Oasis Desert Farming Selects Environment-Specific Date Palm Root Endophytic Communities and Cultivable Bacteria that Promote Resistance to Drought

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2 **and Cultivable Bacteria that Promote Resistance to Drought**

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20

21 **Running title:** Oasis Palm Endophytes Promote Drought Resistance

22

23 **Summary**

24

25 Oases are desert-farming agro-ecosystems, where date palm (*Phoenix dactylifera* L.) plays a
26 keystone role in offsetting the effects of drought and maintaining a suitable microclimate for
27 agriculture. At present, abundance, diversity and plant growth promotion (PGP) of date palm
28 root-associated bacteria remain unknown. Considering the environmental pressure determined
29 by the water scarcity in the desert environments, we hypothesized that bacteria associated
30 with date palm roots improve plant resistance to drought. Here, the ecology of date palm root
31 endophytes from oases in the Tunisian Sahara was studied with emphasis on their capacity to
32 promote growth under drought. Endophytic communities segregated along a north–south
33 gradient in correlation with geo-climatic parameters. Screening of 120 endophytes indicated
34 that date palm roots select for bacteria with multiple PGP traits. Bacteria rapidly cross-
35 colonised the root tissues of different species of plants, including the original Tunisian date
36 palm cultivar, Saudi Arabian cultivars and *Arabidopsis*. Selected endophytes significantly
37 increased the biomass of date palms exposed to repeated drought stress periods during a nine-
38 months greenhouse experiment. Overall, results indicate that date palm roots shape
39 endophytic communities that are capable to promote plant growth under drought conditions,
40 thereby contributing an essential ecological service to the entire oasis ecosystem.

41

42 **Keywords:** Oasis, Date palm (*Phoenix dactylifera*), Endophytes, Plant Growth Promoting Potential,
43 Drought, Desert farming.

44

45 **Introduction**

46

47 Desert oases are fragile ecosystems, where environmental conditions are milder than those in the
48 surrounding desert ensuring fertility and allowing desert farming (Mekki *et al.*, 2013). Date palm
49 (*Phoenix dactylifera* L.) is a keystone species in the oasis ecosystem because it conditions the oasis
50 microclimate by controlling air temperature, humidity and soil-water dynamics, making it suitable for
51 agriculture (de Grenade *et al.*, 2013). However, date palm cultivation is threatened by low rainfall,
52 high temperatures, water resources often high in salt content, and high incidence of pests (Downer,
53 2004; El-Juhani *et al.*, 2010; Barreveld, 1993).

54 Plant-associated microbes (rhizobacteria and endophytes) benefit the host by positively affecting
55 paedogenesis and nutrient availability, stimulating growth, suppressing diseases, inducing abiotic
56 stress tolerance and influencing crop yield and quality (Mapelli *et al.*, 2012; Berg *et al.*, 2013; Puente
57 *et al.*, 2004). Changes in the structure of plant-associated bacterial communities towards the selection
58 of assemblages that are metabolically and physiologically adapted to abiotic stress, improve the
59 resistance to stressors such as drought by providing different services to plants: (i) production of
60 exopolysaccharides that protect roots from mechanical stress determined by dry soil compactness; (ii)
61 promotion of osmolyte accumulation that contribute to reduce cell dehydration; (iii) enzymatic and
62 non-enzymatic alleviation of oxidative stress; and (iv) synthesis of hormone-like substances that
63 modulate root development and hormone homeostasis (Ahmad *et al.*, 2008; Balloi *et al.*, 2010; Berard
64 *et al.*, 2012; de Zelicourt *et al.*, 2013; Lau *et al.*, 2012).

65 Despite evidence that endophytic bacteria are capable of supporting plant growth under stress (Rolli *et*
66 *al.*, 2015), no data exist on the date palm endophytic bacteria and their role in the protection against
67 drought conditions.

68 Here, we aim to assess the ecology, rhizocompetence and capability of date palm endophytic bacteria
69 to support plant resistance to drought. We will address the following questions: (i) are geo-climatic
70 factors involved in shaping the endophytic community structure in date palm root?, (ii) do endophytes

on date palm roots have plant growth promoting (PGP) functional traits linked to drought resistance?,
 (iii) are cultivable endophytes rhizocompetent and do they present cross-colonisation capacities in
 different plants? and (iv) do date palm root endophytes favour plant tolerance to drought stress?

