FUNCTIONAL PLANT BREEDING JOURNAL

Scientific Journal. ISSN 2595-9433
Volume 5, Article n. 4, Jan/December
D.O.I. http://dx.doi.org/10.35418/2526-4117/v5a4
Received: 06/25/2022 - Accepted: 09/13/2022

SELECTION BETWEEN WATERMELON (*CITRULLUS* LANATUS) ACCESSIONS VIA THEIR ASSOCIATED MICROBIOTA

Rania Aydi-Ben-Abdallah[1]*, Hela Chikh-Rouhou[1], Hayfa Jabnoun-Khiareddine[1], Mejda Daami-Remadi[1]

[1] *LR21AGR03-Production and Protection for a Sustainable Horticulture (2PHD), IRESA-University of Sousse, Regional Research Centre on Horticulture and Organic Agriculture, Chott Mariem, Tunisia.*

**Corresponding author: Rania Aydi Ben Abdallah (raniaaydi@yahoo.fr)*

Abstract: Microbiome engineering, which can improve the functional capabilities of native microbial species under challenging agricultural ambiance, is an emerging biotechnological strategy to improve crop yield and resilience against biotic and abiotic environmental constraints. In this study, the microbial community structure in the rhizosphere of 7 watermelon accessions was monitored using the soil dilution plating technique on specific media. All accessions tested were screened for their root growth before planting to select accessions with an improved root system. The fruit production was determined at four months post-planting. The total soluble solids (TSS) content was measured on flesh of sampled fruits. The dendrogram of hierarchical ascending classification clustered watermelon accessions into two main groups. The 1st cluster comprised two accessions P1 and P8 which were characterized by the highest abundance of actinomycetes and *Aspergillus* spp. communities in their rhizopshere, the highest weight of fruits with sweet taste. The current study clearly demonstrated that the soil microbial community structure has been shaped by *Citrullus lanatus* accessions. Future watermelon breeding programs will be focused on the selection of accessions that are quite able to exploit these associated beneficial microbial communities for enhanced growth and improved resistance to associated biotic stresses.

Keywords: Breeding, *Citrullus lanatus*, rhizosphere microbial community

Introduction

Watermelon (*Citrullus lanatus* [Thunb.] Matsum. & Nakai) is an horticultural species of the *Cucurbitaceae* family, widely cultivated and consumed throughout the world (González et al., 2020), with global annual planting covering more than 3 million hectares and production exceeding 100 million tons (Sorokina et al., 2021). This species is a naturally rich source of the non-protein α-amino acid citrulline known by its antioxidant and vasodilatation activities (Rimando and Perkins-Veazie, 2005; Zamuz et al., 2021). Its fruits contain a wide range of bioactive compounds including glycosides, carotenoids, flavonoids alkaloids, carbohydrates, fatty acids, and essential oils (Tlili et al., 2011; Zamuz et al. 2021). However, watermelon fruit has undergone significant changes in quality traits, mainly those associated with flesh color, texture, nutrient and sugar content (Yuan et al.,

2021). Therefore, developing varieties with good yield, desirable fruit characteristics and high nutritional value is a priority for watermelon breeding programs (Chikh-Rouhou et al., 2019).

Watermelon crop was highly affected by climate change (Paroon et al., 2019). Its fruit was predominantly composed of water (>90%), therefore water and heat stress can significantly affect fruit yield and quality (Hatfield et al., 2008; Paroon et al., 2019). The root system was the major plant organ involved in water and nutrient acquisition and uptake (Zhu et al., 2011). Thus, developing varieties with an improved root system was critical for enhancing plant survival and performance of watermelon crops during periods of reduced water availability (Katuuramu et al., 2020; Luo et al. 2020; Lombardi et al. 2021). Resilient root architecture could also be important for protection of watermelon crops from root-associated pathogens, such as *Verticillium*, *Fusarium*, and root-knot nematodes thus allowing the affected plant to grow optimally with minimal decreases in their yields (Wimer et al., 2015). A screening of root traits across *C. lanatus* accessions can help identify superior genotypes able to tolerate more and better biotic and abiotic stresses.

The rhizosphere was defined as the soil region under the influence of root exudates and associated soil microorganisms i.e. root microbiome (Philippot et al., 2013). The establishment of plant-rhizosphere microbiome interaction is highly influenced by the host plant and soil properties (Zhu et al., 2011; Li et al. 2021). Plant microbiota influences both plant health and productivity and is considered nowadays as a second plant genome targeted in many breeding programs (Berendsen et al., 2018). Members of plant microbiome can be recovered from the soil (Walsh et al., 2021) and/or seeds (Jonkers et al., 2022). The interactions between these microorganisms might regulate several physiological processes in the host (Andreote et al., 2014). The understanding of the functional roles of these communities in plant health and development and in the evolution of plant phenotype (White et al., 2019) has gained growing interest in agriculture's innovated breeding strategies (Wei and Jousset, 2017). In fact, plants can actively recruit soil microbial community for positive feedbacks, but the underlying mechanisms and plant traits that drive microbiome assembly and functions are largely unknown (Pérez-Jaramillo et al., 2016). Plants employ diverse mechanisms to modulate their microbiome including structural modifications, the exudation of secondary metabolites and the coordinated action of different defense responses (Rolfe et al., 2019). Root-derived exudates, apart from supporting microbial proliferation in the rhizosphere, are also responsible for the formation of distinct microbial assemblages between plant and the rhizosphere (Pascale et al., 2020). Some of the root-associated microbiota can assist plants in nutrient assimilation, leading to the enhancement of their growth and defensive potential, but others can be detrimental for plant health and responsible of various soilborne diseases (De Coninck et al., 2015). Furthermore, soil microorganisms are indirectly involved in the determination of fruit quality (Reganold et al., 2010). Indeed, differences of rhizospheric and endophytic bacteria are recruited by different watermelon phenotypes relating to rind colors formation (Xiao et al., 2022). The knowledge of the rhizosphere microbiome, offering genetic variability to plants, opens up new horizons for plant breeding that could usher in cultivation of next-generation crops depending less on inorganic inputs, resistant to diseases and resilient to climatic changes (Gopal and Gupta, 2016).

