SNP-Based Genetic Structure of Tunisian Durum Wheat Landraces and Distribution of the Resistance to Septoria Tritici Blotch

Maroua Ouaja

Institut National Agronomique de Tunisie

Bochra Bahri

University of Georgia

Sahbi Ferjaoui

Regional Field Crop Research Center,

Maher Medini

Banque Nationale des Gènes

Udupa M. Sripada

International Center of Agricultural Research in Dry Areas, Rabat

Sonia Hamza (✉ hamza.sonia@inat.agrinet.tn)

Institut National Agronomique de Tunisie

Research Article

Keywords: Durum wheat landraces, genetic structure, admixture, Zymoseptoria tritici, resistance.

Posted Date: November 3rd, 2021

DOI: <https://doi.org/10.21203/rs.3.rs-957863/v1>

License: © ⓘ This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Abstract

Background: Septoria tritici blotch (STB) has marked durum wheat production worldwide. This fungal disease is until today a challenge for farmers, researchers and breeders all united in the aim of reducing its damage and improving wheat resistance. Tunisian durum wheat landraces were reported to be valuable genetic resources for resistance to biotic and abiotic stresses and are therefore prominently deployed in breeding programs to develop new varieties adapted to fungal diseases as STB and to climate change constraints overall.

Results: A total of 366 local durum wheat accessions were assessed for resistance to two virulent Tunisian isolates of *Zymoseptoria tritici* Tun06 and TM220 under field conditions. Population structure analysis of the durum wheat accessions, performed with 286 polymorphic SNPs (PIC >0.3) covering the entire genome, identified three genetic subpopulations (GS1, GS2 and GS3) with 22% of admixed genotypes. Interestingly, all of the resistant genotypes were among GS2 or admixed with GS2.

Conclusions: This study revealed the population structure and the genetic distribution of the resistance to *Z. tritici* in the Tunisian durum wheat landraces. The grouping pattern of accessions appear to be associated, to some extent, with the geographical pattern of the landraces. We suggested that GS2 accessions were mostly introduced from eastern Mediterranean populations, unlike GS1 and GS3 that originated from the west. Resistant GS2 accessions belonged to landraces Taganrog, Sbei glabre, Richi, Mekki, Badri, Jneh Khotifa and Azizi. Furthermore, we suggested that admixture contributed to transmit STB resistance from GS2 resistant landraces to initially susceptible landraces such as Mahmoudi (GS1), but also resulted in the loss of resistance in the case of GS2 susceptible Azizi and Jneh Khotifa accessions.

Background

Durum wheat (*Triticum. turgidum* L. ssp. *durum*) is a tetraploid species, originated in the Fertile Crescent about 10,000 BP. It evolved from the domestication of hulled tetraploid wheat subspecies as emmer (*Triticum turgidum* L. ssp. *dicoccum*) in the eastern Mediterranean, notably at the mountains of the Fertile Crescent (Iran, Turkey, Syria and Jordan) and at the Tigris and Euphrates basin [1-6]. Thereafter, the geographical expansion of durum wheat had closely followed human migration [4], where Phoenicians, Greeks and Romans contributed crucially in the pervasion and adoption of durum wheat cultivation around the Mediterranean Basin [5]. During their migration, domesticated wheat populations underwent strong natural and human selection processes, after which they have adapted specifically to local environments and developed to become landraces [7, 8]. Howbeit, durum wheat followed two dispersal pathways from its area of origin in the Mediterranean Basin, over the north side via South Europe (Turkey, Greece, and Italy) and over the south side via North Africa [1, 9]. The spread of durum wheat populations occurred by land through the Balkans and by maritime route through the Mediterranean Sea [5]. Along these pathways, wheat landraces have traced a complex history of dissemination, adaptation and genetic differentiation in time and space [10]. Therefore, the eastern–western dispersal of the Mediterranean

durum landraces favored their divergence into genetically distinct groups. In fact, Moragues et al [9] classified a collection of 63 durum wheat landraces from the Mediterranean basin in a north dispersal group (including south European and south-western Asian landraces) and a south dispersal group (including landraces from North Africa and the Iberian Peninsula). Soriano et al [11] also structured a collection of 172 durum wheat landraces from 21 Mediterranean countries into four genetic populations related to their geographical origin, namely eastern Mediterranean, eastern Balkans and Turkey, western Balkans and Egypt, and western Mediterranean.

