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Molecular description of *Blumeria graminis* f. sp. *hordei* isolates

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INTRODUCTION

The air-born fungus *Blumeria graminis* f. sp. *hordei* (*Bgh*) is a causal agent of barley powdery mildew. The pathogen attracts substantial attention due to its destructiveness (Fig. 1). However, molecular diversity studies based on "house-keeping" genes do not provide sufficient resolution when applied to isolates from geographically limited regions. This study focuses on developing a more efficient genotyping system capable to discriminate between closely related isolates. Furthermore, it demonstrates application of new markers on a set of isolates including Czech Bgh population together with selected isolates originating from different parts of the world.

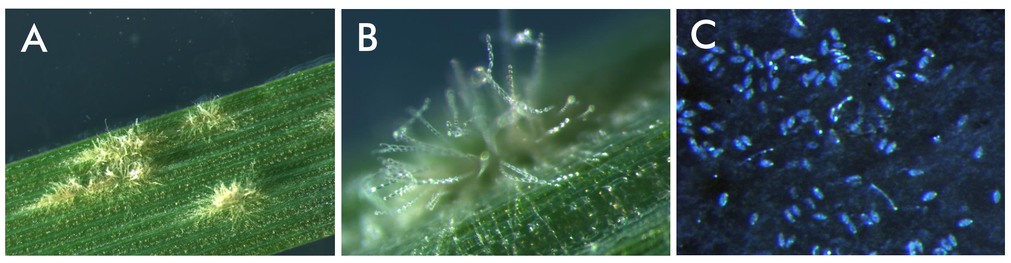

Figure 1: *Blumeria graminis* DC., currently considered to be the 6th most important fungal plant pathogen (Dean *et al.*, 2012).
A) Colonies of fungus visible on leaf surface. B) Conidiophores. C) Conidia.

MATERIAL & METHODS

Whole genome sequence data of *Bgh* strain DH14 (Spanu *et al.*, 2010)

Searching microsatellites using WebSat tool (Martins *et al.*, 2009)

Searching transposable elements junctions: BLAST with RepBase (Jurka *et* Kohany, 2011)

Designing primers, testing polymorphism on 14 *Bgh* isolates, sequencing

Set of new markers – SSR, ISBP/RJM, SNP

DNA extraction from conidia, genotyping, phylogenetic analysis by neighbor-joining algorithm using software package PHYLIP 3.69 (Felsenstein, 2005)

RESULTS

Based on the approach described above, a genotyping marker panel comprising 16 SSR, 14 SNP and 2 ISBP/RJM markers was developed (Fig. 2).

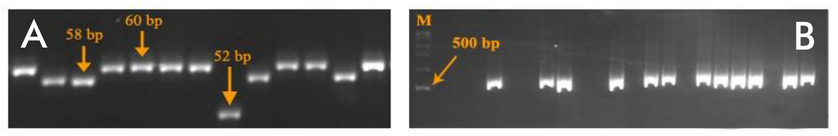

Figure 2: Examples of new markers amplified from *Bgh* isolates.
A) SSR marker *obm28*. **B)** ISBP/RJM marker *obm14.* PCR products were visualized by ethidium bromide staining after electrophoretic separation on 6% and 4% polyacrylamid gel, respectively.

The final marker panel comprising 32 polymorphic markers provided significant resolution of 158 studied isolates, most of them showed unique genotype profiles. The analysis of phylogenetic relationship performed by neighbor-joining algorithm for 97 Czech isolates resulted in 87 separate clades and revealed high diversity of the pathogen population within a small geographical area (Fig. 3, 4).

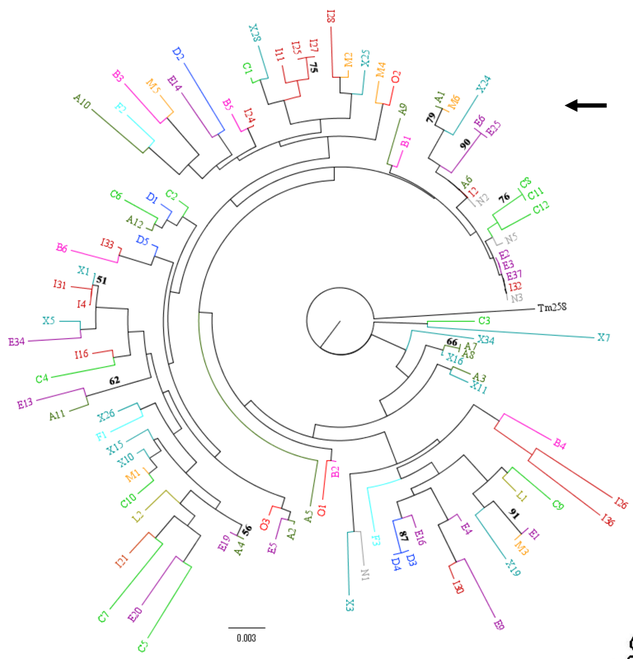

Figure 3: Tree phylogram obtained from neighbor-joining inference analysis performed for 97 Czech *Bgh* isolates.

Bootstrap values exceeding 50 % are indicated above branches. Groups of isolates are separated by different colours corresponding to the region of origin within the Czech republic (Fig. 4).

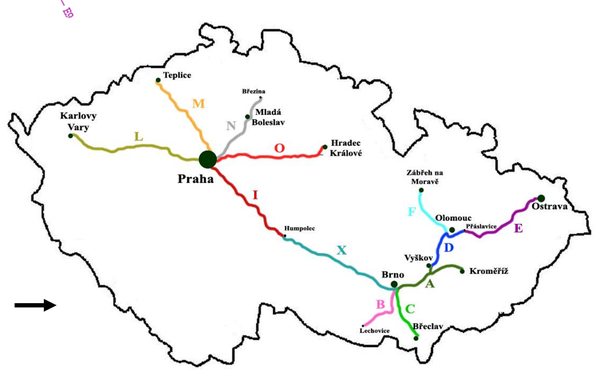

Figure 4: Distribution of studied isolates within the Czech republic.

Colours of individual sections correspond to colours of isolates on Fig. 3. Letters (A, B, C, D, E, F, I, L, M, N, O, X) represent groups of isolates collected in respective region.

CONCLUSIONS

- Whole genome sequence data of *Blumeria graminis* f. sp. *hordei* were employed to design a panel of molecular markers based on microsatellites and insertion sites of transposable elements. Altogether, 32 polymorphic markers of three different types (SSR, SNP, ISBP/RJM) were developed and used for further analysis.
- Genotyping of 158 Bgh isolates revealed high genetic variability allowing unambiguous identification in most of the cases. Resulting genotype profiles were used for phylogenesis inference analysis to demonstrate one of possible applications of these data.
- After supplementing with data on virulence of individual isolates, this study might open new opportunities of studying the host-pathogen relationship and patterns of the pathogen spatial and time distribution.

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ÚEB C. R. HANÁ