CSIRO PUBLISHING

COLLECTION | RESEARCH PAPER
https://doi.org/10.1071/FP23166

Functional Plant Biology

Seed bacterial microbiota in post-submergence tolerant and sensitive barley genotypes

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Handling Editor:
Daan Weits

Received: 27 July 2023
Accepted: 3 January 2024
Published: 25 January 2024

Cite this:
Gómez-Álvarez EM *et al.* (2024) *Functional Plant Biology* **51**, FP23166. doi:10.1071/FP23166

ABSTRACT

Flooding is a predominant abiotic stress for cultivated plants, including barley. This cereal crop shows a large adaptability to different environmental conditions, suggesting the presence of key traits to tolerate adverse conditions. During germination, genetic variations account for dissimilarities in flooding tolerance. However, differences in the seed microbiota may also contribute to tolerance/sensitivity during seedling establishment. This work investigated differences in microbiome among the grains of barley accessions. Two barley phenotypes were compared, each either tolerant or sensitive to a short submergence period followed by a recovery. The study used a metataxonomic analysis based on *16S* ribosomal RNA gene sequencing and subsequent functional prediction. Our results support the hypothesis that bacterial microbiota inhabiting the barley seeds are different between sensitive and tolerant barley accessions, which harbour specific bacterial phyla and families. Finally, bacteria detected in tolerant barley accessions show a peculiar functional enrichment that suggests a possible connection with successful germination and seedling establishment.

Keywords: abiotic stress, barley, flooding, germination, *Hordeum vulgare*, hypoxia, microbiota, submergence.

Introduction

Barley (*Hordeum vulgare* ssp. *vulgare*) is an important cereal crop, cultivated in a wide range of environments throughout the world (Bustos-Korts *et al.* 2019). Its plasticity and adaptability to different climatic conditions suggest that it has key traits for responding to abiotic and biotic stresses.

Flooding is one of the predominant abiotic stresses for plants. When a rise of water due to heavy rains in unexpected areas or seasons is associated with the poor capacity of the soil to drain water, this can have a severe impact on sensitive plant species. In fact, flooding consequences include less oxygen (O_2) for respiration, less carbon dioxide and poor light for photosynthesis in turbid water (Geigenberger 2003; Voesenek and Bailey-Serres 2015; Loreti and Perata 2020; Cho *et al.* 2021).

In a previous study, we compared barley accessions characterised by sensitivity or tolerance to submergence stress followed by a recovery period during germination (Gómez-Álvarez *et al.* 2023). The different phenotypic outcome was associated with the presence of secondary dormancy in sensitive barley accessions, in which a more lignified seed coat was the result of transcriptional regulation during seed development. Sensitive accessions of barley were associated with sequence variation in a locus harbouring a laccase gene, possibly resulting in a lower seed permeability and O_2 diffusion in the seed. This, in turn, results in a reduced seed germination during the recovery period (Gómez-Álvarez *et al.* 2023).

Although plant genetic differences account for variations in flooding tolerance, dissimilarities in the seed microbiota may also contribute to this trait. Seed-borne bacteria can be extremely important for germination and seedling establishment because potential effects might be expressed in the initial developmental stage. Hypoxic conditions might

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shape the microbiome composition, favouring the presence of facultative anaerobic and strict anaerobic microbes.

The microbiome is an integral part of the resilience to flooding stress in plants, due to its high adaptability to change (Martínez-Arias *et al.* 2022). In this sense, the release of plant substances from root exudates to the endosphere would seem to attract certain microbes and recruit some of them through a 'cry for help' mechanism (Rizaludin *et al.* 2021; Martínez-Arias *et al.* 2022). Despite this, during flooding stress, beneficial bacteria in the rhizosphere of spring wheat have been found to decrease, while the abundance of potential detrimental bacteria increases (Francioli *et al.* 2022).

Our previous results on rice, characterised by either short or long coleoptiles under submergence, identified a very specific microbiome in the variety showing a long coleoptile under submergence (Ahumada *et al.* 2022). In parallel, the isolation of bacteria species and the analysis of plant growth promoting activities, identified bacteria that possibly support rice tolerance to submergence. In fact, some strains were found to have amylase activity and the capacity to produce indole-related compounds, such as auxins, *in vitro* (Ahumada *et al.* 2022). Indeed, rice's capacity to germinate under submergence depends on the activation of a set of enzymes involved in starch degradation to fuel the growing embryo (Perata *et al.* 1992; Guglielminetti *et al.* 1995; Kretzschmar *et al.* 2015), and auxin contributes to the coleoptile elongation under water (Nghi *et al.* 2019, 2021).

