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Diversity and incidence of plant-parasitic nematodes associated with saffron (*Crocus sativus* L.) in Morocco and their relationship with soil physicochemical properties

Fouad MOKRINI [1], Salah-Eddine LAASLI [1,2], Youssef KARRA [1], Aicha EL AISSAMI [2] and Abdelfattah A. DABABAT [3,*]

[1] *Biotechnology Unit, Regional Centre of Agricultural Research, National Institute of Agricultural Research (INRA), Rabat, Morocco*
[2] *Faculty of Science, Mohammed V University, Laboratory of Botany, Mycology and Environment, Rabat, Morocco*
[3] *International Maize and Wheat Improvement Center (CIMMYT), P.K. 39 06511, Emek, Ankara, Turkey*

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Summary – Saffron (*Crocus sativus*) fields in Morocco's Taliouine and Taznakht regions were surveyed between January and April 2018 to study the diversity and incidence of plant-parasitic nematodes and assess the effects of soil physicochemical properties on the nematodes. Fourteen nematode genera were identified in soil and root samples collected from 66 saffron fields. The most common plant-parasitic nematodes in the Taliouine region were *Pratylenchus* spp. and *Helicotylenchus* spp. In the Taznakht region, the most common nematodes were *Pratylenchus* spp., *Tylenchorhynchus* spp. and *Ditylenchus dipsaci*. Nematodes, particularly *Pratylenchus* spp. and *Ditylenchus* spp., were abundant and frequent throughout the region. Several nematode genera were significantly associated with soil texture and mineral content, indicating that soil properties play an important role in plant-parasitic nematode communities. This description of plant-parasitic nematode assemblages associated with saffron fields in Morocco and their relationship with soil physicochemical properties provides a starting point from which appropriate nematode management strategies can be implemented.

Keywords – *Ditylenchus* spp., ecology, nematode survey, *Pratylenchus* spp., soil texture.

Saffron (*Crocus sativus* L.), the costliest spice of the world, is an autumn-flowering geophyte species that belongs to the Iridaceae family. This triploid ($2n = 3x = 24$) and sterile plant is propagated by vegetative reproduction through the formation of daughter corms from the mother corm as the flowers are sterile and fail to produce viable seeds (Fernández & Pandalai, 2004). Dried stigmas of saffron flowers have been valuable since ancient times for their odoriferous, colouring and medicinal properties (Winterhalter & Straubinger, 2000). Saffron is cultivated more or less intensively in different countries of the world such as Iran, Greece, Morocco, India, Spain, China, Turkey, Azerbaijan and Italy (Fernández *et al.*, 2010). More than 90% of its global production takes place in Iran (300 tons year^{-1}) followed by Greece (5-7 tons year^{-1}), Morocco (5 tons year^{-1}), India (3 tons year^{-1}) then Spain (2 tons year^{-1}) (Fernández *et al.*, 2010). In Morocco, the main saffron production is concentrated in the Taliouine and Taznakht regions, which cover more than 1650 ha (MAPM, 2017).

Biological stress caused by various pathogens is one of the main reasons causing reduction in the yield of saffron (Sharma *et al.*, 2005; Castillo López & Gómez-Gómez, 2009). Among these pathogens, plant-parasitic nematodes (PPN), attack the saffron crop and cause a significant reduction in its production (Ahrazem *et al.*, 2010). Several plant-parasitic nematodes have been reported to be associated with saffron disease (Schenk, 1970; Ahrazem *et al.*, 2010; Mahdikhani & Alvani, 2013). These include *Pratylenchus* spp. (Schenk 1970; Mahdikhani & Alvani 2013), *Aphelenchoides subtenuis*, *A. besseyi*, *A. curiolis* (Ortuño & Oros, 2002; McCuiston *et al.*, 2007), *Ditylenchus dipsaci*, *Filenchus pratensis*, *Geocenamus tenuidens*, and *Merlinius* spp. (Mahdikhani & Alvani, 2013).

In Morocco, saffron is cultivated in the south of the country, which is characterised by a wide diversity of soil

* Corresponding author, e-mail: a.dababat@cgiar.org

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types (sandy and clay loam), climates (arid and semi-arid) and vegetation (perennial vegetation). However, there is no information available on the occurrence and distribution of PPN associated with saffron and their relation with soil physicochemical properties. In view of this paucity of information and in order to orientate further nematological research, we present an extensive overview concerning the incidence of PPN associated with saffron from both the Taliouine and Taznakht regions in Morocco. Therefore, the main objectives of this study were: *i*) to study which taxa of PPN are associated with saffron in Morocco; *ii*) to determine the frequency of occurrence and abundance of nematodes in Taliouine and Taznakht regions; and *iii*) to investigate the effect of soil physicochemical properties on the community structure of PPN.

Materials and methods

Nematode survey

An intensive survey was conducted to determine the distribution and occurrence of PPN in saffron fields from rain-fed and irrigated lowlands in the Taliouine and Taznakht regions of Morocco. Those two provinces are the main saffron-producing areas in Morocco and represent the total production in the country. Surveyed fields were selected based on their production importance, soil type utilised and geographical location. The number of fields sampled per region was determined as a function of the importance (surface planted) of the saffron crop. A total of 66 representative fields were visited and sampled (Fig. 1) representing eight provinces of the saffron production areas. In each locality, 5-8 saffron fields were randomly selected for sampling. From each field, a total of 15 subsamples were arbitrarily collected then mixed thoroughly to form a representative sample of 2 kg including soil and root. The samples were kept in plastic bags and stored at 4°C before analysis to minimise changes in nematode populations (Barker *et al.*, 1969). Analyses of soil texture and composition from each surveyed field were carried out at the Regional Center of Agricultural Research (INRA) in Agadir. Soil characteristics and properties of the surveyed fields in both Taliouine and Taznakht regions of Morocco are summarised in Table 1.

Nematode extraction and identification

Representative samples from each surveyed field were carefully mixed and processed for extraction no later than

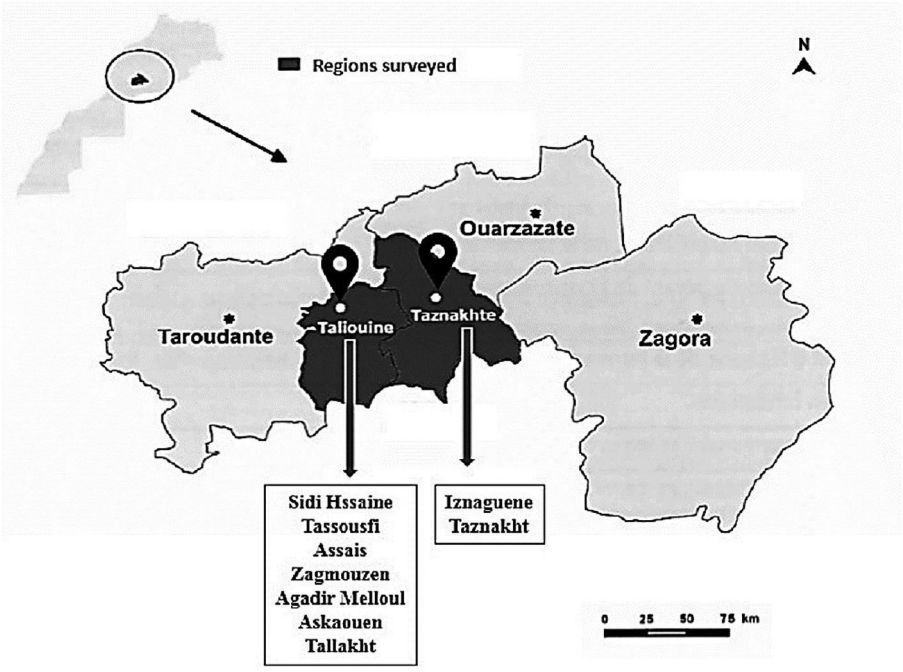

Fig. 1. Map of the agro-ecological regions in Morocco where samples were collected from saffron fields.

