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Pumpkins (*Cucurbita* spp.) diversity and their associated microbiota

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Abstract: Root-associated microbiota play a key role in plant growth, resilience, and health. In this study, the microbial community structure in the rhizosphere of 12 pumpkins accessions belonging to three *Cucurbita* species i.e. *C. pepo*, *C. maxima*, and *C. moschata*, was monitored using the soil dilution plating technique on specific media. All accessions tested were also screened for their production and yield parameters. Based on Principal Component Analysis (PCA), 4 accessions of *C. maxima* (namely C5, C23, C14.2 and C6.2) were characterized by the greatest average fruit weight and yield, the highest actinomycetes, bacterial, *Trichoderma* spp. and *Aspergillus* spp. communities, and the lowest total fungal population in their rhizosphere. Positive correlations were noted between fruit fresh weight, culturable bacteria and *Trichoderma* spp. populations in the rhizopshere of pumpkins accessions. Negative correlations were noted between fruit weight and yield parameters and the total culturable fungal populations. The current study clearly demonstrated that the rhizosphere soil microbial communities have been shaped by *Cucurbita* species and accessions. Based on the significant links observed between soil microbiota and yield parameters, future pumpkin breeding programs could be focused on the selection of accessions that are quite able to exploit these associated beneficial microbial communities.

1. Introduction

Plants through their root system and surrounding soil influenced by root exudates represent an interesting ecological niche for the development of soil microbiota which are able to colonize the rhizosphere, roots and eventually move to the above-ground plant parts (Compant *et al*., 2019). Due to its ecological importance and functional diversity, the rhizosphere microbiome was intensively explored for various features (Marques *et al*., 2014; Edwards *et al*., 2015; Gopal and Gupta, 2016; Compant *et al*., 2019). Microbiotas associated to roots are derived from the soil environment which contains highly diverse microorganisms including Acidobacteria, Verrucomicrobia, Bacteroidetes, Proteobacteria,

Planctomycetes, and Actinobacteria (Fierer, 2017). Seeds may be colonized by various microorganisms which proliferate later in the roots of the developing plant and colonize the rhizosphere (Compant *et al*., 2019).

Soil microbial communities play key roles in plant development and health (Philippot *et al*., 2013; Adam *et al*., 2018). In fact, they may be associated to growth promotion, improved nutrient uptake, and enhanced tolerance to various abiotic and/or biotic stresses (Trivedi *et al*., 2020). The below-ground microbial composition is influenced by many abiotic and biotic factors including soil traits (pH, salinity, structure, moisture, organic matter, environmental conditions), relative abundance of soilborne bio-aggressors, plant species, genotypes, and agricultural and disease management practices (Hardoim *et al*., 2015; Fierer, 2017; Compant *et al*., 2019). All the above-mentioned factors contribute, at variable degrees, to the definition of the root microbial community structure together with the host-related factors like plant age and developmental stage, health status and the composition of root exudates (Bulgarelli *et al*., 2012). Based on Carelli *et al*. (2000) investigation, the rhizosphere community composition varies between plant species and even within the same species between plant genotypes. Also, the root exudates play a key role in recruiting and shaping the soil microbial population structure as they serve as nutrient sources for rhizosphere microorganisms (Sung *et al*., 2006) and represents an important component of communication with rhizosphere-inhabiting microorganisms (Haichar *et al*., 2014). Hence, the variation in the chemical composition of root exudates between and within plant species (Grayer *et al*., 2004) may lead to the development and the proliferation of a phylogenetically diverse array of microorganisms. The chemical composition of root exudates, resulting of different below-ground interactions and factors (soil chemical and physical properties, plant species, age, etc), may impact the soil microbial community structure and function by influencing plant physiology and development (Griffiths *et al*., 1999). In fact, among the members of the rhizosphere microbiome, some are beneficial for plant growth and resilience but others may be phytopathogenic exhibiting capacity to overcome the innate plant defense system and to cause devastating diseases (de Faria *et al*., 2021).

Pumpkin (*Cucurbita* spp.) is an extraordinary vegetable species that may be exploited for medicinal and nutritional features (Tlili *et al*., 2020; Hosen *et al*., 2021; Chikh-Rouhou *et al*. 2023 b). However, pumpkin cultivation is still ignored in some countries. *Cucurbita* pepo L., *C. maxima* Duchesne, and *C. moschata* Duchesne are three pumpkin species economically important which are grown over various agricultural regions worldwide (Maynard *et al*., 2002). In Tunisia, pumpkin has significant economic importance especially as familiar agriculture because of its rusticity, high nutritional values and long post-harvesting conservation. There is no improved cultivar in Tunisia and the production of *Cucurbita* is based on local accessions and landraces. Chikh-Rouhou *et al*. (2019, 2023 a, 2023 b) evidenced that pumpkin landraces collected from farmers of the Centre-East of Tunisia belongs to three species namely *C. maxima*, *C. pepo*, and *C. moschata* with a predominance of *C. maxima*. Pumpkins face a number of constraints including a shortage of genetically improved seeds, infections with various pests and pathogens (Ndinya, 2019) in addition to the plant parasitic nematode *Pratylenchus* (Zhao *et al*., 2022). Developing new cultivars with superior qualities, higher mineral contents, important yield and average weight of fruits, potential resistance towards pests and fungal diseases, tolerance to environmental difficulties, shelf lives enhancement is highly required (Paris, 2016; Seymen *et al*., 2016; Hosen *et al*., 2021).

