Deficit irrigation differentially modulates rhizosphere microbial community and metabolites of two potato genotypes differing in drought tolerance

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Key words: Actinobacteria; Microbiome; Proteobacteria; Specific root length; Tuber initiation

Abstract

Beneficial interactions between plant root exudates and the rhizosphere microbial community can alleviate the adverse effects of environmental stress on crop yields, but these interactions remain poorly understood in potato growing in drying soil. We investigated the responses of rhizosphere soil microorganisms and metabolites, and biochemical and physiological responses of two potato genotypes with contrasting drought tolerance (drought tolerant 'C93' and drought sensitive 'Favorita'), to two different irrigation treatments imposing contrasting soil water availability in the field. Deficit irrigation altered rhizosphere soil bacterial communities and metabolites of C93 more than Favorita. While the abundance of *Nitrosospira* and *Nitrobacter* belonging to the Proteobacteria increased in C93, in Favorita the *Streptomyces* and *Nocardioides* belonging to the Actinobacteria increased. These microbial changes were significantly correlated with rhizosphere organic acid concentrations, with 3-phenyllactic acid increasing in C93, and citric acid increasing in Favorita. Although deficit irrigation restricted shoot growth of C93 at the tuber initiation stage (unlike Favorita), its specific root length was 41% greater than Favorita irrespective of irrigation treatment. Deficit irrigation significantly increased foliar chlorophyll and proline

accumulation of both genotypes, with the latter 28% higher in Favorita. Independent of irrigation treatment, yield of the more vigorous C93 (producing 22 and 89% more shoot biomass under deficit and full irrigation respectively) was 84% higher than Favorita. It was concluded that different potato genotypes selectively recruit beneficial microorganisms by secreting different organic acids to alleviate the adverse effects of deficit irrigation.

1. Introduction

Potato is the fourth most important food crop after rice, wheat and corn (FAOSTAT 2022), but its shallow root system makes it susceptible to drought stress, that restricts key physiological and biochemical processes and decreases tuber yields (George et al., 2017). Although sometimes grown as a rainfed crop (Martínez et al., 2021), its drought susceptibility means it is more commonly irrigated. While supplying crop water requirements (full irrigation) is possible when water resources are adequate, regional water scarcity means it is sometimes necessary to supply less water (deficit irrigation) than optimal, which dries the soil and can restrict yields (Ahmadi et al., 2017; Niu et al., 2024). Such yield limitations can be ameliorated by growing more drought tolerant potato genotypes, that close their stomata to restrict transpiration and/or more extensively proliferate their roots during deficit irrigation to maintain water uptake to fill the tubers (Huntenburg et al., 2023). More drought-tolerant potato genotypes that better maintained tuber yields upon suspending irrigation had more sensitive stomatal closure, but similar leaf relative water content than less drought-tolerant genotypes (Gervais et al., 2021), implying genetic variation in non-hydraulic signaling of drying soil. Under restricted irrigation, genotypes with greater root proliferation in deeper moister soil layers maintained water uptake and tuber yield (Puértolas et al., 2014), yet other genotypes increased root mass at the expense of tuber yield (Ahmadi et al., 2017). Nevertheless, few drought-tolerant potato cultivars have been bred as it is an autotetraploid species (Xu et al., 2017) and both traditional and modern breeding technologies are cost-, labor- and time-consuming. Thus, other drought resistant technologies should be developed to alleviate the adverse effects of deficit irrigation on potato production.

45 One such technology is to apply certain soil microbes as soil inoculants to enhance plant productivity in dry
46 environments by: 1) producing extracellular polymers to form biofilms on the roots, which can retain water (Costa
47 et al., 2018), 2) producing extracellular enzymes degrading organic compounds to enhance soil nitrogen cycling and
48 crop nutrition (Zhao et al., 2023) and (3) secreting or metabolizing plant hormones thereby altering root morphology
49 to increase water and nutrient uptake (Zhou et al., 2016) to promote plant growth (Belimov et al., 2015). Many plant
50 growth promoting rhizobacteria (PGPR) degrade 1-aminocyclopropane-1 carboxylic acid (ACC), the precursor of
51 the stress hormone ethylene, via the bacterial enzyme ACC deaminase, thereby enhancing potato root growth, shoot
52 vegetative vigor and tuber yield. Furthermore, auxin-producing bacteria increased potato root growth, with some
53 associatively fixing nitrogen to increase plant N content (Naqqash et al., 2020). Despite considerable promise,
54 relatively few microbial inoculants have been developed to ameliorate plant drought responses, in part because
55 indigenous microbes out-compete them (Teijeiro et al., 2020), thereby diminishing rhizosphere colonization of the
56 inoculant with time (Naqqash et al., 2020). Understanding how deficit irrigation shapes the rhizosphere microbial
57 community of potato genotypes with contrasting drought tolerance may identify candidate strains with high root
58 colonization that may be suitable to develop as microbial inoculants.
59 Alternatively, increased organic acid exudation from plants exposed to environmental stresses (Kang et al., 2019)
60 could recruit PGPR strains to enhance plant drought tolerance (Kang et al., 2019). Osmotic stress increased root
61 exudation of organic (malic, lactic, acetic, citric) acids from a drought-tolerant maize cultivar more than a drought-
62 sensitive cultivar (Song et al., 2012), with citric acid stimulating microbial chemotaxis and biofilm formation (Saleh
63 et al., 2020) that might contribute to drought tolerance (Song et al., 2012). These microbes produce a diversity of
64 compounds that affect plant growth and physiology, such as lactic acid bacteria and *Bacillus* that produce
65 phenyllactic acid (Zheng et al., 2011), that increased lateral root density of Arabidopsis (Maki et al., 2022) and plant
66 height and root length of wheat (Shi et al., 2018). While potato root exudates have been studied in response to pest

and pathogen infection (Hoysted et al., 2018; Amponsah et al., 2023), the few studies considering potato root exudation in response to deficit irrigation measured specific compounds such as amino acids (Belimov et al., 2015). To our knowledge, no study has used nontargeted soil metabolomics to comprehensively understand how deficit irrigation affects potato rhizosphere metabolites.

