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Impact of rhizosphere microbial communities on yield-related traits of six onion (*Allium cepa* L.) genotypes

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Abstract: Microbial communities play an important role in plant functioning and development. The microbial population structure in the rhizosphere of six onion genotypes CRB, OPNB, CRR, OPNR, CRJ and OPNJ was monitored at three sampling times using the soil dilution plating technique. Onion genotypes were also screened for their plant growth and bulb production parameters. The total bacterial, *Pseudomonas fluorescens* and actinobacterial populations increased from 38 to 71% with the increase of plant growth stages compared to the status before planting. As for the fungal population, a decrease by 43-51% was recorded at harvest. Only bacterial and actinobacterial populations varied upon onion genotypes. The average bulb weight was 24.2-46.8 and 32.8-52.8% significantly higher in the OPNR and OPNJ genotypes compared to OPNB, CRJ and CRB and similar to CRR. Based on Principal Component Analysis, the total and average bulb weights were positively correlated to the total fungal and Aspergillus spp. populations in the first group and to *P. fluorescens* and actinobacteria populations in the second one.

Keywords: Breeding, bulb production, onion, rhizosphere microbial population

Introduction

Onion (*Allium cepa* L.) is one of the most important bulbous vegetable crops worldwide and grown in more than 140 countries (Ochar and Kim, 2023). Annual onion production was estimated at 100 million tons of dry bulbs (Fao stat, 2019). In Tunisia, onion production is low (about 188.473 tons) probably due to the genetic erosion and biodiversity loss (Chalbi et al., 2023a, b). In addition, environmental changes such as droughts, heat waves and/or floods were reported as main factors threatening seed production around the globe (Moloto, 2022). Therefore, the collection and the preservation of local genetic resources may constitute an important tool to conserve and regenerate onion crop in Tunisia (Chalbi et al., 2023a, b). In front of the increasing demand of onions for culinary and medical purposes (Ochar and Kim, 2023), breeding new cultivars with high bulb yields and good quality are becoming of increased interest.

Nowadays, breeding programs are based on innovative tools and methods relying on systems-based approaches such as agroecology, microbiology, integrated pest management, sociology, and economy (Nuijten al., 2020). Among the explored innovative breeding strategies, plant-associated soil microbiome opens up new horizons for the evolution of plant phenotype and the cultivation of next-generation crops less-dependent on inorganic inputs, more tolerant

to diseases and resilient to climatic changes while leading to enhanced crop yields (Tian et al., 2020). In fact, according to Dlamini et al. (2022), for better understanding ecosystem responses to the changing environments, it is important to elucidate the associated microbial diversity, distribution and functions. The diversity of microbial community's structures and their functional activities are influenced by various root exudates such as amino acids, sugars, siderophores, and enzymes that may vary depending on plant species and genotypes (Wei et al., 2017). In fact, Chen et al. (2021) demonstrated that plants able to produce bioactive secondary metabolites are usually associated to highly specific microbial communities that are shaped by those compounds.

Plants of different species or genotypes of the same plant species recruit distinct microbial communities (Li et al., 2021). Therefore, scientific studies are more focused on finding quick and inexpensive tools to monitor soil microorganisms that play a key role in improving crop quality and in limiting the use of synthetic inputs. The elucidation of the nature of interactions occurring between roots and associated microorganisms are relevant to better using them in cultivation (Prisa, 2023).

Therefore, the present study was conducted in order to select high yielding onion genotypes cultivated in their native environment and to determine their associated culturable soil microbial populations.

Materials and methods

Plant material

Six (06) onion genotypes with different colors (white, yellow and dark red) were used in this study (Chalbi et al., 2023a, b). They were obtained from the onion breeding program at the Regional Research Centre on Horticulture and Organic Agriculture (CRRHAB), Chott-Mariem, Tunisia. Seeds were sown, mid-October, in cell trays and regularly watered to avoid stress.