Durum wheat cultivation history in North Africa and particularly Tunisia, involved the intervention of Phoenicians importing wheat from Lebanon to Carthage, along with the development of Carthage trade maritime activity in the Mediterranean Sea favoring seed exchanges between Tunisia and the Mediterranean countries. North African landraces were also introduced and diffused by Romans, who greatly influenced durum wheat cultivation in this area by setting up modernized irrigation systems [5, 12, 13]. Recently, Robanna et al [13] studied the structure of six Tunisian durum wheat landraces, and reported their genetic similarity with landraces from North African countries and landraces from Greece, Italy, and Lebanon. Durum wheat was prevalent and well established in North Africa in the classical times [5]. Accordingly, North Africa and Abyssinian regions are considered as secondary centers of diversity for durum wheat [14, 15]. Ren et al [16] also suggested that North Africa should be considered as a microcenter of wheat diversity. Tunisia, being part of the secondary center of diversity for durum wheat, has a rich collection of old local durum wheat landraces [14]. To date, around 40 old durum wheat landraces are known in Tunisia that were morphologically characterized and classified by Bœuf [14] and Dghais et al [17]. Several studies emphasised high levels of genetic and agro-morphological diversity [13, 18-22], phenological features [23-26] and resistance to biotic and abiotic stresses i.e. drought, heat and fungal diseases [21, 27-29] within the Tunisian old durum wheat germplasm. Recently, Ben Krifa et al [30] showed a complex structure of 14 Tunisian durum wheat populations that was not entirely related to their geographic origin and variety name. However, Ouaja et al [22] identified a strong correlation between the genetic structure of 11 Tunisian durum wheat landraces and their morphological characterisation and nomenclature. Therefore, various interacting factors were reported that have influenced the structure and evolutionary dynamics of durum wheat in Tunisia and the Maghreb region overall, among which complex selection trajectory, the significance of variety names, the occurrence of heterogeneous mixtures within populations, local adaptation, local and regional exchanges between farmers and, loss and misidentification [22, 30, 31]. After the Green revolution, old durum wheat landraces were mainly grown and managed by smallholder farmers under low-input traditional agrosystems in the marginal areas of Mediterranean region, notably in southern Europe and North Africa [4, 32] as they were progressively abandoned from the early 1970s and replaced by improved genetically uniform modern varieties/cultivars [33, 34]. In Tunisia, durum wheat landraces are still cultivated by low-input farmers, in northern and central mountainous areas, under traditional farming systems. These landraces, transmitted by farmers from one generation to the next, are designated by a variety name linked to a historical origin, regional location and specific phenotypic characteristics [22, 30].

Septoria Tritici Blotch (STB) caused by the fungus *Zymoseptoria tritici* (*Z. tritici*) (Desm.) (Formerly *Mycosphaerella graminicola*) is currently considered among the most damaging and worldwide distributed fungal disease on cultivated wheat [35, 36]. The appearance of *Z. tritici* as a host-specialized wheat pathogen occurred about 10,500 years ago via host tracking throughout the time of wheat domestication [37-39]. Comparative genomic analysis between *Z. tritici* and its close relatives highlighted strong adaptive evolution of *Z. tritici* in relation to specialization on wheat [40]. Howbeit, Stukenbrock et al [37] demonstrated that wheat-adapted *Z. tritici* was derived from an ancestral population infecting wild grasses in the Middle East and that the domestication of an agricultural crop was concomitantly accompanied by the domestication of a fungal pathogen. Accordingly, the Fertile Crescent is considered a hotspot of *Z. tritici* genetic diversity [37, 41]. Therefore, wheat landraces and their wild relatives from the Fertile Crescent, having co-evolved for a long time with *Z. tritici*, must harbor the greatest diversity for resistance to STB [42]. Moreover, a host species specialization was highlighted in *Z. tritici* populations to either bread or durum wheat [43-45] making bread wheat-derived *Z. tritici* strains not suitable to decipher *Stb* genes in durum wheat, as studies into the genetic basis of STB resistance were entirely based on the *Z. tritici*-bread wheat pathosystem [46-51].