Barley grains contain a complex microbial population with a genotype-dependent endophytic seed microbiome but also a consistent core (Bziuk *et al.* 2021). In fact, despite genotype specificity, a core of seed endophytic bacteria was identified, with *Enterobacteriaceae* members being the dominant group and a high abundance of *Curtobacterium*, *Paenibacillus* and *Pantoea* (Bziuk *et al.* 2021). An investigation of the active part of barley seed microbiome in modern cultivars, using rRNA based sequencing, confirmed a core microbiome that includes *Paenibacillaceae* and *Enterobacteriaceae* (Yang *et al.* 2017). *Paenibaciullus* and *Pantoea* genera, observed in the seed endosphere of barley accessions from different geographical locations, were also shown to have plant growth promoting activities (Rahman *et al.* 2018).

Extensive studies have been conducted on the bacterial composition of barley and its associations with the genotype. Wild and domesticated barley accessions have a different bacteria root and rhizosphere microbiota composition (Bulgarelli *et al.* 2015) and a correlation is reported between the rhizosphere microbiota diversity and the host genetic diversity (Alegria Terrazas *et al.* 2020). The selection of the barley rhizosphere microbiota is likely driven by nitrogen shortage, suggesting a plant-driven shaping in growth-limiting conditions (Alegria Terrazas *et al.* 2022). Finally, a small number of loci appear to be responsible for the composition of the barley rhizosphere microbes, suggesting new possibilities in crop improvement (Escudero-Martinez *et al.* 2022).

Knowledge of seed microbiota composition, and possibly the *in vitro* isolation of some microbes with plant growth promoting activities, could lead to innovative solutions based on selected microbial applications at the first stage of plant development. This may enhance food security while using natural resources.

In this study, we conducted a metataxonomic analysis based on *16S* ribosomal RNA (rRNA) gene sequencing (Marchesi and Ravel 2015) on barley grains in two barley accessions showing a tolerant phenotype (ability to germinate) versus two barley accessions with a sensitive phenotype (inability to germinate), in relation to a submergence period followed by recovery. The aim was to assess whether bacterial seed endophytes show a dissimilar association with sensitive and tolerant barley accessions, and whether they might help during germination. We thus conducted a metataxonomic analysis by investigating the composition of putative endophytic bacteria immediately after submergence stress. The results of our analysis suggest that tolerant accessions of barley have a different microbiome than that found in sensitive accessions, and that this difference may support tolerance.

Materials and methods

Plant materials

Accessions of barley (*Hordeium vulgare* L. ssp. *vulgare*) WB-433, WB-281, WB-050 and WB-352 were sourced from the Whealbi project (https://www.whealbi.eu/). Seeds were derived from lines obtained from two rounds of single seed descent (Bustos-Korts *et al.* 2019), and subsequently reproduced during the 2020 growing season at Fiorenzuola d'Arda in Northern Italy (44°56′N 9°54′E, 80 m.a.s.l.). The soils in Fiorenzuola d'Arda have the following composition: silt 50%, clay 36%, sand 14%; the soil is slightly basic with pH 7.85 and an organic mass of 3.0% in the upper 30 cm of topsoil. They are classified as fine silty, mixed and mesic Udic Ustochrepts (Rizza *et al.* 2018).

Accessions were selected in terms of previously identified contrasting phenotypes in relation to the Submergence Tolerance Index (STI, Gómez-Álvarez *et al.* 2023), i.e. whether they have a capacity to germinate after 2 days of submergence followed by 5 days of recovery. This index is high in WB-281 (Tajikistan) and WB-433 (Montenegro) and low in WB-050 (Greece) and WB-352 (Syria).

Seeds were stored at 4°C in a LAB-MIDI refrigerator (Desmon Scientific, Nusco, Italy). Prior to the submergence treatment, hulled seeds were surface sterilised with 75% ethanol for 2 min, 3.5% sodium hypochlorite for 2 min, and 75% ethanol for 1 min (Bertani *et al.* 2016). Seeds were washed with sterile water at each step, and at the end of the process.

Sampling and submergence experiment

Sterile seeds were placed in Magenta GA-7 (Merck, 77 mm × 77 mm × 97 mm) boxes. They were germinated in a growing incubator (Percival Scientific, Perry, IA, USA) at 20°C and 50% relative humidity (RH) in the dark. In air, seeds were placed on wet sterile filter paper. In submergence, they were transferred to Magenta GA-7 boxes filled with sterile water. The experiments included four replicas for each barley accession under air and submergence, with 15 seeds for each replicate. After 48 h, 1 g of barley seeds for each replicate was stored at −80°C. DNA extraction, library preparation and sequencing were performed at IGATech Technology Services (Udine, Italy). Possible external contamination of submergence water was checked by incubation on LB (Luria–Bertani) plates at 30°C for 48 h under dark aerobic conditions.