Breeding plants for beneficial plant-microbe interactions is an emerging field mainly focusing the below-ground interactions in the rhizosphere and their valorization for the development of economically and ecologically interesting plant material (Bakker *et al*., 2012; Adam *et al*., 2018). In fact, breeding shapes the composition of the root-associated microbial communities including the antagonistic potential towards the encountered pathogens (Peiffer and Ley, 2013; Bouffaud *et al*., 2014; Cardinale *et al*., 2015). Thus, breeding strategy is recently focused on genotypes-microbial holobiont interactions in order to generate diverse new phenotypes without altering plant genomic information (Wei and Jousset, 2017; Adam *et al*., 2018; Wille *et al*., 2018).

Therefore, this study aimed to select the most productive pumpkin accession among 12 tested, to determine their associated culturable soil microbial community and to search for an eventual link between fruit and yield parameters and their associated microorganisms and soil traits.

2. Materials and Methods

Plant material

Twelve (12) pumpkin (*Cucurbita* spp.) accessions belonging to three *Cucurbita* species (*C. maxima*, *C. moschata*, and *C. pepo*) are used in this study. Their main traits are detailed in Table 1 and figure 1. They were obtained from the Cucurbits breeding program at the Regional Research Centre on Horticulture and Organic Agriculture (CRRHAB), Chott-Mariem, Tunisia.

For each accession, seeds were sown in cell trays and maintained at 25°C under greenhouse conditions. At the two-true-leaf growth stage, they were further transplanted (end of March) to an open field at the experimental station of CRRHAB of Sahline, Tunisia (N35° 45'05'', E10°42'39'').

Experimental design

Pumpkins seedlings were transplanted into rows with a distance of 120 cm between seedlings within the same row and 80 cm between rows. The trial was conducted under drip irrigation system without inputs. Cattle manure was applied at a rate of 500 Kg ha^{-1} before planting. The experimental design was a completely randomized block design. Two replicates of six seedlings each were used per each accession tested.

Soil sampling

Composite soil samples from each replicate were collected at the initial state (before planting) (Table 2) and four times post planting i.e. at 30, 60, 90, 150 days post-planting (DPP).

After planting, three soil cores (7 cm in diameter ×

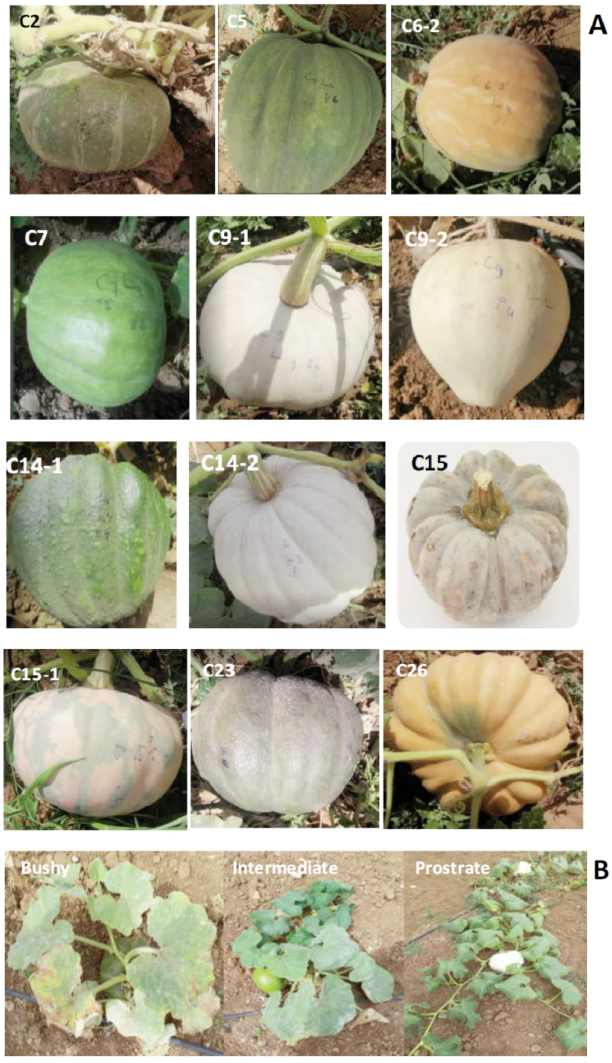

Fig. 1 - Diversity of Pumpkin (*Cucurbita* spp.) accessions used in the study (A) and their plant growth habits (B). C7, C15: *Cucurbita pepo*. C2, C5, C6.2, C9.1, C9.2, C14.2, C15.1, C23: *Cucurbita maxima*. C14.1, C26: *Cucurbita moschata.*