Table 1. Locality, characteristics and codes of sampling sites in the Taliouine and Taznakht regions, Morocco.

Region	Locality (Province)	GPS information (Latitude, Longitude)	Altitude (m a.s.l.)	Soil texture	Organic matter (%)	pH (H_2O)	Samples per site	Code
Taliouine	Sidi Hssaine	+30°27′58.06″, −7°43′33.31″	1630	Clay loam	2.1	8.7	10	SH
	Tassousfi	+30°29′16.01″, −7°52′21.01″	1570	Sandy clay loam	1.8	8.7	6	TA
	Assais	+30°34′26.01″, −7°36′48.03″	1700	Medium loam	2.9	9.1	5	AS
	Zagmouzen	+30°38′34.30″, −7°49′34.98″	1846	Sandy loam	2.1	8.0	5	ZE
	Agadir Melloul	+30°45′22.99″, −7°22′17.29″	1663	Clay loam	2.7	8.6	15	AM
	Askaouen	+30°44′24.85″, −7°46′56.14″	1947	Sandy loam	1.8	7.7	4	ASK
	Tallakht	+30°26′06.54″, −7°46′01.70″	1619	Clay loam	2.5	7.8	2	TAL
Taznakht	Taznakht	+30°34′20.03″, −7°12′09.03″	1459	Sandy loam	3.5	8.8	7	TAZ
	Iznaguene	+30°41′08.09″, −7°41′44.00″	2466	Sandy loam	3.8	8.7	11	IZN

48 h after being stored. For each sample, nematodes were extracted separately from roots and soil. Roots of each sample were gently washed in tap water to free adhered soil particles, chopped into pieces (*ca* 0.5 cm) and then nematodes were extracted from a sub-sample of 20 g using a modified Baermann technique (Hooper, 1986). Nematodes were also extracted from 100 cm^3 of soil using the same modified Baermann technique used for extraction of nematodes from roots. After 48 h, nematode suspensions from both soil and roots were collected in beakers, allowed to settle for 2 h and concentrated to about 20 ml by removing excess water (supernatant) using the settling-siphon method (Caveness, 1975). Nematodes were identified to genus level using dichotomous keys (Mai & Lyon, 1975; Mai & Mullin, 1996). The Seinhorst (1959) method as modified by De Grisse (1969) was used to kill and fix the nematodes by adding 4% hot (60-80°C) formaldehyde to a small drop of water in a glass cavity vessel that contained the nematodes. The nematodes were transferred to solution I (99 parts 4% formaldehyde + 1 part pure glycerin) in a square 7 cm diam. watch glass. This square watch glass dish was placed in a desiccator containing about one tenth of its volume of 96% ethanol. The next day, the watch glass containing the nematodes was removed from the desiccator and placed in an incubator at 37°C. Then 3 ml of solution II (95 parts 96% ethanol + 5 parts pure glycerin) was added to the watch glass. This was repeated three times at intervals of 3 h, while the watch glass was partially covered by a glass slide to allow evaporation. Finally, 2 ml of solution III (50 parts 96% ethanol + 50 parts pure glycerin) was added and the watch glass was left overnight at 37°C in the incubator. The nematodes in pure glycerin from each sample were mounted on glass slides for identification to species level under a light microscope. Species of root-lesion nematodes were identified using the keys of Castillo & Vovlas (2007) and Ryss (1988).

Multiplication and identification of *Meloidogyne* spp.

Populations of *Meloidogyne* species collected from soil and roots were increased on susceptible tomato plants (n = 2) (*Solanum lycopersicum* 'Prystila F1') in a glasshouse at 25.5°C, 16 h of artificial light and 60-90% relative humidity. Sixty days after inoculation, plants were removed from pots, and the root systems gently washed to free adhering soil particles. Egg masses (n = 10) were picked individually from infested roots using a small needle. These egg masses were surface-sterilised in 0.5% NaOCl (Dababat & Sikora, 2007) then rinsed in tap water three times and prepared for inoculation. To obtain pure cultures, seedlings of susceptible tomato plant (*S. lycopersicum* 'Prystila F1') were transplanted singly into 500 ml plastic pots containing sterilised sandy loam soil and sand (2:1, v/v) and allowed to grow for 5-7 days before each was inoculated with a single egg mass. After 2 months, galled roots were harvested and egg masses of each population were recovered from roots. To perform the molecular identification, DNA of each nematode population was extracted from 1-5 freshly hatched second-stage juveniles (J2). Nematodes were transferred to an Eppendorf tube containing 25 μl double distilled water and 25 μl nematode lysis buffer (final concentration: 200 mM NaCl, 200 mM Tris-HCl (pH 8), 1% mercaptoethanol and 800 μg proteinase K). The tubes were incubated at 65°C for 1.5 h and then at 99°C for 5 min, consecutively (Holterman *et al.*, 2006). The J2 DNA was amplified *via* PCR using SCAR primers developed from RAPD markers (Zijlstra *et al.*, 2000).

Assessment of nematode population densities

Nematode diversity and incidence were assessed by calculating prevalence, mean intensity and maximum density (Boag, 1993). Prevalence (*i.e.*, number of samples having a particular nematode species divided by the number of samples examined, expressed as a percentage), mean intensity (*i.e.*, number of individuals of a particular nematode species in the positive samples divided by the number of positive samples), and maximum density (*i.e.*, maximum number of individuals of a particular nematode species recovered from a sample).

Physicochemical analyses of soil

The soil analyses were carried out at the INRA soil laboratory in Agadir, using standard methods (Anderson & Ingram, 1993). The following soil properties were analysed including soil texture: proportions of clay (0-2 μm), silt (2-50 μm) and sand (50- >200 μm); pH and electrical conductivity EC (μS cm^{-1}) using 1:2.5 soil:water ratio methodology described by Richards (1954); exchangeable cations: potassium, manganese and magnesium; exchangeable acidity; total soil organic matter; nitrogen content; soil solution including iron, copper, zinc, sodium and phosphorus.

Diversity of plant-parasitic nematodes

The diversity of PPN was conducted using the Shannon-Wiener index (Krebs, 1985).

$$H' = -\sum_{i-1}^{s} pi \ln pi$$

Where s is the number of genera, i represents species from the studied environments, pi is the proportion of characters belonging to the corresponding number of genera, and H' is commonly used to characterise species diversity in a community. H' accounts for both number of species and the evenness J. A separate measure of the evenness is usually given:

$$J = \frac{H}{H\,\max}, \quad H\,\max = \log_2 s$$

The taxon dominance parameter was calculated for each nematode genus in prospected localities together with the frequency. This parameter represents the regression between abundance and frequency for each genus sampled (Fortuner & Merny, 1973). The distribution diagram of nematode communities was applied as abundance variables were transformed to $\log_{10}(x + 1)$ before analysis. In addition, a heatmap analysis was conducted to describe the population structure of nematodes at the corresponding localities surveyed using Ward's clustering algorithm in the R package.