DNA extraction, library preparation and sequencing

DNA was extracted from barley seeds using the kit Dneasy Mericon Food (Qiagen, Venlo, NL) IGATech standard protocol. The hypervariable regions V3 and V4 of the *16S* rRNA gene were amplified with the 16S-341F 5′-CCTACGGGNBGCASCAG-3′ and 16S-805R 5′-GACTACNVGGGTATCTAATCC-3′ primer set. An initial peptide nucleic acid (PNA) clamping was applied during the first polymerase chain reaction (PCR) step to block amplification of the host mitochondrial and chloroplast *16S* sequences (Lundberg *et al.* 2013), following the PNA Bio protocol (Newbury Park, CA, USA). The amplification included 25 PCR cycles using locus-specific primers, and a subsequent amplification that integrated the relevant flow-cell binding domains and unique indices (NexteraXT Index Kit, Illumina, San Diego, CA, USA). The sequencing of libraries was performed with the 300 bp paired-end mode using MiSeq (Illumina).

Bioinformatic analysis

The quality of the sequencing process was verified with FastQC (Andrews 2010). The removal of low-quality reads was performed with ERNE-FILTERING (Del Fabbro *et al.* 2013). After denoising using the QIIME2 ver. 1.9.0 DADA2 plugin (Caporaso *et al.* 2010), high quality sequences with a minimum length of 275 bp were converted into amplicon sequencing variants (ASVs). Chimeras were removed using the QIIME2 UCHIME plugin. ASVs sequences were aligned to the SILVA (Ribosomal RNA Gene Database Project Data1) database (Quast *et al.* 2013) (ver. 132), using a 99% sequence identity. The taxonomy matrix and ASVs table were used for subsequent studies.

Statistical analysis

R (R Core Team 2021) was used to perform statistical analyses. Briefly, a phyloseq object was obtained with R/Phyloseq (McMurdie and Holmes 2013). After the removal of reads of chloroplasts, mitochondria and unassigned features, the rarefraction of the ASVs table was performed to the minimum library size. The 'estimate_richness' function of R/Phyloseq was used to calculate Chao1 and Shannon indexes related to alpha-diversity. After the Shapiro–Wilk test, Student's *t*-test was used to calculate differences between treatments and phenotype.

Cumulative sum scaling (CSS) was used to normalise data with cumNorm() from the R/MetagenomeSeq v.3.8 (Paulson *et al.* 2013). For the calculation of the beta-diversity, the canonical analysis of principal coordinates (CAPs) feature of the ordinate() function of the R/Phyloseq was utilised, with constraining of the phenotype and treatment variables. Weighted Unifrac, Bray–Curtis and Jaccard distances were used to analyse differences, which were confirmed using PERMANOVA analysis for the three distance matrixes. Venn diagrams were created using the ps_venn() function from the MicEco package (Russel 2023), taking into account the number of ASVs belonging to each group. Pheatmap() function from Pheatmap() package (Kolde and Kolde 2015) was utilised to perform the heatmaps, where the data was transformed to log $(1 + x)$.

Functional prediction based on *16S* rRNA gene sequence analysis

Functional prediction based on *16S* rRNA gene sequence was performed by using the MicFunPred resource (Mongad *et al.* 2021).The ASVs table and the representative reads were utilised, maintaining borderline chimeras. The analysis was performed using Pfam and KEGG Orthology (KO) databases (Li *et al.* 2014; Kanehisa *et al.* 2016*a*, 2016*b*; Keller-Costa *et al.* 2021; Mistry *et al.* 2021). The analysis of results was conducted using R (R Core Team 2021). ANOVA analysis (P-value < 0.05) corrected by the False Discovery Rate (FDR) was applied to detect any significant differences among KO groups and Pfam of identified bacteria. The visualisation of MicFunPred results was obtained with the *Pheatmap*() function, using the viridis colour palette from R/Viridis (Garnier *et al.* 2021).

Results and discussion

Seed microbiota differs in tolerant and sensitive barley accessions

Two tolerant (WB-281, WB-433) and two sensitive (WB-050, WB-352) barley accessions were previously found to be able or not to germinate after 2 days of submergence followed by 5 days of recovery (Gómez-Álvarez *et al.* 2023). Seeds belonging to these accessions were collected after 48 h under air and complete submergence before the recovery period (Fig. 1*a*). In a previous work, we found that a differential lignification during seed development is responsible for the

subsequent phenotype observed during germination (Gómez-Álvarez *et al.* 2023) (Fig. 1*a*). Our goal was to study whether seed characteristics could be linked to differences in seed microbial communities, which in turn may play a role in the dissimilar responses observed during the recovery period.