In contrast, many studies evaluated the impacts of drought on rhizosphere and bulk soil metabolite profiles in various species in seeking to explain drought-induced changes in the rhizosphere microbial community. Withdrawing irrigation for several week stimulated recruitment of Gram-positive bacteria in the sorghum root microbiome, but rewatering favored Gram-negative bacteria (Xu et al., 2018). Further detailed measurements correlated the concentrations of drought-associated plant metabolites in the sorghum rhizosphere with Actinobacteria abundance, but rewatering rapidly (within 24 hours) re-established rhizosphere metabolite profiles coincident with rhizosphere depletion of Actinobacteria and enrichment of Gemmatimonadetes (Cadell et al. 2023). Collectively, these observations highlight that almost complete soil moisture depletion dramatically altered the rhizosphere metabolome and microbiome, but whether such pronounced shifts occur in regularly, although suboptimally, irrigated crops is less certain.

While root exudation can selectively recruit microorganisms to improve plant stress resilience (Liu et al., 2020), whether this occurs in potato genotypes exposed to different irrigation treatments in the field is not clear. Applying four different deficit irrigation treatments (maintaining a range of soil matric potentials from -15 to -45 kPa) to 3 different pot-grown potato cultivars had similar, cultivar-independent effects on the relative abundance of the soil microbial community (Gumiere et al., 2019), perhaps because the selected cultivars were expected to show similar physiological responses to deficit irrigation. While two potato cultivars of contrasting drought tolerance were selected to investigate how different soil water contents altered the soil microbiome, limited treatment duration (2 weeks of deficit irrigation applied to pot-grown vegetative plants) meant yield was not measured (Martins et al.,

2023). We hypothesized that potato genotypes with contrasting drought tolerance would exude different organic acids to recruit different microbial communities to mitigate physiological and agronomic impacts of deficit irrigation. Accordingly, we investigated growth (shoot and root vigor) and physiology (foliar chlorophyll concentrations and antioxidant enzyme activities) of two genotypes with contrasting drought tolerance to a field deficit irrigation treatment that restricted tuber yields. Relationships between rhizosphere microorganisms and soil metabolites were explored to determine if the drought-tolerant genotype recruited a different soil microbial community.

2. Materials and methods

2.1. Plant materials and experimental treatment

Two genotypes differing in drought resistance were used: "Favorita" (Fav, drought sensitive) and "C93" (high drought tolerant). Favorita was classified as drought sensitive based on *in vitro* screening of 11 different cultivars in 15% PEG-8000 (w/v) (Deng et al., 2014), and was less able to maintain leaf water content with partial or full altered than other cultivars (Yao et al., 2023). C93 was selected based on two-years of drought evaluation trials (Qin et al., 2019a), with greater yield stability in pot trials (Qin et al., 2019b) than locally adopted cultivars. Furthermore, two years of field trials with the same irrigation treatments implemented here (in 2023 and 2024) indicated C93 had 29% higher tuber yields than Favorita under deficit irrigation (Fig. S1).

The experiment was conducted in a rain-proof shelter in 2021 (with fallow soil in 2020) in Chabei Administrative District (41°25′N, 114°56′E), Zhangjiakou City, Hebei Province, China. Plastic was used to close the top and sides of the shelter only when it rained. As there was free air exchange between the shelter and the outside air at all other times, air temperature and relative humidity were assumed to be similar, averaging 16°C and 70% during the growing season. The experiment comprised a split-plot experimental design including two irrigation level (main plots) and two genotypes (sub-plots) with three replications. Thus, there were 4 treatments and 12 plots, and each plot was 2.7 m × 5 m (three ridges, Fig. S2). The ridge and plant distance were 0.9 m and 0.18 m, respectively. Following local

irrigation recommendations (Men and Liu, 1995), the irrigation treatments were full irrigation (FI) and deficit irrigation (DI). Full irrigation kept the soil relative water content at 55-65%, 65-75%, 70-80%, 50-60% for seedling stage, tuber initiation stage, tuber bulking stage and starch accumulation stage, respectively, reflecting the relative drought sensitivity of different potato phenological stages (Wei et al., 2021). For deficit irrigation, soil relative water content was 20% lower at each growth stage, with supplementary irrigation applied when the average soil water content at a depth of 0-40 cm was lower than the target, measured by TDR (TRIME-BT, IMKO Micromodultechnik GmbH, Germany) every four days (Fig. S3). A drip irrigation system with drippers spaced every 0.3 m on the top of the ridges supplied water at a flow rate of 1.0 L h^{-1}. Supplementary irrigation was suspended when the canopy began to senesce (Fig. S3): on 6 Sep. (DI and FI) for C93 and 21 Aug. (DI) and 2 Sep. (FI) for Favorita, respectively. Thus, total irrigation volume supplied differed between genotype/treatment combinations, comprising 1275 (DI) and 2572 (FI) $m^3 \cdot ha^{-1}$ for C93 and 1154 (DI) and 2388 (FI) $m^3 \cdot ha^{-1}$ for Favorita, respectively.