Results

Inter-oases beta-diversity of the endophytic bacterial community in the root of date palm.

The oases selected for this study (Supporting Figure 1A) were private agriculture farms, characterized by a traditional management: old date palm tree plantations, organic fertilization and irrigation by submersion using deep aquifer fossil water. The oases located along north-south aridity transect were characterized by poorly fertile soils, with loess soils in the northern oases (BD-16, BD-B, and BD-C) and sandy-silt soil in the southern ones (BD-1, BD-5, BD-8 and BD-9).

Similar numbers of 16S rRNA gene copies of endophytic bacteria were measured by quantitative Real Time PCR in the root tissues of date palms from seven Tunisian desert oases (PERMANOVA; $df = 6$, 20 ; $F = 1.1$; $p = 0.4$; Supporting Figure 1B).

Multiple-band PCR-DGGE profiles of the 16S rRNA gene were observed in the root tissues, demonstrating discrete endophytic bacterial communities inhabiting the date palm root tissues along the examined north-south aridity transect (Supporting Figure 2). Principal coordinate analysis (PCoA) of PCR-DGGE gel-band profiles explained 81.5% of the total variability and indicated a north-south segregation pattern of bacterial communities relative to the latitudinal position of the oases with respect to the Chott El Jerid saline system (Fig. 1A); these results were confirmed by canonical analysis of principal coordinates (CAP), where a clear separation between northern-southern oases is evident (Fig. 1B). Endophytic bacterial communities segregated between oases located to the North (BD-16, BD-B and BD-C) and South (BD-1, BD-5, BD-8 and BD-9) of the Chott El Jerid as supported by PERMANOVA Pair Wise test (PERMANOVA, $df = 1$, 20 ; $F = 20.19$; $p < 0.001$;

Supporting Table 2). Among the environmental factors examined (Supporting Table 1), a marginal test revealed that latitude, longitude, altitude, temperature and rainfall were statistically significant driving forces determining the structure of root-associated bacterial communities (Table 1A). However, latitude and temperature proved to be the dominant geo-climatic variables shaping the structure of endophytic bacterial communities (Table 1B). We assessed the linkage of the environmental factors through Multiple Correspondence Analysis that showed they were related each other with the only exception for the minimum rainfall (Supporting Figure 3).

103

104 *PGP functionality of cultivable endophytic bacteria associated with date palm roots.*

A total of 120 bacterial strains were isolated from the root tissues of date palms cultivated in the oases sampled. Isolates were assigned to five phyla, Proteobacteria (76%), consisting of Beta- (6%) and Gamma-Proteobacteria (70%), Actinobacteria (17%), Firmicutes (6%) and Bacteroidetes (1%) (Fig. 2A), consistent with the cultivation-independent approach (Supporting Table 3). Phylogenetic analysis performed on the isolates showed that species belonged to 11 bacterial genera (Fig. 2B), where *Pseudomonas* were the most common, representing 27% of the collection (35% in the southern oases and 16% in the northern oases).

The 28 different genotypes identified following dereplication of the collection by internal transcribed spacer-PCR (ITS-PCR) fingerprinting were characterized for PGP activities *in vitro* (Fig. 2C). Potentially beneficial strains, according to their PGP scores indicating multiple PGP and biocontrol traits and resistances to different abiotic stresses, were recovered from all the date palm roots samples. While none of the isolates displayed all the assayed PGP traits, 13 strains presented 12 or more and 15 strains ranged between 8 and 11 PGP traits. All isolates showed potential adaptation to unfavourable environmental conditions typical of arid soils through halotolerance (26 strains grew in media with 5% NaCl added) and resistance to low-water availability (28 strains) and variable temperature ranges (10 strains). None of the strains grew at 50°C and only 6 strains tolerated 10% NaCl in the growth medium. PGP potential properties, such as those related to nutrient provision (phosphate solubilisation

and siderophore release) and auxin synthesis were equally distributed among isolates. Exopolymers (EPS) production was primarily presented by *Pseudomonas* strains. Seventy five percent of the strains were active against both *Botrytis cinerea* and *Aspergillus niger*.