One sampling point was analysed: 48 h in air (control, both tolerant and sensitive barley accessions subsequently germinated in this condition) and 48 h under submergence (treatment, only tolerant varieties subsequently germinated during the recovery period) (Fig. 1*a*). Our hypothesis is that the differences observed during the recovery period between sensitive and tolerant accessions might be associated with the presence of dissimilar microbial communities after submergence.

The *16S* rRNA gene analysis generated 5 016 648 paired-end reads. After *in silico* removal of chimeras and unassigned sequences and querying the SILVA database (99% sequence similarity), 2010 ASVs were assigned. Alpha-diversity was estimated using the Shannon and Chao1 indexes, comparing the different treatments (air and submergence), the different phenotypes (sensitive and tolerant) and the interaction between them.

The Shannon index of diversity revealed a high range of variability among samples in both air and submergence, whose difference was not significant (Fig. 1*b*). Although the Shannon index distribution among samples was large in both sensitive and tolerant barley accessions, the difference between phenotypes was significant (Student's *t*-test, $P < 0.05$). This revealed that tolerant accessions have a higher diversity than sensitive accessions regardless of the growth conditions (Fig. 1*b*). The Chao1 index of richness also showed a large range of variation and the difference between air and submergence conditions was not significant (Fig. 1*b*). On the contrary, the Chao1 index was significantly different between sensitive and tolerant accessions, although there was a large variation in the tolerant samples (Fig. 1*b*). For both the indexes, the interaction between treatments and phenotypes was not significant.

Beta-diversity analysis of community composition was performed. Visualisation of the data distribution was computed using the canonical analysis of principal components (CAP) with a constraint for the treatment condition and phenotype (Fig. 1*c*). The Bray–Curtis CAP was followed by the permutation analysis of variance (PERMANOVA), which found an effect for phenotypes ($P < 0.05$) but not for treatments and phenotype-by-treatment interactions. The use of weighted Unifrac and Jaccard distances showed the same pattern, albeit not significant (Supplementary Table S1).

These results support the hypothesis of a putative endophytic bacterial microbiota difference between sensitive and tolerant barley accessions, irrespective of the air/submergence conditions. This conclusion differs with the result previously found for rice under submergence (Ahumada *et al.* 2022), where the tolerant rice accessions under submergence were part of a separate cluster, suggesting a specific bacterial community. In fact, rice and barley differ in their response to submergence in that rice is able to germinate under water whereas barley is not. The Arborio rice tolerant variety is able to elongate much of the coleoptile toward the water surface where it acts as a snorkel in order to allow O_2 diffusion to underwater organs (Narsai *et al.* 2015; Shiono *et al.* 2022). This particular characteristic may be associated with a different bacterial community than the one in the variety that elongates the coleoptile less. However, this does not happen with barley, where none of the accessions germinate under water.

Sensitive and tolerant barley accessions show specific bacteria phyla and families

A Venn diagram was used to highlight the distribution of the ASVs in barley accessions characterised by different phenotypes, showing significant differences in alpha and beta-diversity indexes. The results show that sensitive and tolerant accessions harbour unique communities with only 117 ASVs in common. Tolerant barley accessions had double the amount of ASVs relative to sensitive accessions (Fig. 2*a*), reflecting the results obtained with the Chao1 index (Fig. 1*b*).

The heatmap of the ten most abundant phyla (representing 96% of the community) and the most abundant families (representing 46% of the community) for tolerant and sensitive accessions (Fig. 2*b*–*e*) showed the predominance of the phyla Proteobacteria and Actinobacteria (alt. Actinomicetota) in both sensitive and tolerant accessions. Both phyla were previously found to be abundant in the rhizosphere of *H. vulgare* ssp. *vulgare* elite cultivars (Escudero-Martinez *et al.* 2022). Proteobacteria and Actinobacteria were also found to be predominant in the rhizosphere and root of the *H. vulgare* ssp. *vulgare* landrace, in modern cultivars and in *H. vulgare* ssp. *spontaneum* (Bulgarelli *et al.* 2015) and in the barley seed microbiome of different genotypes (Bziuk *et al.* 2021). The phylum of Proteobacteria remained high under submergence only in sensitive accessions.