Statistical analysis

Principal component analyses (PCA) were applied to explore PPN community patterns and physicochemical soil factor patterns in relation to the localities (provinces) surveyed. After data normalisation using the Anderson-Darling normality test (Stephens, 1974), PPN and soil variables associated with the principal component analyses were subjected to a one-way ANOVA performed using XLSTAT 2016.02.28451 software (Addinsoft). Significant differences among variables were tested using protected least significant difference and by the Tukey test at $P < 0.05$. Differences obtained at levels of $P < 0.05$ were considered to be significant. Log-linear regression analyses were established to describe the relationship between nematodes and soil organic matter.

Results

Density and diversity of PPN associated with saffron

PPN were detected in 95 and 90% of the fields surveyed in the Taliouine and Taznakht regions, respectively. PPN prevalence, mean intensity and maximum densities are shown in Table 2. We detected 14 PPN genera associated with saffron: 12 genera in Taliouine and 14 genera in Taznakht, while 12 genera were common to both regions. A sample of randomly selected specimens contained the following species: *Pratylenchus thornei*, *P. crenatus*, *P. coffeae*, *P. penetrans*, *Meloidogyne incognita*, *D. dipsaci*, *Helicotylenchus vulgaris* and *Hoplolaimus indicus*. The root-lesion nematodes, *Pratylenchus* spp., were the most prevalent group of nematodes in both regions and four species dominated: *P. thornei*, *P. penetrans*, *P. crenatus* and *P. coffeae*. Mean intensity and maximum density of *Pratylenchus* spp. were generally greater in the Taliouine region (2-11 nematodes 100 cm^{-3}) compared to the Taznakht region (2-5 nematodes 100 cm^{-3}) (Table 2). No disease symptoms were observed on the stems, corms or leaves of nematode-infected planting stocks. However, plants infected by root-lesion nematodes had lesions on the roots of saffron. *Meloidogyne* spp. (45%) and *Helicotylenchus* spp. (60%) were more frequently detected in Taliouine than Taznakht. *Pratylenchus* spp., *Tylenchorhynchus* spp. and *Ditylenchus* spp. were more frequently detected in Taznakht (>50% of samples) than in Taliouine. The detection frequency of *Tylenchus* was similar in both regions (46%). *Longidorus* spp., *Hemicriconemoides* spp., *Paratylenchus* spp., *Aphelenchoides* spp., *Xiphinema* spp. and *Rotylenchus* spp. were generally detected less frequently than other genera, though they were more common in Taznakht than Taliouine. *Longidorus* spp., *Criconema* spp. and *Hoplolaimus* spp. were also found, but with low distribution.

PPN abundance and frequency in each region surveyed is shown in Figure 2. Of the 12 PPN genera identified in Taliouine, *Tylenchus* spp., *Tylenchorhynchus* spp., *Helicotylenchus* spp. and *Pratylenchus* spp. were shown to be more abundant and more frequent. *Ditylenchus* spp. and *Meloidogyne* spp. were less abundant but with a high frequency (Fig. 2A). In Taznakht (Fig. 2B), *Hoplolaimus* spp., *Criconema* spp., *Longidorus* spp., *Paratylenchus* spp., *Xiphinema* spp., *Rotylenchus* spp. and *Helicotylenchus* spp. were the most abundant and frequent of the 14 genera identified. Other frequent (but less abundant) ge-

Table 2. Prevalence, mean and maximum density of plant-parasitic nematodes from soil (100 cm^3) and root (20 g) from saffron in Morocco.

Nematode taxa/ genus and species	Taliouine					Taznakht				
	Prevalence (%)	Mean intensity		Maximum density		Prevalence (%)	Mean intensity		Maximum density	
		Root	Soil	Root	Soil		Root	Soil	Root	Soil
Meloidogyne spp. (J2) (root-knot)	45	2	5	4	11	10	1	3	3	5
M. javanica	+					+				
Pratylenchus spp. (lesion)	68	8	11	21	18	75	3	7	6	9
P. penetrans	+					+				
P. coffeae	–					+				
P. thornei	–					+				
P. crenatus	+					+				
Ditylenchus spp. (stem)	36	7	5	–	7	55	2	2	5	7
D. dipsaci	+					+				
Helicotylenchus spp. (spiral)	59	–	6	–	22	30	–	2	–	3
H. vulgaris	+					–				
Paratylenchus spp. (pin)	13	2	5		7	20	1	2	3	4
Aphelenchoides (foliar)	9	–	2	–	3	20	1	2	1	1
Tylenchus spp.	46	–	6	–	11	45	–	2	–	5
Tylenchorhynchus spp.	40	3	7		12	60		2		3
Longidorus (needle)	4	–	1	–	3	15	–	1	–	2
Hemicriconemoides spp.	4.5		2		2	5	–	2	–	2
Xiphinema spp. (dagger)	13	–	5	–	8	20	–	3	–	4
Rotylenchus spp. (spiral)	21.7	–	2	–	5	25	–	1	–	3
Criconema spp. (ring)	–	–	–	–	–	10	–	1	–	2
Hoplolaimus spp.	–	–	–	–	–	5	–	2	–	2
H. indicus						+				

nera in this region included *Tylenchus* spp., *Ditylenchus* spp. and *Meloidogyne* spp.

An advanced heatmap was used to infer PPN population structure in saffron fields in nine provinces of Taliouine and Taznakht regions (Fig. 2C). This analysis confirmed the widespread distribution of *Pratylenchus* spp., *Ditylenchus* spp., *Helicotylenchus* spp. and *Meloidogyne* spp. throughout the provinces studied. Provinces grouped into two distinctive clusters: saffron fields in Tallakht, Askaoun, Assais, Zagmouzen and Tassousfi provinces (Taliouine region) clustered together with Taznakht province (Taznakht region). Soil samples from these localities did not have *Hoplolaimus* or *Criconema* populations, except for Taznakht province. The other cluster contained provinces with an abundance of all PPN genera identified.

PPN genera also grouped into two main clusters, with the smaller cluster consisting of *Pratylenchus* spp., *Helicotylenchus* spp. and *Tylenchorhynchus* spp., which were present in most localities. Table 3 shows the number of genera and the Shannon diversity index (H') and evenness (J) values for the sites surveyed. The number of genera present was significantly ($P < 0.05$) higher in Zagmouzen province ($H' = 2.01$), compared to the other provinces. The Shannon diversity index was significantly ($P < 0.05$) higher in the same locality. Therefore, some provinces showed an improved diversity index (SH, TA, and AS) with $H' = 1.98$. There was no significant difference in evenness between all the fields studied.

Both regions cultivating saffron exhibited differences in PPN density in term of sampled localities (Fig. 3). PPN density was generally lower in Taznakht (Fig. 3A). Within this region, Iznaguene province had the highest PPN density 37 nematodes (100 g soil)$^{-1}$, followed by Taznakht province 27 nematodes (100 g soil)$^{-1}$. PPN densities were generally higher in Taliouine, especially in Agadir Melloul (AM) and Sidi Hssaine (SH) provinces, which had 270 and 188 nematodes (100 g soil)$^{-1}$, respec-