Two strains with contrasting PGP scores (Fig. 2C), *Pseudomonas frederickbergensis* E102 and *Pseudomonas brassicacearum* E141 strains, were selected for further studies on the rhizocompetence and the *in vivo* PGP potential on date palm.

In vitro root adherence and co-colonisation assay on date palm and Arabidopsis thaliana.

Date palm root colonization by strain E141 was studied using a *gfp*-labelled mutant. Colonisation was observed to persist after 72 h (Fig. 3A). Co-colonisation competition assay with an exogenous bacterial competitor demonstrated a selective colonisation capacity of strain E141. After 72 h exposure to *dsRED*-labelled *E. coli* and *gfp*-labelled E141, only cells of the latter were detected on date palm root rhizoplane (Fig. 3B). Root colonization by the *gfp*-labelled E141 strain was also observed on *Arabidopsis* root. Along time course experiments, microscopic observations of *Arabidopsis* roots exposed for 1 h to the *gfp*-labelled *P. brassicacearum* strain E141 revealed green fluorescent cells along the primary root (Supporting Figure 4A). After 3 h of exposure, the organisation of bacterial cell microcolonies was observed both on primary roots and root hairs (Supporting Figure 4B). Five hours after exposure to the strain, the expected arrangement of *gfp*-labelled cell clusters continued to adhere to the rhizoplane (Supporting Figure 4C). The *gfp*-labelled E141 strain had considerably colonised the primary root after 24 (Supporting Figure 4D) and 48 hours, while no cells of the *dsRED*-labelled *E. coli* were observed on the root after 48 h from the initial bacterization (Fig. 3C). The experiment confirmed the ability of strain E141 to establish on the root system of a plant species other than its original host. Fluorescent *gfp*-labelled E141 cells were detected in abundance on the rhizoplane of both the studied plant root systems, and were also visible in longitudinal-sections of the roots, suggesting its ability to penetrate root tissues (Fig. 3B and C).

147 In addition, closer quantitative evaluation showed that both *Pseudomonas* spp. strains E102 and E141
148 efficiently colonised the roots of all three date palm cultivars (Deglet Nour, Safawy and Sofry) at high
149 density (Fig. 3D). The strain E102 maintained a constant population density at about 10^4 CFU g⁻¹ of
150 tissue on all three cultivars. Similar values were observed with E141-treated roots, with the exception
151 of the Sofry cultivar that had a density of 10^3 CFU g⁻¹ on root tissue. No kanamycin-resistant isolates
152 other than the strains E102 and E141 were obtained from inoculated plants or non-inoculated control
153 plants, indicating that no contamination by spontaneous kanamycin-resistant bacteria occurred.

154

155 *Date palm growth promotion under controlled drought stress.*

156 Strains E102 and E141 were tested for their *in vivo* PGP potential during a nine months-long growth
157 experiment that included ten successive periods of induced water stress. During the experiment field
158 capacity was maintained at 50% while during the water stress periods it was decreased down to 15-
159 20% by interrupting irrigation (Fig. 4A). Bacteria-treated date palm plantlets had significantly more
160 fresh aerial and root biomasses than non-inoculated plants (Fig. 4B and C, Supporting Table 4 and 5).
161 Both strains increased fresh biomass of date palm shoot and root between 25-30% and 20-23%,
162 respectively (Fig. 4B and C). Dry biomass (Fig. 4B and C) and number of leaves (Fig. 4D) confirmed
163 that bacterial inoculation significantly increased plant growth (Supporting Table 4). No statistical
164 differences were observed for root or shoot length (Fig. 4E, Supporting Table 4 and 5).