The phylum Cyanobacteria was abundant only in tolerant barley accessions (Fig. 2*c*). Cyanobacteria have been found in association with terrestrial plants, and their capacity to fix CO_2 and atmospheric nitrogen can have a large impact on the host plant, with a growing interest when available in the rice field (Álvarez *et al.* 2020). On the other hand, the phylum Deferribacterota (alt. Deferribacteres) was abundant only in the list of the sensitive accessions (Fig. 2*b*). Deferribacterota are halotolerant and frequently found in marine niches (Alauzet and Jumas-Bilak 2014). Interestingly, they are preferentially anaerobes and were found mostly in sensitive seeds, which are likely hypoxic (Gómez-Álvarez *et al.* 2023).

Among the families, *Paenibacillaceae*, *Oxalobacteraceae*, *Bacillaceae*, *Erwiniaceae* and *Beijerinckiaceae* were common to both tolerant and sensitive barley accessions (Fig. 2*d*, *e*). *Paenibacillaceae* and *Oxalobacteraceae* were among the most abundant in barley seeds also in previous works

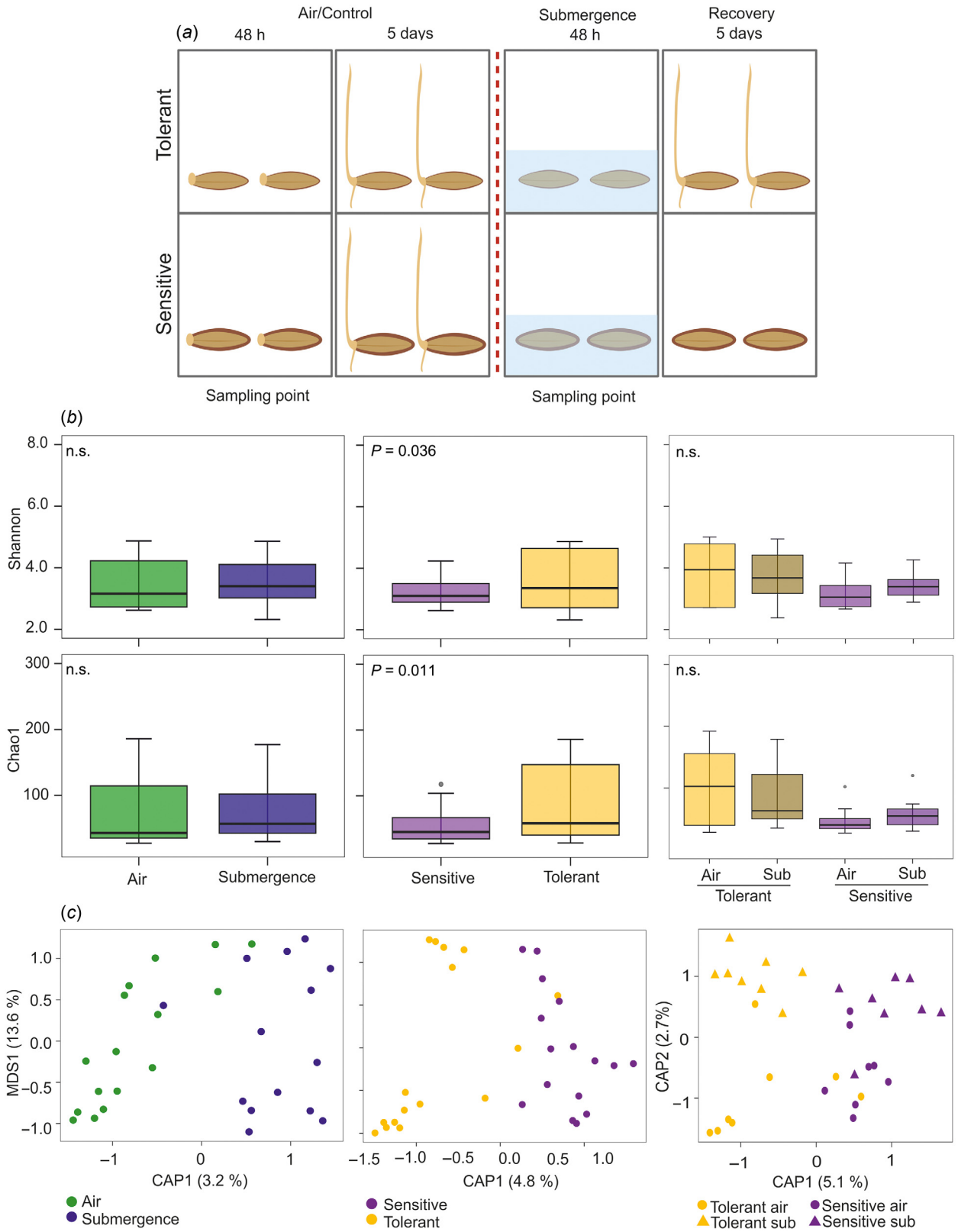

Fig. 1. (*a*) The experimental set up, showing the sampling point and differential phenotypes of tolerant and sensitive barley accessions after the recovery period. (*b*) Alpha-diversity analysis performed by using Shannon and Chao1 indexes (Student *t*-test) on samples collected before the recovery period. (*c*) Beta-diversity analysis performed using the canonical analysis of principal coordinates (CAP) constrained for variables on the Bray–Curtis distance (PERMANOVA test). n.s., not significant; sub, submergence.

