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Spatio-temporal analysis of post-harvest moulds genera distribution on stored durum wheat cultivated in Tunisia

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ABSTRACT

Wheat represents a principal ingredient in traditional Tunisian diet including couscous, bread, pasta and biscuits. Northen Tunisia is an important growing area of wheat which after harvest is stored in silos and on farm. The cereal grains can become contaminated by post-harvest moulds during storage in silos under unfavorable conditions leading to a decrease in quality, packing and marketing of wheat. In this study, a mycological survey was undertaken to determine the biodiversity of post-harvest moulds on durum wheat stored in silos localized in five regions of Northern Tunisia and to investigate changes during the storage period. A total of 127 samples were obtained from Oued Mliz, Jendouba, Ksar Mezouar, Mateur and Ghezala silos during 2010–2011 and 2011–2012 wheat seasons. After sampling, seeds were placed on Potato Dextrose Agar medium (PDA) for 7 days of incubation at 28 °C. A total of 6035 strains of filamentous fungi were isolated.

The quantitative and qualitative changes on wheat mycoflora during storage were statistically explored by multivariate methods including correspondence and hierarchical cluster analysis. The most predominant post-harvest moulds genera isolated were *Alternaria* (28%), *Fusarium* (19%), *Penicillium* (19%), *Aspergillus* (14%), *Mucor* (8%) and *Rhizopus* (7%). Various genera of fungi imperfecti, including *Ulocladium*, *Geotrichum*, *Chaetomium*, *Trichothecium*, *Paecilomyces*, *Aureobasidium* and *Chrysonilia* (anamorphic *Neurospora*), and the Mucorales genera *Lichtheiia* and *Syncephalastrum* accounted for the remainder of about 6% of the total. Statistical data analysis revealed six mycological patterns corresponding to six distinct communities as characterized by the prevalence of different moulds. Such patterns clearly showed different spatio-temporal variability indicating that distribution and evolution of moulds during storage was sensitive to geographic location, year of sampling and short or long-term storage.

1. Introduction

In Tunisia, wheat is one of the most important crops being cultivated on 1.6 million hectares of the total agricultural land (5 million hectares). In 2012, wheat production reached 1.1 million metric tons including 900,000 metric tons of durum wheat and 200,000 metric tons of bread wheat. Northern Tunisia represents the main wheat production area where harvested wheat is stored in silos and in farms. Good agricultural practices during cultivation and harvesting must be used for protection against fungal attacks that could become a source of post-harvest fungi during storage. At harvesting and during the different phases of wheat processing from harvest to marketed products (including transport, storage and processing conditions for grain and grain products), seeds are often contaminated by both fungi and their metabolites (Riba et al., 2008).

Under unfavorable conditions, stored wheat grains can be subjected to post-harvest mould deterioration caused by *Alternaria*, *Aspergillus*, *Fusarium*, *Mucor*, *Rhizopus*, and other genera especially if drying and storage phases are poorly managed (Magan et al., 2003). Fungal contamination of wheat may affect kernel quality like germination capacity, discoloration, and modification of nutritional matrices leading to mycotoxin production. Factors like water availability, temperature, humidity and atmospheric

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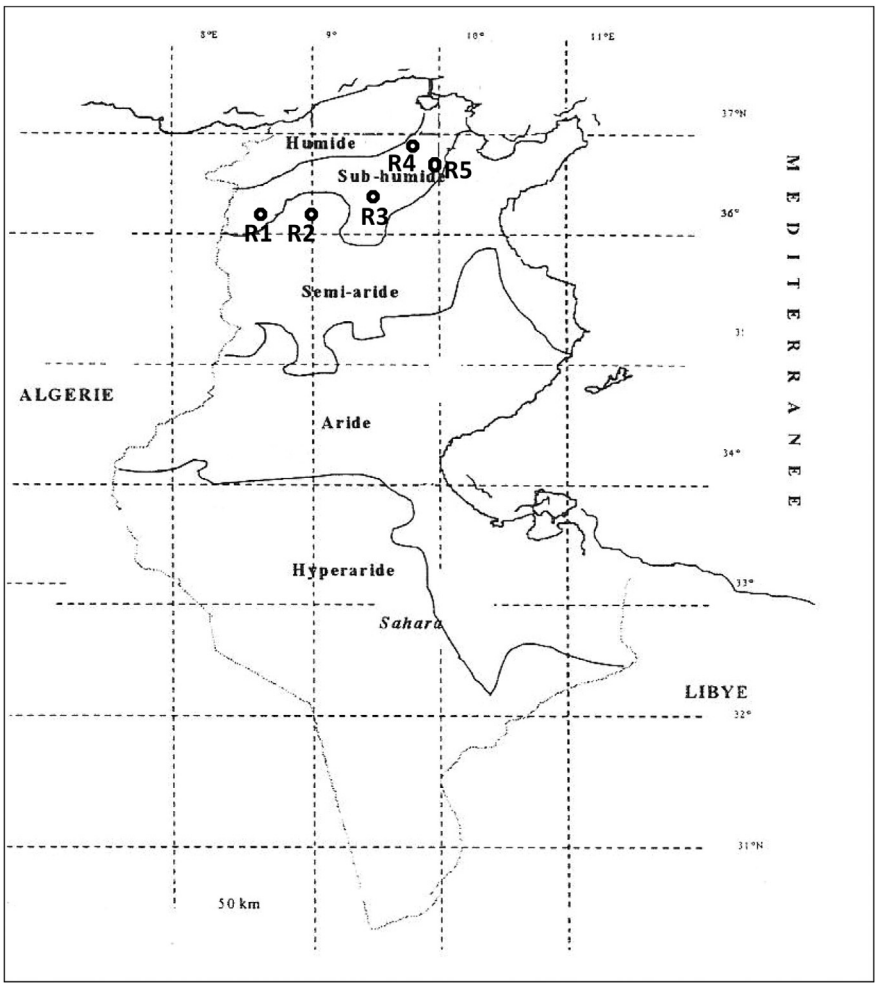

Fig. 1. Map of Tunisia. Durum wheat has been collected in five regions of Northern Tunisia. R1: Oued Mliz; R2: Jendouba; R3: Ksar Mezouar; R4: Ghezala; R5: Mateur.

composition represent key parameters in safe storage and affect the rate of proliferation of post-harvest moulds (Magan et al., 2003; Dantigny et al., 2006; Gregori et al., 2013).