Table 1 - Pumpkin accessions (species and characteristics) used in this study and their main traits

Accession codes	*Cucurbita* species	Plant growth habit	Fruit shape	Flesh color
C2	*C. maxima*	Bushy	Transverse broad elliptic	Yellow
C5	*C. maxima*	Prostrate	Medium elliptic	Orange
C6.2	*C. maxima*	Intermediate	Globular	Yellowish orange
C7	*C. pepo*	Bushy	Globular	Cream
C9.1*	*C. maxima*	Prostrate	Transverse medium elliptic	Yellow
C9.2	*C. maxima*	Intermediate	Heart shaped	Yellowish orange
C14.1	*C. moschata*	Intermediate	Top shaped	Yellowish orange
C14.2	*C. maxima*	Prostrate	Medium elliptic	Yellow
C15*	*C. pepo*	Bushy	Transverse elliptical	Yellow
C15.1*	*C. maxima*	Intermediate	Transverse medium elliptic	Orange
C23	*C. maxima*	Prostrate	Transverse elliptical	Orange
C26	*C. moschata*	Prostrate	Transverse broad elliptic	Orange

* Highly susceptible to powdery mildew (data not shown).

Table 2 - Soil characteristics estimated at the initial state (before pumpkin planting) as determined by soil dilution [z] plating on selective media

Soil characteristic	Data
Initial soil characteristics	
pH	7.42
EC (dS m^{-1})	0.56
Culturable microbial population (CFU [y] g^{-1} fresh	
Total bacteria (× 10^7)	2.99
Actinomycetes (× 10^4)	0.95
Total fungi (× 10^4)	1.62
Aspergillus spp. (× 10^3)	0.12
Trichoderma spp. (× 10^3)	1.12
Fusarium spp. (× 10^3)	0.18

[z] Soil sample was a composite soil from twenty soil cores collected before planting and soil dilution was made from a concentration of 10% (w v^{-1}).
[y] CFU= Colony-Forming Unit.

15 cm in depth) were removed from the rhizosphere soil of each sampled plant and were combined to make one composite soil per accession. At the initial state (before planting), ten soil cores were removed and were combined to make one composite soil sample. Two replicates were considered for each soil sampling.

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. Two subsamples were processed from each soil sample.

Determination of soil pH and electrical conductivity (EC)

Each composite soil sample was air-dried and suspended into distilled water (1:10 soil H_2O^{-1} ratio). Soil filtrates obtained by filtration through Whatman paper No. 1 were analyzed for the determination of their pH and electrical conductivity (EC) using a glass electrode (VWR sympHony®) and a digital conductivity meter (HANNA®), respectively.

Estimation of soil microbial community structure

General populations of culturable soil microorganisms were determined using the soil dilution plating techniques on various agar media according to Larkin and Honeycutt (2006) with some modifications. For each subsample taken from each composite soil, 10 g were added to 90 ml of sterile 0.2% water agar, vigorously stirred for 30 min, serially diluted and a-100 µl sample was plated on 10% Tryptic Soy Agar (TSA) for total bacterial counts, Yeast Malt Agar (ISP medium No. 2) amended with 75 mg l^{-1} of nalidixic acid and 100 mg l^{-1} of cyclohexamide for actinomycete counts, and Potato Dextrose Agar (PDA) amended with 300 mg l^{-1} of streptomycin sulphate for total fungal counts. Three replicates of one plate each were used for each soil subsample.

Bacterial and actinomycete plates were incubated at 28°C for 2 and 14 days, respectively, and fungal plates were maintained at 25°C for 7 days. Colonies of *Trichoderma* spp., *Aspergillus* spp., and *Fusarium* spp. were identified based on their macro- and micro-morphological traits (Barnett and Hunter, 1987) under light microscope and counted separately. Colony-forming units (CFU) were counted to estimate the microbial density on each selective medium (Marin *et al*., 2013). The soil microbial population counts were estimated per 1 g of fresh soil.

Yield parameters

The average fruit weight and the average yield per plant were noted at five months post-planting. The average fruit weight parameter was determined for three randomly sampled plants.

Statistical analysis

Data were subjected to a one-way analysis of variance (ANOVA) using Statistical Package for the Social Sciences (SPSS) software for Windows version 16.0. Data for pH, EC of soil samples and rhizosphere microbial population counts were analyzed according to a completely randomized factorial model with two factors (Accessions tested × Sampling times). As for yield parameters, data were analyzed according to a completely randomized block design. Experiments were repeated twice. Means were separated using Tukey test to identify significant pair-wise differences at $P \leq 0.05$.

Correlations between fruit weight and yield parameters and soil characteristics (pH, EC and microbial community structure) were carried out using Pearson's test at $P \leq 0.05$.

For an overview of pumpkins accessions distribution, and to explore soil microbial community contributing to classification, a Principal Component Analysis (PCA) was also performed using SPSS.