In Tunisia and under suitable environmental conditions for infection, STB causes considerable yield losses up to 50-60% [52, 53]. The introduction of the modern cultivar 'Karim' in 1980 displaced the cultivation of landraces, resulting in a reduction of the genetic diversity (genetic erosion) and therefore, enhancing the susceptibility to STB [54, 55]. Durum wheat landraces, characterized by a substantial level of genetic diversity [20, 22, 32], represent the main sources of resistance to *Z. tritici* to be incorporated into breeding programs for a sustainable STB disease control and management [21, 56, 57]. The present study consisted on a) analyzing the genetic structure of 366 Tunisian durum wheat accessions belonging to 13 landraces using 286 single nucleotide polymorphic markers (SNPs), b) evaluating the resistance of these accessions to two durum-wheat derived *Z. tritici* isolates (Tun06 and TM220) and, c) relating genotyping and disease screening data of the Tunisian durum wheat accessions to describe the life history of STB resistance in local durum wheat landraces.

Results

1. Genetic structure of durum wheat landraces

The genetic structure of the durum wheat accessions was determined using the Bayesian clustering model implemented in STRUCTURE [58]. The maximum likelihood (LnP (K)) and delta K (ΔK) methods [59]) showed that the most likely number of genetic subpopulations (K) was 3 (**Figure 1, a and b**). The inferred population structure at K=3 showed that 78% of the genotypes have a membership coefficient higher than 0.7 to one of the genetic subpopulations (GS1, GS2 and GS3), the rest were admixed. GS1, GS2, GS3 and admixed genotypes represented 20%, 45%, 13% and 22% of the entire collection, respectively. Among admixed genotypes, 53%, 27% and 20% were admixed between G1-G2, G1-G3 and G2-G3, respectively (**Figure 1c, Table S4**).

PCAs were performed using the 286 SNPs on the 366 genotypes (**Figure 2**). Axes 1 and 2 of the PCAs accounted for 24.33% and 16.54% of the total genetic variation, respectively. **Figure 2a** showed that PCA grouping corroborated the genetic structure inferred by STRUCTURE, pointing a clear differentiation between GS1, GS2 and GS3. Admixed genotypes were essentially distributed between GS1 and GS2 and between GS2 and GS3, reflecting ongoing hybridization and allele exchanges between these groups. Pairwise *Fst* values also showed considerable genetic differentiations between GS1, GS2 and GS3 (**Table 1**). The highest *Fst* value (0.751) was observed between GS1 and GS3. *Fst* values between GS1 and GS2 and between GS2 and GS3 were both around 0.400. Similarly, *Nm* indices between GS1 and GS2 and between GS2 and GS3 were close; while *Nm* between GS1 and GS3 was the lowest (0.078) reflecting an almost absence of genetic exchange between these two populations. The AMOVA (**Table 2**) was consistent with pairwise *Fst* and *Nm* analysis revealing that the genetic variation between subpopulations (61%) was higher than the variation within subpopulation (39%).

GS1 was solely composed by the landrace Mahmoudi. GS2 was composed of the landraces Azizi, Badri, Biskri, Jneh Khotifa, Mekki, Richi, Sbei glabre and Taganrog. GS3 was composed by the landrace Biada and two modern cultivars (Khlar and Maali). Twenty accessions of Mahmoudi, 11 of Jneh Khotifa, 8 of Richi, one Azizi and one Roussia accession were admixed between GS1 and GS2. Admixed genotypes between GS2 and GS3 included one accession of Azizi, 4 of Biada, 2 of Biskri, 5 of Souri, 5 of Roussia and 2 modern cultivars (Om Rabia and Nasr). Finally, one accession of Mahmoudi, 2 of Biada, 8 of Bidi, 6 of Souri, 2 of Roussia and 2 modern cultivars were admixed between GS1 and GS3 (Salim and Karim) (**Figure 2b**, **Table S4**).

In addition, population structure was investigated from K=13 (corresponding to the number of landraces studied) to K=20 (**Table S5**). At K=13, landraces Azizi, Badri, Biada, Bidi, Jneh Khotifa, Mekki, Richi and Sbei glabre were assigned to separate genetic subpopulations. However, landraces Souri and Roussia were grouped in the same genetic subpopulation. Landraces Mahmoudi and Biskri were both divided into two genetic subpopulations. Taganrog landrace was entirely composed of admixed genotypes (between Sbei glabre, Jneh Khotifa, Azizi and Badri). The STRUCTURE assignment that matched exactly the landraces denomination was obtained for K=15. Indeed, at K=15, all 13 landraces were discriminated and assigned to genetically distinct subpopulations, except for Mahmoudi and Biskri, which were both divided into two genetic subpopulations as for K=13. In fact, Mahmoudi subpopulations corresponded to two morphological types of Mahmoudi namely Mahmoudi-122 and Mahmoudi-986. Biskri subpopulations corresponded to two morphological types of Biskri namely Biskri-Ac1 and Biskri-glabre (**Figure S1**).