In addition to the need of optimal physical conditions for safe storage, infestation of wheat grains by insects must be controlled to safeguard quality and safety of the product. Insects in silos produce metabolic heat, dust and fecal material which contribute to grain deterioration and fungal proliferation (Fourar-Belaifa et al., 2011; Sinha and Sinha, 1990). Use of aeration for cooling stored seeds can reduce the effect of high moisture, dramatically reduce pest development, slow mould growth and prevent chemical changes in wheat grains during long term storage (Da-Wen and Woods, 1997). Aeration alone does not guarantee insect-free grain, so fumigation may also have to be carried out. If insect pests are not controlled their populations may persist in silos and infest the grain harvested in subsequent years. In Tunisia, many authors have studied the prevalence of filamentous fungi and their mycotoxins on harvested and marketed cereals (Bensassi et al., 2009; Zaied et al., 2009). However, research on Tunisian stored wheat mycoflora from silos is limited. The objectives of this study were focused on (i) identification and quantification of fungal genera in stored wheat in Northern Tunisia followed by (ii) statistical analysis to highlight inherent mycological patterns and their spatio-temporal variations through different sampling sites, periods and years. Thus any interaction between the contaminant mycoflora of wheat and storage time, taking into account geographical location and sampling year, could be discerned.

2. Materials and methods

2.1. Study regions and silos

Five wheat-producing regions, characterized by a semi-arid and a sub-humid climate, were chosen for the study: Oued Mliz (R1), Jendouba (R2), Ksar Mezouar (R3), Ghezala (R4) and Mateur (R5) (Fig. 1). One silo in each region was randomly chosen to provide the wheat samples for the study.

2.2. Wheat sample collection

A total of 127 durum wheat samples, intended for food production, were collected during the post-harvest storage period in silos. 85 out of 127 wheat samples were collected from August to March in 2010–2011 (year A), while 42 samples were collected during 2011–2012 (year B). Samples (1 kg) were obtained at the beginning of each storage month from Oued Mliz (R1), Jendouba (R2), Ksar Mezouar (R3), Ghezala (R4) and Mateur (R5) regions. Each site was investigated for eight periods extending from 1 to 210 days of wheat storage: 1, 30, 60, 90, 120, 150, 180, 210 days.

2.3. Isolation and identification of wheat grain mycoflora

From each sample, 50 grains of wheat were placed in a Petri dish containing PDA medium supplemented with chloramphenicol (100 mg/l) according to the direct plating technique described by Botton et al. (1990) and Wareing (1997). Wheat grains were surface-sterilized by immersion in 1.5% sodium chloride for 2 min. Seeds were then rinsed in sterile distilled water and surface dried before direct plating. Plating was carried out in duplicate and plates were incubated at 28 °C for 7 days (Fig. 2). The isolated strains were sub-culturing on PDA, Malt Extract Agar (MEA) and Czapek Dox Agar (Cz) followed by macro and microscopic identification and apical fungal growth of pure colonies. Determination of fungal genera was done using the identification keys of published guidelines (Botton et al., 1990; Samson et al., 1996).

2.4. Dataset preparation prior to statistical analysis

After fungal identifications and counting, quantitative data corresponding to the occurrence levels of different genera were organized into a table containing 37 rows and 14 columns: rows corresponded to the five sites R1-R5 followed for eight periods (1–210 days) minus 3 rows (site R4 at 180 and 210 days, site R5 at 210 days) for which some sampling problems occurred leading to absence of data. The 14 columns corresponded to 7 fungus types (6 separated major genera and one block of grouped minor genera) × 2 years (A and B).

2.5. Topological and typological analysis

Fungal biodiversity in different sampling sites and periods was statistically studied by means of multivariate methods including correspondence and hierarchical cluster analysis. Correspondence analysis (CA) is a dual approach advantageously applied on abundance datasets to extract extreme relative levels both along rows and columns (Escofier and Pagès, 1991; Greenacre, 1993). From such extreme values, extreme taxonomic profiles can be defined for some sampling sites and periods in which some fungal genera are relatively favored at the expense of others. The different mycological patterns (MPs) represented taxonomic bases for the spatio-temporal study of fungal biodiversity.

After identification of the different mycotypes, the CA coordinates of the 37 samples were used in hierarchical cluster analysis (HCA) to classify them into the different MPs (Everitt et al., 2001). For that, Euclidean distances between samples were calculated followed by cluster construction using the Ward aggregation rule.

The multivariate analyses were carried out using ADE-4 software (Chessel et al., 1998).

Table 1
Distribution of fungi isolated from stored wheat of 5 regions in North of Tunisia during 7 months in silos (2010–2011 and 2011–2012).