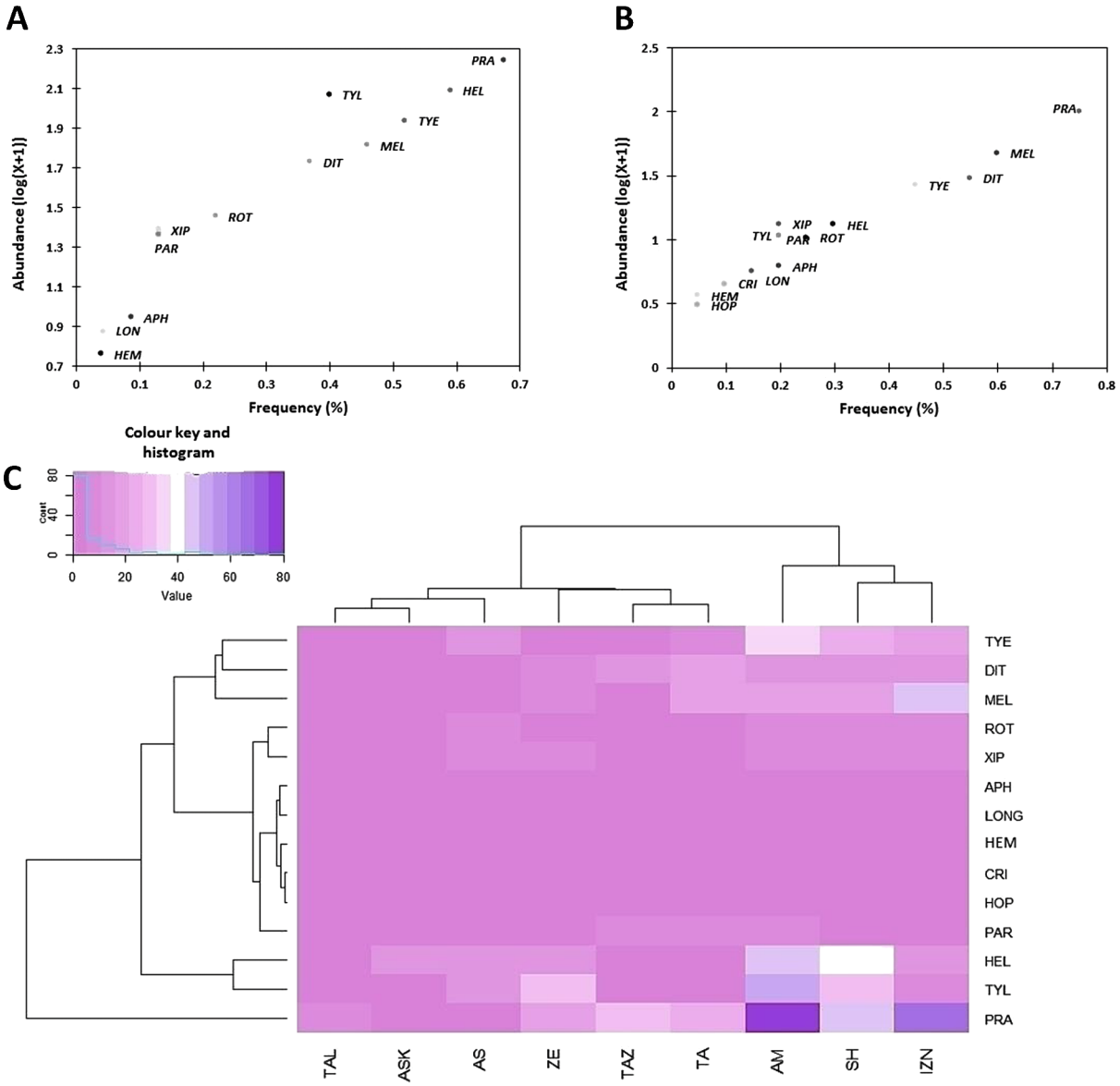

Fig. 2. Distribution diagram (Abundance-Frequency) of nematode communities in saffron fields of the Taliouine region (A) and the Taznakht region (B). C: Heatmap of population structure of nematode genera in saffron fields in different provinces of Taliouine and Taznakht. Ward's clustering algorithm was applied to the Spearman dissimilarity matrix of nematode distribution in saffron fields in nine provinces. The upper dendrogram represents the provinces where saffron fields were sampled; left dendrogram represents the nematode genera. The colour key scale represents normalised nematode frequencies with intensity of colour representing nematode frequency $(100\ \text{g soil})^{-1}$. Region codes: TAL = Tallakht; ASK = Askaouen; AS = Assais; ZE = Zagmouzen; TAZ = Taznakht; TA = Tassousfi; AM = Agadir Melloul; SH = Sidi Hssaine; IZN = Iznaguene. Genus codes: *APH* = *Aphelenchoides*; *CRI* = *Criconema*; *DIT* = *Ditylenchus*; *EL* = *Helicotylenchus*; *HEM* = *Hemicriconema*; *HOP* = *Hoplolaimus*; *LONG* = *Longidorus*; *MEL* = *Meloidogyne*; *PAR* = *Paratylenchus*; *PRA* = *Pratylenchus*; *ROT* = *Rotylenchus*; *TYE* = *Tylenchus*; *TYL* = *Tylenchorhynchus*; *XIP* = *Xiphinema*.

Table 3. Diversity of plant-parasitic nematode communities of saffron in the different localities.

Region	Localities (province)	Diversity parameters		
		Number of species	Shannon diversity index (H')	Evenness (J)
Taliouine	SH	18.80 ab	1.99 ab	0.678
	TA	14.66 cd	1.98 ab	0.73
	AS	13.20 d	1.98 ab	0.76
	ZE	21.00 a	2.10 a	0.68
	AM	16.87 c	1.91 b	0.67
	ASK	6.50 e	1.46 c	0.77
	TAL	5.50 ef	1.47 c	0.86
Taznakht	TAZ	14.21 cd	1.95 ab	0.73
	IZN	13.40 d	1.57 b	0.6
P		<0.001	0.0287	0.684

Means in the same column followed by different letters are significantly different by Tukey's test. For locality codes see Table 1.

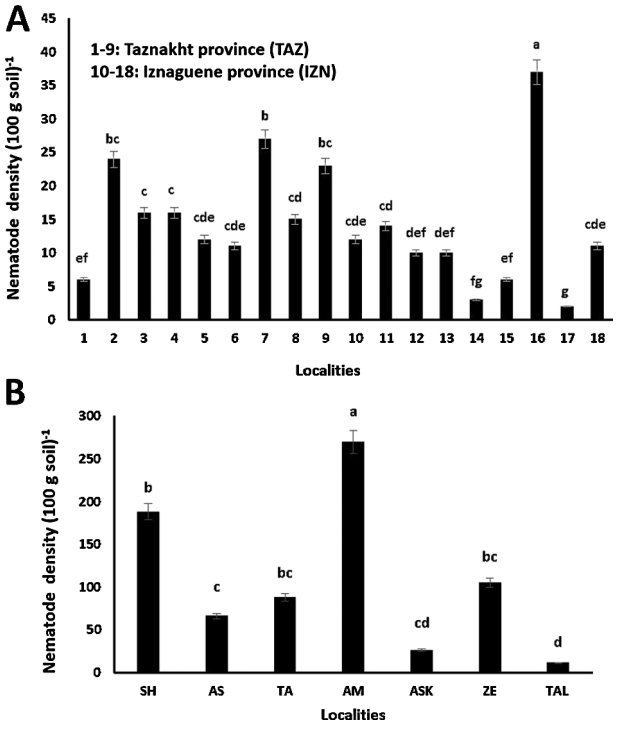

Fig. 3. Nematode density of saffron (100 g soil)$^{-1}$ in prospected localities of Taznakht (A) and Taliouine region (B). Localities (1-9) and (10-18) represent Taznakht (TAZ) and Iznaguene (IZN) provinces, respectively. Letters represent homogeneous groups based on protected least significant difference test (LSD) for each variable at ($P < 0.001$). Error bars represent the standard error. Region codes are given in Table 1.

tively (Fig. 3B). The lowest densities were found in Tallakht (TAL) province 11 nematodes (100 g soil)$^{-1}$, followed by Askaoun (ASK) province (26 nematodes (100 g soil)$^{-1}$).