165

166 Discussion

167

168 The environmental conditions of the oases were found to influence the diversity of the endophytic
169 bacterial communities in the date palm roots, but not the cell abundance, differently from what was
170 observed in the rhizosphere (Ferjani et al., 2014). The selection of endophytic bacteria by root tissues
171 is a complex process controlled by several factors including, the plant physiological features, the soil

172 geochemical characteristics, the agriculture management practices and the geoclimatic conditions
 173 (Angel *et al.*, 2010; Bachar *et al.*, 2010; Berg and Smalla, 2009; Chaparro *et al.*, 2013; Ding *et al.*,
 174 2013; Hardoim *et al.*, 2008; Schläeppli *et al.*, 2014). We found that latitude and temperature influenced
 175 beta-diversity of root-associated endophytic bacteria, such that they could clearly be separated into
 176 two groups, those South and those North of the Chott El Jerid saline lake in southern Tunisia. Results
 177 support the conjecture that beta-diversity of date palm endophytic communities varies along a north-
 178 south environmental gradient and indicate that PCR-DGGE of 16S rRNA gene was able to detect such
 179 variations in beta-diversity. Even though PCR-DGGE is not anymore adequate for assessing alpha-
 180 diversity in comparison with next generation sequencing (NGS), several recent studies have shown
 181 that community fingerprinting techniques, including PCR-DGGE, are still useful for analysing beta-
 182 diversity, where they support the same conclusions as NGS techniques (Cleary *et al.* 2012; Gobet *et*
 183 *al.*, 2014; Van Dorst *et al.*, 2014).

184 To improve the effectiveness of isolation of root endophytes from the inner part of date palm root, we
 185 have initially tested a root-peeling approach (Sessitsch *et al.*, 2002). However, since we were not able
 186 to obtain intact root cores, we have adopted a surface sterilization procedures with bleach and ethanol
 187 treatments (Sun *et al.*, 2008) that did not affect the root structure. Cultivable date palm endophytic
 188 bacteria were primarily from the class Gammaproteobacteria. Gammaproteobacteria-rich root tissues
 189 have been documented in other arboreal plants growing in arid regions such as grapevines (Marasco *et*
 190 *al.*, 2013a), banana trees (Souza *et al.*, 2013) as well as trees grown in conventional environments
 191 (Lodewyckx *et al.*, 2002; Taghavi *et al.*, 2009). Gammaproteobacteria of the genus *Halomonas* were
 192 observed only in Ksar Ghilane, the southern-most oasis examined in this study. The majority of soil in
 193 arid and semiarid areas is salt-affected, particularly in oases, where salt accumulation is caused by a
 194 combination of factors including poor quality of irrigated water, poor or no drainage, shallow saline
 195 water tables, and salinization of soil and groundwater (Marlet *et al.*, 2009). *Halomonas* strains with
 196 PGP properties have previously been observed in association with halophytes such as *Salicornia* spp.
 197 (Argandonña *et al.*, 2005; Jha *et al.* 2012) including those in the same area of the oases studied here
 198 (Mapelli *et al.*, 2013).

199 The most frequent endophytic bacteria among the isolates belonged to the genus *Pseudomonas* well
 200 known for its PGP properties (Ali et al., 2013; Roca et al., 2014). Pseudomonads from the date palm
 201 root endosphere were capable to enhance phosphate solubilisation, release siderophores, fix nitrogen,
 202 produce phytohormones and ACC deaminase, produce EPS and grow at low water activities in
 203 presence of 20% polyethylene glycol (PEG). These features may support, directly or indirectly, plant
 204 growth under drought favouring water uptake (Compant et al., 2010; Daffonchio et al., 2015).

205 The above-mentioned isolates represent bacterial species capable of forming colonies after 48 hours
 206 incubation on agar plates. However, these fast growing bacteria represent a fraction of the root system
 207 microbiome (Hardoim et al., 2008). It should be considered that several slow growing bacteria such as
 208 many actinobacteria are also an important component of the root system microbiome and they would
 209 deserve further studies in the date palm (Sessitsch et al., 2002; Quin et al., 2015).

210 We have demonstrated that date palm roots provide a suitable microenvironment for bacteria with
 211 multiple PGP traits. Fully developed metabolic interactions require physical contacts between plants
 212 and their endophytes (Brader *et al.*, 2014). Before bacteria can express any PGP traits, endophytes
 213 must adhere to the rhizoplane, colonise the root system and finally spread throughout the plant's
 214 tissues (Hardoim *et al.*, 2008; Marasco et al., 2013b; Rodriguez-Navarro *et al.*, 2007). The
 215 *Pseudomonas* strains E102 and E141 efficiently cross-colonized different plant species and date palm
 216 cultivars, confirming their endophytic lifestyle.