Genus	2010–2011		2011–2012		Total of fungi	% Total of isolates
	Number of isolates	Fungi %	Number of isolates	Fungi %		
Aspergillus sp.	528	16.7	289	10.2	817	14
Penicillium sp.	671	21.2	457	16	1128	19
Fusarium sp.	535	16.9	618	21.7	1153	19
Alternaria sp.	775	24.4	899	31.5	1674	28
Rhizopus sp.	236	7.5	199	7	435	7
Mucor sp.	249	7.9	209	7.2	458	8
Other moulds	184	5.4	186	6.4	370	6
Total	3178	100	2857	100	6035	100

2.6. Spatio-temporal analysis of fungal biodiversity

Spatio-temporal variation of fungal biodiversity was analyzed from stacked columns plots showing the percentages of the six MPs along (i) the five geographical sites (R1–R5) on one hand and (ii) the eight sampling periods on the other hand.

3. Results

3.1. Mycological analysis and identification of fungal isolates

A total of 6035 post-harvest moulds strains were isolated revealing that all the stored grains were highly contaminated by the so-called “field fungi” and “storage fungi”. This mycological analysis showed a predominance of *Alternaria*, *Penicillium*, *Fusarium*, *Aspergillus*, *Rhizopus* and *Mucor* genera. The highest level of wheat fungal infection concerned *Alternaria* species, comprising 28% of the total for post-harvest moulds isolated during the two sampling years (Table 1).

During 2010–2011 storage period, the predominant fungi of all wheat samples were *Alternaria* (24.4%) and *Penicillium* genera (21.2%). *Fusarium* and *Aspergillus* had similar contamination levels in the stored wheat (16.9% and 16.7% respectively). Our analyzed samples showed a much lower percentage of contamination by the Mucorales genera *Mucor* (7.9%) and *Rhizopus* (7.5%). During the second sampling year (2011–2012) a similar order of predominance as the first sampling year was seen, except that *Fusarium* species with 21.7% of samples prevailed over *Penicillium* species, which were only found in 16% of samples. *Alternaria*, which is considered as a field fungi, was isolated at the very high frequency of 31.5%. Contamination of stored samples by *Aspergillus* (10.2%), *Mucor* (7.2%) and *Rhizopus* (7%) genera was significantly lower than in the first year.

The other groups of fungi encountered and isolated from stored durum wheat represented a very low percentage with an average of

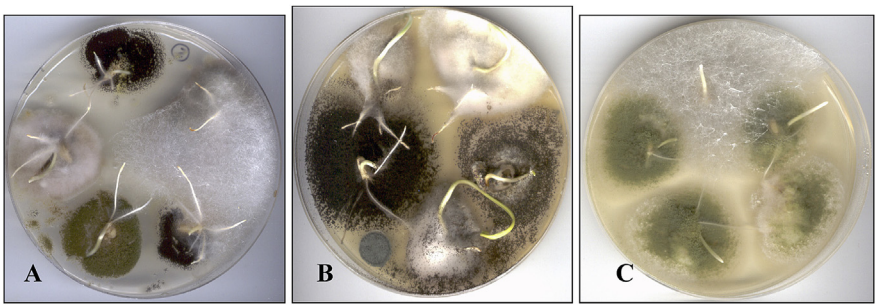

Fig. 2. A–C. Direct plating of durum wheat seeds on PDA medium.

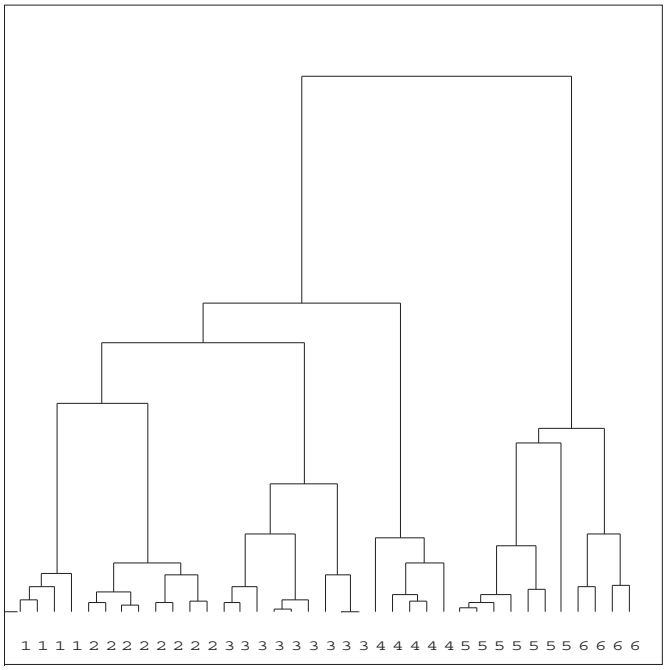

Fig. 3. Dendrogram showing 6 systematic groups of fungal communities.

6% during both storing periods 2010–2011 and 2011–2012. They belonged to the fungi imperfecti genera *Ulocladium*, *Geotrichum*, *Chaetomium*, *Trichothecium*, *Paecilomyces*, *Aureobasidium* and *Chrysonilia* (anamorphic *Neurospora*), and the Mucorales genera *Lichtheiia* and *Syncephalastrum* (Table 1).

3.2. Topological and typological analyses of mycological patterns

After application of Correspondence analysis (CA) on the dataset (37 × 14), the first four principal components (PCs) explained 70% of total variability. Therefore, the four first factorial coordinates were used in HCA to highlight six systematic groups (Fig. 3). On the dendrogram, the six groups showed relatively high distinctness compatible with a high differentiation between them.

To identify the mycological characteristics of each systematic group, CA-factorial coordinates plots and box plots were used to visualize the relative abundances of the different fungal genera (Figs. 4 and 5).

Group 1 was characterized by high relative levels of *Penicillium* (more particularly during the year B) (Fig. 5a). In the CA plot, this was highlighted by extreme projections of fungus points PB and PA with low F2 values (Fig. 4a).