The UPGMA tree, generated with 286 SNPs data of 366 genotypes, differentiated three subclusters that were mainly in agreement with the genetic grouping defined by STRUCTURE (**Figure 3**). Subcluster « SC-I » grouped all Mahmoudi GS1 and admixed genotypes, jointly with all GS2 and admixed genotypes of Jneh Khotifa, 2 Azizi admix, 3 Biada admix, one Roussia admix, 8 Richi admix and one Richi GS2. Subcluster « SC-II » included all Azizi, Badri, Biskri, Mekki, Sbei glabre, Taganrog and Richi GS2 genotypes, along with 5 Souri admix and 3 Mahmoudi admix. Subcluster « SC-III » grouped all GS3 genotypes notably Biada, together with 8 admixed genotypes of Bidi, 6 of Roussia, 6 of Souri and 2 of Mahmoudi.

2. Distribution of the resistance to *Zymoseptoria tritici* among subpopulations

The 366 durum wheat accessions were evaluated for their resistance to two Tunisian *Z. tritici* isolates Tun06 and TM220. Based on disease scoring, the landraces were categorized into three classes of response to *Z. tritici* (R, I and S) as defined by Ouaja et al [21]. Overall, 27%, 15% and 55% of the collection were R, I and S genotypes to isolate Tun06, respectively. In addition, 28%, 7% and 43% of the collection were R, I and S genotypes to isolate TM220, respectively.

The ANOVA (**Table 3**) revealed that the genetic subpopulation effect is highly significant, indicating that the variation in the responses to infection by *Z. tritici* isolates relied on the population genetic structure. However, a non-significant effect of isolates on the RAUDPC scores was revealed. Genetic subpopulations x isolates interaction was also non-significant. Accordingly, isolates Tun06 and TM220 did not significantly varied in their severity (RAUDPC scores) towards wheat accessions. Therefore, the rest of the analysis were pursued with solely Tun06 isolate, a reference isolate with the lowest missing data.

The distribution of R, I and S classes by genetic subpopulation was uneven (**Figure 2.c, Table S4**). GS1 and GS3 were mainly formed by accessions of class S. In fact, 93% and 85% of the accessions were susceptible to Tun06 within GS1 and GS3, respectively. Interestingly, 44% and 31% of resistant accessions to Tun06 belonged to GS2 and admixed genotypes with GS2, respectively. Genotypes with intermediate responses were also mainly among GS2 or admixed with GS2. While GS2 comprised 35% of susceptible accessions. About 44% of admixed genotypes (mostly G1-G2 and G2-G3) were susceptible to Tun06.

The PCA of 366 Tunisian durum wheat accessions sorted by classes of response to isolate Tun06 (**Figure 2c**) showed that resistant genotypes were mainly laying among GS2 and admixed genotypes G1-G2 and G2-G3. Susceptible genotypes were spread over the three subpopulations. UPGMA tree (**Figure 3**) showed that the three subclusters grouped genotypes of different resistance classes. All susceptible Mahmoudi GS1 genotypes and mostly resistant admixed genotypes between GS1 and GS2 (Mahmoudi, Jneh Khotifa, Badri and Richi) were in SC-I. SC-II essentially comprised resistant GS2 landraces (Taganrog, Sbei glabre, Richi, Mekki, Badri and Azizi). However, 100% of Biskri accessions, 54% of Azizi, 7% of Mekki, 5% of Sbei glabre and 45% of Souri admix were within SC-II and showed susceptibility. SC-III is entirely composed of susceptible genotypes of Biada GS3, Biada admix, Bidi admix, Souri admix and Roussia admix.

Discussion

Old durum wheat germplasm represents a precious genetic heritage. Understanding the genetic and phenotypic structure of old local landraces will help retracing their life history of struggle for resistance and survival over time. Durum wheat population structure study also help deciphering new sources of resistance to cope with challenging abiotic and biotic stresses, notably STB, one of the most devastating fungal disease on durum wheat crop. In the present study, we genotyped 366 Tunisian durum wheat accessions belonging to 13 old Tunisian landraces [22], collected from three central and two southern

regions in Tunisia, using 286 SNPs derived from a High-density 90K wheat SNP array [60]. This study revealed the population structure and the genetic distribution of the resistance to *Z. tritici* in the Tunisian durum wheat landraces.