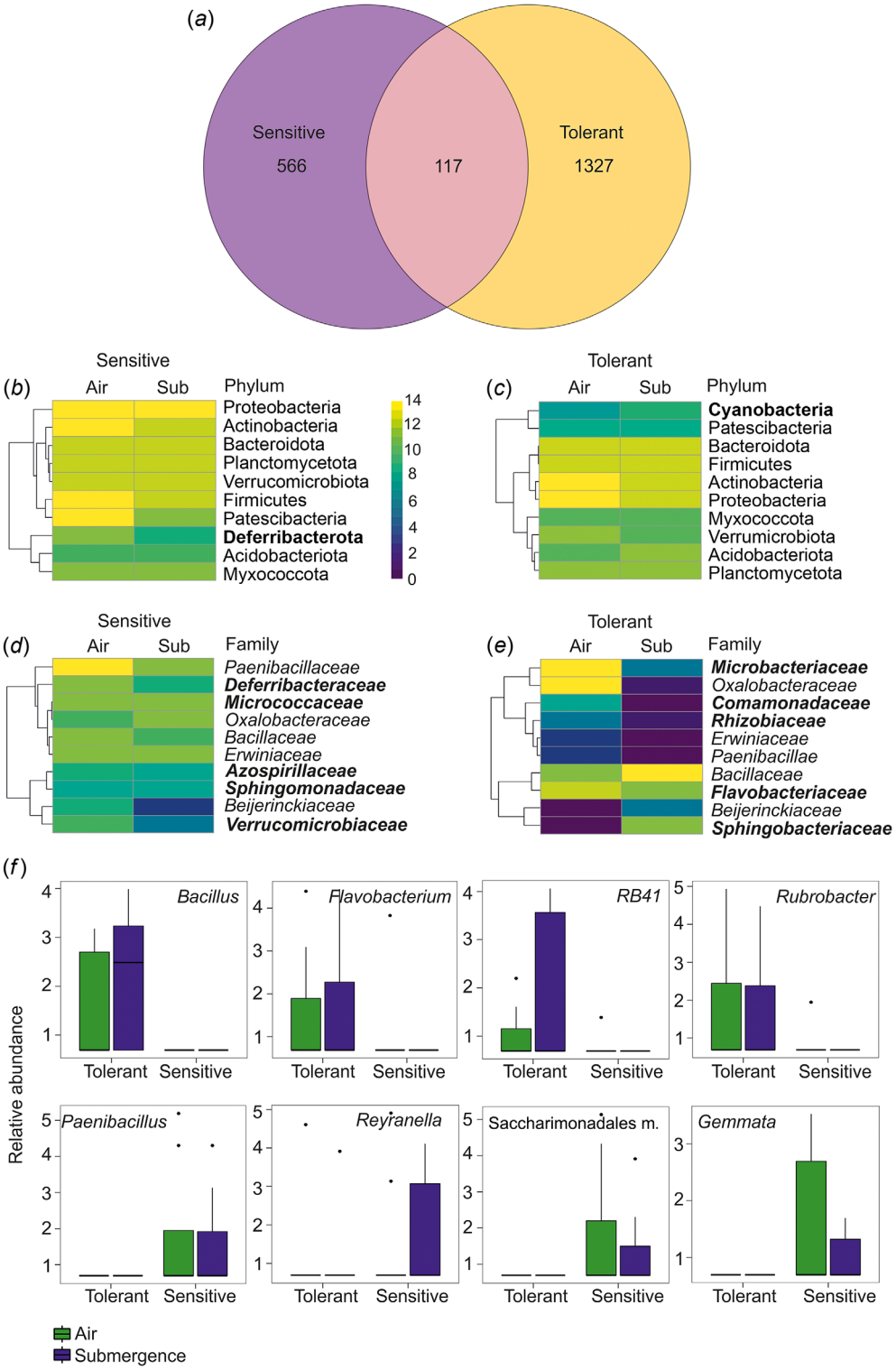

Fig. 2. (*a*) Venn diagram for the variable phenotype representing the total number of ASVs identified as described in the 'Bioinformatic Analysis' section. (*b*–*e*) Heatmap of the relative abundance for the 10 most abundant unique ASVs belonging to sensitive and tolerant varieties grouped by phylum (*b*, *c*) and family (*d*, *e*). Unique ASVs for sensitive and tolerant accessions are in bold. (*f*) Boxplots of the significant genera depending on the phenotype variable (FDR < 0.05) and showing differences between air and submergence samples. Relative abundance is calculated as $-\log(1 + X)$ of number of counts for given ASVs. m., members.

