Genomic fingerprinting of *Camelina* species based on γ-tubulin gene intron length polymorphism

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Introduction

Plants from genus *Camelina* are considered to be one of the most promising sources of oil for biofuel production. Thus false flax (*C. sativa* L. Crantz.) have been the focus of many investigations to date, but the limitation on broader use of *C. sativa* as a genetic model and for gene editing is its hexaploid nature. Beside that other species of this genus are underused despite their potential utility as germplasm donors for camelina genetic improvement.

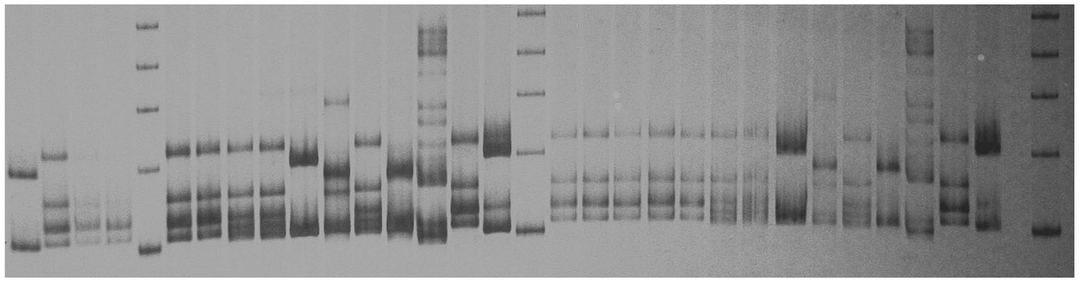
Materials and methods

In this study, DNA profiling of six *Camelina* species (*C. microcarpa*, *C. rumelica*, *C. hispida* var. *grandiflora*, *C. alyssum*, *C. laxa*, which were received from GRIN-Global, USDA (http://www.ars-grin.gov/) and *C. sativa*) was conducted using assessment of γ-tubulin intron length polymorphism. PCR was performed using degenerated primers, amplification products were analyzed via electrophoresis in non-denaturing acrylamide gel.

1. Search for the annotated γ-tubulin nucleotide and amino acid sequences of the *Arabidopsis thaliana*
2. Search for homologous DNA sequences in the genomes of *Zea mays* and *Linum usitatissimum*.
3. Primers design to conserved regions of exons, polymerase chain reaction with primers derived
4. Electrophoresis of the reaction products in 6% non-denaturing polyacrylamide gel, fragments visualization by silver nitrate staining, introns length polymorphism detection in plant genomes

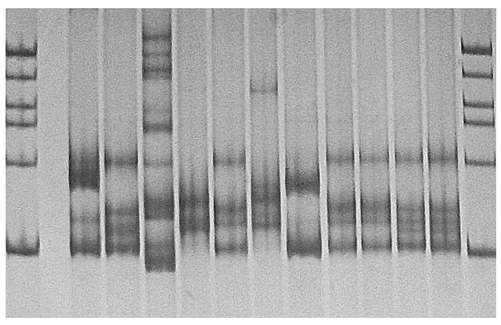
Results

Among seven *C. microcarpa* accessions obtained from USDA (http://www.ars-grin.gov/), two samples (PI650134, PI650135) possessed atypical γ-tubulin intron patterns. *C. microcarpa* had 4 amplicons of 507 bp, 528 bp, 557 bp, 620 bp. The PI650134 accession contained four amplicons, two being similar to *C. rumelica* - 510 bp and 578 bp, as well as 553 bp and 700 bp fragments. The PI650135 accession had two fragments only (507 bp and 600 bp), which are unique compared to other *Camelina* species.



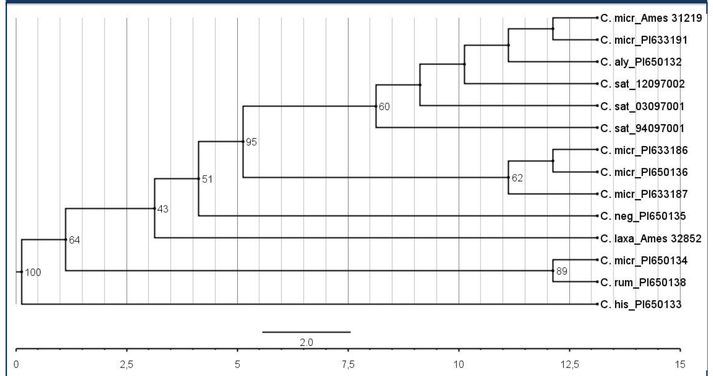
The analysis with SSR markers confirmed differences among all camelina species. Previously, Galasso et al. (2015) described accessions characterized by atypical β-tubulin intron length polymorphism. Brock et al. (2019) described the PI650135 accession as a new species *C. neglecta*.

γ-Tubulin intron profiles of *C. alyssum* and *C. sativa* were very similar to *C. microcarpa*, which could be explained by their common origin. In *C. rumelica,* two fragments (510 bp and 578 bp) were found, while *C. hispida* possessed high number of fragments (about 10) of 498-956 bp length.



Together, these results demonstrate high polymorphism of this species. Because only two copies of γ-tubulin gene are represented in a diploid genome, ploidy can also be evaluated with this method.

Dendrogram



The UPGMA dendrogram based on fragments obtained with the intron lenght polymorphysm of γ-tubulin genes method of *Camelina*.

Conclusions

- Our results confirm that *C. neglecta* and *C. rumelica* are diploid species and, as such, their use could simplify transformation or genome editing versus use of the hexaploid *C. sativa*.
- High similarity of *C. sativa* and *C. microcarpa* γ-tubulin intron amplicons patterns give extra evidence for their close evolutionary relationship. The PI650134 *C. microcarpa* accession and *C. rumelica* are located very close on dendrogram within having different amplicons pattern at same time, which could be explained by their potential joint origin.

References

Brock J.R., Mandáková T., Lysak M.A., Al-Shehbaz I.A. (2019) *Camelina neglecta* (*Brassicaceae*, *Camelineae*), a new diploid species from Europe. PhytoKeys, 115, 51-57.

Galasso I., Manca A., Braglia L., Ponzoni E., Breviario D. (2015) Genomic fingerprinting of *Camelina* species using cTBP as molecular Marker. Am. J. Plant Sci., 6, 1184-1200.