Experimental design

From each genotype, onion seedlings were transplanted to an open field at the experimental station of Sahline, Tunisia (N35° 45'05", E10°42'39") in December 2022. Seedlings were transplanted into rows with a distance of 40 cm between seedlings within the same row and 60 cm between rows. The experimental design was a completely randomized block design. Each genotype was replicated four times (04) and each replication consists of one hundred and twenty-five (125) seedlings. They were subjected to agricultural practices commonly adopted by farmers in the region and irrigated using the drip irrigation system. They were fertilized with 28 Kg ha^{-1} NPK (13:40:13). Cattle manure (200 Kg ha^{-1}) was used as soil amendment before onion seedlings transplantation.

Soil sampling

Composite soil samples consisted of five soil cores (7 cm in diameter × 15 cm in depth) collected from each replicate. Composite soil samples were collected at the initial state (before planting), 3 months post-planting (MPP) and 6 MPP (at harvest). Four replicates were considered for each soil sampling per genotype. Once brought to laboratory, soil samples were passed through a 2-mm sieve to remove rocks and large organic debris. They were stored in plastic bags at 10°C until use.

Determination of soil pH and electrical conductivity (EC)

Each composite soil sample was air-dried and suspended into distilled water at 1:5 (soil/water) ratio. Soil samples were then filtrated using Whatman paper No. 1. The pH and EC of each soil sample were measured using a glass electrode (VWR sympHony®) and a digital conductivity meter (HANNA®), respectively. Each soil sample was replicated three times.

Determination of soil microbial population structure

The culturable soil microbial populations were determined using the soil dilution plating methods on specific agar media according to Larkin and Honeycutt (2006) protocols. Bacterial and actinobacteria plates were incubated at 28°C for 2 and 14 days, respectively, and fungal plates were maintained at 25°C for 7 days. Each soil sample was replicated three times and each replication consisted of one agar plate. Colonies of *Pseudomonas fluorescens* were identified based on their yellow fluorescent color under UV light when grown on KB medium. Colonies of *Aspergillus* spp. were identified based on their macro- and micro-morphological traits when examined under light microscope and counted separately. Colony-forming units (CFU) were counted to estimate the microbial density on each specific medium per 1 g of fresh soil (Marin et al., 2013).

Determination of growth parameters

The number of leaves, length of the pseudostem and the plant weight were determined in fully grown plants (at 3 MPP). The growth parameters were determined for 10 randomly sampled plants for each genotype.

Determination of onion bulb production parameters

The total bulb number, the double bulb number, the total bulb weight and the bulb average weight were determined at harvest (6 MPP to extend the storage life of onions). For each onion genotype, the bulb production parameters were noted for 10 randomly sampled plants per each replication. The bulb average weight was determined as the total bulb weight divided by the numbers of total onion bulb.

Statistical analysis

A one-way ANOVA was performed using the Statistical Package for the Social Sciences (SPSS) version 16.0 software's general linear model (GLM) procedure. Data for pH, EC and soil microbial population of soil samples were analyzed according to a completely randomized factorial model with two factors (Sampling times× Genotypes tested). As for growth and onion production parameters, data were analyzed according to a completely randomized design. Means were separated using Tukey test to identify significant pair-wise differences at $P \leq 0.05$. Experiments were repeated twice in 2022-2023. For an overview of onion genotypes distribution, and to explore soil microbial population structure contributing to classification, a principal component analysis (PCA) was also performed using SPSS software

Results

Variation of soil pH and EC

ANOVA analysis of pH and EC values of the composite soil collected from the rhizosphere of six onion genotypes varied significantly (at $P \leq 0.05$) depending on sampling times, genotypes tested and their interaction (Table 1).