217 Under drought conditions Deglet Nour date palms treated with *Pseudomonas* spp. E102 or E141
 218 appeared healthier, with increased fresh and dry biomass of the roots and the whole plant, compared to
 219 non-inoculated plants. Because of the increase in plant dry weight rather than in water content,
 220 bacteria can be accredited for a capacity of stimulating growth and increasing biomass. Plants with
 221 more root biomass can efficiently extract residual soil water during drought-stress periods, favouring
 222 overall plant growth as observed in shoot weights. Therefore, in addition to the nutritional and
 223 protective effects that have been shown for oil, banana and coconut palms (Andrade *et al.*, 2014;
 224 Bakhtiar *et al.*, 2013; Garcia *et al.*, 2012; George *et al.*, 2013; Mia *et al.*, 2010), endophytic bacteria

also help palms cope with water stress and drought, two essential obstacles to desert farming. The versatility of these strains in root colonization, general PGP activities and specific promotion of resistance to drought suggests that such drought-protective activity likely takes place naturally in oasis ecosystems.

Experimental procedures

Study sites and preparation of date palm root tissues.

During March 2010, root samples of date palm (*Phoenix dactylifera* L. cv. Deglet Nour) were collected from seven different oases located along an aridity-latitude gradient in Tunisia (Supporting figure 1A) characterized by different geo-climatic traits (Supporting Table 1). In each sampling station, the roots of three healthy Deglet Nour date palms of similar age were collected at 40-60 cm depth, where the root system is most dense and active. All root samples were collected using sterile tools. In the laboratory, the root tissues were immediately surface-sterilized as described by Sun *et al.* (2008) and the efficacy of the sterilization method was verified by plating the water from the last washing step and by plating pieces of the sterilized root on Tryptic Soy Agar (TSA). No colonies were obtained from all the control plates after 10 days incubation at 30°C. Recovered samples were stored at -20°C for molecular analysis and at 4°C for isolation.

DNA extraction, Real Time-PCR and PCR-DGGE analyses.

Three grams of surface-sterilised roots were ground in liquid nitrogen using a sterile mortar and pestle to obtain a homogenous root tissue powder. Total DNA was extracted from 1 g of powdered root tissue using the DNeasy Plant kit (Qiagen), according to the manufacturer's protocol. The DNA was quantified and stored at -20°C until use.

Quantitative Real Time-PCR (q-PCR) was performed on a Chromo4 real-time detector (Bio-Rad) to measure the presence and concentration of bacterial populations in root tissues following the method described in Merlino *et al.* (2013).

Primers 907R and 357F with a GC-clamp were used in this study for the amplification of bacterial 16S rRNA genes (Muyzer *et al.*, 1993). DGGE fingerprint analysis was performed as described in Marasco *et al.* (2012). The DGGE bands were excised from the gels using a sterile cutter extracted from the gel plug and amplified as reported in Marzorati *et al.* (2006). Amplification products were sequenced by MacroGen Inc. (South Korea), and sequences were deposited in the GenBank database under accession numbers KM355719 to KM355744. The DGGE band patterns were converted into line plots using Image J software to generate a matrix of x/y values (Schneider *et al.*, 2012). Statistical analyses of the data are reported in the Supporting Material under the heading “Statistical Analysis”.

260

Isolation, identification and in vitro PGP activities of cultivable endophytes.

One gram of sterilised root from each sampling station was ground in phosphate buffer saline (10 mM K_2HPO_4 , 10 mM KH_2PO_4 , 0.14 M NaCl) using a sterile mortar and pestle. The suspension was diluted in 10-fold series and plated onto TSA medium. After incubation at 30°C for 48 h, fast-growing bacterial colonies representing different morphotypes were selected and spread three times on the original medium. A total of 120 purified isolates were frozen in 25% glycerol at -80°C until use.

DNA was extracted from isolates by boiling lysis as described by Marasco *et al.* (2012). Isolates were dereplicated using the ITS-PCR fingerprinting protocol (Daffonchio *et al.*, 1998). Representative isolates from each genotype were identified by partial sequencing of the 16S rRNA gene at MacroGen Inc. (South Korea) after PCR amplification according to Marasco *et al.* (2012). Sequences were deposited in the GenBank database under accession numbers KM355745 to KM355772.