3. Results

Variation of soil pH and EC

ANOVA analysis of pH values varied significantly

(at $P \leq 0.05$) depending on sampling times only. No significant difference was noted between pumpkins accessions and between both factors (Table 3). A significant decrease of pH values of about 9.9 to 12.7% was noted at harvest (150 DPP) as compared to soil samples collected at 30, 60 and 90 DPP (Table 3).

Table 3 - pH and electrical conductivity (EC) of soil samples removed from the rhizosphere of pumpkins depending on accessions tested and sampling times

Soil samples	pH	EC (dS m^{-1})
Accessions [z] means [y]		
C2	7.30 a	0.507 ef
C5	7.22 a	0.45 f
C6.2	7.27 a	0.61 bc
C7	7.18 a	0.63 abc
C9.1	7.24 a	0.69 a
C9.2	7.24 a	0.508 def
C14.1	7.17 a	0.57 cd
C14.2	7.21 a	0.66 ab
C15	7.14 a	0.62 abc
C15.1	6.69 a	0.56 cde
C23	7.19 a	0.47 f
C26	7.27 a	0.503 ef
Sampling times means [x]		
30 DPP [w]	7.44 a	0.62 a
60 DPP	7.25 a	0.61 ab
90 DPP	7.48 a	0.4 c
150 DPP	6.53 b	0.5 b
Source of variation	p-values	
Accessions (Acc)	0.27	P ≤ 0.001
Sampling times (ST)	P≤0.001	P ≤ 0.001
Acc × ST	0.48	P ≤ 0.001

[z] C7 and C15= *Cucurbita pepo*. C2, C5, C6.2, C9.1, C9.2, C14.2, C15.1 and C23= *C. maxima.* C14.1 and C26= *C. moschata*.
[y] Accessions means (for all sampling times combined) followed by the same letter are not significantly different according to Tukey test at P≤0.05.
[x] Sampling times means (for all accessions combined) followed by the same letter are not significantly different according to Tukey test at P≤0.05.
[w] DPP= Days post-planting.

ANOVA analyses revealed a significant variation in EC values among accessions, sampling times and their interaction (Table 3). The highest EC values were recorded in the rhizopshere of *C. maxima* C9.1, *C. maxima* C14.2, *C. pepo* C7 and *C. pepo* C15. As for the sampling time effect on this parameter, the EC of the rhizosphere soil associated to the twelve pumpkins accessions was 34.4-35% and 18.1-19% higher at 30-60 DPP than at 90 and 150 DPP, respectively (Table 3).

Variation of the culturable soil microbial structure

The number of bacterial and actinomycetes colonies varied significantly (at $P \leq 0.05$) among pumpkins accessions, sampling times and their interaction (Table 4). The highest population of culturable bacteria was obtained from the rhizosphere of *C. maxima* C23 and *C. pepo* C15 which was 33.1-55.8% and 15.8-44.4% more abundant than those of the remaining accessions (Table 4). The abundance of culturable bacteria in the rhizosphere of all the remaining accessions was significantly comparable. Concerning the effect of the sampling times (all accessions combined) on this parameter, bacterial colonies counts

Table 4 - Culturable bacterial, actinomycetes and fungal population densities in soil samples (CFU g^{-1} of fresh soil) removed from the rhizosphere of pumpkins plants depending on accessions tested and sampling times

Culturable microbiome population	Bacteria	Actinomycetes	Fungi
Accessions [z] means [y]			
CFU [x] g^{-1} of fresh soil	× 10^8	× 105	× 105
C2	1.68 bc	0.96 bc	1.36 a
C5	1.62 bc	1.68 a	0.93 a
C6.2	2.24 bc	1.20 abc	1.22 a
C7	1.48 c	1.08 bc	1.69 a
C9.1	1.59 bc	1.08 bc	1.59 a
C9.2	1.80 bc	0.96 bc	1.58 a
C14.1	1.77 bc	1.05 bc	1.63 a
C14.2	2.04 bc	1.13 abc	1.32 a
C15	2.66 ab	0.82 c	1.45 a
C15.1	1.98 bc	0.90 c	1.28 a
C23	3.35 a	1.53 ab	1.02 a
C26	1.93 bc	0.74 c	1.20 a
Sampling times means [w]			
CFU g^{-1} of fresh soil	× 108	× 105	× 105
30 DPP [v]	3.15 a	0.93 b	1.57 ab
60 DPP	3.08 a	2.86 a	1.86 a
90 DPP	1.06 b	0.27 c	1.41 b
150 DPP	0.74 b	0.32 c	0.58 c
Sources of variation		p-values	
Accessions (Acc)	P≤0.001	P≤0.001	0.06
Sampling times (ST)	P≤0.001	P≤0.001	P≤0.001
Acc × ST	P≤0.001	P≤0.001	0.15

[z] C7 and C15= *Cucurbita pepo*. C2, C5, C6.2, C9.1, C9.2, C14.2, C15.1 and C23= *C. maxima.* C14.1 and C26= *C. moschata*.
[y] Accessions means (for all sampling times combined) followed by the same letter are not significantly different according to Tukey test at P≤0.05.
[x] CFU= Colony forming unit.
[w] Sampling times means (for all accessions combined) followed by the same letter are not significantly different according to Tukey test at P≤0.05.
[v] DPP= Days post-planting.

from the rhizosphere of all pumpkins accessions noted at 30 and 60 DPP were 65.6-66.3 and 75.9-76.5% significantly higher than those recorded at 90 and 150 DPP, respectively.