Identification of a population structure in the Tunisian durum wheat landraces related to their potential introduction pathways

The genetic structure of 366 durum wheat accessions was investigated using 286 SNPs. Three major genetic subpopulations (K=3: GS1, GS2 and GS3) were obtained under STRUCTURE [58]. At K= 15, we were able to attribute each landrace to a distinct genetic group, with the exception of Mahmoudi and Biskri landraces that were both divided into two groups. This result is in agreement with our previous study [22], where 8 out of 11 Tunisian durum wheat landraces corresponded to distinct genetic groups using 10 SSR markers. AMOVA analysis showed a high genetic variability (61%) between subpopulations, suggesting that the three subpopulations were derived from different gene pools. This is consistent with previous studies of tetraploid wheats [2, 61] and barley [62] showing that local landraces were derived from multiple ancestral populations and had reticulated phylogenetic relationships. However, Soriano et al [32] detected only 13% of genetic variability between four genetic subpopulations that were tracing distinct geographical pattern of the Mediterranean durum wheat germplasm. The formation of distinct domesticated gene pools were also reported for several other crops such as common bean, which underwent parallel evolution and spread further through the development of landraces with distinct characteristics and specific adaptations [63-66].

Informations about putative origins and years of introduction in Tunisia of the 13 herein studied landraces, along with their agro-morphological characteristics as described by Ouaja et al [22], were analyzed to explain their inferred genetic structure. In fact, the grouping pattern of accessions appear to be associated, to some extent, with the geographical pattern of the landraces. In this study, the subpopulation GS2 mainly included landraces of North African and East Mediterranean origins. GS2 includes landrace Mekki from Morocco, landrace Taganrog from Cyprus, landraces Azizi, Jneh Khotifa and Sbei glabre that were considered as local populations and Richi which was reported as foreign [17, 34, 67]. Robbana et al [13] also reported that Tunisian landraces were genetically associated to North African landraces. However, using a set of DArtSeq markers to describe the genetic diversity of Tunisian landraces, Robbana et al [13] reported that Jneh Zarzoura, a close relative to Jneh Khotifa [17] clustered distinctly with accessions from Jordan. Moreover, local landraces names have been traditionally selected by natives, generally according to morphological features or locality, and are often consciously used by farmers for management, selection or exchanges [14, 68]. Sahri et al [68] particularly highlighted the significance of variety name, which have largely influenced the structure and evolutionary dynamics of durum wheat in Morocco. Therefore, nominal analogies were frequently reported between landraces of different Mediterranean regions, probably reflecting trade and migration of the same landraces around the Mediterranean basin. Xynias et al [6] reported that the Italian cultivar « Senatore Capelli » was selected in 1915 from the local North African landrace « Jean Retifah » which was very prominent and marked the cultivation of durum wheat in Italy. The landrace « Jean Retifah » must be the known Jneh

Khotifa in Tunisia [14, 17]. In addition, Soriano et al [32] studied the structure of durum wheat landraces from 21 Mediterranean countries, using SSR markers, and reported two Italian landraces named « Hymera » and « Aziziah » that were associated to eastern Mediterranean genetic group. Knowing that Tunisia was the former bread basket of the Roman Empire [69, 70], these latter could be the landraces known Hmira and Azizi in Tunisia [14, 17, 20, 22], suggesting though that Azizi landrace may also have an eastern origin. Nevertheless, Boeuf [14] mentioned that the landraces of North Africa had dominant characters, specific to Abyssinian wheats, such as red, purple or black spikes, pubescent glumes and red or dark-colored grains. These characters were totally absent and unknown in Europe. Herein it was noted that landraces of GS2, with the exception of Biskri, shared features of spikes and grains [22] corresponding to the Abyssinian wheats as Bœuf [14] reported.

