

Cold plasma seed treatment drives transgenerational microbiome changes in buckwheat

Jure Mravlje^{1,2}, Marjana Regvar¹, Mateja Germ¹, Aleksandra Golob¹, Miran Mozetič², Rok Zaplotnik², Katarina Vogel-Mikus^{1,2}, Matevž Likar¹

¹*Department of Biology, Biotechnical Faculty, University of Ljubljana, Jamnikarjeva 101, SI-1000 Ljubljana, Slovenia*

²*Jožef Stefan Institute, Jamova cesta 39, SI-1000 Ljubljana, Slovenia*

Cold plasma (CP) seed treatment is increasingly explored as an environmentally friendly alternative to chemical seed treatments, yet its influence on seed-associated microbiomes under field conditions and across generations remains insufficiently understood. Here, we evaluated the effects of low-pressure CP treatment (50:50 O₂:N₂) on common (*Fagopyrum esculentum*) and Tartary (*Fagopyrum tataricum*) buckwheat by combining laboratory experiments (in vitro) and field performance assessments with shotgun metagenomic profiling of grain-associated bacterial and fungal communities across two successive generations. Our results showed that CP treatment delayed in vitro germination in both species and reduced the final germination of Tartary buckwheat by approximately 20%, but had a limited impact on its overall field performance. Notably, despite reduced seedling emergence, CP-treated plants exhibited substantially higher grain yields, increasing by 56% in Tartary and even 70% in common buckwheat relative to the control groups. Cultivation-based microbiological assays indicated a 30–50% reduction in viable fungal colonisation following CP exposure. Metagenomic analyses revealed significant shifts in the first-generation seed microbiome, including decreased relative abundance of Actinobacteria and increased relative abundance of Proteobacteria. Among fungi, the most notable shift was an approximately 10% reduction in Pleosporaceae. However, second-generation grains displayed pronounced restructuring of fungal communities, with Pleosporaceae abundance nearly doubling irrespective of parental CP treatment, suggesting strong environmental reassembly. Overall, CP treatment induced measurable but partially transient alterations in seed-associated microbiomes. While bacterial community shifts showed limited persistence across generations, fungal communities were largely reshaped by environmental factors in the second generation. These findings indicate that CP acts as a short-term ecological disturbance to the seed microbiome and underscore the importance of field-based, multigenerational approaches for evaluating microbiome responses to CP and similar physical seed treatments.

This research was funded by the Slovenian Research and Innovation Agency (ARIS): program groups (P1-0212-Plant biology and P2-0082-Thin-film structures and plasma surface engineering), and projects J1-3014 and J7-60126.

We also thank Rangus Mill for providing us with buckwheat grains for our experiments. Ana Mezinec Urbanija, Neja Bizjak Štrus, Janez Trtnik and Matic Resnik are acknowledged for technical assistance and routine treatments.