Group 2 showed high relative levels of *Alternaria* (years A and B) and *Fusarium* (year B) (Fig. 5b). Its representative samples and their characteristic fungi (AlA, AlB, FB) were grouped on the extreme positive side of the first factor F1 of CA (Fig. 4a, b).

Group 3 was relatively more dispersed than the other ones because of its lower compactness of dendrogram (Fig. 3). It was generally characterized by higher relative levels of the genera *Penicillium*, *Aspergillus* and *Alternaria* during the year A (Fig. 5c). In CA, the points PA, AsA and AlA showed extreme projections along the third factor F3 (Fig. 4c).

Group 4 highlighted particularly high relative levels of *Alternaria* and *Fusarium* (years A and B) in addition to marked relative levels of *Mucor* (year A) (Fig. 5d). This mycological particularity was highlighted in CA by extreme projections of the column point MA on the positive side of F2 (Fig. 4a) and negative side of F4 (Fig. 4c).

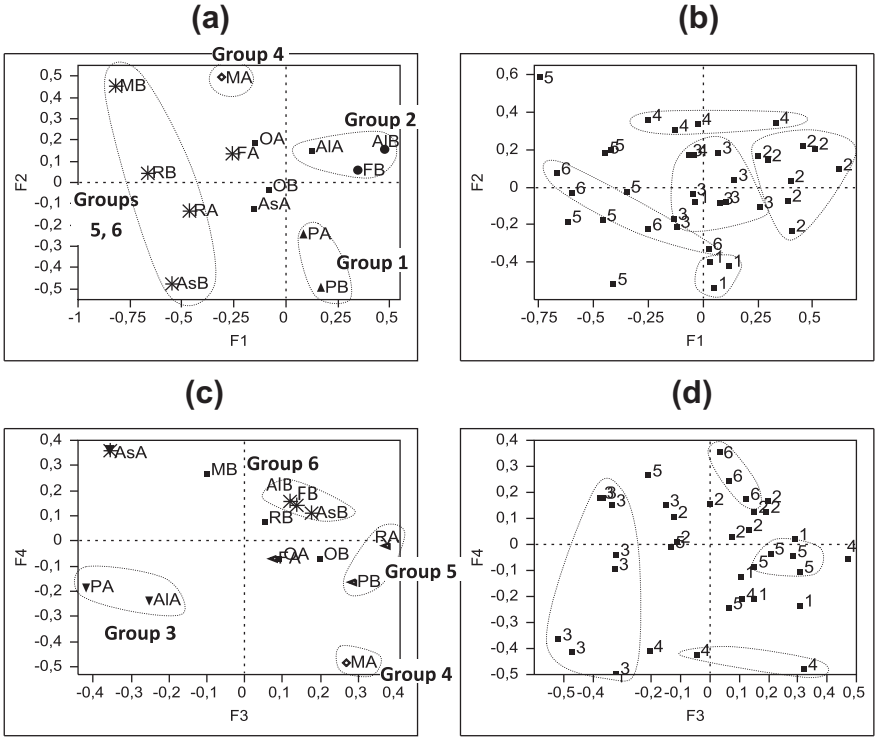

Fig. 4. Correspondence analysis (CA) factorial coordinates of columns (a, c) and rows (b, d) projected in the principle components plans F1–F2 (a, b) and F3–F4 (c, d). The 37 mycological profiles (rows) were encoded by the six systematic groups to which they belong (b, d); the 14 column points were encoded by genera initials followed by A or B for sampling years (a, c).

Group 5 was particularly characterized by high relative levels of *Rhizopus* (years A and B), *Mucor* (year B) and *Fusarium* and *Alternaria* (year A) (Fig. 5e). In CA, the row points of this group projected both along F1 (negative side) and F3 (positive side) (Fig. 4a, c): In negative side of F1, representative points of group 5 projected in a same subspace than the column points MB, RB, RA, AsB and FA (Fig. 4a, b). This was indicative of relatively high levels of *Rhizopus* (years A and B), *Mucor* and *Fusarium* (year A) in group 5. Along F3, group 5 occupied a small space containing the column points RA, PB and FA (Fig. 4c); this indicated high relative levels of *Rhizopus*, *Fusarium* (year A) and *Penicillium* (year B) in group 5. Moreover, along F1, the points of group 5 were opposite to those of group 2 (i.e. to points AlB, FB) indicating relatively low levels of *Alternaria* and *Fusarium* in group 5 (particularly in year B) (Fig. 4a).

Group 6 was dominated by *Aspergillus* followed by *Alternaria* and *Fusarium* during both years A and B (Fig. 5f). In CA, the upper right-hand quadrant of the F3–F4 plot highlighted a small area in which group 6 was characterized by the column points AlB, FB and AsB (Fig. 4c). This was indicative of a mycological pattern in which the relative development of *Alternaria*, *Fusarium* and *Aspergillus* were particularly advantaged in the year B compared to the five other patterns.

3.3. Temporal analysis of mycological patterns

From day 1 to day 210, the six MPs showed gradual succession associated with some preferential development periods for different genera (Fig. 6a).

Group 2 (characterized by *Alternaria* and *Fusarium*) was dominant for the 60 first days. During this period, group 2 was accompanied by group 4 (characterized by *Mucor* in addition to *Alternaria* and *Fusarium*), and group 5 (showing relatively high level of *Rhizopus* in presence of *Alternaria* and *Fusarium*).

The period 90–180 days was characterized by dominance of group 3 showing particularly high relative levels of *Penicillium* and *Aspergillus* (year A) with a strong presence of *Alternaria* and *Fusarium* (years A and B). Group 5 appearing in 1–60 days reached a peak at 90 days and disappeared at 150 days. In addition to group 3, the period 120–180 days was characterized by group 6 in which *Aspergillus* was better represented than the other genera. In summary, the period 120–180 days was more particularly favorable for *Aspergillus* and *Penicillium*.