2.2. Plant measurements

At tuber initiation stage, leaf relative chlorophyll content was measured by SPAD-502 (Minolta Crop, Tokyo, Japan) on the young, fully expanded third and fourth leaves, counting from the main stem apex. These leaves were then sampled for superoxide dismutase (SOD), proline (Pro) and malondialdehyde (MDA) measurements using assay kits according to the manufacturer's instructions (Beijing Solarbio Co., Ltd. Beijing, China). Then the whole above-ground part of the plant was removed and dried at 80°C until constant weight. The entire root system was excavated with a shovel, shaken gently, and then the soil adhering to the root was collected as previously described (Qin et al., 2022). Samples were passed through a 2-mm sieve and divided into two parts—one was stored at −80°C for microorganism and metabolites analysis and the other was used for biochemical analysis. At the same time, any roots remaining in the soil were collected by hand, with all soil sieved to recover the roots to determine fresh root weight and root length (WinRhizo version 2008a, Regent Instruments Inc., Quebec). Roots were then dried at 80°C

until constant weight, with specific root length calculated as root length divided by root dry biomass. All these measurements comprised three plants from each plot. Potato yield was determined from each plot at final harvest. Irrigation water use efficiency (IWUE) was calculated as tuber yield divided by total irrigation amount.

2.3. Soil biochemical properties

Soil pH (water:soil = 2.5:1 g/g), organic matter (OM, dichromate oxidation method), nitrate nitrogen (NO_3-N, KCl solution extraction ultraviolet spectrophotometry method), ammonium nitrogen (NH_4-N, KCl solution extraction spectrophotometric method), available phosphorus (AP, $NaHCO_3$ extraction molybdenum antimony anti-colorimetric method) and available potassium (AK, NH_4OAc extraction-flame photometry) were measured as previously described (Bao, 2000). Soil enzyme activity assay kits measured alkaline phosphatase (ALP), sucrase (SUC) and urease (URE) according to the manufacturer's instructions (Beijing Solarbio Co., Ltd. Beijing, China)

2.4. Rhizosphere soil 16S rRNA gene sequencing and bioinformatic analysis

Rhizosphere soil DNA (500 ng) was extracted by a PowerSoil DNA Isolation Kit (MoBio Laboratories, Carlsbad, CA, USA). DNA purity and quality was measured on 1% agarose gels and with a NanoDrop spectrophotometer (Thermo Scientific). The V3-V4 variable regions of bacterial 16S rRNA gene were amplified with primers 338F (5′-ACTCCTACGGG AGGCAGCAG-3′) and 806R(5′-GGACTACNNGGGTATCTAAT-3′) (Qin et al., 2022). An Illumina MiSeq platform (Beijing Allwegene Co., Ltd., Beijing, China) performed deep sequencing. Qualified sequences with 97% similarity threshold were assigned to the same operational taxonomic units (OTUs) (Bi et al., 2022). The BLAST tool classified all OTU representative sequences into different taxonomic groups against Silva138 Database. QIIME software (v1.8.0) calculated richness and diversity indices. Principal coordinates analysis (PCoA) and heatmap figures were performed by R (v3.6.0) based on the Bray Curtis algorithms distance. The Metastats by mothur program (v1.34.4) compared treatment differences in bacterial communities. Functional prediction of the microbial communities was based on FAPROTAX. Python software (v2.7) conducted LEfSe (linear

discriminant analysis (LDA) Effect Size) analysis.

2.5. Rhizosphere soil metabolome detection and analysis

Nontargeted soil metabolomics was evaluated by LC–MS/MS in Allwegene (Beijing Allwegene Co., Ltd., Beijing, China). Soil samples (2.0 g) were removed from -80℃, thawed at 4°C and added to MeOH: ACN: H_2O (v: v: v=2: 2: 1) solution containing internal standard at 60 Hz for 120 s and ultrasonicated for 10 min. After placing at -20°C for 1 h, samples were centrifuged at 13000 rpm at 4°C for 15 min, before removing the supernatant for freeze-drying. Samples were reconstituted with ACN:H_2O solution (v: v=1: 1), vortexed for 30 s, ultrasonicated for 10 min and centrifuged at 13,000 rpm at 4°C for 15 min. The supernatant was transferred to the injection bottle including 10 μl of each sample mixture (a quality control samples (QC)) for LC-MS/MS analysis. Sample metabolic analytes flowing from the column were collected in positive and negative mode by high-resolution mass spectrometry Triple TOF 5600+ was used as previously described, with identical raw data management and metabolite annotation (KEGG and HMDB database) (Ma et al., 2023). Metabolites with VIP (variable importance in the projection) >1 and $p<0.05$ (student's test) were considered as significantly differential metabolites (DMs). Commercial databases including KEGG (http://www.kegg.jp) and MetaboAnalyst (http://www.metaboanalyst.ca/) identified the pathways of metabolites.

2.6. Data analysis

After checking plant and soil variables for normality and homogeneity of variance (logarithmic transformation applied to Pro), two-way analysis of variance (ANOVA) determined effects of genotype and irrigation treatment in SPSS v22.0 (SPSS Inc., Chicago, IL, United States). Treatment means were discriminated ($p<0.05$) by LSD test, except the MDA data that were analyzed separately by Kruskal-Wallis test. All other analyses used R (v3.6.0), including partial least squares-discriminant analysis (PLS-DA) of treatment effects on soil metabolites and redundancy analysis (RDA) of how soil biochemical properties affected the bacterial community and soil metabolites.