(Yang *et al.* 2017; Bziuk *et al.* 2021), whereas the other families seem to be specific to the accessions that we analysed. In the sensitive barley accessions, the *Paenibacillaceae* family was the most abundant in air and was reduced under submergence (Fig. 2*d*).

The unique ASVs of sensitive barley accessions belong to the *Deferribacteraceae*, *Micrococcaceae*, *Azospirillaceae*, *Sphingomonadaceae* and *Verrucomicrobiaceae*, which are absent from the list of predominant families in tolerant accessions (Fig. 2*d–e*). At the family level, in tolerant accessions there is an enrichment of ASVs from *Microbacteriaceae*, *Comamonadaceae*, *Rhizobiaceae*, *Flavobacteriaceae* and *Sphingobacteriaceae*, not observed in the 10 most represented groups of sensitive barley accessions (Fig. 2*d–e*). Of these, *Microbacteriaceae*, *Comamonadaceae* and *Flavobacteriaceae* have been found to be abundant in barley root (Bulgarelli *et al.* 2015). *Sphingobacteriaceae* are known to be endophytes of maize (Kämpfer *et al.* 2016). *Microbacteriaceae* and *Oxalobacteriaceae* are reduced under submergence, whereas there were more *Bacillaceae* (Fig. 2*e*).

Air and submergence samples show a similar amount of unique ASVs (Fig. S1), reflecting the result of the alpha and beta-diversity analyses. The ASVs that were only present in the submergence samples belong to *Chloroflexi*, whereas those present only in air are *Patescibacteria*, which have been found to dominate in the rhizosphere and roots of spring wheat microbiota respectively (Francioli *et al.* 2021).

At the family level, *Sphingobacteriaceae*, *Xanthobacteriaceae*, *Bacteroidaceae Vicinamibacteraceae* and *Pseudomonadaceae* are specifically present in submergence (Fig. S1). These families are different from what has been found in spring wheat microbiota under flooding conditions at different growing stages, suggesting a specific response of these plant species (Francioli *et al.* 2021, 2022).

The most significant genus difference (FDR, for all the single comparisons P-value < 0.05) between sensitive and tolerant barley accessions were analysed (Fig. 2*f*). *Bacillus*, *Flavobacterium*, *RB41*, and *Rubrobacter* were found only in tolerant samples both in air and in submergence conditions. *Bacillus* is one of the twenty most abundant genera in seed and rhizosphere microbiome in barley (Bziuk *et al.* 2021). *Bacillus* species with an endophytic lifestyle can provide plants with protection from phytopathogens, increased resistance to abiotic stresses, and plant growth promotion (Lopes *et al.* 2018). *Flavobacterium* has been described in Norwegian barley germinated seeds (3–4 days) (Jiao *et al.* 2022). RB41 has also been previously observed in barley (Lewin *et al.* 2021).

In our study, the genera *Gemmata*, *Paenibacillus*, *Reyranella* and Saccharimonadales members were found only in sensitive varieties. *Paenibacillus* has been reported as one of the most abundant in barley seeds (Yang *et al.* 2017; Bziuk *et al.* 2021) and, when isolated, it is able to produce protease, β-1-3-glucanase, cellulase and indole-3-acetic acid (IAA) (Bziuk *et al.* 2021). It has also been reported in ungerminated Norwegian barley seeds (Østlie *et al.* 2021).