Furthermore, according to Bœuf [14], the geographical expansion and domestication of wheats from Abyssinia enhanced the accumulation of recessive characters, in particular white spikes, hairless glumes and light-colored grains widely adopted in Europe. During crop domestication process, several changes were induced for major morphological, structural and functional traits associated with adaptation and cultivation in order to meet human needs, as reported for the common bean (*Phaseolus vulgaris*) [66]. Bœuf [14] mentioned that the whiteness of the spike and light-colored grains were among the most sought after and preferred criteria in wheat by European farmers during commercial trade in North Africa. So far, these traits are characteristics of the landraces Mahmoudi (GS1), Biskri (GS2), Biada and Bidi (GS3) [22]. Landraces Biskri, Biada and Bidi were introduced in Tunisia from Algeria, while Mahmoudi was considered as a local landrace population with various reported origins including Algeria and Italy. Another example reflecting nominal analogy between Mediterranean landraces is the Tunisian landrace Biada and the Spanish landraces « Blancal » and « Blanco de Baleares » meaning, among others, the white wheats [17, 22, 71], thus indicating that such phenotypic characteristic was probably derived from western Mediterranean. Based on these findings/statements, although the majority of Tunisian landraces are North Africa, we suggest that subpopulations GS1 and GS3 were introduced to North Africa and particularly to Tunisia from Europe/western Mediterranean, unlike subpopulation GS2 which may be originated from the Middle East. Ben Krima et al [30] also agree that the combination of both genetic and agro-morphological approaches are essential for retracing the history, origin and dynamic lifestory of Tunisian durum wheat landraces. In the same context, Moragues et al [9] highlighted two dispersal pathways of the Mediterranean durum wheat landraces which had contributed to the divergence of these landraces into distinct genetic groups following their adaptation to different local environments. A first pathway through the North-East of the Mediterranean basin to Europe, and a second pathway through the South of the Mediterranean basin to North Africa reaching the Iberian Peninsula.

Genetic distribution of the resistance of *Z. tritici* in the Tunisian durum wheat populations

Tunisian durum wheat landraces have been reported to exhibit valuable sources of resistance to STB, useful to include in breeding programs and to develop varieties with durable and broad spectrum of resistance [21, 56, 57]. In the present study, the 366 genotyped durum wheat accessions were also screened for *Z. tritici* resistance, under field conditions, using two *Z. tritici* isolates Tun06 and TM220

collected from two Tunisian regions, Bizerte and Manouba, respectively. The analysis of variance showed a non-significant variation between isolates Tun06 and TM220 towards the durum wheat accessions, suggesting that they likely have similar virulence profile. This result agrees with Ferjaoui et al [56] findings, detecting only two virulence profiles among 55 Tunisian *Z. tritici* isolates screened at seedling stage.

Overall, 60% of the accessions were susceptible, showing that Tun06 and TM220 isolates were virulent on the majority of the accessions, which reflect the adaptation of *Z. tritici* virulence to durum wheat landraces in Tunisia. Likewise, Ouaja et al [21] suggested that the Tunisian *Z. tritici* isolate Tun06 still preserve virulences against old durum wheat landraces even though they are currently marginally grown in wheat production areas in Tunisia. In addition, several studies reported that *Z. tritici* undergoes frequent sexual reproduction on durum wheat in Tunisia [72, 73]. In fact, sexual reproduction plays a key role in the evolution of pathogenicity traits, including virulence and aggressiveness [74], enabling the fungus to quickly evolve and circumvent the resistance genes by creating new combinations of alleles and, in combination with the asexual reproduction allowing frequent generation of the new genotypes [42, 73]. Nevertheless, about 27% of the collection was resistant, suggesting that Tunisian durum wheat landraces still carry effective STB resistance genes.

The analysis of variance showed a large and significant variation between GS1, GS2 and GS3 regarding the resistance to isolate Tun06. Subpopulations GS1 (composed of Mahmoudi accessions) and GS3 (Biada accessions) showed higher frequencies of susceptible responses than GS2. The distribution of the resistance within each landrace observed in the UPGMA tree, indicate that the resistance relies on the landrace instead of the genetic structure ($K=3$), as GS2 grouped both resistant and susceptible genotypes. These results also indicate that although landraces of GS2 formed an individualized genetic group, they may harbor combination of resistance genes that differ in nature, number, structure, chromosomal localisations and type of interaction that still need to be depicted and revealed by a genome wide association study (GWAS).