Co-occurrence networks for the microbial community with differential OTUs used a Kruskal-Wallis test (p<0.05, RA>0.1%). Those networks were visualized by Gephi v0.9.7 (Web Atlas, Paris, France) with corresponding node and edge files based on the Spearman correlation (p<0.05, |r|>0.7) between OTUs. Spearman correlation analysis explored relationships among key genera of the soil microbial community, plant variables and soil metabolites. Correlation networks were constructed with by Cytoscape (v 3.9.1) with absolute r values > 0.7.

3. Results

3.1. Microbial community diversity and composition

Deficit irrigation generally decreased the α diversity indices, but all indices were lower in C93 than in Favorita irrespective of soil moisture (Fig. 1). Genotype also significantly affected observed_species ($F=9.5$, $F_{0.05}=7.7$) and PD_whole_tree indices ($F=8.7$, $F_{0.05}=7.7$). PCoA analysis based on the Bray-Curtis distance demonstrated that deficit irrigation significantly affected bacterial community structure (Fig. S4), with the PCo1 axis (31%) separating the two treatments and the PCo2 axis (19.9%) separating the two genotypes.

At the phylum level, the rhizosphere bacterial communities in all soil samples were dominated mostly by Proteobacteria (41.2-51.2%), Actinobacteriota (11.8-18.6%), Bacteroidetes (7.4-10.7%), Acidobacteria (7.9-8.5%), Chloroflexi (3.2-4.8%), Gemmatimonadota (3.5-4.7), Patescibacteria (2.5-5.5), Verrucomicrobiota (2.1-2.9%), Myxococcota (1.3-2.0%) (Fig. 2a). Metastats analysis revealed that soil moisture significantly affected the abundance of seven phyla on roots of Favorita, with deficit irrigation significantly decreasing the Proteobacteria, Armatimonadota, SAR324_clade_Marine_group_B and WPS-2, while fully irrigated plants had significantly more Actinobacteriota, Patescibacteria, Deinococcota (Fig. S5a). Interestingly, deficit irrigation significantly changed the abundance of six phyla on C93 with the Armatimonadota, Dependentiae, Firmicutes, Planctomycetota, Cyanobacteria and Nitrospirota decreasing with relative abundance (RA) less than 1% (Fig. S5b). At the genus level, the most abundant 20 bacteria belonged to the phyla Actinobacteriota and Proteobacteria. Deficit irrigation increased

199 the relative abundance of *Streptomyces*, *Nocardioides* and *Nitrobacter* in both genotypes, and *Nitrosospira* only in
200 C93 (Fig. 2b). Thus, deficit irrigation affected rhizosphere bacterial communities differently in each genotype.
201 LEfSe analysis further distinguished biomarkers. *Streptomyces* was the common genus for both genotypes, while
202 *Nocardioides* and *Nitrosospira* were the dominant biomarkers for C93 (Fig. 2c, 2d). Functional prediction based on
203 FAPROTAX database showed that most of the bacterial microbes were related to nitrogen cycling with deficit
204 irrigation (Fig. 2e). Interestingly, deficit irrigation enhanced the chitinolysis related pathway of C93 along with the
205 two nitrogen cycling pathways including aerobic_nitrite_oxidation and nitrification (Fig. 2e). Microbes related to
206 the chitinolysis pathway mainly comprised the genera *Lysobacter* (order *Xanthomonadales*, phylum Proteobacteria).
207 Microorganisms related to nitrogen cycling pathways included many genera including *Pseudomonas*, *Rhodoplanes*,
208 *Alcaligenes*, *Paracoccus* and *Xanthobacter*, which mainly belonged to phyla Proteobacteria, Firmicutes and
209 Myxococcota. Thus, deficit irrigation affected the bacterial community (especially the key genera belonging to the
210 phyla Actinobacteriota and Proteobacteria) of C93 more than Favorita.
211 *3.2. Bacterial community network*
212 To further explore how deficit irrigation affected the rhizobacterial communities, a microbial co-occurrence
213 network for genotypes and treatments was constructed (Fig. 3). Deficit irrigation had fewer nodes (19) linked by
214 similar edges (36) and higher average degree (3.8) than full irrigation plants (26, 35, and 2.7, respectively) (Table
215 S1). The genotypes responded similarly referring to the number of nodes, although C93 had similar negative and
216 positive edges as well as lower average clustering coefficient (Fig. 3a, b). The genera *Nitrobacter*, *Ramibacter*,
217 *Microvirga*, *Lysobacter* and *Devosia* had more edges with deficit irrigation (Fig.3c, d) with the first four along with
218 *Rhizobacter*, *Pseudomonas Nocardioides* and *Streptomyes* had more edges in C93 than Favorita (Fig. 3a, b).
219 Complex bacterial community relations existed particularly for C93, suggesting more potential interactions within
220 the community.