Table 1. Variation of the pH and electrical conductivity of soil samples collected at three sampling times from the rhizosphere of six onion genotypes

		pH	EC (dS m^{-1})
Sampling time (ST) / Onion genotype (G)*			
0 MPP	CRB	7.66 a ± 0.01	0.20 a ± 0.003
	OPNB	7.35 a ± 0.1	0.19 a ± 0.005
	CRR	7.69 a ± 0.03	0.20 a ± 0.005
	OPNR	7.67 a ± 0.007	0.17 a ± 0.001
	CRJ	7.62 a ± 0.03	0.17 a ± 0.007
	OPNJ	7.63 a ± 0.001	0.20 a ± 0.001
3 MPP	CRB	7.84 b ± 0.06	0.20 a ± 0.009
	OPNB	7.68 b ± 0.003	0.20 a ± 0.007
	CRR	8.15 a ± 0.04	0.22 a ± 0.003
	OPNR	7.62 b ± 0.005	0.20 a ± 0.005
	CRJ	7.68 b ± 0.02	0.22 a ± 0.005
	OPNJ	7.61 b ± 0.02	0.23 a ± 0.005
6 MPP	CRB	7.28 a ± 0.01	0.36 a ± 0.01
	OPNB	6.97 b ± 0.08	0.35 a ± 0.009
	CRR	7.19 ab ± 0.005	0.29 b ± 0.007
	OPNR	7.07 ab ± 0.001	0.30 ab ± 0.001
	CRJ	7.14 ab ± 0	0.30 ab ± 0.009
	OPNJ	7.15 ab ± 0.01	0.30 ab ± 0.001
Sources of variation			
		P values	
ST		$P \leq 0.001$	$P \leq 0.001$
G		$P \leq 0.001$	0.014
ST × G		0.007	$P \leq 0.001$

* *For each sampling time, values within each column followed by the same letter are not significantly different according to Tukey test at $P \leq 0.05$. pH: Hydrogen potential. EC: Electrical conductivity. CRB, OPNB, CRR, OPNR, CRJ, OPNJ: Onion genotypes. MPP: Month Post-Planting.*

The pH values were significantly higher (1.01 and 1.08 times) at 3 MPP than at initial state (before planting) and 6 MPP, respectively. At initial state, the pH values varied from 7.35 to 7.67 and were similar in the rhizosphere of all six genotypes. However, at 3 MPP, it changed in the rhizosphere of CRR genotype and significantly increased to 8.15 compared to 7.61-7.84 recorded in the rhizosphere of remaining genotypes. A significant variation in soil pH values between CRB and OPNB genotypes was determined at 6 MPP (Table 1).

In all genotypes, the EC values were 1.52 and 1.68 times higher at 6 MPP than at 3 MPP and at initial state (0 MPP), respectively. At the initial sate and 3 MPP, the EC values were similar. However, at 6 MPP, the EC recorded in the rhizopshere of OPNB and CRB were significantly 17.1 and 19.4% higher than that of CRR and similar to OPNR, CRJ and OPNJ (Table 1).

Variation of the culturable soil microbial population structure depending on sampling times

The total bacteria, *P. fluorescens*, actinobacteria, total fungi and *Aspergillus* spp. counts of colonies growing from plated soil samples varied significantly (at $P \leq 0.05$) upon sampling times (Figure 1).

The total bacterial population was 39.2 and 51.9% significantly higher at 3 MPP compared to 6 MPP and 0 MPP (initial state), respectively (Figure 1a). Similarly, the *P. fluorescens* colonies count was 37.7 and 50.9% significantly higher at 3 MPP compared to 6 MPP and 0 MPP, respectively (Figure 1b). Concerning the actinobacterial population, this parameter was 64.9-70.6% significantly higher at 3 MPP and 6 MPP compared to the initial soil state (Figure 1c). The total fungal colonies recovered from the rhizosphere of all onion genotypes at 6 MPP were 43.3-51.3% significantly lower than those recovered at 3 MPP and at initial state (Figure 1d). The Aspergillus spp. populations were 27.1 and 53.7% significantly lower at 3 MPP and 6 MPP than at the initial soil state (0 MPP), respectively (Figure 1e).