The susceptibility found within GS1, GS3 and Biskri and Azizi of GS2 genotypes is due to large-scale use and commercial share of these landraces, which were extensively cultivated especially in northern Tunisia where they have lost resistance [14, 17]. In fact, *Z. tritici* is mainly prevalent on durum wheat in northern and northwestern regions of Tunisia, where sub-humid zones represent hot spots of STB disease with high infection levels [52, 54, 75]. The landraces that were widely grown in northern zones ended up being susceptible as a consequence of the rapid adaptation of *Z. tritici* isolates [42, 49, 51, 76]. Moreover, rapid adaptation of *Z. tritici* to landraces from western mediterranean origin (GS1 and GS3) could be facilitated by domestication process mainly accompanied by a strong reduction in genetic diversity and/or high levels of gene loss compared to wild ancestors or wild gene pool [77-79]. These events reduced the adaptation of cultivated wheat to erratic environmental variations, where wild traits show much greater fitness over domesticated ones [66]. This scenario might be consistent with the significant variability of the resistance to *Z. tritici* observed between subpopulations in the present study, suggesting that resistance (R) genes of the three subpopulations may have evolved divergently and crucially under a combination of environmental and human pressures. Therefore, we hypothesize that major genes

conferring specific resistance (as dominant characters) have undergone modifications and alterations by mutations during the geographic expansion and with the wide exchange network of durum wheat landraces among Mediterranean regions, which resulted in the loss of dominant R genes/alleles and the spread of susceptibility as observed within landraces of subpopulations GS1 and GS3. Alternatively, the results suggest that landraces of GS1 and GS3 were initially susceptible to *Z. tritici* when introduced to Tunisia. Nevertheless, unlike GS1 and GS3, most landraces of GS2 (except for Biskri and Azizi landraces) were resistant because they had recourse to different geographical pattern/pathway as they were local or directly derived from an eastern origin and did not pass via Europe. Western Europe farmers were among the first to create and adopt modern methods of plant breeding and exert wheat genetic improvement, involving direct selection for homogeneous material, thus, indirectly reducing the variability of the genetic sources of resistance or even unintentionally selecting for recessive genes [14, 80, 81]. In contrast, smallholder farmers in North Africa have been preserving the local durum wheat diversity with on-farm conservation practices over generations [14, 17, 22, 68].

In this study, 22% of the accessions were admixed, among which 51% of admixed genotypes between GS2 and GS1 and 24% admixed between GS2 and GS3. Admixtures occurs mainly by gene flows, through the frequent introduction of new genotypes into fields and seed exchange network within and between farmer communities [2, 16, 32]. In fact, *Nm* between GS1 and GS2 and between GS2 and GS3 were both around 0.4. All the resistant accessions of Mahmoudi were admixed between GS1 and GS2 and all the resistant accessions of Biada, Souri and Roussia were admixed between GS3 and GS2; suggesting that resistant landraces in GS2 were probably the sources transmitting resistance to *Z. tritici* via admixture. Indeed, resistant Mahmoudi genotypes may have acquired their resistance from Jneh Khotifa, as they appeared phylogenetically close in the UPGMA tree. On the other hand, admixture may have also caused loss of resistance as some admixed Jneh Khotifa and Richi accessions were susceptible to Tun06. This result indicated that admixture between genetically distinct landraces/populations and frequently recurring gene exchanges (or gametic association between gene loci) [82], may have elicited susceptibility within initially resistant accessions throughout an alteration or loss of the resistance genes/alleles.

Conclusion

The present study revealed that Tunisian durum wheat life history of resistance to STB involve the interaction of miscellaneous factors, including the landrace genetic structure and introductory pathways, the local commercial share defining the geographic and regional distribution of the landraces and the occurrence of admixtures within these landraces. In fact, landraces were subjected to genetic differentiation in time and space during their introductory pathways in the Mediterranean area, their adaptation to specific environments and the Human selection pressure/domestication, contributing to their divergence in their resistance spectra. In addition, the regional distribution of the landraces across Tunisia influenced the evolutionary history of resistance genes under different climatic constraints in relation to hotspots of STB disease. Admixtures also represent one of the main driver factors of the resistance to *Z. tritici* involving old or ongoing exchanges that contributed to the introgression and/or the

loss of resistance genes. Our results suggest the presence of diverse resistance sources towards two *Z. tritici* isolates Tun06 and TM220 in the Tunisian durum wheat landraces, which implies the need for more in-depth research to investigate and characterize the related resistance genes. Continuous identification of new sources of resistance to STB is required for the development of wheat cultivars with sustainable field resistance. Therefore, old local durum wheat landraces can play an important role as donor of resistance genes in breeding programs. In addition, further investigation and study of virulence patterns in *Z. tritici* populations in Tunisia will provide new insights and understanding of the *Z. tritici*-durum wheat interactions and trace their co-evolution.