Physicochemical properties of soils under saffron production

All study sites in the Taliouine region were located at moderate to high altitude (1570-1947 m a.s.l.) (Table 1). Physicochemical analyses of soil samples indicated two types of soil texture: sandy clay loam and clay loam. Soil was generally 80% calcareous brown with a clay-loam texture, 50 cm deep with a pH range of 8.0 to 9.1. The lowest organic matter content (1.8%) was found in samples from Tassousfi province and the highest organic matter content was detected in samples originating from Assais (2.9%) (Table 1). Problematic salinity levels were not detected in any sample. In the Taznakht region, the soil is sandy-loam with a slightly alkaline pH of 8.8. The climate is semi-arid with an annual precipitation >250 mm. In both studied regions, the rainy period lasts from November to April. Annual sunshine exceeds 300 days, but fog and dew are fairly regular. Average temperatures range from 10 to 26°C, though the region sometimes experiences surges of Saharan air that can raise temperatures above 40°C.

Principal component analyses of the soil characteristics (Tables 4 and 5) across the Taliouine and Taznakht regions (Fig. 4) showed that the fraction of variance accounted for by the first two PC axes is 36.87% and 30.09% (eigenvalues), respectively. A loading plot of the soil factors (Fig. 4A) indicated that the PC1 axis was related to nitrogen and silt content in negative PC values and to sand, organic matter, electrical conductivity

Table 4. Physicochemical soil characteristics analysed in the PCA and canonical analyses and corresponding codes.

Soil characteristics	Code
Granulometry	
Clay	Cla
Sand	San
Silt	Sil
Organic matter	OM
Nitrogen	N
pH H_2O	pH
Limestone	Lim
Soil solution	
Phosphorus	P
Iron	Fe
Magnesium	Mg
Manganese	Mn
Potassium	K
Sodium	Na
Zinc	Zn
Copper	Cu
Conductivity	Ec

Table 5. Plant-parasitic nematode genera analysed in the PCA and canonical analyses and corresponding codes.

Genus	Code
Aphelenchoides	*APH*
Criconema	*CRI*
Ditylenchus	*DIT*
Helicotylenchus	*HEL*
Hemicriconemoides	*HEM*
Hoplolaimus	*HOP*
Longidorus	*LONG*
Meloidogyne	*MEL*
Paratylenchus	*PAR*
Pratylenchus	*PRA*
Rotylenchus	*ROT*
Tylenchus	*TYE*
Tylenchorhynchus	*TYL*
Xiphinema	*XIP*

and mineral content in positive PC values. The PC2 axis was related to clay content, pH, copper, potassium and, to a lesser extent, sodium and magnesium content. The bi-plot of soil factors interacting with sampling regions indicated a significant differentiation between all provinces surveyed in term of soil patterns (Fig. 4B). Five provinces of the Taliouine region were related to soil granulometry characteristics such as clay and silt, and to nitrogen mineral (phosphorus, copper and zinc) content. Zagmouzen and Askaouen provinces (Taliouine region) had a good affinity with soil iron content. Moreover, the Taznakht region indicated a strong affiliation with organic matter, electrical conductivity and soil limestone content. Linear regressions of soil organic matter and total PPN in Taliouine and Taznakht revealed a negative correlation between those two parameters ($R^2 = 0.3502$ and 0.1905, respectively) (Fig. 4C).

Nematode community patterns

Principal component analyses of the nematode genera distribution across the Taznakht region (Fig. 5) showed that the fraction of variance accounted for by the first two PC axes is 27.20 and 19.92% (eigenvalues), respectively (Iznaguene province). In Taznakht province, the fraction of variance accounted for by the first two PC axes was 23.11 and 19.44%, respectively (Fig. 5A). In Iznaguene province, the PC1 axis related to *Longidorus* spp., *Meloidogyne* spp., *Criconema* spp., *Hoplolaimus* spp. and *Ditylenchus* spp. (positive PC values), and *Pratylenchus* spp., *Helicotylenchus* spp. and *Hemicriconemoides* spp. (negative PC values). The PC2 axis related to *Rotylenchus* spp., *Paratylenchus* spp., *Aphelenchoides* spp. and *Xiphinema* spp. (positive PC values), and, to a lesser extent, *Tylenchorhynchus* spp. (negative PC value).

The root-knot nematode *Meloidogyne* spp. localised with *Xiphinema* spp. in the PC1 axis with positive corresponding values. *Tylenchus* species had negative values on the same axis. The PC2 axis is diversified in this province with six genera in the positive part (Fig. 5B). The projection of the sample eigenvalues on the PC axes indicated that *Meloidogyne*, *Helicotylenchus* and *Tylenchus* species were significantly more abundant in Iznaguene province, while *Pratylenchus*, *Xiphinema* and *Ditylenchus* species were more abundant in Taznakht province (Fig. 5C).

Principal component analysis across mean localities of the Taliouine region (Fig. 6) showed that the fraction of variance accounted for by the PC axes was 27.74% (Agadir Melloul) to 46.07% (Tassousfi) for PC1, and 20.09% (Agadir Melloul) to 30.52% (Tassousfi) for PC2 (Fig. 6A). PC1 was clearly related to *Aphelenchoides* spp. (Sidi Hssaine), *Meloidogyne* spp., *Pratylenchus* spp. (Tassousfi), *Longidorus* spp. (Zagmouzen), *Tylenchus* spp., *Helicotylenchus* spp. (Assais), *Xiphinema* spp. and *Ditylenchus* spp. (Askaouen) (positive PC values). The PC2 axis was related to *Helicotylenchus* spp. and *Ditylenchus* spp. (Sidi Hssaine), *Hemicriconemoides* spp. (Tassousfi), *Paratylenchus* spp. (Zagmouzen), *Roty-*