In this study, the potential influence of rhizosphere microbiome assembly and function on watermelon breeding will be discussed through the establishment of beneficial interactions with the rhizopshere microbiome. Therefore, this study aimed to select the most productive and sweet taste watermelon accessions among seven tested, to determine their associated culturable soil microbial community and to search for an eventual link between root growth, fruit

production and quality and their associated microorganisms.

Material and methods

Plant material

Seven (07) watermelon (*Citrullus lanatus*) accessions are used in this study (Chikh-Rouhou and Garcés-Claver, 2021). They were obtained from the *Cucurbitaceae* 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 Sahline, Tunisia (N35° 45'05'', E10°42'39'').

Experimental design

Watermelon seedlings were transplanted into rows with a distance of 120 cm between seedlings within the same row and 80 cm between rows. The experimental design was a completely randomized block design. Two replicates of six seedlings each were used per accession tested. They were subjected to agricultural practices commonly adopted by farmers in the region and irrigated and fertilized as needed.

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, 120 days post-planting (DPP). At the initial state, ten soil cores were removed and were combined to make one composite soil sample. After planting, three soil cores (7 cm in diameter × 15 cm in depth) were removed from the rhizosphere soil of each sampled plant and were combined to make one composite soil per accession. 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 dH_2O^{-1} ratio). Soil filtrates obtained by filtration through Whatman paper No. 1 were analyzed for the determination of their pH and 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 (bacteria, actinomycetes and fungi) were determined using the soil dilution plating techniques on various agar media. 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 specific agar media (Larkin and Honeycutt, 2006). Three replicates of one plate each were used for each soil subsample. Colony-forming units (CFU) were counted to estimate the microbial density on each selective medium per 1 g of fresh soil (Marin et al., 2013).

Root growth and fruit production parameters

The maximum root length and the root fresh weight were determined before planting. The average fruit weight was noted at the last harvest (four months post-planting). The root growth parameters and the average fruit weight were determined for three randomly sampled plants.

Determination of total soluble solids (TSS)

Total soluble solids (TSS) content was measured by cutting a wedge of flesh and squeezing the juice into the prism of a digital refractometer (Atago PR-100, Tokyo, Japan). Three replicates were used per each accession.

Data analysis

Data were analyzed 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 root growth, fruit production and TSS parameters, data were analyzed according to a completely randomized design. Experiments were repeated twice. Means were separated using Tukey test to identify significant pair-wise differences at $P \leq 0.05$. For an overview of watermelon accessions distribution, and to explore soil microbial community contributing to classification, a Principal Component Analysis (PCA) and a dendrogram of hierachical ascending classification were performed.

Results and Discussion

Variation of soil pH and EC

ANOVA analysis of pH and EC values of the rhizosphere soil collected around the seven watermelon accessions varied significantly (at $P \leq 0.05$) depending on accessions tested, sampling times and their interaction (Table 1). The highest pH value (6.79) was recorded in the rhizosphere of P16 accession followed by 6.69 to 6.72 in the rhizosphere of P8, P14 and P15 accessions. The pH values were significantly 1-1.01 times higher at 60 and 120 DPP than at 30, 90 DPP and initial state (before planting) (Table 1). Regarding EC, the highest values (0.48 and 0.52 dS m^{-1}) were recorded in soil samples collected from the rhizopshere of P2 and P6, respectively. As for the sampling time effect on this parameter, the EC of the rhizosphere soil associated to the seven watermelon accessions was 36.5-40% and 50-52.7% higher at 90 and 120 DPP than at 30 and 60 DPP, respectively (Table 1). Significant increments of the rhizosphere EC values of 38.4-41.8% were recorded at 90 and 120 DPP compared to the initial state soil (before planting).

Table 1. pH and electrical conductivity (EC) of soil samples removed from the rhizosphere of watermelon plants depending on accessions tested and sampling times.

Accessions* (Acc)+	pH	EC (dS m^{-1})
P1	6.53 d	0.39 bc
P2	6.59 cd	0.48 a
P6	6.62 c	0.52 a
P8	6.69 b	0.29 e
P14	6.70 b	0.35 cd
P15	6.72 b	0.41 b
P16	6.79 a	0.33 d
Sampling times (ST)++		
0 DPP** (before planting)	6.64 b	0.32 b
30 DPP	6.62 b	0.33 b
60 DPP	6.70 a	0.26 c
90 DPP	6.64 b	0.52 a
120 DPP	6.71 a	0.55 a
Source of variation	P-values	
Acc	$P \leq 0.001$	$P \leq 0.001$
ST	$P \leq 0.001$	$P \leq 0.001$
Acc × ST	$P \leq 0.001$	$P \leq 0.001$

+ Accessions (for all sampling times combined) followed by the same letter are not significantly different according to Tukey test at $P \leq 0.05$.

++ Sampling times (for all accessions combined) followed by the same letter are not significantly different according to Tukey test at $P \leq 0.05$.

**DPP: Days post-planting.

The rhizosphere was largely influenced by the plant genotype via root activities (York et al., 2016). Indeed, living roots release a wide range of organic compounds to the soil that transform the physicochemical properties of the rhizosphere (Cantó et al., 2020). Indeed, plant roots and associated microorganisms can also alter rhizosphere pH via redox-coupled reactions (Canarini et al., 2019). Edaphic factors of soil such as pH, EC, texture and salinity are important determinants of community structure and diversity of soil microbiome (Min et al., 2016).

Variation of the culturable soil microbial structure

The number of bacterial, actinomycetes and fungal colonies growing from plated soil samples varied significantly (at $P \leq 0.05$) upon tested watermelon accessions, sampling times and their interaction (Table 2).