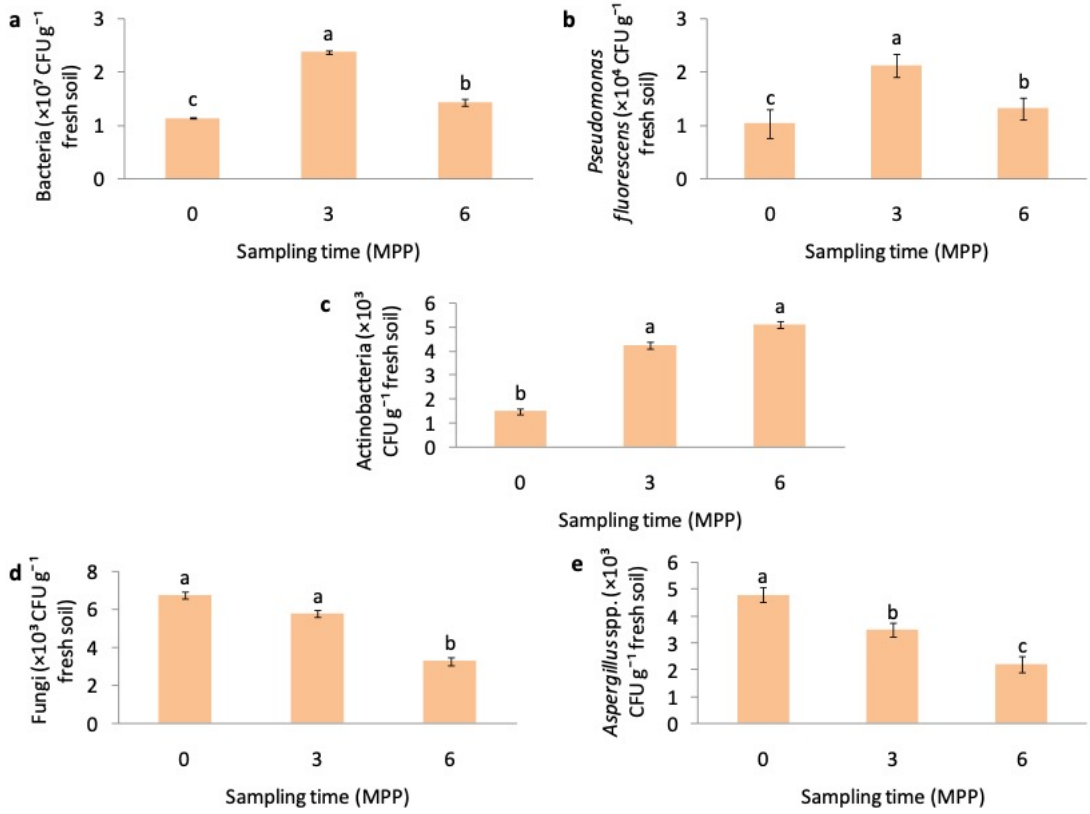

Figure 1: Variation in the microbial population densities depending on soil sampling times: a: Total bacterial population density. b: Pseudomonas fluorescens population density. c: Actinobacterial population density. d: Total fungal population density. e: Aspergillus spp. population density. CFU: Colony-Forming Unit. Bars sharing the same letter are not significantly different according to Tukey test at $P \leq 0.05$.

Variation of the culturable soil microbial population structure depending on onion genotypes

ANOVA analysis revealed a significant (at $P \leq 0.05$) variation in the CFU of total bacterial and actinobacterial populations upon onion genotypes (Figure 2).

The population of total culturable bacteria estimated on soil samples collected from the rhizosphere of CRB and OPNJ genotypes were significantly more abundant (13.6-18.4%) than

those of CRR and similar to OPNB, OPNR and CRJ (Figure 2a). The actinobacterial population was significantly higher (46.4%) in CRR and OPNR than in OPNJ and similar to CRB, OPNB and CRJ (Figure 2b). The P. fluorescens, total fungal and Aspergillus spp. populations did not vary significantly depending on the six genotypes tested.