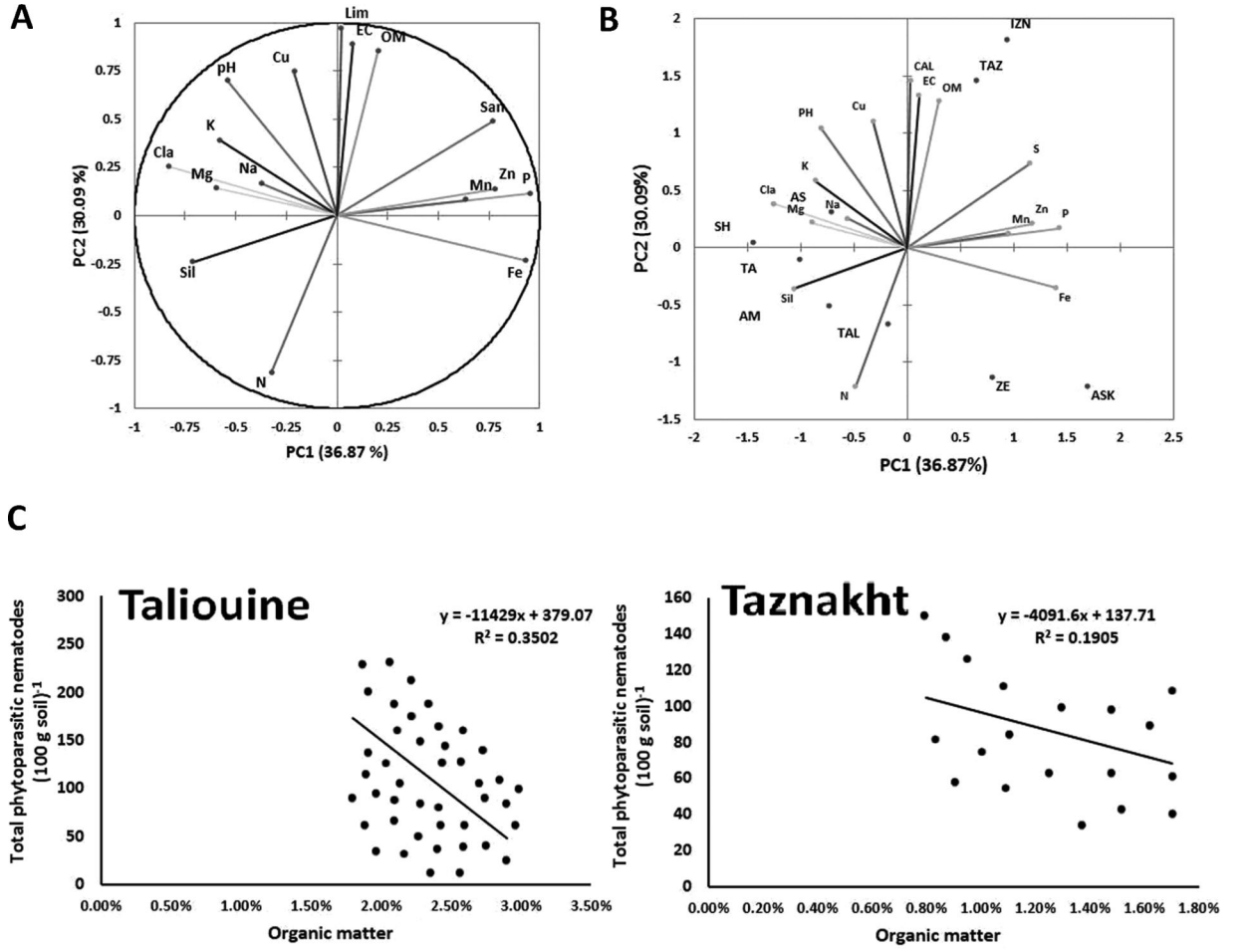

Fig. 4. Physicochemical soil characteristics in all the regions surveyed. A: PCA loading plot for the soil characteristics; B: Biplot of soil characteristics in interaction with the regions prospected; C: Linear regressions of soil organic matter and total PPN in saffron regions (Taliouine and Taznakht). All R^2 values were significant at $P < 0.001$. Localities and soil codes are given in Tables 1 and 4.

lenchus spp. (Agadir Melloul), *Meloidogyne* spp. (Assais) and *Pratylenchus* spp. (Askaouen) (positive PC values). The projection of the sample eigenvalues on the PC axes indicated that *Helicotylenchus*, *Pratylenchus* and *Tylenchus* species were significantly more abundant at Sidi Hssaine and Agadir Melloul provinces (Fig. 6B). *Meloidogyne* and *Ditylenchus* species were more abundant in Tassousfi province. Zagmouzen province seems to be an optimal place for *Tylenchus* species to occur on saffron fields.

Relationship between soil factors and abundance of plant-parasitic nematodes

A canonical correspondence analysis (Fig. 7) was used to reveal the relationship between soil factors and PPN abundance. The first axis explains 37.13% of the variance and the second axis explains 25.28%. The nematode genera *Pratylenchus* spp., *Paratylenchus* spp., *Meloidogyne* spp. and *Xiphinema* spp. indicated a significant ($P < 0.05$) positive relationship with sandy soils and mineral content (especially Fe, P and Zn). *Rotylenchus* spp., *Helicotylenchus* spp., *Tylenchorhynchus* spp. and *Hoplolaimus* spp. were significantly ($P < 0.05$) correlated with clay and silt soil texture and magnesium content.

Discussion

This study provides baseline information on genera diversity, incidence and distribution of plant-parasitic nematodes associated with saffron in Morocco. To our knowl-

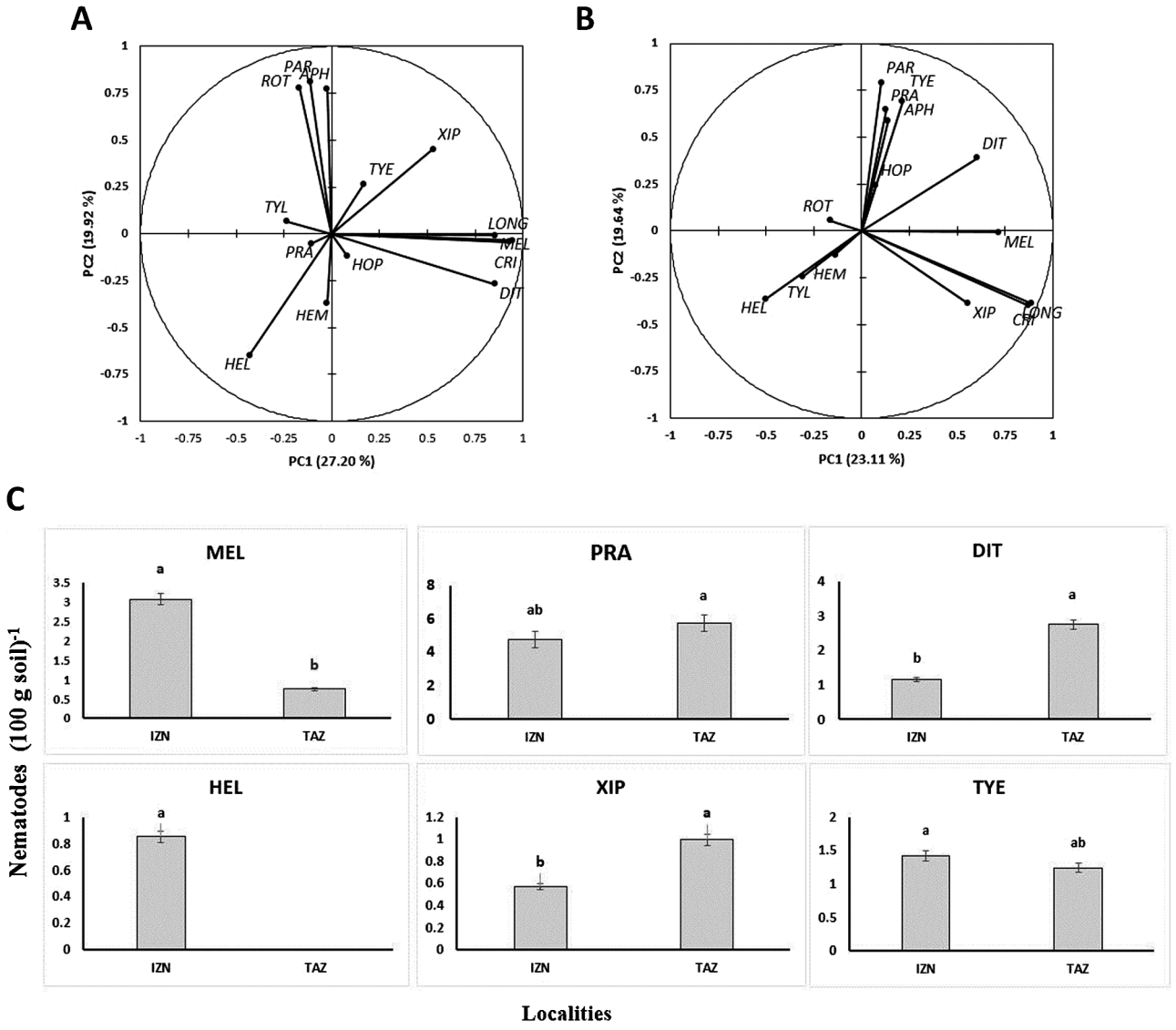

Fig. 5. Plant-associated nematode community patterns in the localities surveyed in Taznakht. A: PCA loading plot for the nematode genera in Iznaguene province; B: PCA loading plot for the nematode genera in Taznakht region; C: Mean value of the discriminant nematode variables according to the PCA analysis. Region and genus codes are given in Table 1 and Figure 2. Letters represent homogeneous groups based on protected least significant difference tests (LSD) for each variable at ($P < 0.001$). Error bars represent the standard error.