Finally at 210 days, fungal biodiversity revealed to be dominated by group 1 showing relatively high levels of *Penicillium* in presence of *Alternaria* and *Fusarium*.

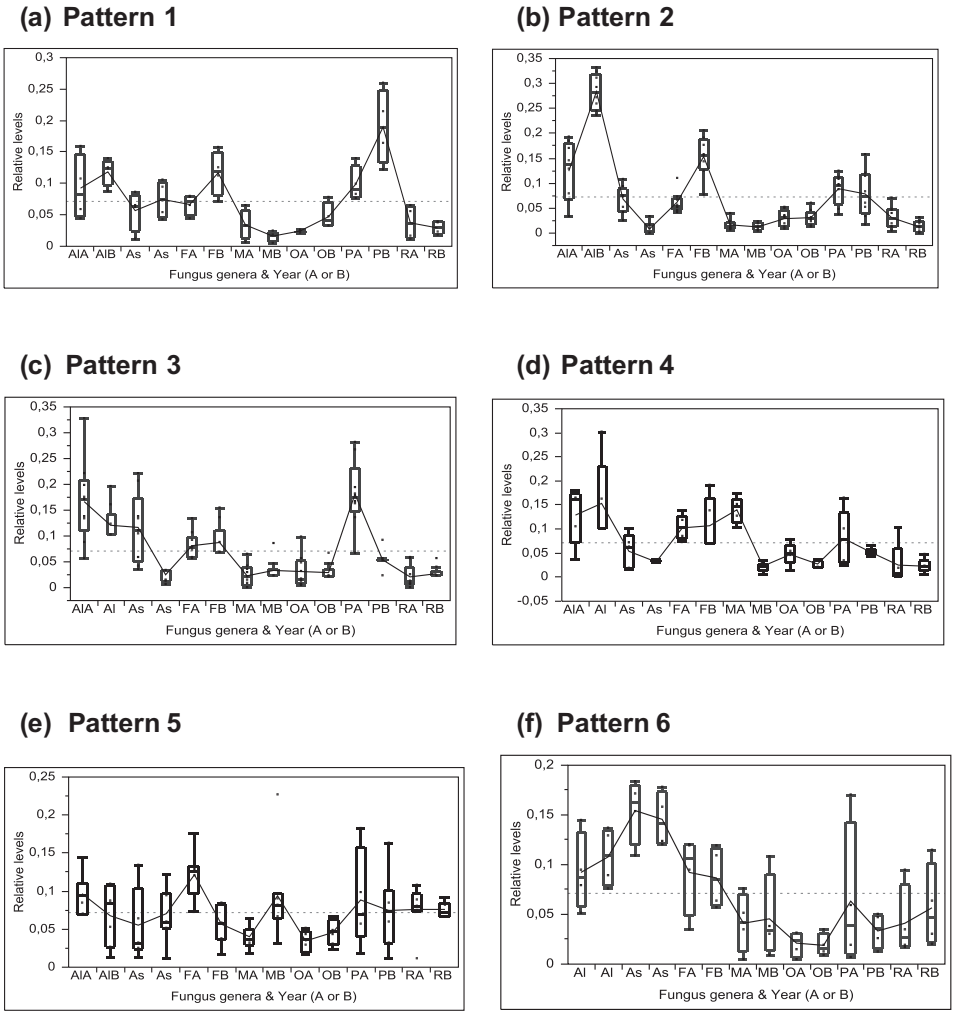

Fig. 5. Box plots showing six mycological patterns highlighted by correspondence and hierarchical cluster analyses. Legend: Al: *Alternaria*; As: *Aspergillus*; F: *Fusarium*; M: *Mucor*; P: *Penicillium*; R: *Rhizopus*; O: Others. The end letters A and B designate years A and B respectively.

