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# **Plant Science**

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## Application of different molecular marker systems for the evaluation of genetic diversity and rhizomania resistance in sugar beet

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Statement of the Problem: Rhizomania belongs to the most devastating sugar beet diseases in terms of economical losses. The most efficient method of symptoms prevention and restriction of the disease spread turned out to be resistance breeding. Despite a significant progress in this field, there is still a risk of the disease outbreak due to the appearance of rhizomania-resistance breaking strains of Beet Necrotic Yellow Vein Virus, the causative agent of rhizomania. The purpose of this study is to evaluate different molecular marker systems in breeding materials of sugar beet, including rhizomania-resistance associated markers along with single nucleotide polymorphisms (SNPs), selected minisatellites and microsatellites of beet. This has been realized as a part of the multiyear program of the PBAI-NRI, aiming at enhancement of sugar beet resistances. Methodology & Theoretical Orientation: Sugar beet breeding materials representing different phenotypic responses to the virus were characterized by the following methods: DNA isolation according to Davis (1986), PCR, electophoresis on LE agarose gels stained with ethidium bromide (HR agarose was used for minisatellite and microsatellite fragments), documentation and evaluation of the products (Gel DocTM 2000, BIO-RAD; QuantityOne). Genetic similarity was estimated using Nei and Li formula (1979) and these coefficients applied for the construction of dendrograms. For recognition of polymorphic SNPs, a restriction fragment length polymorphism (RFLP) procedure was employed and further verified. Findings: In the populations studied, the identified SNPs corresponded to their phenotypic manifestations and fully confirmed the sequencing results, and as such may constitute candidate markers for selection. Using other molecular sequences, appropriate dendrograms were constructed, allowing for precise evaluation of relationships between materials under the study. Conclusion & Significance: Further significance of the markers obtained is being performed on subsequent generations of the breeding materials in order to recognize a linkage of these variants with rhizomania resistance sources.



Figure 1: Application of molecular markers for discrimination of sugar beet breeding materials differying in rhizomania resistance.  $A_iA^i$  identification of polymorphic SNPs with RFLP allowed for distinction of homo- and heterozygotes with regard to rhizomania-resistance accompanying loci; B\_C - dendrograms obtained by UPGMA-based clustering using arbitrary sequences and tandem repeats (micro- and minisatellites), respectively, illustrating genetic distances between materials under the study.

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#### **Recent Publications**

- 1. Biancardi E, Lewellen RT (2016) Introduction. In: Rhizomania, Biancardi E, Tamada T (eds). Springer International Publishing Switzerland pp.22-24.
- 2. Davis LG, Dibner MD, Battey JF (1986) Preparation of DNA from eukaryotic cells. In: Basic methods in molecular biology, Davis LG, Dibner MD, Battey JF (eds). Elsevier New York pp.42–50.
- 3. Nei M, Li WH (1979) Mathematical model for studying genetic variation in terms of restriction endonucleases. Proceedings of the National Academy of Sciences of the United States of America 76:5269–5273.
- 4. Richardson KL, Mackey B, Hellier B (2019) Resistance in *Beta vulgaris* L. subsp. *maritima* (L.) Thell. to the Rz1-breaking strain of rhizomania. Genetic Resources and Crop Evolution 66:929-939.
- 5. Litwiniec A, Gośka M, Choińska B, Kużdowicz K, Łukanowski A, Skibowska B (2016) Evaluation of rhizomania-resistance segregating sequences and overall genetic diversity pattern among selected accessions of *Beta* and *Patellifolia*. Potential implications of breeding for genetic bottlenecks in terms of rhizomania resistance. Euphytica 207: 685-706.
- 6. Broccanello C, McGrath JM, Panella L, Richardson K, Funk A, Chiodi C, Biscarini F, Barone V, Baglieri A, Squartini A, Concheri G, Stevanato P (2018) A SNP mutation affects rhizomania-virus content of sugar beets grown on resistance-breaking soils. Euphytica 214:14.

#### Biography

Anna Litwiniec develops multidisciplinary approach in research on biodiversity of Beta/Patellifolia genetic resources. The comprehensive model established includes not only studies on genetic bases of rhizomania-resistance sources and identification of associated molecular markers, but also evaluation of overall genetic diversity and application of specifically-designed biomarkers in differentiation of resistance responses. Apart from that in the Laboratory of Biotechnology at the Department of Genetics and Breeding of Root Crops, Plant Breeding and Acclimatization Institute – National Research Institute in Poland, Bydgoszcz Research Center, evaluation of bioactivity of selected plant derivatives is being performed in terms of their potential health-promoting and pro-ecological effects in cooperation with medical environment.

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