221 *3.3. Soil metabolites*

222 A total of 271 metabolites were identified from the rhizosphere soil samples. Deficit irrigation significantly
223 differed from full irrigation only for C93 along PC1 (partial least squares-discriminant analysis), accounting for 34.2%
224 of the total variance. Interestingly, PC1 successfully discriminated fully irrigated C93 plants from fully irrigated
225 Favorita plants (Fig. 4). Based on the parameters VIP > 1 and $p < 0.05$, deficit irrigation up-regulated 14 and down-
226 regulated 37 differential metabolites (DMs) in Favorita (Fig. S6a) with 69 up-regulated and 20 down-regulated DMs
227 in C93 (Fig. S6b). The top 20 DMs of the two pairwise comparisons were organic acids and derivatives, organic
228 oxygen compounds, amino acids and lipids and lipid-like molecules. Deficit irrigation significantly increased citric
229 acid in Favorita by 5.7-fold (Fig. 5a), and 3-phenyllactic acid in C93 by 11.1-fold (Fig. 5b). KEGG pathway analysis
230 revealed no common significant pathways between the irrigation treatments for either genotype. In Favorita, deficit
231 irrigation altered 3 pathways including beta-alanine metabolism, carbon fixation pathways in prokaryotes and
232 nicotinate and nicotinamide metabolism (Fig. 5c). Deficit irrigation altered 10 pathways in C93, including starch and
233 sucrose metabolism, ABC transporters and phosphotransferase system (PTS) (Fig. 5d). Citric acid in Favorita was
234 correlated with carbon fixation pathways in prokaryotes, but 3-phenyllactic acid in C93 did not involve any of the
235 remarkable KEGG pathways.

236 *3.4. Soil physical and chemical properties*

237 For most of the irrigation season, average soil volumetric water content at a depth of 40 cm did not vary between
238 genotypes, but was significantly lower under deficit irrigation (Fig. S3). Favorita was exposed to a lower soil water
239 content in the last 20 days of the experiment (before soil and plant sampling), because irrigation ceased due to its
240 earlier canopy senescence. Irrigation treatment affected soil OM, NO_3-N, NH_4-N, ALP and URE (Table 1). Under
241 full irrigation, soil NO_3-N was 85% higher for C93 than Favorita, while NH_4-N showed the opposite change. Deficit
242 irrigation significantly decreased NO_3-N, OM and ALP by 71%, 36% and 95%, respectively in C93, while decreased

243 NH_4-N by 76% and significantly increased soil URE by 27-fold in Favorita. Soil pH, AP, AK and SUC showed
244 similar values in the two treatments for both genotypes. Deficit irrigation affected soil chemical properties to a
245 greater extent in C93 than Favorita.

246 *3.5. Plant biochemical and morphological parameters*

247 Root vigor (dry weight, length, surface area) was greater in C93, with specific root length 41% higher than Favorita
248 (Fig. 6a) independently of irrigation treatment. Under full irrigation, shoot biomass of C93 was 89% higher than
249 Favorita. Deficit irrigation decreased shoot biomass of C93 by 31% but had no effect on Favorita (Fig. 6b),
250 generating a significant genotype x treatment interaction (Table S2). Thus, shoot growth of C93 was more vigorous,
251 but more sensitive to deficit irrigation.

252 Deficit irrigation increased leaf chlorophyll concentration by 5% and 4% (Fig. 6c) and proline concentration by
253 2.5-fold and 1.3-fold in C93 and Favorita, respectively (Fig. 6d). While deficit irrigation did not affect SOD activity,
254 which was 39% higher in C93 irrespective of irrigation treatment (Fig. 6e), it also tended to ($P=0.08$) increase
255 malondialdehyde (MDA) concentration, especially in C93 (Fig. 6f). Therefore, the greater SOD activity of C93 did
256 not prevent a drought-induced increase in MDA concentration.

257 Tuber yield of C93 was 84% higher than Favorita irrespective of irrigation treatment. Deficit irrigation decreased
258 tuber yield by 33% irrespective of genotype, indicating similar sensitivity to soil water deficit (Fig. 6g). Irrigation
259 water use efficiency (IWUE) of C93 was twice as high as that of Favorita under deficit irrigation and 52% higher
260 under full irrigation, and deficit irrigation increased IWUE by 54% in C93 (Fig. 6h). Thus, greater root vigor of C93
261 likely increased water uptake to sustain higher shoot biomass and yield than Favorita.

262 *3.6. Interactions among plants, rhizosphere soil properties, microbes and metabolites*

263 Redundancy analysis (RDA) illustrated relationships between rhizosphere soil properties, microbes and
264 metabolites. Soil ALP and URE significantly influenced bacterial community composition. The first axis explained

23.2% of the variation, and the second axis explained 14.9% (Fig. 7a). Soil NH_3-N、OM、NH_4-N and AK significantly influenced soil metabolites. The first axis explained 45.5% of the variation and the second axis explained 19.2% (Fig. 7b). Thus, soil bacterial community was significantly affected by soil enzyme activities, while soil nutrient status significantly affected the metabolites.

Four potential beneficial microbial genera were identified and further analyzed. *Nitrosospira* was negatively correlated with OM. *Pseudomonas* was positively correlated with NH_3-N and NH_4-N, while negatively with pH. *Nocardioides* was negatively correlated with NH_3-N and ALP, while positively with Pro. *Streptomyces* was negatively correlated with tuber yield, NH_4-N and ALP, while positively with URE and Pro (Fig. 8a). *Streptomyces* and *Nocardioides* were more significantly associated with soil metabolites than *Pseudomonas* and *Nitrosospira*, being positively correlated with organic acid and derivatives (e.g., citric acid, aspartic acid and 3-hydroxypropionic acid) and negatively correlated with organo-heterocyclic compounds (e.g., thymine, 3-hydroxypyridine and 3-methyloxindole). All the organic oxygen compounds and lipids and lipid-like molecules were significantly negatively correlated with *Streptomyces*, *Nocardioides* and *Nitrosospira* (Fig. 8b). Potentially beneficial soil microbes and metabolites were remarkably affected by soil nitrogen, URE and ALP, with soil nitrogen closely related to concentrations of organic acid and other organic compounds.