Actinomycetes community was abundant on the rhizopshere of *C. maxima* C5, *C. maxima* C6.2, *C. maxima* C14.2 and *C. maxima* C23 which was 35.7-55.9%, 10-38-3%, 4.4-34.5% and 29.4-51.6% higher than that associated to the remaining accessions. For all pumpkins accessions combined, the actinomycetes population was 67.5, 90.5 and 91.9% significantly higher at 60 DPP than at 30, 90 and 150 DPP, respectively.

Data given in Table 4 showed that the total culturable fungal community varied significantly (at $P \leq 0.05$) depending on sampling times only and that all accessions tested exhibited significantly comparable fungal community populations. Fungal colonies recovered from the rhizosphere of all pumpkins accessions at 60 DPP were 15.6, 24.2 and 68.8% significantly higher than those recovered at 30, 90 and 150 DPP, respectively.

As for fungal community structure, culturable *Aspergillus* spp. and *Trichoderma* spp. populations varied significantly (at $P \leq 0.05$) in the rhizosphere of pumpkins plants depending on tested accessions, sampling times and their interaction (Table 5). For instance, the rhizospheric *Aspergillus* spp. community associated to *C. maxima* C14.2 was significantly 40-48.8% more abundant than that associated to *C. moschata* C26 and *C. maxima* C2 accessions. Furthermore, *Trichoderma* spp. population was significantly 75.9-84.1% higher at the rhizosphere of *C. maxima* C14.2 than at that of *C. pepeo* C7 and *C.*

Table 5 - Culturable fungal population structure in soil samples (CFU g^{-1} of fresh soil) removed from the rhizosphere of pumpkins plants depending on accessions tested and sampling times

Culturable fungal population	*Aspergillus* spp.	*Trichoderma* spp.	*Fusarium* spp.
Accessions [z] means [y]			
CFU [x] g^{-1} of fresh soil	$\times 10^4$	$\times 10^4$	$\times 10^4$
C2	1.92 b	1.33 abc	0.07 a
C5	3 ab	2 ab	0.83 a
C6.2	3.08 ab	0.66 abc	1.5 a
C7	3.33 ab	0.50 bc	0.07 a
C9.1	3.25 ab	0.33 c	0.83 a
C9.2	3 ab	0.83 abc	1.66 a
C14.1	2.42 ab	1.50 abc	0.08 a
C14.2	3.75 a	2.08 a	0.09 a
C15	2.58 ab	1.58 abc	0.08 a
C15.1	2.83 ab	1.17 abc	0.07 a
C23	2.75 ab	1.75 abc	0.09 a
C26	2.25 b	1.17 abc	0.08 a
Sampling times means [w]			
CFU g^{-1} of fresh soil	$\times 10^4$	$\times 10^4$	$\times 10^4$
30 DPP [v]	0.83 c	1.08 ab	0.27 a
60 DPP	0.55 c	0.72 b	0.08 a
90 DPP	1.44 b	1.58 a	1.38 a
150 DPP	9.81 a	1.58 a	0.27 a
Sources of variation		p-values	
Accessions (Acc)	$P \leq 0.01$	$P \leq 0.01$	0.14
Sampling times (ST)	$P \leq 0.001$	$P \leq 0.01$	0.68
Acc × ST	$P \leq 0.001$	$P \leq 0.001$	0.35

+[z] C7 and C15= *Cucurbita pepo*. C2, C5, C6.2, C9.1, C9.2, C14.2, C15.1 and C23= *C. maxima.* C14.1 and C26= *C. moschata*.
[y] Accessions means (for all sampling times combined) followed by the same letter are not significantly different according to Tukey test at $P \leq 0.05$.
[x] CFU= Colony forming unit.
[w] Sampling times means (for all accessions combined) followed by the same letter are not significantly different according to Tukey test at $P \leq 0.05$.
[v] DPP= Days post-planting.

maxima C9.1 (Table 5).

Aspergillus spp. colonies recovered from the rhizosphere of all pumpkins accessions at 150 DPP were 85.3 and 94.4% significantly higher than those recovered at 90 and 30-60 DPP, respectively. *Trichoderma* spp. population estimated was significantly higher (+54.4%) at 150 and 90 DPP than at 60 DPP. Concerning *Fusarium* spp. populations, no significant differences were detected between pumpkins accessions and sampling times nor their interaction (Table 5).