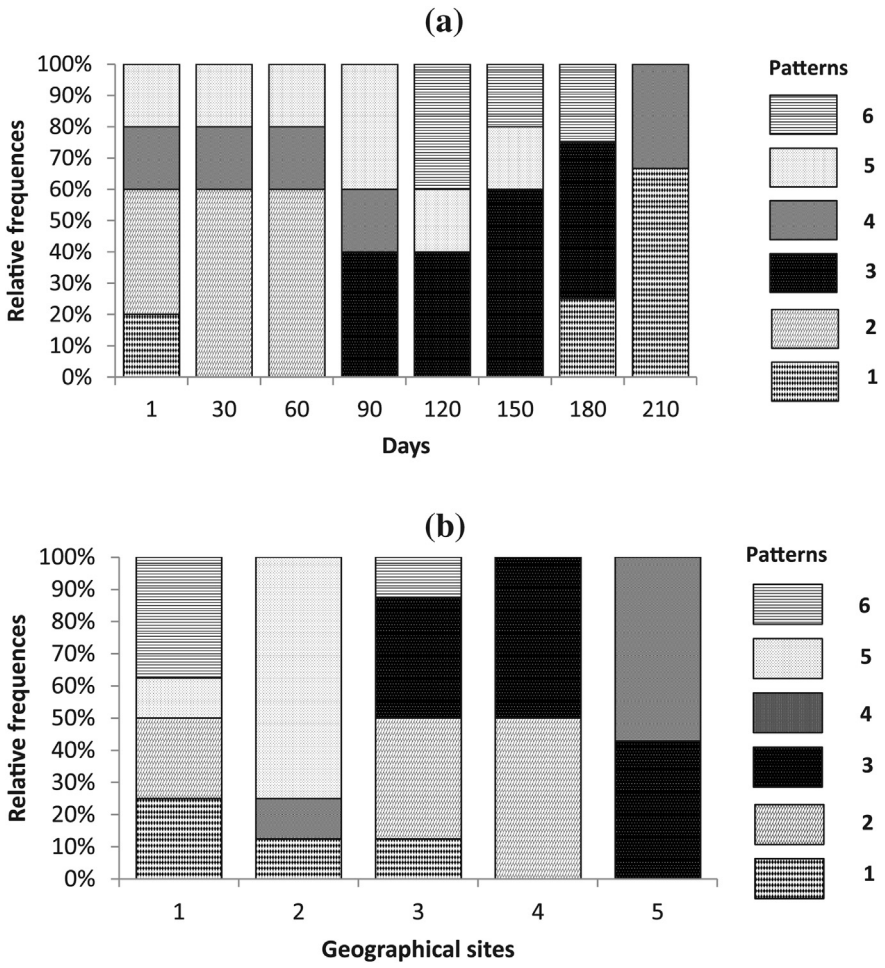

Fig. 6. (a) Stacked columns plot showing the relative frequencies of the six mycological patterns through the eight sampling periods (1, 30, 60, 90, 120, 150, 180, 210 days). (b) Stacked columns plot showing the geographical distribution of the six mycological patterns through the five west–east sampling sites R1–R5 corresponding to Oued Mliz, Jendouba, Ksar Mezouar, Ghezala and Mateur, respectively.

In general, temporal analysis of fungal biodiversity highlighted strong development of *Alternaria* and *Fusarium* in the first sampling period (1–60 days) followed by *Penicillium* and *Aspergillus* developments in later sampling period (120–210 days).

3.4. Geographical analysis of mycological patterns

Among the five studied sites, the six MPs showed differential frequencies associated to a West–east gradient (Fig. 6b). Moreover, most of sites showed dominance trends of some fungal genera.

The most Western site R1 showed relatively high occurrences of MPs 6 and 1 which were characterized by relatively high developments of *Aspergillus* and *Penicillium*, respectively. The first mycological pattern (MP1) extended from the site R1 to R3 and disappeared in the Eastern sites R4 and R5.

The site R2 was particularly dominated by MP5 in which the *Rhizopus* and *Mucor* genera were relatively well developed.

At the other geographical extremity, the most Eastern site R5 appeared to be particularly favorable for MP4 in which the *Mucor* genus was relatively well developed (year A) in contrast to all the other sites.

At wider geographical scale, the Eastern sites R3, R4, R5 revealed to be favorable for the development of MP3 characterized by high relative levels of *Penicillium* and *Aspergillus* (year A). This pattern was absent in the Western sites R1, R2. Moreover, the *Alternaria* genus was relatively more developed in the sites R3, R4, R5 compared to the Western sites R1, R2.

The neighboring intermediate sites R3 and R4 showed high similarity linked to their domination by the MPs 2 (*Alternaria* and *Fusarium* in the year B) and 3 (*Penicillium* and *Aspergillus* in the year A).

4. Discussion

Cereals are natural substrates for fungal development due to their nutritional composition rich in starch and protein, proving necessary carbohydrate and nitrogen for development of micromycetes (Magan and Aldred, 2007). In this work, we characterized the biodiversity of fungal flora in stored durum wheat locally produced in Northern Tunisia. The study focused on monitoring fungal variability in five regions (Oued Mliz, Jendouba, Ksar Mezouar, Ghezala and Mateur) for two years (2010–2011 and 2011–2012) during eight storage periods (extending from 1 to 210 days). Results showed that mycoflora was diversified by six major fungal genera belonging to two distinct groups, those so-called “field group” such as *Alternaria*, *Fusarium* and Mucorales (*Rhizopus* and *Mucor*) and those belonging to the so-called group of “storage” such as *Aspergillus* and *Penicillium*.

Occurrence of these fungal genera in cereals has been reported in several studies in many countries, viz. in Europe (Gregori et al.,

2013; Magan et al., 2003; Medina et al., 2006; Tabuc et al., 2009), South Africa (Rabie et al., 1997), in nearby countries to Tunisia such as Algeria and Morocco (Moghtet et al., 2012; Riba et al., 2010). The genus *Alternaria* was the most dominant among the total mycoflora isolated during the two sampling years. However, this finding differed from the findings obtained in previous studies, where *Aspergillus* was the dominant genus isolated from stored products (Hajjaji et al., 2006; Houssou, 2009).

Variability of fungal community in durum wheat during the storage period in silos showed high development of *Alternaria* and *Fusarium* genera during the two years of wheat sampling. Dominance of these two fungal genera was important at the beginning of the storage period (1st–60th day) (MP2), with a greater presence in North-Eastern sites of Tunisia, specifically in Ksar Mezouar, Mateur and Ghezala silos. *Alternaria* and *Fusarium* genera are considered as field and soil moulds but they can also be detected in storage condition (Magan et al., 2003). Kosiak et al. (2004) reported a huge post-harvest contamination of wheat by *Alternaria* and *Fusarium* genera. However, this contamination was positively correlated with site and storage period but not with the sampling year. Kammoun Gargouri et al. (2009) and Bensassi et al. (2009) have also highlighted the dominance of these two wheat genera cultivated in regions of Northern Tunisia, specifically in sub-humid and higher semi-arid bioclimatic regions. Indeed, high contamination levels in silos may be due to the initial high level of pre-harvest moulds in wheat grains.

From 90 to 180 storage days, fungal contamination was characterized by a dominant co-occurrence of *Penicillium* and *Aspergillus* during 2010–2011 (MP3) and a significantly better development of *Aspergillus* between 120 and 180 days in comparison to the other genera (MP6). Usually, storage moulds can develop on substrates whose moisture content is quite low (10–18%); they are responsible for deterioration of stored wheat grain with a possible production of mycotoxins (Ghosh and Nandi, 1986; Mills et al., 1995; Rahman, 2006). In addition, *Penicillium* requires high humidity and low temperature to grow, in opposition to *Aspergillus* genus; but *Aspergillus amstelodami*, *Aspergillus niger* and *Aspergillus versicolor* species, can also develop in high humidity (Magan and Aldred, 2007).