4. Discussion

4.1. Deficit irrigation enhanced rhizosphere microbiome differences between genotypes

Selecting genotypes that better associate with soil microbes confers drought tolerance (Zolla et al., 2013), but requires a drought resilient soil microbial community. A more diverse and complex microbial community structure better resisted low soil moisture (Gumiere et al., 2019), with more complex relationships among OTUs within a co-occurrence network (Fig. 3) potentially contributing to greater drought stress tolerance. Here, the microbial community of the drought-tolerant C93 in drying soil had more edges and higher average degree with deficit

287 irrigation, indicating more potential interactions among the key genera than those in full irrigation and Favorita,
288 respectively.

289 Deficit irrigation decreased median values of α diversity of bacterial community for both genotypes especially for
290 C93, as in previous field studies of potato (Faist et al., 2023). In contrast, pot trials indicated a significant increase
291 in the α diversity of the drought sensitive genotype but no change in the drought tolerant genotype (Martins et al.,
292 2023). Deficit irrigation significantly affected the microbial community composition here (Fig. S4), by decreasing
293 the first dominant phyla (Proteobacteria) and increasing the second (Actinobacteriota) in both genotypes (Fig. 2a).
294 These important bacterial phyla can enhance plant resistance to abiotic stress (Palaniyandi et al., 2013; Fitzpatrick
295 et al., 2018). Proteobacteria decompose many kinds of organic matter as energy sources (Bi et al., 2022) and, grow
296 fast in nutrient-rich environments (Ling et al., 2022), with lower soil N concentrations under deficit irrigation (Table
297 1) potentially explaining their reduction for both genotypes. Similarly, when three potato genotypes were grown
298 under deficit irrigation the Proteobacteria were less enriched (Gumiere et al., 2019), as here with 16 of the 20 most
299 abundant bacterial genera belonging to the Proteobacteria and more than half of them decreased under deficit
300 irrigation (Fig. 2b). While deficit irrigation could suppress bacterial community activity in Favorita by diminishing
301 root exudation, the Actinobacteriota (especially *Streptomyces* and *Nocardioides*) significantly increased. Likewise,
302 *Streptomyces* was enriched in the rhizosphere of the more drought-sensitive cultivar (Martins et al., 2023).
303 *Streptomyces* inoculants increased foliar proline concentrations of deficit and fully irrigated tomato plants (Abbasi
304 et al., 2020), and might enrich proline content of both genotypes exposed to deficit irrigation (Fig. 6d). Deficit
305 irrigation enriched *Streptomyces* and *Nocardioides* abundance, two nitrogen-fixing genera (Nafis et al., 2019) that
306 might enhance nitrogen uptake of Favorita, attenuating drought stress.

307 In C93, deficit irrigation increased the abundance of 3 genera (Proteobacteria phylum) (Fig. 2b): *Nitrosospira* and
308 *Nitrobacter* (which participate in nitrification - Daims et al., 2016) more than doubled and *Lysobacter* increased 1.3-

309 fold. In agreement, functional prediction indicated enrichment of the nitrification and aerobic_nitrite_oxidation
310 pathways in C93, which could alleviate the lower soil N status with deficit irrigation (Table 1). *Lysobacter* abundance
311 increased in potato cultivars that were more tolerant of deficit irrigation (Faist et al., 2023). This genus stimulated
312 compensatory growth of grapevine after re-watering (Zhang et al., 2019), enhanced antioxidant (SOD and POD-
313 peroxidase) enzyme activities in soybean (Zhang et al., 2017) and secreted chitinase to degrade chitin and other
314 organic compounds into amino sugars involving soil N-cycle (Zhang and Yuen, 2000). Functional prediction
315 indicated enrichment of the chitinolysis pathway was mainly related to *Lysobacter*. Thus, C93 may recruit *Lysobacter*
316 to degrade chitin providing energy for other microorganisms (e.g. *Nitrosospira*, *Nitrobacter*) that enhance soil
317 nitrogen availability. Isolating these organisms and supplying them as inoculants to potato will help understand
318 whether these genera mediate plant drought stress responses.

319 *4.2. Deficit irrigation promoted rhizosphere organic acid accumulation that may recruit beneficial microorganisms*

320 Plant root exudation and the microbial community determine rhizosphere metabolite profile (Liu et al., 2020),
321 with deficit irrigation causing significantly more differential metabolites (DMs) in C93 than Favorita rhizospheres.
322 Concentrations of 3-phenyllactic acid increased by 11.1-fold in C93, with this compound (50-200 μM) inducing
323 auxin-responsive root growth in Arabidopsis and wheat plants *in vitro* (Shi et al., 2018; Maki et al., 2022) which
324 might enhance nutrient foraging in the soil. Concentrations of 3-phenyllactic acid were significantly correlated with
325 the abundance of two organisms closely related to nitrogen cycling, *Nitrosospira* and *Sphingomonas* (Huang, 2000;
326 Liu et al., 2023), suggesting this molecule altered microbial recruitment even if the mechanisms are not clear. The
327 higher foliar chlorophyll (a nitrogen-containing molecule) concentrations of C93 (Fig. 6c) suggests increased
328 nitrogen uptake by the plant and/or these microbes mediating plant nitrogen relations. Alternatively, 3-phenyllactic
329 acid, which the food industry has tested as an antimicrobial compound (Ning et al., 2017; Wu et al., 2021), may
330 indirectly enhance plant drought tolerance by degrading membrane integrity and inhibiting biofilm formation of

certain bacteria (Dieuleveux et al., 1998; Lin et al., 2024), thereby allowing other bacteria that are resistant to drought stress to proliferate. Therefore, applying 3-phenyllactic acid to potato plants grown in sterile conditions seems necessary to resolve whether this compound stimulates root proliferation, and/or recruits these microbial genera to promote potato drought tolerance.