Table 2. Culturable microbiome population densities in soil samples (log CFU g^{-1} of fresh soil) removed from the rhizosphere of watermelon plants depending on accessions tested and sampling times.

Culturable microbiome (log CFU* g^{-1} fresh soil)	Bacteria	Actinomycetes	Fungi	*Aspergillus* spp.	*Trichoderma* spp.	*Fusarium* spp.
Accessions (Acc) +						
P1	5.49 c	3.71 a	3.99 ab	3.09 a	1.53 b	0.65 a
P2	5.89 a	3.60 abc	3.75 bc	2.21 ab	0.67 bc	1.04 a
P6	5.69 abc	3.43 cd	3.50 c	2.08 ab	0.84 bc	0.40 a
P8	5.81 ab	3.66 ab	3.52 c	2.05 ab	0.40 c	0.60 a
P14	5.59 bc	3.48 bcd	3.65 c	2.35 ab	0.90 bc	0.40 a
P15	5.76 ab	3.56 abcd	4.02 a	2.25 ab	2.94 a	0.23 a
P16	5.47 c	3.37 d	3.51 c	1.78 b	0.67 bc	0.62 a
Sampling times (ST) ++						
0 DPP* (before planting)**	6.15 a	3.26 c	3.53 b	1.60 b	0.76 a	0.89 ab
30 DPP	5.15 d	3.26 c	3.49 b	0.45 c	1.43 a	1.33 a
60 DPP	5.62 c	3.67 b	3.67 b	2.02 b	1.25 a	0.30 bc
90 DPP	5.61 c	3.57 b	3.92 a	3.48 a	1.15 a	0.28 bc
120 DPP	5.82 b	3.96 a	3.92 a	3.75 a	1.07 a	0.10 c
Sources of variation	**P-values**					
Acc	$P \leq 0.001$	$P \leq 0.001$	$P \leq 0.001$	0.05	$P \leq 0.001$	0.44
ST	$P \leq 0.001$	$P \leq 0.001$	$P \leq 0.001$	$P \leq 0.001$	0.27	$P \leq 0.001$
Acc × ST	$P \leq 0.001$	$P \leq 0.001$	$P \leq 0.001$	$P \leq 0.05$	$P \leq 0.001$	$P \leq 0.05$

+ Accessions means (for all sampling times combined) followed by the same letter are not significantly different according to Tukey test at $P \leq 0.05$. ++ Sampling times means (for all accessions combined) followed by the same letter are not significantly different according to Tukey test at $P \leq 0.05$. *CFU: Colony forming unit. ***DPP: Days post-planting.

The population of culturable bacteria estimated on soil samples collected from the rhizosphere of the accessions P2, P8 and P15 were significantly (1.07, 1.05-1.06 and 1.04-1.05) more abundant than those of P1 and P16 accessions (Table 2). Concerning the effect of the sampling times, bacterial colonies recovered from the rhizosphere of all watermelon accessions noted at 30, 60-90, and 120 DPP were 16.2, 8.6-8.7 and 5.3% significantly lower than those recorded at the initial state, respectively.

Actinomycetes community was abundant on the rhizopshere of the accessions P2, P8 and P1 which was significantly 6.3, 7.9 and 9.2% higher than P16 accession, respectively. For all watermelon accessions, the actinomycetes population was 7.3, 9.8, and 17.6% significantly higher at 120 DPP than at 60, 90 and 30 DPP. The actinomycete population was 17.6% significantly higher at 120 DPP compared to the initial soil state (Table 2).

The total culturable fungal community estimated on the rhizosphere of P1 and P15 accessions was 8.5-12.2 and 9.2-12.9% significantly higher than the remaining accessions (P6, P8, P14, P15, and P16), respectively (Table 2). Fungal colonies recovered from the rhizosphere of all watermelon accessions at 90 and 120 DPP were 7.3 and 11.8% significantly higher than those recovered at 30 and 60 DPP. The fungal community was 10.9% significantly higher at 90 and 120 DPP than at the initial soil state (before planting).

As for fungal community structure, culturable *Aspergillus* spp., *Trichoderma* spp., and *Fusarium* spp. populations varied significantly (at $P \leq 0.05$) in the rhizosphere of watermelon plants depending on accessions tested and sampling times (Table 2). Only *Trichoderma* spp. population varied significantly (at $P \leq 0.05$) upon tested watermelon accessions only. For instance, the rhizospheric *Trichoderma* spp. community associated to P15 accession was 47.9 to 86.3% significantly more abundant than the remaining accessions (Table 2). Concerning the effect of

the sampling times (for all accessions combined), *Aspergillus* spp. and *Fusarium* spp. populations varied significantly (at $P \leq 0.05$) upon this factor. *Aspergillus* spp. colonies from the rhizosphere of all watermelon accessions noted at 90 and 120 DPP were 41.9-46.1 and 87.1-88% significantly higher than those recorded at 60 and 30 DPP, respectively. *Aspergillus* spp. colonies recovered from the rhizosphere of all watermelon accessions were 54-57.3% significantly higher at 90 and 120 DPP as compared to the initial soil state (Table 2). However, *Fusarium* spp. colonies recovered from the rhizosphere of all watermelon accessions was 77.4 to 92.4% significantly higher at 30 DPP than those recovered at 60, 90 and 120 DPP. *Fusarium* spp. population estimated at 30 DPP was similar as that noted at the initial soil state (Table 2).