Variation of fruit production and yield among pumpkins accessions tested

Analysis of variance revealed a significant (at $P \leq 0.05$) variation of the average fruit weight between the pumpkin accessions. The highest average fruit weights ranging between 4.18 and 8.48 Kg were noted in the accessions C5, C14.2, C15.1 and C23 of *C. maxima* and C14.1 of *C. moschata* whereas for the remaining seven pumpkins accessions, this parameter varied between 2.53 and 3.9 Kg (Fig. 2A).

The average fruit yield produced per plant varied significantly (at $P \leq 0.05$) among pumpkins accessions. Four *C. maxima* accessions (namely C5, C6.2, C14.2, and C23) and one *C. moschata* accession (C26) produced significantly the highest fruit yields per plant (5.31-6.21 Kg plant^{-1}) than the remaining ones (0.91-3.96 Kg plant^{-1}) (Fig. 2B).

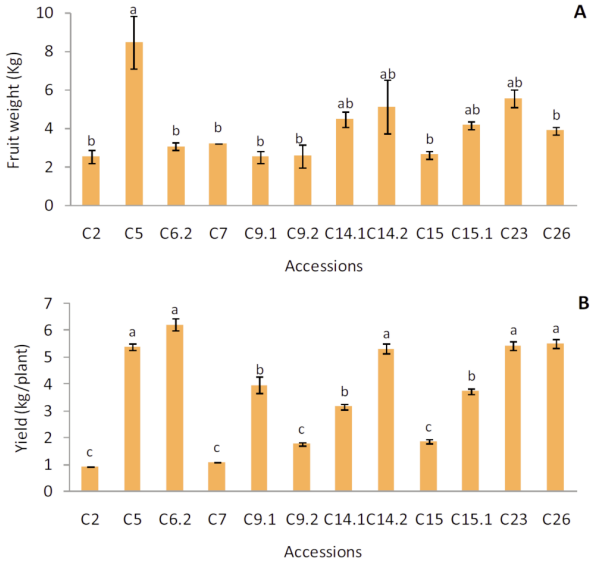

Fig. 2 - A, B Average fruit weight and yield per plant of pumpkins accessions noted five months post-planting. Bars sharing the same letter are not significantly different according to Tukey test at $P \leq 0.05$. The average fruit weight (A) and the average yield (B) per plant were determined at harvest. C7 and C15: *Cucurbita pepo*. C2, C5, C6.2, C9.1, C9.2, C14.2, C15.1, and C23: *C. maxima.* C14.1 and C26: *C. moschata.*

Correlation between production and yield parameters and soil characteristics

Pearson's correlation analysis indicated that the average fruit weight was significantly and positively correlated to the associated actinomycetes community (r= 0.765, *P*= 0.004) and *Trichoderma* spp. population (r= 0.697, *P*= 0.012) but it was significantly and negatively (r= -0.700, *P*= 0.01) linked to the total culturable fungal population in the analyzed soil samples (Fig. 3).

Pearson correlation analysis, also, revealed a significant and negative correlation between the average fruit yield per plant and the fungal population (r = -0.701; *P* = 0.011) colonizing the rhizosphere of pumpkins accessions (Fig. 3).

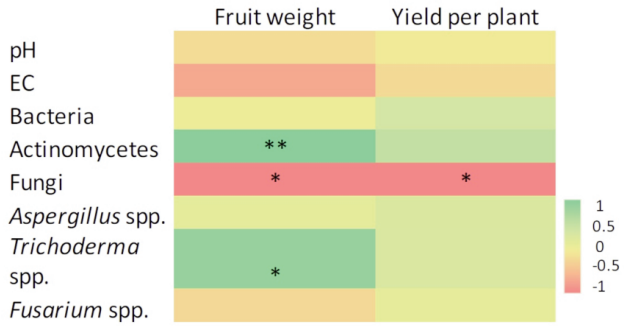

Fig. 3 - Heat map of Pearson's correlation (r) between the average fruit weight and the average yield per plant of pumpkins accessions and soil characteristics. Asterisks indicate statistically significant correlation values, negative or positive at * P≤0.05 and ** P≤0.01.

Multicriteria analysis via PCA

Based on the PCA analysis performed, the first two main components (PC) comprised about 68.31% of the variability existing in the analyzed genotypes. PC-1 explained 47.05% of the total variability. The most important traits related to this axis were: the fruit fresh weight, the yield per plant, and actinomycetes and *Trichoderma* spp. population. The most important traits of PC-2, which explained 21.25% of the total variation, were EC values and *Aspergillus* spp. community (Fig. 4A).

