere positions should be clearly marked, especially in case of the chromosome I. The narrow white stripe at the section 2 looks like a centromere while the real centromere is covered by color dots. Additionally, the color-coding should be changed since some colors are too similar (e.g. three hues of orange color are difficult to distinguish).
2. I assume that the position of VFB_m3_Fok was already known. If it is so, the first paragraph of chapter 4.1 should be rephrased since there were actually only three satellites analyzed.
3. It would have been nice to see pictures of hybridizations of all mapped satellites, not only a selection. In scientific articles it is nowadays quite usual to show all the data, at least in a supplementary material.

Questions:

1. Is there any plant tribe where all three types of centromere (i.e. monocentric, meta-polycentric, and holocentric) occur?
2. Why did you use different annealing temperatures for PCR amplification and PCR labeling (sections 3.4.2.2 and 3.4.2.3)?
3. For satellites VFBm200 and LASm3 two different probes were used. Why in these cases and not in the others?
4. I believe an important outcome of FISH experiments is the striking difference between

hybridization of the oligo probe LASm3_H1 and cloned probe LASm3_c355 which are designed according to the same satDNA, but gave very different hybridization signals. In light of these results, would you prefer using oligo probes or cloned probes for mapping of putative centromeric satellites?

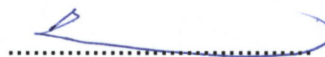
Conclusion:

In conclusion, I

r e c o m m e n d

the thesis for the defense and I suggest the grade 1.

In **České Budějovice** date **12. 9. 2016**

A handwritten signature in blue ink, consisting of a stylized, elongated shape with a small loop at the end.

signature