Bacteria identified in tolerant barley accessions are functionally enriched

Significantly different functions between bacteria identified in sensitive and tolerant barley accessions were predicted, using the KEGG ORTHOLOGY (KO) database (Fig. 3). Tolerant barley accessions showed a bacterial functional enrichment compared to sensitive accessions in all the categories analysed. Interestingly, these include several categories involved in sugar-related mechanisms, such as the alpha-glucoside transport system, pentose phosphate pathway, maltogenic alpha-amylase, starch and sucrose metabolism, multiple sugar transport system and D-allose transport system (Fig. 3). Such activities might be involved in the use of sugar when the plant germinates after submergence during the recovery

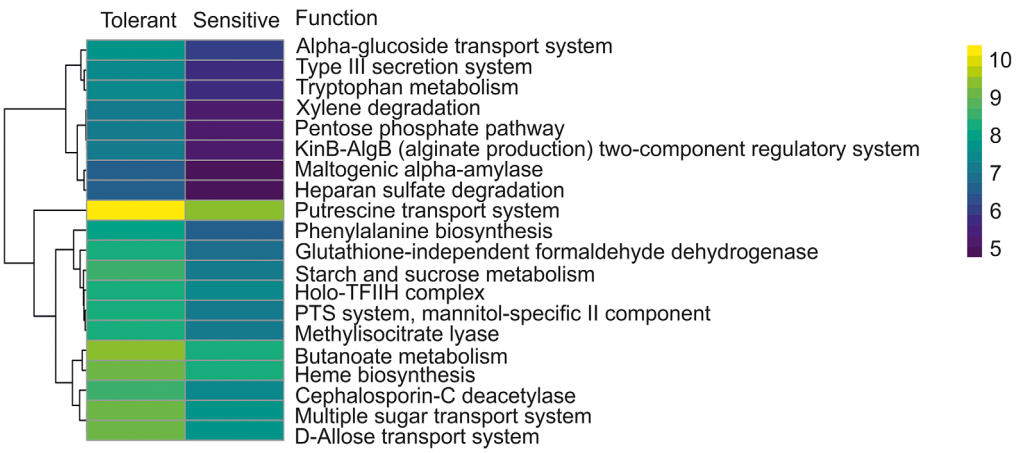

Fig. 3. Functional analysis with MicFunPred using the KEGG ORTHOLOGY (KO) database depending on the phenotype variable (FDR, $P < 0.05$). Relative abundance is calculated as $-\log(1 + X)$ of number of counts for given ASVs. PTS, phosphotransferase system.

period and thus help the plant to establish the seedlings. On the other hand, they may suggest that the availability of sugars from seeds in germination enriches the bacterial community.

Significantly different functions between air and submergence were also predicted using the KO database (Fig. S2). Interestingly, samples from submerged seeds were enriched in bacteria with putative functions involved in starch and sucrose metabolism, which is crucial in overcoming submergence stress. On the contrary, nitric oxide synthase function is reduced under submergence. Nitric oxide is crucial for seeds to germinate, and submergence stress has been found to induce secondary dormancy in sensitive barley accessions (Gómez-Álvarez *et al.* 2023), suggesting a potential link with plant phenotype.

Conclusions

The identification of a peculiar microbiome associated with crops that are tolerant to environmental constraints may facilitate an innovative approach in which these bacteria are isolated and applied to sensitive crop species. In this framework, our study aimed to assess possible differences in the microbiota of barley accessions characterised by tolerance/sensitivity to a short submergence stress followed by recovery. Indeed, a further number of accessions, characterised by different relatedness, would help in deciphering whether the shape of microbiota in this context is associated with barley genetics. The results of this study suggest that at the end of submergence, tolerant barley accessions have a different microbiome from the sensitive accessions. This microbiome is present whether the barley is in air or submerged. Tolerant barley accessions are richer and more diverse than sensitive accessions, and have specific communities of amplicon sequence variants. Finally, at the functional level, bacteria associated with tolerant barley accessions are enriched in sugar-related categories. These results, which predict bacteria functions on the basis of a taxonomic representation, suggest a possible connection with a prompt barley germination during the recovery period after submergence.

Supplementary material

Supplementary material is available online.

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Data availability. Original data are available at https://www.ncbi.nlm.nih.gov/PRJNA996914.

Conflicts of interest. The authors declare that there are no conficts of interest.

Declaration of funding. This work was financially supported by Sant'Anna School of Advanced Studies and by the microbING project, Italian Ministry for University and Research (MUR) (PNR, EU regulation 241/2021, PNRR 2021-2026).

Acknowledgements. The authors thank Alessandro Tondelli from CREA Research Centre for Genomics and Bioinformatics (Fiorenzuola D'Arda, Italy) for kindly providing barley seeds. They are grateful to Iris Bertani from the International Centre for Genetic Engineering and Biotechnology (Trieste, Italy) for critically reading the manuscript.

Author contributions. CP and PP conceived the work, CP designed and supervised the experiments. MSJ performed the submergence experiment with the support of GDA. EMGA performed bioinformatic, statistical and functional analysis. MDA supervised the bioinformatics analysis. CP and EMGA wrote the article, which was revised and approved by all the authors.

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