At 210 days (end of storage period), *Penicillium* species showed up in relatively high levels compared with other fungi (MP1). This result is probably due to bad storage conditions promoted by high moisture of stored wheat. Indeed, recent studies have highlighted the influence of water activity and temperature on *Penicillium verrucosum* development. Cabañas et al. (2008) highlighted the dominance and strong competition of *Penicillium* genus compared to other fungi under moister conditions of storage.

Contamination rate of stored wheat by Mucorales was relatively higher at 60 days of storage in Jendouba and Mateur sites (R2, R5) (MPs 5, 4). Mucorales are naturally present on field crops and soils. However, their presence in grain stocks can also be a sign of bad storage conditions. In fact, proliferation of fungal genera in silos during the two studied years showed high variability probably linked to variability of conditions within silos (humidity, temperature and O_2/CO_2 ratio) that may influence the good conservation of wheat grains. Indeed, proliferation of fungi in cereals occurred on the grain surface, and temperature, humidity and atmospheric burden of CO_2 in silos may enhance or inhibit development of some fungal genera (Fleurat-Lessard, 2002; Magan et al., 2003; Cairns-Fuller et al., 2005). It is clear that lack of ventilation coupled with a high temperature, promotes growth of storage moulds such as *Aspergillus* and *Penicillium* (Riba et al., 2010). Gregori et al. (2013) highlighted (i) the influence of some parameters (water availability, temperature and atmospheric composition) on fungal growth and mycotoxin production during wheat storage in bags and (ii) the interaction between sampling year and sampling date on fungal contamination rate.

Initial fungal contamination can occur in silos before storing wheat. Indeed, all the surfaces of the storage structures and materials of wheat reception are not easy to clean. They retain cereal dusts and residues that harbor fungal spores that can affect the microbiological and mycotoxicological quality of stored grains the following production year (Tangni and Pussemier, 2007). In addition, insects are active vectors of mould spores both in the field and in storage areas (Dunkel, 1988; Sinha and Sinha, 1990). Consequently, bad management of fumigation of structures to control insects before and during storage may have an impact on insect development and on post-harvest mould proliferation during storage.

5. Conclusion

Our results showed that stored durum wheat in Tunisia was contaminated with two major fungal flora groups, a “field group” such as *Alternaria*, *Fusarium*, *Rhizopus* and *Mucor*, and “storage group” such as *Aspergillus* and *Penicillium*. Mycoflora have a particular profile depending on geographical and temporal factors during storage. This last point highlights the importance of developing an assurance quality system throughout the period of grain storage in silos.

It is also important that farmers and silo’s managers adopt appropriate practices to limit proliferation of pre-harvest and post-harvest moulds before and during storage. This can be accomplished by using appropriate techniques such as good drying of grain coming into storage, cleaning all silos before harvest and burning or burying any residues, preventing insects invasion by applying insecticides to the cleaned surfaces and on wheat at reception, adopting good management of storage by monitoring physical conditions with detectors, and aerating silos correctly when necessary. A study of the impact of storage period on mycotoxin levels in wheat would significantly contribute to quantifying the influence of short or long time storage on production of toxins by post-harvest moulds.

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References

Bensassi, F., Zid, M., Rhouma, A., Bacha, H., Hajlaoui, M.R., 2009. First report of *Alternaria* species associated with black point of wheat in Tunisia. Anal. Microbiol. 59, 465–467.

Botton, B., Breton, A., Fèvre, M., Gauthier, S., Guy, P., Larpent, J.P., Reymond, P., Sanglier, J.J., Vayssier, Y., Veau, P., 1990. Moisissures utiles et nuisibles, Importance industrielle. Ed. Masson, Paris, p. 512.

Cabañas, R., Bragulat, M.R., Abarca, M.L., Castellá, G., Cabañes, F.J., 2008. Occurrence of *Penicillium verrucosum* in retail wheat flours from the Spanish market. Food Microbiol. 25, 642–647.

Cairns-Fuller, V., Aldred, D., Magan, N., 2005. Water, temperature and gas composition interactions affect growth and ochratoxin A production by isolates of *Penicillium verrucosum* on wheat grain. J. Appl. Microbiol. 99, 1215–1221.

Chessel, D., Dolédec, S., Thioulouse, J., Olivier, J.M., 1998. ADE-4 Software. <http://pbil.univ-lyonl.fr/ADE-4/ADE-4F.html>.

Dantigny, P., Bensoussan, M., Vasseur, V., Lebrihi, A., Buchet, C., Ismaili-Alaoui, M., Devlieghere, F., Roussos, S., 2006. Standardisation of methods for assessing mould germination: a workshop report. Int. J. Food Microbiol. 108, 286–291.

Da-Wen, S., Woods, J.L., 1997. Deep-bed simulation of the cooling of stored grain with ambient air: a test bed for ventilation control strategies. J. Stored Prod. Res. 33, 299–312.

Dunkel, F.V., 1988. The relationship of insects to the deterioration of stored grain by fungi. Int. J. Food Microbiol. 7, 227–244.

Escofier, B., Pagès, J., 1991. In: Devillers, J., Karcher, W. (Eds.), Applied Multivariate Analysis in SAR and Environmental Studies. Kluwer Academic Publishers, Dordrecht, pp. 1–32.

Everitt, B.S., Landau, S., Leese, M., 2001. Cluster Analysis. Arnold Publishers, London.