The distribution of pumpkins accessions among the two axes showed the variability and allowed distinguishing 3 main groups (Fig. 4B). The 1st group included 4 accessions (C5, C23, C14.2 and C6.2 belonging to *C. maxima*) characterized by the highest average fruit weight, the highest yield per plant, the

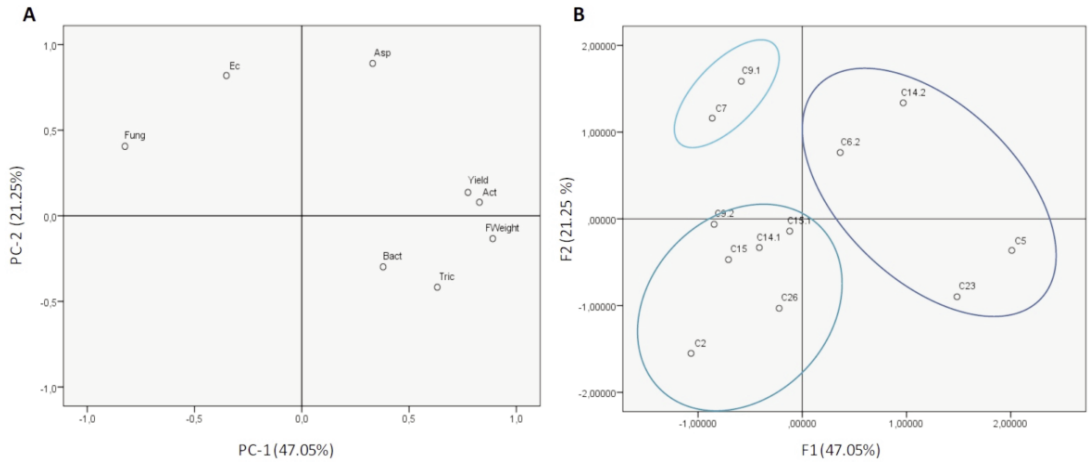

Fig. 4 - PCA biplot the variability existing in the analyzed traits (A) and the distribution of pumpkins accessions (B). Ec: Electrical conductivity. Fung: Fungi. Asp: *Aspergillus* spp. Yield: Yield per plant. Act: Actinomycetes. Fweight: Fruit weight. Bact: Bacteria. Tric: *Trichoderma* spp. C7 and C15: *Cucurbita pepo*. C2, C5, C6.2, C9.1, C9.2, C14.2, C15.1 and C23: *C. maxima*. C14.1 and C26: *C. moschata.*

highest actinomycetes and bacterial communities, the highest *Trichoderma* spp. and *Aspergillus* spp. populations, and the lowest fungal community in their rhizosphere. The 2nd group was comprised of C7 (*C. pepo*) and C9.1 (*C. maxima*) accessions characterized by the lowest fruit weight and the lowest *Trichoderma* spp. populations, and the 3rd group was composed of the remaining 6 accessions exhibiting intermediate yield per plant and *Trichoderma* spp. populations.

4. Discussion and Conclusions

Plant-associated microbiome plays a fundamental role in plant growth and health (Wei and Joussset, 2017). Breeding programs focusing genotype-associated beneficial microbiome help achieve ecologically desired plant phenotype traits (Adam *et al*., 2018; Wille *et al*., 2018). The current study aimed to select the most productive pumpkins accessions based on the variability of their soil microbial community structure and to investigate the presence of eventual links between *Cucurbita* spp. production and yield parameters and their rhizosphere soil associated microorganisms. Our results clearly demonstrated that *Cucurbita* species and accessions shaped their own soil microbial community structure. Some microbes have a particular affinity for certain pumpkins accessions in determining rhizosphere communities. The variation of composition of microbial distribution in the rhizosphere of *Cucurbita* spp. accessions may be explained by the differences in their root morphology and the composition and content of their root exudates which play a fundamental role in the recruitment of plant holobiont. Plant-associated microbiome and their interactions are highly diverse and multiple factors shape the microbial community assembly and functioning. In fact, the microbial community's structure varies significantly depending on plant species and/or genotypes growing in the same soil environment (Kang and Mills, 2004; Yao and Wu, 2010; Berendsen *et al*., 2012; Aydi Ben Abdallah *et al*., 2023) and even on plant growth stage (Chaparro *et al*., 2014; Compant *et al*., 2019). The variation in the soil-associated microbiome communities has been assigned to the differences in the root morphology, the type of rhizodeposits, the amount and the composition of root exudates and mainly carbon sources which are limiting factors for microbial activity and proliferation (Marschner *et al*., 2007; Broeckling *et al*., 2008; Compant *et al*., 2019). Moreover, edaphic factors such as soil pH, electrical conductivity (EC), soil texture, soil parental material, and soil salinity are important determinants of community structure and diversity of soil microbiome (Lozupone and Knight, 2007; Lauber *et al*., 2008; Xu *et al*., 2014; Sun *et al*., 2015; Min *et al*., 2016).