The microbial community's structure varies significantly depending on plant species and/or genotypes, growing in the same soil environment, and even on plant growth stage (Compant et al., 2019). Some microbes have a particular affinity for certain watermelon accessions in determining rhizosphere communities which can be explained by the variation in their root exudates composition. The microbial rhizosphere assembly was extremely dynamic and mostly influenced by rhizodeposits that can act as major carbon sources for microbes (Kandaswamy et al., 2017). Plant roots influence soil microbial communities leading to a very specific rhizosphere microbiome characterized by a larger active microbial community, but exhibiting reduced diversity compared with bulk soil (Lopes et al., 2019). The rhizosphere composition varied for the same plant species, according to the available source of microbes in each soil, possibly indicating that the functioning of the rhizosphere components drives the selection of the organisms (Andreote et al., 2014). It was a strong interdependence between plant genotype and rhizosphere microbiome composition however, the plant genes underlying this process was little unknown (Jacoby et al., 2017). Cantó et al. (2020) highlighted the significant role of organic acids and genes controlling their transport in the establishment of the rhizosphere microbiome. Additionally, plant life cycle imposes a temporal pattern in exudates secretion that sculpts the dynamics of its root-associated microbiota (Edwards et al., 2018). Cantó et al. (2020) studies suggest the important coordination between plant developmental stage and changes in root-associated microbiota to counterbalance plant immunity and nutrition needs. Zhalnina et al. (2018) demonstrate that the fluctuations in chemical composition of root exudates during plant growth respond to the substrate metabolite preferences of rhizosphere-associated microorganisms. The relative abundance of bacteria, actinomycetes and *Trichoderma* spp. populations shaped the 7 tested watermelon accessions which let to explore accessions with highest microbial community in their rhizosphere. Yang et al. (2017) found that plant holobiont including *Trichoderma* spp. and non pathogenic *Aspergillus* spp. could act as key network, thus reducing the chance of further plant soil-borne pathogen invasion. A possible implication for breeding programs could be the selection of accessions enriching less pathogenic microorganisms and/or expressing a higher microbial diversity in their rhizosphere (Compant et al., 2019).

Variation of root growth parameters, fruit production and total soluble solids of fruit flesh among watermelon accessions

Analysis of variance revealed a significant (at $P \leq 0.05$) variation of the root growth parameters, the maximum root length and the root fresh weight, between the seven watermelon accessions tested (Figures 1a and 1b). The maximum root length recorded in the accession P2 was 33.3 to 50% significantly higher than that of the accessions P1, P6 and P8 and similar to that noted in the accessions P14, P15 and P16 (Figure 1a). The root fresh weight of P14, P15 and P16 accessions was significantly 52.2-54.6 and 60.6-62.6% higher than those of the accessions P2 and P6 and similar to those of P1 and P8 (Figure 1b).

Bars sharing the same letter are not significantly different according to Tukey test at $P \leq 0.05$. The maximum root length (a) and the root fresh weight (b) were determined on

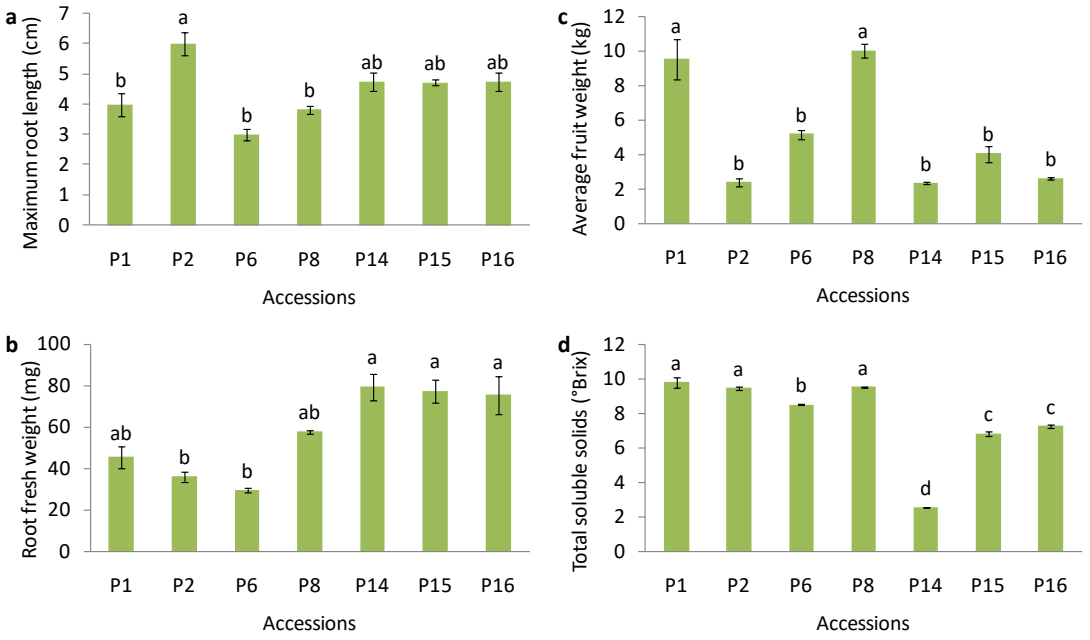

Figure 1. Variation of root growth parameters, fruit production and fruit total soluble solids between watermelon accessions tested.

watermelon seedlings before planting. The average fruit weight (c) and the total soluble solids (d) were determined at the last harvest (four months post-planting).

Root architecture and anatomy traits are starting to become an integral target of crop breeding programs (Lynch, 2019; Lombardi et al. 2021). Indeed, root phenotype has a profound impact on plant fitness and it was likely to be under strong selection (Schmidt et al., 2016). In addition, root development modifies soil structure around the root and contributes to the formation and dynamic of the rhizosphere in which the root-associated soil microbiome plays a crucial role in plant growth and health (Cantó et al., 2020). Regarding the selection exerted in the rhizosphere, a question that needs to be robustly addressed remains concerning the selection of the taxonomical affiliation of rhizosphere organisms or their ability to perform specific roles when closely associated with plant roots (Andreote et al., 2014). The variation of the microbial structure and distribution in the rhizosphere of tested watermelon 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.