The 28 isolates obtained after dereplication were screened for production of IAA, siderophores, mineral phosphate solubilisation, nitrogen-fixation activity, EPS and ammonia production, protease

274 activity and tolerance to drought, salt and temperature variation as described in the Supporting
275 Methods 1.

276

277 *In vivo recolonization experiment of date palm and Arabidopsis root system.*

278 The isolates in the collection were transformed with the pHM2-*gfp* plasmid (Favia *et al.*, 2007). Stable
279 transformants were obtained with *P. frederikbergensis* E102 and *P. brassicacearum* E141 strains as
280 described in the Supporting Methods 2.

281 The *gfp*-labelled strain E141 was selected for colonisation experiments on date palm and *Arabidopsis*
282 following the protocol described in the Supporting Methods 3. A co-colonisation competition assay
283 was performed using *gfp*-labelled E141 and *dsRED*-labelled *E. coli*, as described in the Supporting
284 Method 4. At time intervals, bacterized roots of date palm and *Arabidopsis* seedlings were analysed by
285 confocal laser-scanning microscopes (Leica TCSNT) with Leica Confocal Software, using BP530/30
286 GFP (excitation at 488 nm) and LP590 TRITC filters (excitation length at 568 nm). For some
287 experiments (as explained in the figure legends) a Zeiss LSM 710 Upright Confocal Microscope has
288 been used with GFP and Propidium Iodide (PI) lasers and Bright Field light (BF) and the software
289 ZEN2009.

290 Quantification of the colonization of date palm roots by strains E102 and E141 were performed by
291 selective plating of the strains transformed with plasmid pHM2-*gfp* as described in Supporting
292 Methods 5.

293

294 *The ability of Pseudomonas sp. to promote date palm growth in vivo under drought stress.*

295 Date palm seeds (*Phoenix dactylifera* cv. Deglet Nour) were soaked in water for 48 h to soften the
296 mesocarp, sterilized with 90% ethanol for 2 min and then 1% sodium hypochlorite for 15 min
297 followed by three rinses in sterile distilled water. Surface-sterilized seeds were sown in a pot
298 containing 3 Kg of sterilized soil mixture (3:1 soil to sand). The soil mixture was sterilized by the

tyndallisation method: three cycles of sterilization at 100°C for 60 min were alternated with overnight incubation at 30°C. After tyndallisation, 1 g of the soil mixture was serially diluted and spread on a TSA petri dish to evaluate the CFU. After 48 h at 30°C, the CFU g⁻¹ of mixed soil had bacterial counts < 10¹. Seeds were coated once with the bacterial suspension strains E102 and E141 at a concentration of 10⁸ cells g⁻¹ of soil; non-inoculated seeds were watered with sterile tap water. Seeds were maintained at 30°C for 1 week and after germination the pots were transferred to a growth chamber with ~100 µmol photons m⁻² s of light for 12 h during the day; the average temperature is reported in Fig. 4A. The *in vivo* experiment continued for approximately 9 months (281 d). Date palm plantlets experienced a variable degree of controlled water stress reported as a percentage of field capacity (Fig. 4A). After 281 d shoot and root fresh weight, dry weight and length and number of leaves were measured on each of the 19 replicate plants per treatment. Statistical analyses of the data are reported in the Supporting Material under the heading “Statistical Analysis”.

311

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313

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TABLES AND TABLE LEGENDS

Table 1. Environmental factors shaping the structure of date palm root endophytic bacterial communities from oases in southern Tunisia. DistLM analysis was performed on individuate geo-climate variables influencing the structure of the bacterial community associated with date palm root tissues. Geo-climatic variables tested included latitude (Lat N), longitude (Long E), altitude (Alt); minimum temperature (T min), maximum temperature (T max), minimum rainfall (Rainfall min) and maximum rainfall (Rainfall max). (A) The marginal test considers each geo-climatic variable and its contribution to explaining the total variability. (B) Sequential test to explain the total variation from variables. F = statistic F; p = probability (statistically significant variables are shown in bold, p<0.05); Prop = proportion of total variation explained; AIC = Akaike information criterion; Cumul = cumulative variation explained by the listed variables; Res df = residual degrees of freedom.