Fleurat-Lessard, F., 2002. Qualitative reasoning and integrated management of the quality of stored grain: a promising new approach. J. Stored Prod. Res. 38, 191–218.
Fourar-Belaifa, R., Fleurat-Lessard, F., Bouznad, Z., 2011. A systemic approach to qualitative changes in the stored-wheat ecosystem: prediction of deterioration risks in unsafe storage conditions in relation to relative humidity level, infestation by *Sitophilus oryzae* (L.), and wheat variety. J. Stored Prod. Res. 47, 48–61.
Ghosh, J., Nandi, B., 1986. Deteriorative abilities of some common storage fungi of wheat. Seed Sci. Technol. 14, 141–149.
Greenacre, M.J., 1993. Correspondence Analysis in Practice. Academic Press, London.
Gregori, R., Meriggi, P., Pietri, A., Formenti, S., Baccarini, G., Battilani, P., 2013. Dynamics of fungi and related mycotoxins during cereal storage in silo bags. Food Control 30, 280–287.
Hajjaji, A., El Otmani, M., Bouya, D., Bouseta, A., Mathieu, F., Collin, S., Lebrihi, A., 2006. Occurrence of mycotoxins (ochratoxin A, deoxynivalenol) and toxigenic fungi in Moroccan wheat grains: impact of ecological factors on the growth and ochratoxin A production. Mol. Nutr. Food Res. 50, 494–499.
Houssou, P.A., 2009. Natural infection of cowpea (*Vigna unguiculata* (L.) Walp.) by toxigenic fungi and mycotoxin contamination in Benin, West Africa. J. Stored Prod. Res. 45, 40–44.
Kammoun Gargouri, L., Gargouri, S., Hajlaoui, M.R., Marrakchi, M., 2009. Occurrence and distribution of *Microdochium* and *Fusarium* species isolated from Durum wheat in Northern Tunisia and detection of mycotoxins in naturally infested grain. J. Phytopathol. 177, 546–551.
Kosiak, B., Torp, M., Skjerve, E., Andersen, B., 2004. *Alternaria* and *Fusarium* in Norwegian grains of reduced quality—a matched pair sample study. Int. J. Food Microbiol. 93, 51–62.
Magan, N., Hope, R., Cairns, V., Aldred, D., 2003. Post-harvest fungal ecology: impact of fungal growth and mycotoxin accumulation in stored grain. Eur. J. Plant Pathol. 109, 723–730.
Magan, M., Aldred, D., 2007. Post-harvest control strategies: minimizing mycotoxins in the food chain. Int. J. Food Microbiol. 119, 131–139.
Medina, A., Valle-Algarra, F.M., Mateo, R., Gimeno-Adelntado, J.V., Mateo, F., Jimenez, M., 2006. Survey of the mycobiota of Spanish malting barley and evaluation of the mycotoxin producing potential of species of *Alternaria*, *Aspergillus* and *Fusarium*. Int. J. Food Microbiol. 108, 196–203.
Mills, J.T., Seifert, K.A., Frisvad, J.C., Abramson, D., 1995. Nephrotoxigenic *Penicillium* species occurring on farm-stored cereals grains in Western Canada. Mycopathologia 130, 23–28.
Moghtet, S., Kadi, H., Nahal, B., Nora-Moussaoui, A., Amrouche, A., Lazouni, A.H., 2012. Physicochemical factors' effect on fungal and mycotoxical contamination of French soft wheat marketed in Algeria. In: Sciences du Vivant, Biologie, vol. 4. ScienceLib (Mersenne), p. 120802. ISSN: 2111–4706.
Rabie, C.J., LÜbben, A., Marais, G.J., Jansen van Vuuren, H., 1997. Enumeration of fungi in barley. Int. J. Food Microbiol. 35, 117–127.
Rahman, Z.U., 2006. Storage effect on nutritional quality of commonly consumed cereals. Food Chem. 95, 53–57.
Riba, A., Bouras, N., Mokrane, S., Mathieu, F., Lebrihi, A., Sabaou, N., 2010. *Aspergillus* section *Flavi* and aflatoxins in Algerian wheat and derived products. Food Chem. Toxicol. 48, 2772–2777.
Riba, A., Mokrane, S., Mathieu, F., Lebrihi, A., Sabaou, N., 2008. Mycoflora and ochratoxin A producing strains of Aspergillus in Algerian wheat. International Journal of Food Microbiology 122, 85–92.
Samson, R.A., Hoekstra, E.S., Frisvad, J.C., Filtenborg, O., 1996. Introduction to Foodborne Fungi. Centraal Bureau voor Schimmelcultures, Baarn.
Sinha, A.K., Sinha, K.K., 1990. Insect pests, *Aspergillus flavus* and aflatoxin contamination in stored wheat: a survey at North Bihar (India). J. Stored Prod. Res. 26, 223–226.
Tabuc, C., Marin, D., Guerre, P., Sesan, T., Bailly, J., 2009. Molds and mycotoxin content of cereals in Southeastern Romania. J. Food Prot. 72, 662–665.
Tangni, E.K., Pussemier, L., 2007. Ergosterol and mycotoxins in grain dusts from fourteen Belgian cereal storages: a preliminary screening survey. J. Sci. Food Agric. 87, 1263–1270.
Wareing, P.W., 1997. Incidence and detection of thermotolerant and thermophilic fungi from maize with particular reference to *Thermoascus* Species. Int. J. Food Microbiol. 35, 137–145.
Zaied, C., Abid, S., Zorgui, L., Bouaziz, C., Chouchane, S., Jomaa, M., Bacha, H., 2009. Natural occurrence of ochratoxin A in Tunisian cereals. Food Control 20, 218–222.