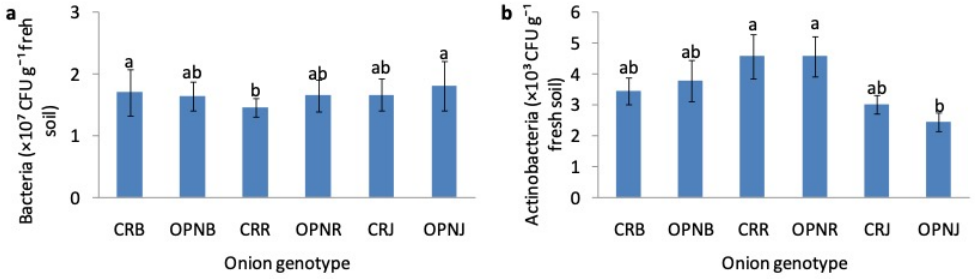

Figure 2: Variation of the bacterial and actinobacterial populations densities depending on onion genotypes: a: Total bacterial population density. b: Actinobacterial population density. CFU: Colony-Forming Unit. Bars sharing the same letter are not significantly different according to Tukey test at $P \leq 0.05$. CRB, OPNB, CRR, OPNR, CRJ, OPNJ: Onion genotypes.

Variation of growth and bulb production parameters between onion genotypes

ANOVA revealed a significant (at $P \leq 0.05$) variation of the number of leaves, number of double bulbs, total bulb weight, and bulb average weight between the six tested genotypes (Figure 3).

The number of leaves recorded in the OPNR genotype was 38.6 and 50% significantly higher than that of the CRR and CRB genotypes, respectively and similar to that of the OPNB, CRJ and OPNJ genotypes (Figure 3a). The pseudo stem length and the plant height did not vary significantly among the six genotypes.

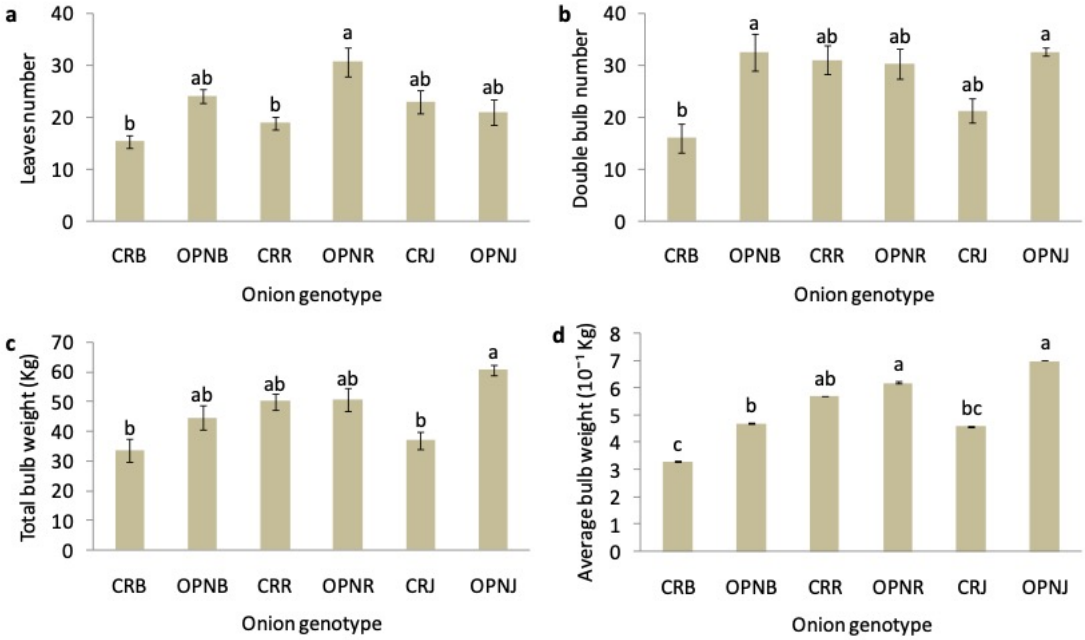

Figure 3: Variation of growth and bulb production parameters between onion genotypes: a: Leaves number of onion plants. b: Double bulb number. c: Total bulb weight. b: Average bulb weight. Bars sharing the same letter are not significantly different according to Tukey test at $P \leq 0.05$. CRB, OPNB, CRR, OPNR, CRJ, OPNJ: Onion genotypes.