edge, this is the first comprehensive study on PPN in saffron fields in Morocco. A total of 14 genera of both endoparasitic and ectoparasitic plant-parasitic nematodes were recorded. The presence of these nematodes indicates potential damage to saffron and the need for appropriate management strategies. Lower number of genera (5 and 12) were identified in previous surveys reported from India and Iran, respectively (Mahdikhani & Alvani, 2013; Sheikh *et al.*, 2014). Sheikh *et al.* (2014) found that the main PPN from saffron in ten districts of Kashmir valley, India, were *Tylenchus* spp., *Helicotylenchus* spp., *Pratylenchus* spp., *Hirschmanniella* spp. and *Psilenchus* spp. The abundance of PPN genera was shown to be the main parameter to estimate their distribution in soil or rhizosphere system of crops based on their frequency.

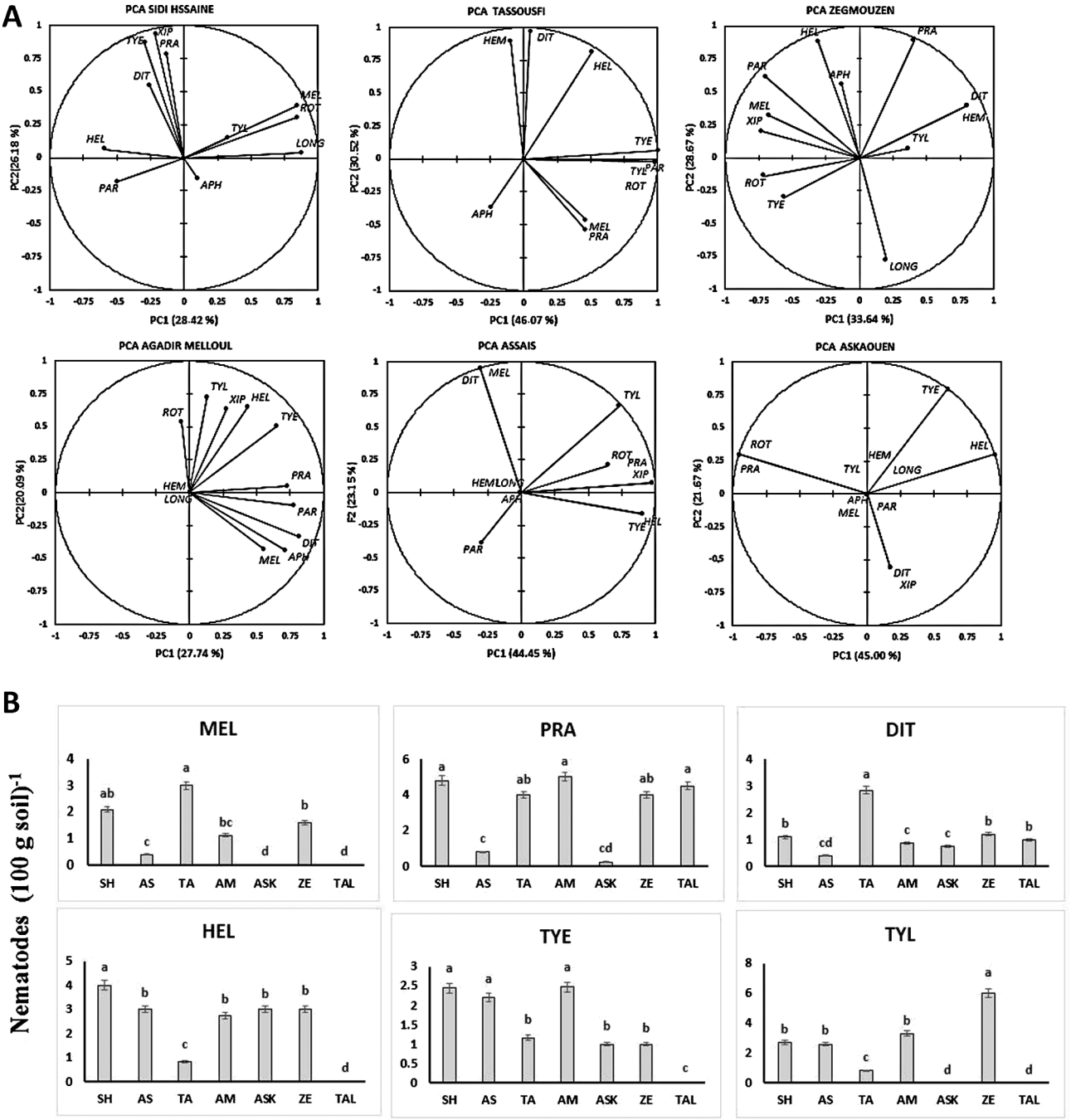

Fig. 6. Plant-associated nematode community patterns in the localities surveyed in the Taliouine region. A: PCA loading plot for the nematode genera in prospected localities; B: Mean value of the discriminant nematode variables according to the PCA analysis. Region and genus codes are given in Table 1 and Figure 2. Letters represent homogeneous groups based on protected least significant difference test (LSD) for each variable at ($P < 0.001$). Error bars represent the standard error.

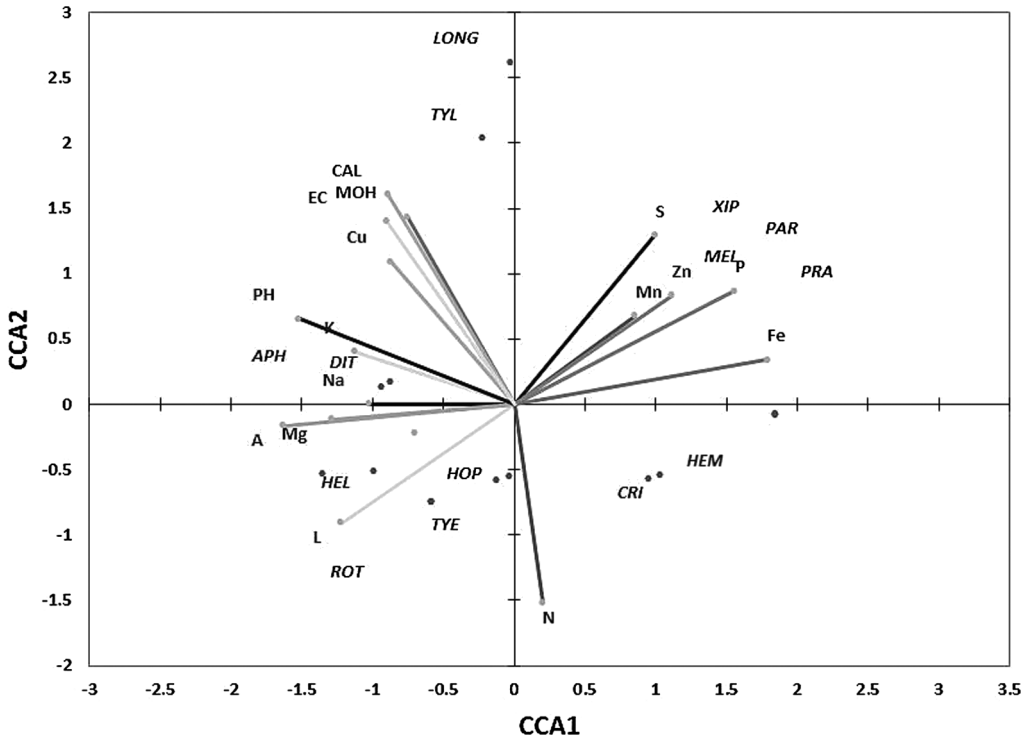

Fig. 7. Canonical correspondence analysis of the relationship between nematode communities of saffron and soil properties in Taliouine and Taznakht. For codes, see Tables 4 and 5.