Deficit irrigation increased rhizosphere citric acid concentrations by 5.7-fold in Favorita, as in maize (Song et al., 2012), which stimulates bacterial chemotaxis (Zhang et al., 2014), solubilizes soil phosphate to improve plant phosphate uptake (Wang et al., 2015) and enhances antioxidant enzyme activity of stressed plants when applied to the soil (Tahjib-Ul-Arif et al., 2021). While citric acid is often the most abundant organic acid in potato tubers, deficit irrigation had little impact on tuber concentrations (Bethke et al., 2009). In contrast, shoots of a more drought tolerant cultivar *in vitro* accumulated citric acid following osmotic stress (Bündig et al., 2016). To our knowledge, there were no studies on the response of citric acid exudation from potato roots to deficit irrigation, but alkaline stress caused significant citric acid accumulation in roots (Lu et al., 2024) and citric acid may exude to the soil and help plant to alleviate stress. Furthermore, deficit irrigation increased rhizosphere 3-hydroxypropionic acid concentrations in Favorita, with a grass (*Achnatherum inebrians*) growing at low N stress having higher leaf concentrations of 3-hydroxypropionic acid when colonized by the endophyte *Epichloe gansuensis* (Hou et al., 2021). Both citric acid and 3-hydroxypropionic acid concentration positively correlated with *Nocardioides* and *Streptomyces* abundance, indicating these organic acids may serve as a carbon source for these two nitrogen-fixing genera (Nafis et al., 2019). Whether theses metabolites alleviate drought stress in potato requires further investigation.

4.3. Decreased soil nutrient status with deficit irrigation may mediate root architecture

Soil carbon and nitrogen (N) cycles are particularly sensitive to water shortage (Deng et al., 2021). Besides the microbial changes discussed above, deficit irrigation decreased rhizosphere organic matter, nitrogen (NH_4-N and NO_3-N) status, alkaline phosphatase and urease activity, especially NO_3-N concentrations of C93 and NH_4-N

353 concentrations of Favorita respectively (Table 1). Genotypic variation in plant response to nitrogen forms (Tang et
354 al., 2018) was associated with nitrate acting as a signal to regulate root architecture, especially stimulating lateral
355 root development (Walch-Liu and Forde, 2008; Wang et al., 2011). Increased specific root length (SRL) of C93 may
356 indicate that NO_3-N is not only supplying plant nutrition but is involved in signal transduction and root growth
357 promotion. In *Arabidopsis thaliana*, nitrate promoted greater first- and second-order lateral root development, while
358 ammonium was involved in more third-order lateral roots elongation (Meier et al., 2020). While potato responds
359 positively to mixed NH_4-N and NO_3-N nutrition (Cao and Tibbitts, 1993), its root architectural responses to different
360 nitrogen forms should be investigated.

361 *4.4. Shoot vigor at tuber initiation determined tuber yield in C93 but not Favorita*

362 Root traits were measured as these can enhance water uptake of potato. Deficit irrigation tended to increase SRL
363 of C93 (Fig. 6a), but this adaptive response did not prevent shoot growth inhibition or decreased tuber yields.
364 Drought- or scion-mediated increases in SRL were sometimes associated with higher stomatal conductance, but
365 inversely correlated with canopy expansion (Jefferies, 1993), suggesting homeostasis of plant water relations. While
366 SRL may indicate the (carbon) efficiency of the root system in extracting water, relatively few deep potato roots can
367 maintain water extraction when the upper soil profile dried (Stalham and Allen, 2004; Puértolas et al., 2014).
368 Measuring vertical gradients in soil moisture might better discriminate cultivar differences in water uptake, with
369 geophysical techniques determining the depth of water uptake in potato fields (Blanchy et al., 2020). Applying these
370 techniques should help identify more drought tolerant cultivars amongst those known to differ in root size (Wishart
371 et al., 2014).

372 Deficit irrigation did not affect shoot growth of the less vigorous Favorita at the tuber initiation stage while
373 decreasing shoot growth of the more vigorous genotype C93 (Fig. 6b). Continued potato canopy development after
374 tuber initiation (Chang et al., 2018) requires weekly measurements to determine treatment effects on both canopy

375 growth and senescence (Huntenburg et al., 2021). While mid-season shoot biomass measurements explained 71% of
376 the variation in tuber yield when a single cultivar was exposed to a factorial combination of soil compaction and
377 drought stress treatments (Huntenburg et al., 2021), leaf area duration measurements explained even more of the
378 variation in tuber yields across different cultivars and mulch management techniques (Boyd et al., 2002). That deficit
379 irrigation limited yield of both genotypes similarly (Fig. 6g) may reflect a similar leaf area duration after tuber
380 initiation, requiring more frequent canopy measurements to explain genotypic differences in yield.