Concerning the yield related parameters, they significantly varied between the six onion genotypes. Indeed, the double bulb number of OPNB and OPNJ genotypes was significantly

higher (50.7%) than those of the CRB genotype and similar to those of CRR, OPNR and CRJ genotypes (Figure 3b). The total bulb weight was 39.2-44.6% significantly higher in the OPNJ genotype compared to CRB and CRJ and similar to those noted in the OPNB, CRR and OPNR genotypes (Figure 3c). The average bulb weight was 24.2 to 46.8% significantly higher in the OPNR genotype compared to OPNB, CRJ and CRB genotypes and similar to CRR genotype (Figure 3d). This parameter was 32.8 to 52.8% significantly higher in the OPNJ genotype compared to OPNB, CRJ and CRB genotypes and similar to CRR genotype (Figure 3d).

Multivariate analysis

The PCA analysis showed that three main components accounted for most total variation of 54.64 % (Table 2). PC-1 explained 23.14 % the greatest relative influence for the distribution of the onion genotypes. The most important traits related to this axis were: the total fungal and Aspergillus spp. population, the number of leaves, the total bulb weight and the bulb average weight. The most important traits of PC-2, which explains 16.57% of the total variation, were the colonies counts of P. fluorescens and actinobacteria, the electrical conductivity of soil samples, the total bulb weight and the bulb average weight. For PC-3, the traits were pH, the total bacterial and actinobacterial populations and the total bulb number (Table 2).

Table 2. Contribution percentage and major characters associated with the three first principal components of onion genotypes

	PC-1	PC-2	PC-3
Explained proportion of variation (%)	23.142	16.578	14.927
Cumulative proportion of variation (%)	23.142	39.720	54.647
Traits		Eigenvectors	
pH	0.294	0.069	**0.820**
EC	-0.500	**0.551**	-0.016
Bacteria	-0.028	0.273	**0.311**
Pseudomonas fluorescens	-0.105	**0.305**	0.267
Actinobacteria	-0.208	**0.539**	**0.414**
Fungi	**0.589**	-0.600	0.065
Aspergillus spp.	**0.556**	-0.659	0.011
Leaves number	**0.502**	-0.108	0.062
Pseudostem length	-0.046	-0.486	0.108
Total bulb number	-0.010	-0.159	**0.487**
Double bulb number	**0.717**	**0.323**	0.073
Total bulb weight	**0.806**	**0.400**	-0.031
Bulb average weight	**0.803**	**0.439**	-0.269

Bold numbers refer to principal characters that contributed to each principal component

Discussion

The elucidation of the microbial diversity in the rhizosphere can provide a better understanding of the interactions between plant-associated microorganisms and roots (Prisa, 2023). This study has highlighted the distribution of culturable microorganisms present in the rhizosphere of six onion genotypes and their benefits in plant growth and bulb production.

The microbial population structure in the rhizosphere of six onion genotypes tested was monitored at three sampling times i.e. before planting, in the fully grown onion plants (3 MPP) and at bulb harvest (6 MPP). The total culturable bacteria, *P. fluorescens*, actinobacteria, total fungi, and *Aspergillus* spp. populations from the rhizosphere of onion plants varied significantly upon sampling times. The lowest bacterial and actinobacterial populations was noted before planting, increasing with plant growth. The highest density was recorded at 3 MPP for total bacterial and P. fluorescens populations and at 3 and 6 MPP for the actinobacterial population. However, the total fungal population decreased at the final onion maturity stage (6 MPP) compared to the full growth stage (3 MPP) and before planting. Furthermore, the abundance of