The average fruit weight and the total soluble solids (TSS) of watermelon fruits varied significantly (at $P \leq 0.05$) among the tested accessions. The highest average fruit weights of 9.5 and 10 Kg were noted in the accessions P1 and P8 whereas for the remaining five watermelon accessions, this parameter varied between 2.35 and 5.17 Kg (Figure 1c). As for fruit quality, the TSS ranged from 2.56 to 9.8 °Brix for all tested accessions. The highest TSS values of 9.46-9.8°Brix were recorded in the accessions P1, P2 and P8 followed by 8.5 °Brix recorded in P6, then 6.83-7.26 °Brix measured in the accessions P15 and P16 (Figure 1d).

The soil-associated microbiome plays a crucial role in enhancing crop yield and fruit quality (Bertola et al., 2021; Kumar et al., 2022). The most studied nutritional functions performed by rhizosphere microbiome are phosphate solubilization, organic phosphorus mineralization and siderophore production (Lally et al., 2017). Moreover, production and excretion of phytohormones by rhizospheric bacteria have been reported to be associated to increased root surface area for more nutrient and water uptake from the soil and consequently enhanced productivity and added fruit nutritional values (Khan et al., 2020; Bertola et al., 2021). Furthermore, suppression of soil-borne pathogens and resilience

to water deficiency and salinity conditions by rhizosphere microbiome indirectly enhance crop productivity (Kumar et al., 2022).

Multicriteria analysis

The PCA analysis showed that three main components accounted for most total variation of 76.08% (Table 3). PC-1 explained 36.14% the greatest relative influence for the distribution of the accessions tested. The most important traits related to this axis were: Actinomycetes community, *Aspergillus* spp. population, fruit fresh weight and TSS. The most important traits of PC-2, which explains 22.36% of the total variation, were fungal population, *Trichoderma* spp. community, and root fresh weight. For PC-3, the traits were EC, bacteria community and maximum root length (Table 3).

Table 3. Contribution percentage and major characters associated with the three first principal components of watermelon accessions

	PC-1	PC-2	PC-3
Explained proportion of variation (%)	36.149	22.362	17.576
Cumulative proportion of variation (%)	36.149	58.511	**76.087**
Traits	**Eigenvectors**		
pH	-0.911	0.115	-0.157
EC	0.357	-0.305	**0.740**
Bacteria	0.222	-0.285	**0.521**
Actinomycetes	**0.821**	0.259	-0.174
Fungi	0.525	**0.801**	0.254
***Aspergillus* spp.**	**0.747**	0.496	-0.137
***Trichoderma* spp.**	0.173	**0.773**	0.244
Maximum root length	-0.198	0.328	**0.601**
Root fresh weight	-0.688	**0.657**	-0.246
Fruit fresh weight	**0.662**	-0.079	-0.702
Total soluble solids	**0.694**	-0.418	-0.055

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

The dendrogram of hierarchical classification (Figure 2) clustered watermelon accessions into two main groups. The 1st cluster comprised two accessions i.e. P1 and P8 characterized by the highest presence of actinomycetes and *Aspergillus* spp. communities in their rhizosphere, the highest weight of fruits with sweet taste. This cluster represents a good source of fruit quality. Hence, P1 and P8 could be selected as promising accessions for further breeding programs. The 2nd cluster contained 5 accessions, namely P2, P14, P16; P15 and P6, which is further divided in two sub-clusters (P2, P14 and P16) characterized by the highest bacterial population and the highest EC values in their rhizosphere, the highest root length, and intermediate to lowest fruit fresh weight and TSS fruit content. The sub-cluster (P6 and P15) was characterized by the highest total fungi and *Trichoderma* spp. populations in their rhizosphere and intermediate fruit fresh weight and fruit TSS content.

We suggested herein, that actinomycetes and *Aspergillus* spp. communities in the rhizosphere of the two most productive and sweet watermelon accessions (namely P1 and P8) may be involved, either individually or in consortium, in promoting watermelon yield and fruit quality via their direct or indirect plant-growth promoting traits. The beneficial effect of actinomycetes on plant growth and health are demonstrated in various vegetable crops (Chaurasia et al., 2018). Furthermore, actinomycetes are known able to synthesize of diverse secondary metabolites which allows them to participate in the metabolism of carbohydrates including polysaccharides that are important in ripe stage of watermelon fruit (Saminathan et al., 2018). *Aspergillus* spp. are multifaceted fungi that the plant benefits with different manner such as plant growth promotion and protection (Hung and Rutgers, 2016). The rhizosphere microorganisms could be attributed to the fruit's ability to use a wide variety of carbon sources such as carbohydrates, amino acids, and lipids, which could help resist different environmental changes that occur during fruit development (Xia et al., 2015). Indeed, an active microbiome could play a major role in the cell wall modifications process with their ability to produce hydrolytic enzymes that are including in de-esterification and depolymerization, and consequently loss of galacturonic acid and neutral sugars followed by solubilization of oligosaccharides and remaining sugar residues (Saminathan et al., 2018).