Our study revealed that *Pratylenchus* spp. and *Helicotylenchus* spp. were significantly abundant in the two surveyed regions, whereas *Meloidogyne* spp. and *Ditylenchus* spp. were significantly frequent occurrences. In the present study, *Pratylenchus* spp. was clearly the dominant genus among PPN, affecting 68 and 75% of samples collected from Taliouine and Taznakht, respectively. Its pest status on saffron needs further study. Four species of *Pratylenchus*, *P. penetrans*, *P. coffeae*, *P. thornei* and *P. crenatus*, were detected among the most abundant nematodes in both regions. Few studies worldwide reported the presence of *Pratylenchus* spp. in the rhizosphere of saffron (Mahdikhani & Alvani, 2013; Sheikh *et al.*, 2014). Population densities of these nematode species in soil and saffron roots ranged from 7 to 18 vermiform stages of *Pratylenchus* spp. (100 cm^3 soil)$^{-1}$ and 6-21 vermiform stages of *Pratylenchus* spp. (2 g root)$^{-1}$. Other species of the genus *Pratylenchus* that parasitise saffron are *P. loosi* and *P. pratensis* (Mahdikhani & Alvani, 2013). *Pratylenchus penetrans* and *P. pratensis* were identified for the first time in *Crocus* spp. and *C. sativus* and showed high abundance (Metcalf, 1903; Schenk, 1970). Although damaging population thresholds for this genus in saffron planting are still unknown, the population densities encountered in our study may not present a potential risk to saffron planting in field conditions when compared with threshold densities reported on other crops (*e.g.*, cereals) (Thompson, 1993; Nicol *et al.*, 1999). Several studies showed that the lesion nematodes are important parasites infecting many crops and are known to form disease complex with many different soil-borne fungi causing root rot, thereby increasing root damage (Sikora & Fernández, 1990).

The spiral nematode (*Helicotylenchus* spp.), particularly *H. vulgaris*, was also commonly encountered in the Taliouine and Taznakht saffron-producing regions as it was found in around 59 and 30% of the sampled fields of Taliouine and Taznakht, respectively. The occurrence of *Helicotylenchus* spp. on saffron has been reported in India (Hussaini *et al.*, 2010; Mahdikhani & Alvani, 2013) and Iran (Sheikh *et al.*, 2014). Species of this genus are often considered to be mild pathogens (Norton, 1974) and can increase susceptibility to plant-pathogenic fungi enabling them to gain access to the host cells. To date there are no records on the pathogenicity of this nematode on saf-

fron. However, the chances of secondary infections of saffron roots and corms are very high when *Helicotylenchus* spp. feed on such roots. Sheikh *et al.* (2014) indicated that *H. chishti* occurred with significant abundance in saffron fields in the valley of Kashmir followed by *Tylenchus* spp. and *Pratylenchus* spp. *Tylenchorhynchus* spp. have been identified in soil samples collected from the Taliouine and Taznakht regions of Morocco and Mahdikhani & Alvani (2013) observed that *T. brassicae* occurred in saffron soils in Southern Khorasan, Iran. Their importance for saffron production has not been evaluated.

Aphelenchoides subtenuis was identified in bulbs (narcissus) by Cobb in 1926 (Steiner & Buhrer, 1932; van Leeuwen & Trompert, 2008) but its distribution (abundance and frequency) was low because it was classified as a bud and leaf nematode, which is confirmed by our current study. However, studies found that *A. subtenuis* has been detected in corms and leaves of *Crocus* spp. in different countries (Koliopanos & Kalyviotis-Gazelas, 1979; Decker, 1989; Ortuño & Oros, 2002; McCuiston *et al.*, 2007). Zijlstra & Van Hoof (2006) indicated that *M. chitwoodi* and *M. fallax* can affect root growth and yield in *Crocus* plants based on PCR-based detection. Ortuño & Oros (2002) found a marked frequency of *D. destructor*, which severely affects bulbs, corms and tubers.

Understanding the effect of soil properties on PPN communities in saffron regions is an important step in their management since the relationship between soil properties and PPN assemblages is complex and it is influenced by climate (Nielsen *et al.*, 2014). Several studies reported the correlation of some PPN with mineral content (especially Fe, P, Zn and Mg) (Fiscus & Neher, 2002; Yavuzaslanoglu *et al.*, 2012; Ardakani *et al.*, 2014). Francl (1993) found a positive correlation between the density of *Heterodera glycines* and the level of magnesium (Mg). Mateille *et al.* (2014) indicated a strong correlation between soil proprieties and nematode communities in European coastal foredunes, and they concluded that nematodes, such as *Hemicycliophora* spp., *Longidorus* spp. and *Merlinius* spp., colonise the carbonated and mineralised soils. The same authors showed that nematodes are more commonly found in coarse textured soils and in oligotrophic conditions. Thus, the texture of soil was also an important factor for nematodes distribution; this is clear from our current study where eight taxa were significantly associated with soil granulometry. The sandy soils seem to be favoured habitats for nematodes such as *Meloidogyne* spp. (Prot &Van Gundy, 1981), *Pratylenchus* spp. and *Tylenchorhynchus* spp. (Prasard & Rao, 1980), which explains their high frequency and abundance in the Taliouine and Taznakht regions. Many studies reported that the distribution and survival of nematodes (especially *Ditylenchus* spp.) were found to be strictly dependent on soil texture and structure (Gerasimow, 1954; Miyagawa & Lear, 1970; Elgin *et al.*, 1975). Other experiments showed significant differences between cropping systems on the basis of some physical and chemical properties, indicating the important role that soil characteristics played in the abundance, distribution and structure of nematode communities (Kandji *et al.*, 2001) and validating the potential of nematodes as bio-indicator organisms of soil status. Our study indicated a negative correlation between soil organic matter and nematodes in both Taliouine and Taznakht. Similar to our findings, Yavuzaslanolgu *et al.* (2015) found a significant negative relationship between hyphal-feeding nematodes and soil organic matter content ($R^2 = 0.26$; $P < 0.05$), which can be explained by high undegraded organic matter content reducing nematode multiplication rates due to the lower abundance of microorganism.

In conclusion, PPN were detected in 95 and 90% of the fields surveyed in Taliouine and Taznakht, respectively, with 14 nematode genera identified in soil and root samples collected from 66 saffron fields. This study provides baseline information on diversity, incidence and distribution of genera of plant-parasitic nematodes associated with saffron in Morocco. This will be of important value for further research as, so far, studies dealing with PPN on the saffron crop in Morocco and world-wide are very limited. Further investigation is also needed to assess PPN, particularly *Pratylenchus* spp. and *D. dipsaci*, dynamics in both regions and their damage on saffron. Farmers are encouraged to plant certified corms and not source their planting materials from nematode-infested fields and they should embrace good and sustainable culture that could promote a good yield and reduce the spread of plant-parasitic nematodes.

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