bacterial and actinobacterial populations varied between onion genotypes. Previous studies demonstrated that the soil microbial community diversity and density were shaped by the plant species and/or genotypes, growing in the same soil environment, and may vary even depending on plant growth stage (Sun et al., 2022; Chen and Liu, 2024). Similarly, Gschwendtner et al. (2011) also noted significant variations in rhizosphere microbial communities depending on potato cultivars and their developmental stages probably to the differences in the composition and the quantity of their root exudates. Chaparro et al. (2013) also demonstrated that the genes related to root exudates metabolism correspondingly changed with plant developmental stages. In fact, plants are constantly able to adjust the composition and quantity of their root exudates according to the changes occurring in the surrounding environment throughout their developmental stages (Sun et al., 2022). The constituents of root exudates varying throughout the plant development stages may either prevent or facilitate microbial colonization (Aleklett et al., 2022). For instance, phenolic acids, many of which have antimicrobial properties (Cueva et al., 2010), increase after the first weeks of germination (Chaparro et al., 2013). In this study, the bacterial and actinobacterial populations increase at 3 MPP and/or 6 MPP may be explained by the decrease in the phenolic acid's concentration in onion root exudates later after plantation. According to De-la-Peña et al. (2010), plants increase their secretion of defense-related proteins such as chitinases, glucanases, myrosinases later in development. This finding may explain the decrease recorded in the current study in the fungal population noted at 6 MPP. Root exudates facilitate communication with soil microorganisms, which in turn affect plant health through various pathways, such as enrichment of their rhizosphere with beneficial microbes (Chen and Liu, 2024).

The beneficial rhizosphere microorganisms are essential for agricultural production and plant protection (Du et al., 2020). In this study, the total onion bulb and the average bulb weights parameters were positively related to the total fungi, *Aspergillus* spp., *P. fluorescens* and actinobacterial populations. The total bulb number parameter was positively linked to the total bacterial and actinobacterial populations in onion rhizosphere. The microbiome was closely related to plant immunity and phenotypic characteristics such as resistance to different stresses and crop yield (Du et al., 2020). The beneficial consortia of beneficial bacteria composed of *Azotobacter* sp., *Sphingobacterium* sp., *Burkholderia* sp. and *Bacillus* sp. significantly improved the onion plant growth parameters (plant height, number of leaves), yield attributes (bulb yield, 20-bulb weight and equatorial diameter of bulb), bulb quality attributes (ascorbic acid, total soluble solids, pyruvic acid and dry matter) and reduced the physiological loss in weight during 15-120 days of storage at room temperature (Tinna et al., 2020). The role of rhizospheric bacteria *Azotobacter chroococcum*, *P. fluorescens* and *B. subtilis* on the growth and yield of onion and on the microorganisms in the rhizosphere of onion was previously demonstrated in Colo et al. (2014) study through their abilities to produce indole-acetic acid (IAA), siderophores and to solubilize tricalcium phosphate. Moreover, actinobacteria were known to improve growth of several plants directly by excreting plant growth-promoting compounds, mineral nutrients acquisition and enhancing the growth of beneficial microorganisms and/or indirectly via antibiotics and cell wall hydrolyzing enzymes production (AbdElgawad et al., 2020). For instance, *Streptomyces coelicolor* HHFA2 enhances the photosynthetic pigments content and foliar growth parameters of onion in pots and field studies (Abdallah et al., 2013). Hung and Rutgers (2016) emphasized the benefits of *Aspergillus* spp. in plant health and production. We suggest herein that actinobacteria, *P. fluorescens* and *Aspergillus* spp. populations estimated in the rhizosphere of onion genotypes may be involved, either individually or in consortium, in promoting onion bulb production and yield through their plant-growth promoting (PGP) features which need to be elucidated in further studies.

Conclusion

The current study clearly demonstrated that the culturable microbiome population structure in the rhizosphere of onion genotypes positively shaped plant performance, growth, and bulb production. These beneficial consortia can be considered in future onion programs to select beneficial microorganisms for their PGP features. Furthermore, the composition and concentration of root exudates should be analyzed to understand the variation in soil microbiome diversity and density between the six onion genotypes and upon the onion growth developmental stages. Profiling soil microbial communities with next-generation sequencing (NGS) in the onion rhizosphere should be also considered.

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