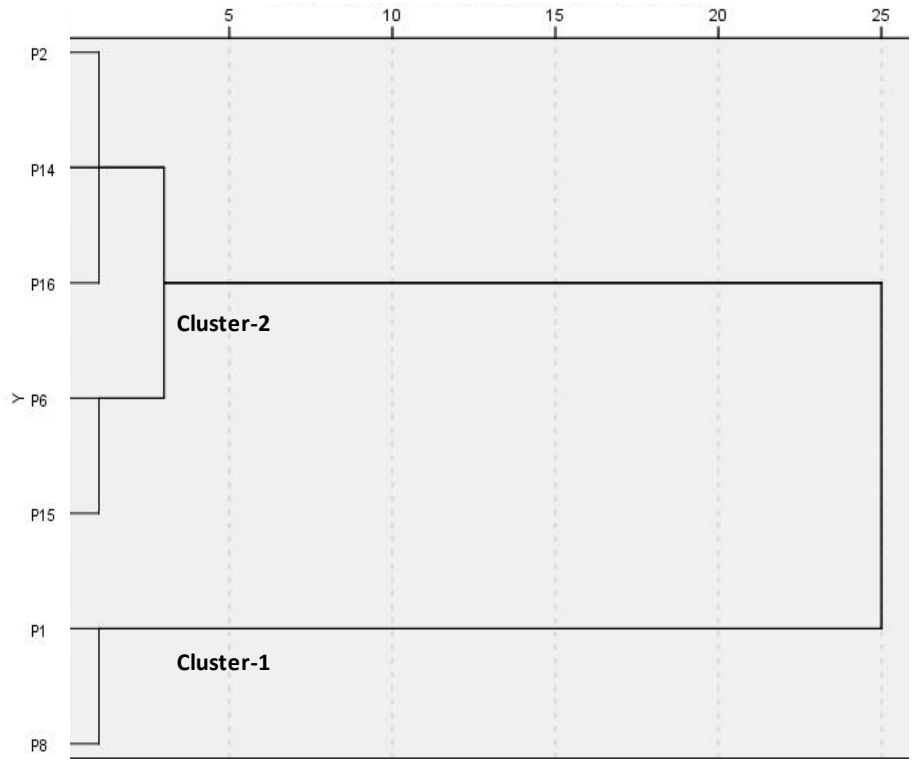

Figure 2. Dendrogram of hierachical ascending classification of watermelon accessions tested.

Conclusion

This study clearly demonstrated the significant role of tested accessions in the variable distribution of microbial community in their rhizosphere leading to differences in fruit production and total soluble solids of fruit flesh between watermelon accessions. The two most productive and sweet watermelon accessions P1 and P8 are quite able to exploit and re-integrate their associated beneficial microbial communities which will be considered in future watermelon breeding programs to enhance watermelon fruit yield and sweet taste into less productive and less sweet taste accessions but characterized by their highly resistance against soil-borne pathogens especially *Fusarium oxysporum* f. sp. *niveum*.

Acknowledgments

This work was funded by the Ministry of Higher Education and Scientific Research of Tunisia through the funding allocated to the research laboratory LR21AGR03-Production and Protection for a Sustainable Horticulture (2PHD), IRESA-University of Sousse, Regional Research Centre on Horticulture and Organic Agriculture of Chott- Mariem, Tunisia.

References

ANDREOTE, F.D.; GUMIERE, T.; DURRER, A. 2014. Exploring interactions of plant microbiomes. **Sciencia Agricola**, 71(6):528-539.

BERENDSEN, R.L.; VISMANS, G.; YU, K.; SONG, Y.; DE JONGE, R.; BURGMAN, W.P.; BURMOLLE, M.; HERSCHEND, J.; BAKKER, P.; PIETERSE, C.M.J. 2018. Disease-induced assemblage of a plant beneficial bacterial consortium. **Multidisciplinary Journal of Microbial Ecology**, 12:1496-1507.

BERTOLA, M.; FERRARINI, A.; VISIOLI, G. 2021. Improvement of soil microbial diversity through sustainable agricultural practices and its evaluation by omics approaches: A perspective for the environment, food quality and human safety. **Microorganisms**, 9(7):1400-1422.

CANARINI, A.; KAISER, C.; MERCHANT, A.; RICHTER, A.; WANEK, W. 2019. Root exudation of primary metabolites: mechanisms and their roles in plant responses to environmental stimuli. **Frontiers in Plant Science**, 10:157-165.

CANTÓ, C.F.; SIMONIN, M., KING, E.; MOULIN, L.; BENNETT, M.J.; CASTRILLO, G.; LAPLAZE, L. 2020. An extended root phenotype: the rhizosphere, its formation and impacts on plant fitness. **The Plant Journal,** 103:951-964.

CHAURASIA, A.; MEENA, B.R.; TRIPATHI, A.N.; PANDEY, K.K.; RAI, A.B.; SINGH, B. 2018. Actinomycetes: an unexplored microorganisms for plant growth promotion and biocontrol in vegetable crops. **World Journal of Microbiology and Biotechnology**, 34:132-147.

CHIKH-ROUHOU, H.; FHIMA, I.; STA-BABA, R.; KHETTABI, M.; GONZALEZ, V.; GARCES-CLAVER, A. 2019. Characterization of Tunisian genetic resources of watermelon (*Citrullus lanatus*). In: Direk, H. (editor). **Proceedings Book of the 6th International Conference on Sustainable Agriculture and Environment (ICSAE)**, Konya-Turkey, pp. 582-585.

CHIKH-ROUHOU, H.; GARCES-CLAVER, A. 2021. *Citrullus* spp. germplasm diversity in Tunisia: An overview. **Cucurbit Genetics Cooperative Reports**, 44:1-3.

COMPANT, S.; SAMAD, A.; FAIST, H.; SESSITSCH, A. 2019. A review on the plant microbiome: Ecology, functions, and emerging trends in microbial application. **Journal of Advanced Research**, 19:29-37.

DE CONINCK, B.; TIMMERMANS, P.; VOS, C.; CAMMUE, B.P.; KAZAN, K. 2015. What lies beneath: belowground defense strategies in plants. **Trends in Plant Science**, 20(2):91-101.

EDWARDS, J.A.; SANTOS-MEDELL, C.M.; LIECHTY, Z.S.; NGUYEN, B.; LURIE, E.; EASON, S.; PHILLIPS, G.; SUNDARESAN, V. 2018. Compositional shifts in root-associated bacterial and archaeal microbiota track the plant life cycle in field-grown rice. **PLoS Biology**, 16(2):1-28.

GONZÁLEZ, V.; ARMIJOS, E.; GARCÉS-CLAVER, A. 2020. Fungal endophytes as biocontrol agents against the main soil-borne diseases of melon and watermelon in Spain. **Agronomy**, 10(6):820-840.

GOPAL, M.; GUPTA, A. 2016. Microbiome selection could spur next-generation plant breeding strategies. **Frontiers in Microbiology**, 7:1971-1980.