The soil-associated microbiomes have an effect on plant growth and yield production. Positive and significant correlations were determined between fruit fresh weight and the culturable bacterial and *Trichoderma* spp. populations in the rhizopshere of pumpkins accessions tested in the current investiga-

tion. Plant-associated microbes with their plant growth-promoting traits play a crucial role in enhancing plant biomass and crop yield (Kumar *et al*., 2022). Halifu *et al*. (2019) demonstrated that inoculation with two *Trichoderma* species on *Pinus Sylvestris* var. *mongolica* seedlings had a positive correlation with growth parameters, soil nutrient content, and soil enzymatic activity in their rhizosphere. *Trichoderma* spp. are able to increase the growth and the extension of the root system and to stimulate the secretion of extracellular enzymes such as sucrase, urease, phosphatase, and organic acids in the rhizosphere. These compounds lead to the improvement of the nutrient cycle and the soil enzymatic activity and consequently the soil nutrient status and availability (Pelagio-Flores *et al*., 2017). Furthermore, *Trichoderma* spp. can secrete the indole 3 acetic acid (IAA) and to promote the growth of many crops as previously demonstrated for cucumber, bottle gourd, and bitter gourd (Kotasthane *et al*., 2015). Furthermore, the volatile and non-volatile secondary metabolites released by *Trichoderma* spp. such as 6-n-pentyl-6H-pyran-2-one (6PP), gliotoxin, viridin, harzianopyridone, harziandione, and peptaibols have a significant growth-promoting effect on plants (José *et al*., 2008). Mohanty *et al*. (2021) also demonstrated that the beneficial bacterial communities may improve crop productivity as part of sustainable agriculture. In fact, *Acidothiobacillus ferooxidans* and *Bacillus cereus* are associated to increased growth and yield and improved soil composition in pumpkin (Ansari *et al*., 2017).

Plant growth improvement may be achieved either directly via the enhancement of nutrient availability and phytohormone modulation and/or indirectly through the biocontrol activity i.e. suppression of associated pathogens and/or the alleviation of biotic and abiotic stresses leading to the improvement of both plant health and crop productivity (Khan *et al*., 2020; Basu *et al*., 2021; Zhang *et al*., 2021; Kumar *et al*., 2022). In the current study, negative and significant correlations were noted between the average fruit weight and yield per plant parameters and the total culturable fungal populations. The fungal population estimated in the rhizosphere of pumpkins accessions may be mainly composed of soilborne pathogens naturally associated to pumpkins plants which may be involved in the recorded decreases in fruit weight and yield. Based on ACP analyses, the 1st group was comprised of 4 accessions of *C. maxima* (namely C5, C23, C14.2 and C6.2) which are characterized with the highest production parameters (average fruit weight and yield per plant) and the highest populations of actinomycetes, bacteria, *Trichoderma* spp. and *Aspergillus* spp., and also the lowest fungal community in their rhizosphere. Hence, these four microbial groups (bacteria, *Aspergillus* spp., *Trichoderma* spp. and actinomycetes) predominant in the rhizosphere of these 4 most productive pumpkins accessions may be involved, either individually or in consortium, indirectly in the promotion of pumpkins yield via their eventual antagonistic potential against their associated fungal pathogens. As demonstrated in Yang *et al*. (2017) study, some potential plant-beneficial microbial agents could act as network key, thus reducing the chance of a given a soil-borne pathogen to invade the target plant species. Also, Chaurasia *et al*. (2018) demonstrated the successful role of actinomycetes on plant protection and growth promotion of Solanaceae, Cucurbitaceae, Brassicaceae, Amaranthaceae, Umbelliferous, Asteraceae, Fabaceae, Asparagaceae, and Amaryllidaceae vegetable crops. Also, as demonstrated in Hung and Rutgers (2016) study, *Aspergillus* spp. are multifaceted fungi that the plant benefits with different manner such as plant growth promotion and protection. Pascual *et al*. (2017) also emphasized the role of *T. harzianum* in reducing the natural infection of melon plants by *F. oxysporum* f. sp. *melonis* and in improving their yields. In Aydi Ben Abdallah *et al*. (2019) study, *Bacillus subtilis* SV41 and *B. amyloliquefaciens* subsp. *amyloliquefaciens* SV65 have successfully decreased the soil infection potential by *Fusarium* species, suppressed Fusarium wilt severity and enhanced tomato growth and production.

In our study, the 4 selected pumpkins accessions are quite able to exploit their associated beneficial indigenous microbial communities which could be considered in the future pumpkin breeding programs.

In conclusion, this study clearly demonstrated the significant role of tested accessions in affecting the distribution of microbial community in their rhizosphere leading to differences in yield parameters between pumpkins accessions. The variation in the microbial community structure with the accessions tested might be due to the changes in the composition of their root exudates which need to be more elucidated in our future investigations. Four accessions of *C. maxima* (namely C5, C23, C14.2 and C6.2) have a great potential as they are characterized by the highest average fruit weight and yield per plant,

the highest populations of actinomycetes, bacteria, *Trichoderma* spp. and *Aspergillus* spp., and the lowest fungal population in their rhizosphere. Thus, the exploitation and the re-integration of the recovered beneficial bacterial and *Trichoderma* spp. populations associated with these four selected accessions of *C. maxima* will be considered in the future pumpkin breeding programs to reduce the threat imposed by their soil-borne pathogens and consequently led to more enhancements in pumpkin fruit yield into the less productive accessions.

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