(A) Marginal test

Variables	F	P	Prop
Lat N	16.256	0.0001	0.46
Long E	11.218	0.0001	0.37
Alt (m)	3.8354	0.0204	0.17
T min (°C)	8.0295	0.0008	0.30
T max (°C)	1.9707	0.1239	0.09
Rainfall min (mm)	7.9872	0.0007	0.30
Rainfall max (mm)	3.6725	0.0230	0.16

(B) Sequential test

Variables	AIC	F	P	Prop	Cumul	Res df
+ Lat N	95.567	16.256	0.0001	0.46108	0.46108	19

+ Long E	95.781	1.5973	0.1725	0.04393	0.50501	18
+ Alt (m)	97.382	0.32608	0.8771	0.00932	0.51432	17
+ T min (°C)	94.856	3.8485	0.0102	0.09417	0.60849	16
+ T max (°C)	93.349	2.7265	0.0363	0.06022	0.66871	15
+ Rainfall min (mm)	92.706	1.8776	0.1068	0.03918	0.70789	14
+ Rainfall max (mm)	92.706	0	1	0.00000	0.70789	14

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486 **FIGURE LEGENDS**

487

488 **Figure 1. Analysis of the bacterial community structure associated with date palm root tissues in**
 489 **different oases of Tunisia.** (A) Principal coordinate analysis represents the structural diversity of
 490 endophytic bacterial communities associated with date palm root tissues. The endophytic communities
 491 of date palm roots from the northern (black symbols) and southern (grey symbols) oases compared to
 492 the Chott El Jerid saline lake exhibit two different groupings that explain 81.5% of the total variation.
 493 (B) The canonical analysis of principal coordinates confirms the separation between northern (black
 494 circles) and southern (grey circles) oases.

495

496 **Figure 2. Diversity and PGP potential of date palm root-associated cultivable endophytic**
 497 **bacteria.** Phylogenetic allocation of the isolates from date palm root tissues at class (A) and genus (B)
 498 levels. (C) PGP potential of the isolates is indicated as a PGP score, determined by the sum of the
 499 number of PGP potential abilities exhibited by each strain. Abiotic stress resists. = abiotic stress
 500 resistance; Bioc. = biocontrol potential; P Sol. = inorganic phosphate solubilization; Sid. = siderophore
 501 production; N fix. = nitrogen fixation; EPS = exopolymers release; IAA = auxin production; Amm. =
 502 ammonia production; Prot. = protease activity; ACCd = ACC deaminase activity; PEG = polyethylene
 503 glycol. The strains marked in grey are those used for the *in vivo* date palm experiment.

504

505 **Figure 3. Rhizocompetence and root recolonization ability of date palm root endophytic bacteria**
 506 **on different plant models.** Colonization of date palm root after 72 h by a *gfp*-labelled E141 strain
 507 alone (A) or supplied together with a *dsRED*-labelled *E. coli* (B). *Arabidopsis thaliana* roots after 48 h
 508 exposure to a *gfp*-labelled E141 strain together with a *dsRED*-labelled *E. coli* (C). The scale bars
 509 correspond to 100 μ m in (A) and (C) and 10 μ m in (B). The *gfp* (panels A-C) and *dsRED* (panels B
 510 and C) fluorescences are visible in green and red, respectively. Images were obtained with a Zeiss

(panels A) or a Leica (panels B and C) confocal microscope (see Experimental Procedures). (D) Reisolation experiments showing the ability of both *P. brassicacearum* E141 and *P. frederiksbergensis* E102 to actively colonize root tissues of different date palm cultivars including the original Tunisian cultivar (Deglet Nour) from which the strains were isolated and two Saudi Arabian cultivars (Safawy and Sofry). CFU g⁻¹ are expressed as mean $\pm$ sd.