HATFIELD, J.L.; BOOTE, K.J.; KIMBALL, B.A.; WOLFE, D.W.; ORT, D.R.; IZAURRALDE, R.C.; THOMPSON, A.M.; MORGAN, J.A.; POLLEY, H.W.; FAY, P.A.; MADER, T.L.; HAHN, G.L. 2008. The effects of climate change on agriculture, land resources, water resources, and biodiversity. Report by the U.S. climate change science program and the subcommittee on global change research., **U.S. Department of Agriculture**, **Washington, DC,** p. 21-74.

HUNG, L.; RUTGERS, S. 2016. Application of *Aspergillus* in plant growth promotion. New and future developments in microbial biotechnology and bioengineering. In: GUPTA, V.K. (editor). *Aspergillus* system properties and applications. **New York**, **Elsevier**, pp. 223-227.

JACOBY, R.; PEUKERT, M.; SUCCURRO, A.; KOPRIVOVA, A.; KOPRIVA, S. 2017. The role of soil microorganisms in plant mineral nutrition-current knowledge and future directions. **Frontiers in Plant Science**, 8: 1-19.

JONKERS, W.; GUNDEL, P.E.; VERMA, S.K.; WHITE, J.F. 2022. Editorial: Seed Microbiome Research. **Frontiers in Microbiology**, 13:943329.

KANDASWAMY, R.; BABURAJ, B.; SANTHANAM, S.; BALASUNDARAM, U. 2017. Plant growth promoting rhizobacteria Bacillus subtilis RR4 isolated from rice rhizosphere induces malic acid biosynthesis in rice roots. **Canadian Journal of Microbiology**, 64:20-27

KATUURAMU, D.N.; WECHTER, W.P.; WASHINGTON, M.L.; HORRY, M.; CUTULLE, M.A.; JARRET, R.L.; LEVI, A. 2020. Phenotypic diversity for root traits and identification of superior germplasm for root breeding in watermelon. **HortScience**, 55(8):1272-1279.

KHAN, N.; BANO, A., ALI, S.; BABAR, M.D.A. 2020. Crosstalk amongst phytohormones from planta and PGPR under biotic and abiotic stresses. **Plant Growth Regulation**, 90(5): 189-203.

KUMAR, S.; DIKSHA, S.; SINDHU, S.; KUMAR, R. 2022. Biofertilizers: An ecofriendly technology for nutrient recycling and environmental sustainability. **Current Research in Microbial Sciences**, 3:100094.

LALLY, R.D.; GALBALLY, P.; MOREIRA, A.S.; SPINK, J.; RYAN, D.; GERMAINE, K.J.; DOWLING, D.N. 2017. Application of endophytic *Pseudomonas fluorescens* and a bacterial consortium to *Brassica napus* can increase plant height and biomass under greenhouse and field conditions. **Frontier in Plant Science**, 8:2193-2203.

LARKIN, R.P.; HONEYCUTT, C.W. 2006. Effect of different 3-year cropping systems on soil microbial communities and Rhizoctonia diseases of potato. **Phytopathology**, 96(1):69-79.

LI, J.; WANG, C.; LIANG, W.; LIU, S. 2021. Rhizosphere microbiome: The emerging barrier in plant-pathogen interactions. **Frontiers in Microbiology**, 12:772420.

LOMBARDI, M.; DE GARA, L.; LORETO, F. 2021. Determinants of root system architecture for future-ready, stress-resilient crops. **Physiologia Plantarum**, 172(4):2090-2097.

LOPES, L.D.; WEISBERG, A.J.; DAVIS, E.W.; VARIZE, C.S.; PEREIRA-SILVA, M.C.; CHANG, J.H.; LOPER, J.E.; ANDREOTE, F.D. 2019. Genomic and metabolic differences between *Pseudomonas putida* populations inhabiting sugarcane rhizosphere or bulk soil. **PLoS ONE**, 14(10):e0223269.

LUO, H.; XU, H.; CHU, C.; HE, F.; FANG, S. 2020. High Temperature can Change Root System Architecture and Intensify Root Interactions of Plant Seedlings. **Frontiers in Plant Science**, 11:160-173.

LYNCH, J.P. 2019. Root phenotypes for improved nutrient capture: an underexploited opportunity for global agriculture. **New Phytopathology**, 223(2):548-564.

MARIN, F.; SANTOS, M.; DIANEZ, F.; CARRETERO, F.; GEA, F.J.; YAU, J.A.; NAVARRO, M.J. 2013. Characters of compost teas from different sources and their suppressive effect on fungal phytopathogens. **World Journal of Microbiology and Biotechnology**, 29(8):1371-1382.

MIN, W.; GUO, H.; ZHANG, W.; ZHOU, G.; MA, L.; YE, J.; LIANG, Y.; HOU, Z. 2016. Response of soil microbial community and diversity to increasing water salinity and nitrogen fertilization rate in an arid soil. **Acta Agriculturae Scandinavica Section B**, 66(2):117-126.

PAROON, S.; YAVARI, G.; REZAZADEH, M. 2019. Impacts of climate change on watermelon and cucumber agriculture in Hormozgan province and prediction of long-term pattern. **Geographical Researches**, 34(3):347-355.

PASCALE, A.; PROIETTI, S.; PANTELIDES, I.S.; STRINGLIS, I.A. 2020. Modulation of the root microbiome by plant molecules: The basis for targeted disease suppression and plant growth promotion. **Frontiers in Plant Science**, 10:1741-1764.

PEREZ-JARAMILLO, J.E; MENDES, R.; RAAIJMAKERS, J.M. 2016. Impact of plant domestication on rhizosphere microbiome assembly and functions. **Plant of Molecular Biology**, 90(6):635-644.

PHILIPPOT, L.; RAAIJMAKERS, J.M.; LEMANCEAU, P.; PUTTEN, W.H. VAN, D. 2013. Going back to the roots: the microbial ecology of the rhizosphere. **Nature Reviews Microbiology**, 11:789-799.

