Univerza *v Ljubljani*, Biotehniška Fakulteta

JAVNA AGENCIJA ZA RAZISKOVALNO DEJAVNOST
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Project

Member of University of Ljubljana	University of Ljubljana, Biotechnical Faculty
Code	J4-9307
Project	Genome editing of selected *Brassica* species with CRISPR/Cas9
Period	1.7.2018—30.6.2021
Range in 2018	1.34 FTE
Head	Dr. Jana Murovec
Research activity	Biotechnical sciences
Research Organisation Partners	National Institute of Chemistry
Co-financing Organisation	/
Abstract	The genus *Brassica* comprises a large number of species and subspecies that are consumed either as shoots, leaves, roots, turnip roots or in the form of seeds. Vegetative plant parts are merchandized mainly as raw products, whereas generative parts in a processed form either as oil, meal, powder, protein, condiment, etc. The species have diversified into a large number of agriculturally important morphotypes

	due to domestication and further breeding. Nowadays, the species B. oleracea comprises morphotypes of cabbage, kale, Chinese kale, savoy cabbage, Brussel sprout, kohlrabi, broccoli, cauliflower; the species B. rapa includes morphotypes of pak choi, Chinese cabbage, turnip, and oilseeds. The species B. napus, an allopolyploid, also includes several morphotypes (rapeseed, rutabaga, fodder rape), with rapeseed (canola) being the economically most important as the third oil crop regarding production quantity. Despite their high economic importance, modern biotechnological approaches for breeding and research of *Brassica* species are still lacking. Few published studies on genome editing relied on stable integration of genome editing vectors with A. tumefaciens, which limits their applicability in horticulture. Although the introduced DNA can be segregated out after mutagenesis, the use of transgenesis during plant variety development can still trigger GMO regulation in countries that rely on process-based regulatory approaches. Therefore, we believe that new protocols for DNA-integration-free genome editing of *Brassica* species are needed and that the use of preassembled sgRNA-Cas9 ribonucleoprotein complexes (RNPs) provides an optimal solution. RNPs are exceptional comparing to other expression technologies as they enable DNA-free genome editing. Moreover, the introduction of RNPs generates genome modifications faster as compared to plasmid delivery since they don't need to be transcribed and translated in cells. They are also degraded faster than other vectors, thus limiting exposure to genome editing reagents. It results in fewer off-target modifications, which are currently the biggest drawback of the CRISPR/Cas9 technique. Delivery of RNPs also prevents insertional mutagenesis as no vector is integrated at random into the genome. The main goal of the project is to develop genome editing approaches for *Brassica* species that will rely on delivery of RNPs into protoplasts and microspores and regeneration of edited plants. The project will combine basic science with a final application in horticulture. The results of the proposed project will have an impact on the implementation of cutting-edge technology in the fields of plant breeding and plant biotechnology and will have a substantial impact on their further development. The project is conceived as a collaboration of distinguished scientist with proven track record relevant to this project, belonging to different complementary scientific fields. The exchange of the knowledge,

	methods and scientific approaches will provide new perspectives and solutions that have not been addressed so far, thus enabling further development of involved scientific fields. This collaboration promises excellent scientific results and technical solutions with possibility of international patent application.
Researchers	https://www.sicris.si/public/jqm/prj.aspx?lang=eng&opdescr=search&opt=2&subopt=402&code1=cmn&code2=auto&psize=1&hits=1&page=1&count=&id=17324&slng=&search_term=murovec%2c+jana&order_by=
The phases of the project and their realization	1 Preparation of RNPs 1.1 Bioinformatics analysis (completed) 1.2 Synthesis of sgRNAs (completed) 1.3 Production of Cas9 (completed) 1.4 Testing in vitro cleavage activity of RNPs (completed) 2 Isolation, transformation and regeneration of *Brassica* protoplasts and microspores 2.1 Optimization of protoplasts isolation and regeneration (completed) 2.2 Isolation and regeneration of microspores (completed) 2.3 Transformation of protoplasts and microspores (in progress) 2.4 Enrichment of protoplast and microspore suspensions (in progress) 2.5 Testing genome editing efficiency (in progress) 3 Modification of target genes of agronomic importance 3.1 Development of *Xcc* resistant cabbage plants (in progress) 3.2 Development of cabbage breeding line (in progress)
Citations for bibliographic records	http://izumbib.izum.si/bibliografije/N20200521111633-J4-9307.html