381 While deficit irrigation affected leaf biochemical measurements of both genotypes similarly, the increased SOD
382 activity and chlorophyll content of C93 didn't affect oxidative damage (MDA accumulation). That MDA
383 accumulates as leaf water status declines (Zhou et al., 2023) suggests C93 was less able to maintain turgor despite
384 its higher specific root length. Since foliar proline accumulation in potato counteracted more than 80% of a decrease
385 in medium osmotic potential (Bussis and Heineke, 1998), the 22% lower concentration in C93 (Fig. 6d) may
386 compromise osmotic adjustment. Cultivars with delayed but sustained proline accumulation better maintained yields
387 when water was withheld during tuberization (Schafleitner et al., 2007). Greater constitutive proline levels may help
388 maintain shoot biomass of Favorita (Fig. 6b), as proline concentrations correlated with whole plant biomass across
389 10 different cultivars grown in pots at 20% of soil available water for a month before harvest (Alhoshan et al., 2019).
390 However, neither of these studies associated higher proline concentrations with greater tuber yield, perhaps due to
391 the metabolic costs of synthesizing this osmoticum.

392 5. Conclusions

393 Potato genotypes differing in drought tolerance (tolerant C93 *versus* sensitive Favorita) accumulated diverse
394 substances (3-phenyllactic acid in C93 and citric acid in Favorita) into the rhizosphere, which were associated with
395 recruiting different beneficial microorganisms (*Nitrosospira* and *Nitrobacter* in C93, *Streptomyces* and *Nocardioides*
396 in Favorita). Despite similar yield losses with deficit irrigation, C93 had higher yields independently of irrigation

397 treatment. Further studies should explore whether applying these organic acids and microorganisms can maintain
398 potato yields when plants receive less irrigation.

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619 Figure Captions

620 Fig.1. Effects of deficit irrigation on the α diversity indices of the bacterial community in potato rhizosphere soil.

621 Fig.2. Effects of deficit irrigation on the bacterial community composition in potato rhizosphere soil. The relative
622 abundance of bacterial community at phylum level (a). The relative abundance of bacterial community of the
623 top 20 genus (b). Bacterial linear discriminant analysis effect size (LEfSe) results in the two potato genotypes
624 under different irrigation treatments (c and d). Functional prediction of bacterial community was based on
625 FAPROTAX (e). The taxa with absolute LDA ≥ 3.5 and $P < 0.05$ was shown.

626 Fig.3. Microbial co-occurrence network for genotypes (Favorita_a and C93_b) treatments (Full irrigation_c and
627 deficit irrigation_d) and. A connection showed a strong correlation (abs. r > 0.6 and $P < 0.05$). The size of each
628 node represented the number of edges that the genus connected with other genera. Green lines and red lines
629 represented negative and positive correlation, respectively.

630 Fig.4. Partial least squares-discriminant analysis (PLS-DA) of potato rhizosphere soil metabolites. Different shape
631 and color points represented different samples from different treatments.

632 Fig.5. The top 20 differential metabolites (a and b) and metabolic pathways (c and d) in different pairwise comparison
633 groups.

634 Fig.6. Effects of deficit irrigation on potato morphological and physiological properties including specific root length
635 (SRL) (a), shoot dry biomass (b), leaf chlorophyll content (SPAD) (c), leaf proline (Pro) content (d), superoxide
636 dismutase (SOD) activity (e), and malondialdehyde (MDA) content (f), tuber yield (g) and irrigation water use
637 efficiency (IWUE) (h). Different lowercase letters at the top of bars indicated significant differences between
638 treatments at $p < 0.05$.

639 Fig.7. Redundancy analysis (RDA) of soil biochemical properties and bacterial community (a), and rhizosphere
640 metabolites (b). pH, Soil pH; OM, organic matter; NO_3-N, nitrate nitrogen; NH_4-N, ammonium nitrogen; AP,

641 available phosphorus; AK, available potassium; ALP, Alkaline phosphatase; SUC, sucrase; URE, urease.

642 Fig.8. The network among four potential beneficial microbes in genus level and potato physiological and
643 morphological characters (a) and soil differential metabolites (b). Only significant correlations ($P < 0.05$) were
644 shown. Pro, proline; pH, Soil pH; OM, organic matter; NO_3-N, nitrate nitrogen; NH_4-N, ammonium nitrogen;
645 ALP, Alkaline phosphatase; URE, urease.

Supporting Information

Supplementary Figure Caption

Supplementary Fig.1. Tuber yield of Favorita and C93 in 2023 (a) and 2024 (b) in a rainproof shelter. Bars were means ± SE of 3 replicates. Different lowercase letters at the top of bars indicated significant differences between treatments at $p < 0.05$.

Supplementary Fig.2. Field layout of the experiment (tuber initiation stage). The dash lines indicate the ridges and the black dots indicate the placement of TDR tubes to measure soil moisture.

Supplementary Fig.3. Average soil volumetric water content of the treatments at a depth of 0-40 cm. Symbols are means of 3 replicates, with error bars omitted for clarity. Vertical dotted lines indicate phenological development of the crops, with orange and yellow symbols indicating when irrigation ceased for Favorita and C93, respectively.

Supplementary Fig.4. Principal coordinate analysis (PCoA) of soil bacterial, with each symbol representing samples from the individual plot.

Supplementary Fig.5. Relative abundance of significant bacterial community in genus level in different pairwise comparison groups. Bars were means ± SE of 3 replicates, with asterisks indicated significant differences between irrigation treatments, with * and ** indicating $p < 0.05$ and $p < 0.01$.

Supplementary Fig.6. Differential metabolites in different pairwise comparison groups for Favorita (a) and for C93 (b), with each symbol representing a different soil metabolite.