REGANOLD, J.P.; ANDREWS, P.K.; REEVE, J.R.; CARPENTER-BOGGS, L.; SCHADT, C.W.; ALLDREDGE, J. R.; ROSS, C.F.; DAVIES, N. M.; ZHOU, J. 2010. Fruit and soil quality of organic and conventional strawberry agroecosystems. **PLoS ONE**, 5(10):1-14.

RIMANDO, A.M.; PERKINS-VEAZIE, P.M. 2005. Determination of citrulline in watermelon rind. **Journal of Chromatography A**, 1078(1-2):196-200.

ROLFE, S.A.; GRIFFITHS, J.; TON, J. 2019. Crying out for help with root exudates: adaptive mechanisms by which stressed plants assemble health-promoting soil microbiomes. **Current Opinion in Microbiology**, 49:73-82.

SAMINATHAN, T.; GARCÍA, M.; GHIMIRE, B.; LOPEZ, C.; BODUNRIN, A.; NIMMAKAYALA, P.; ABBURI, VL.; LEVI, A.; BALAGURUSAMY, N.; REDDY, U.K. 2018. Metagenomic and metatranscriptomic analyses of diverse watermelon cultivars reveal the role of fruit associated microbiome in carbohydrate metabolism and ripening of mature fruits. **Frontiers in Plant Science**, 9:4-17.

SCHMIDT, J.E.; BOWLES, T.M.; GAUDIN, A.C.M. 2016. Using ancient traits to convert soil health into crop yield: impact of selection on maize root and rhizosphere function. **Frontiers in Plant Science**, 7:373-384.

SOROKINA, M.; MC.CAFFREY, K.S.; DEATON, E.E.; MA G.; ORDOVÁS, J.M.; PERKINS-VEAZIE, P.M.; STEINBECK, C.; LEVI, A.; PARNELL, L.D. 2021. A Catalog of Natural Products Occurring in Watermelon-*Citrullus lanatus*. **Frontiers in Nutrition**, 8:729822.

TLILI, I.; HDIDER, C.; LENUCCI, M.S.; ILAHY, R.; JEBARI, H.; DALESSANDRO, G. 2011. Bioactive compounds and antioxidant activities during fruit ripening of watermelon cultivars. **Journal of Food Composition and Analysis**, 24(7):923-928.

WALSH, C.M.; BECKER-UNCAPHER, I.; CARLSON, M.; FIERER, N. 2021. Variable influences of soil and seed-associated bacterial communities on the assembly of seedling microbiomes. **Multidisciplinary Journal of Microbial Ecology**, 15(9):2748-2762.

WEI, Z.; JOUSSET, A. 2017. Plant breeding goes microbial. **Trends in Plant Science**, 22(7):555-558.

WHITE, J.F.J.; KINGSLEY, K.L.; ZHANG, Q.; VERMA, R.; OBI, N.; DVINSKIKH, S.; ELMORE, M.T.; VERMA, S.K.; GOND, S.K.; KOWALSKI, K.P. 2019. Review: Endophytic microbes and their potential applications in crop management. **Pest Management Science**, 75(10):2558-2565.

WIMER, J.; INGLIS, D.; MILES, C. 2015. Evaluating grafted watermelon for verticillium wilt severity, yield, and fruit quality in Washington state. **HortScience**, 50(9):1332–1337.

XIA, B.; GAO, Q.F.; WANG, J.; LI, P.; ZHANG, L.; ZHANG, Z. 2015. Effects of dietary carbohydrate level on growth, biochemical composition and glucose metabolism of juvenile sea cucumber *Apostichopus japonicus* (Selenka). **Aquaculture**, 448:63-70.

XIAO J., CHE, S.Y.; SUN, Y.; YANG, S.D.; HE, Y. 2022. Differences of rhizospheric and endophytic bacteria are recruited by different watermelon phenotypes relating to rind colors formation. **Scientific Reports**, 12:6360-6373.

YANG, H.; LI, J.; XIAO, Y.; GU, Y.; LIU, H.; LIANG, Y.; LIU, X.; HU, J.; MENG, D.; YIN, H. 2017. An integrated insight into the relationship between soil microbial community and Tobacco bacterial wilt disease. **Frontiers in Microbiology**, 8:2179-2189.

YORK, L.M.; CARMINATI, A.; MOONEY, S.J.; RITZ, K.; BENNETT, M.J. 2016. The holistic rhizosphere: integrating zones, processes, and semantics in the soil influenced by roots. **Journal of Experimental Botany**, 67(12):3629-3643.

YUAN, P.; HE, N.; UMER, M.J.; ZHAO, S.; DIAO, W.; ZHU, H.; DOU, J.; KASEB, M.O.; KUANG, H.; LU, X.; LIU, W. 2021. Comparative metabolomic profiling of *Citrullus* spp. fruits provides evidence for metabolomic divergence during domestication. **Metabolites**, 11(2):78-93.

ZAMUZ, S.; MUNEKATA, P.E.S.; GULL´ON, B.; ROCCHETTI, G.; MONTESANO, D., LORENZO, J.M. 2021. *Citrullus lanatus* as source of bioactive components: An up-to-date review. **Trends in Food Science & Technology**, 111: 208-222.

ZHALNINA, K.; LOUIE, K.B.; HAO, Z.; MANSOORI, N. ; DA ROCHA, U.N.; SHI, S.; CHO, H.; KARAOZ, U.; LOQUÉ, D.; BOWEN, B.P.; FIRESTONE, M.K.; NORTHEN, T.R.; BRODIE, E.L. 2018. Dynamic root exudate chemistry and microbial substrate preferences drive patterns in rhizosphere microbial community assembly. **Nature Microbiology**, 3(4):470-480.

ZHU, J.; INGRAM, P.A.; BENFEY, P.N.; ELICH, T. 2011. From lab to field, new approaches to phenotyping root system architecture. **Current Opinion in Plant Biology**, 14(3):310-317.