516

Figure 4. Date palm root endophytic bacteria promote the growth of date palms exposed to drought. (A) Levels of controlled drought stress during a 9-months period of date palm growth. Controlled drought periods were induced by interrupting irrigation for periods of time ranging between 6 and 12 d. Field capacity is indicated in grey; actual temperature in the greenhouse is shown by a black line; annual min (blue line) and max (red line) temperatures in the Ksar Ghilane oasis at the same time as the greenhouse experiment. Date palm growth promotion after a 9-months period of fluctuating water stress following the initial inoculation with two strains (*P. brassicacearum* E141 and *P. frederiksbergensis* E102) isolated from date palm roots sampled in the Ksar Ghilane oasis. Plant biomass after 9-months growth, expressed as mean of shoot (B) and root (C) fresh and dry weights $\pm$ standard error; number of leaves (D) and root and shoot length (E) $\pm$ standard errors. The data (averages, standard deviations and number of plants are reported in Supplementary Table 4) were statistically analysed through a PERMANOVA (Supplementary Table 5); different letters represent statistically significant differences ($p < 0.05$) for each parameter.

Figure 1. Analysis of the bacterial community structure associated with date palm root tissues in different oases of Tunisia. (A) Principal coordinate analysis represents the structural diversity of endophytic bacterial communities associated with date palm root tissues. The endophytic communities of date palm roots from the northern (black symbols) and southern (grey symbols) oases compared to the Chott El Jerid saline lake exhibit two different groupings that explain 81.5% of the total variation. (B) The canonical analysis of principal coordinates confirms the separation between northern (black circles) and southern (grey circles) oases.

62x35mm (300 x 300 DPI)

Figure 2. Diversity and PGP potential of date palm root-associated cultivable endophytic bacteria. Phylogenetic allocation of the isolates from date palm root tissues at class (A) and genus (B) levels. (C) PGP potential of the isolates is indicated as a PGP score, determined by the sum of the number of PGP potential abilities exhibited by each strain. Abiotic stress resist. = abiotic stress resistance; Bioc. = biocontrol potential; P Sol. = inorganic phosphate solubilization; Sid. = siderophore production; N fix. = nitrogen fixation; EPS = exopolymers release; IAA = auxin production; Amm. = ammonia production; Prot. = protease activity; ACCd = ACC deaminase activity; PEG = polyethylene glycol. The strains marked in grey are those used for the in vivo date palm experiment.

200x237mm (300 x 300 DPI)

Figure 3. Rhizocompetence and root recolonization ability of date palm root endophytic bacteria on different plant models. Colonization of date palm root after 72 h by a *gfp*-labelled E141 strain alone (A) or supplied together with a *dsRED*-labelled *E. coli* (B). *Arabidopsis thaliana* roots after 48 h exposure to a *gfp*-labelled E141 strain together with a *dsRED*-labelled *E. coli* (C). The scale bars correspond to 100 μ m in (A) and (C) and 10 μ m in (B). The *gfp* (panels A-C) and *dsRED* (panels B and C) fluorescences are visible in green and red, respectively. Images were obtained with a Zeiss (panels A) or a Leica (panels B and C) confocal microscope (see Experimental Procedures). (D) Reisolation experiments showing the ability of both *P. brassicacearum* E141 and *P. frederiksbergensis* E102 to actively colonize root tissues of different date palm cultivars including the original Tunisian cultivar (Deglet Nour) from which the strains were isolated and two Saudi Arabian cultivars (Safawy and Sofry). CFU g⁻¹ are expressed as mean $\pm$ sd. 141x181mm (300 x 300 DPI)

Figure 4. Date palm root endophytic bacteria promote the growth of date palms exposed to drought. (A) Levels of controlled drought stress during a 9-months period of date palm growth. Controlled drought periods were induced by interrupting irrigation for periods of time ranging between 6 and 12 d. Field capacity is indicated in grey; actual temperature in the greenhouse is shown by a black line; annual min (blue line) and max (red line) temperatures in the Ksar Ghilane oasis at the same time as the greenhouse experiment. Date palm growth promotion after a 9-months period of fluctuating water stress following the initial inoculation with two strains (*P. brassicacearum* E141 and *P. frederiksbergensis* E102) isolated from date palm roots sampled in the Ksar Ghilane oasis. Plant biomass after 9-months growth, expressed as mean of shoot (B) and root (C) fresh and dry weights $\pm$ standard error; number of leaves (D) and root and shoot length (E) $\pm$ standard errors. The data (averages, standard deviations and number of plants are reported in Supplementary Table 4) were statistically analysed through a PERMANOVA (Supplementary Table 5); different letters represent statistically significant differences ($p < 0.05$) for each parameter.

376x316mm (72 x 72 DPI)