Methods

Wheat accessions

A set of 375 durum wheat (*Triticum. turgidum* L. ssp. *durum*) accessions was used in this study. Accessions were collected by the National Gene Bank (BNG) from four regions in Central (the Sahel and Kairoun) and southern (Gabes and Medenine) Tunisia (**Table S1**). Accessions were morphologically characterized, identified and classified into 13 landrace-populations namely Mahmoudi, Biada, Bidi, Biskri, Azizi, Badri, Mekki, Jneh Khotifa, Sbei glabre, Taganrog, Richi, Souri and Roussia as described by Ouaja et al [22] . Information about the origin and year of introduction and selection of these landraces are depicted in **Table S2**.

Field trials and *Z. tritici* isolates

Durum wheat landraces were screened for their resistance to *Z. tritici* at the adult plant stage in the experimental station of CRRGC Beja in northwest Tunisia. Inoculation assays were performed using two durum wheat-derived *Z. tritici* isolates on separate field experimental plots; notably the well-characterized and virulent reference isolate Tun06 (also reported as TunBz-1) sampled from the Bizerte region of Tunisia in 2006 and the isolate TM220 collected from Lansarine region of Tunisia in 2014 [21, 56, 83]. Tun06- and TM220 Field trials were realized as reported by Ouaja et al [21] and followed an Augmented Randomized Complete Block Design (ARCB), including 6 blocks spaced apart of 1 m. Each block was composed of 70 accessions and 7 checks. The checks included six susceptible to moderately resistant modern durum wheat cultivars “Karim, Khia, Om Rabia, Salim, Maali and Nasr” [53, 54] . Durum wheat landraces were evaluated for resistance to Tun06 isolate during two consecutive growing seasons (2015-2016 and 2016-2017), and for resistance to isolate TM220 on the growing season of 2016-2017.

Inoculation and screening for resistance to *Z. tritici*

Tun06 and TM220 isolates were maintained frozen at -80°C. A preculture of the inoculum was prepared by shaking *Z. tritici* isolates (at 100 rpm/ 25°C) for six to seven days, in 100 ml yeast glucose liquid medium (30g glucose, 10g yeast per liter demineralized water). The produced spore suspensions of Tun06 and TM220 were subsequently transferred to 500 ml yeast glucose liquid media and were incubated under the aforementioned conditions to provide sufficient inoculum for the field trials. Spores

of both isolates were collected after overnight settling in static cultures, concentrated by decanting the supernatant medium, and were adjusted to 10^6 spore/ml. Accessions in all experimental plots were inoculated twice, at the three-leaf stage (approximately GS21) and at the stem elongation stage (approximately GS37) [84] as described by Ouaja et al [21].

The disease severity was evaluated by estimating pycnidia coverage percentages. The same leaf layer (F3-F4) of each accession in all experimental plots was assessed for STB resistance, at three time points for isolate Tun06 and at two time points for isolate TM220. Tun06-disease scores were assessed at 18, 38 and 46 days post the second inoculation (dpi) during 2015-2016 growing season, and at 15, 35 and 53 dpi during 2016-2017 growing season. TM220-disease scores were assessed at 20 and 40 dpi during 2016-2017 growing season. Screening data were used to calculate the Area Under the Disease Progress Curve (AUDPC) and the Relative Area Under the Disease Progress Curve (RAUDPC) for quantitative analyses of the temporal differences in disease progress as detailed by Ouaja et al. [21].

Three classes of response to *Z. tritici* at the adult plant stage, defined by Ouaja et al [21], were considered in this study. The resistant class (R) include accessions having pycnidia score <25%, while the susceptible class (S) include accessions showing >47% of pycnidia on infected leaves. Accessions with pycnidia score ranging between 25% and 46% constitute the class of intermediate (I). About 4% and 22.4% of accessions had missing data for Tun06 and TM220, respectively.

Genotyping and data analysis

A total of 20,120 polymorphic SNPs were generated by Illumina sequencing 375 Tunisian durum wheat accessions using a High-density 90K wheat SNP array (iSelect, San Diego, USA) [60], among which 9195 (~ 45.7%) were mapped in the *T. aestivum* reference genome (<http://www.wheatgenome.org/>). The filtered SNPs had < 5% of missing data and a minor allele frequency of 5%. From these markers, a total of 286 SNPs, covering the entire genome with 12 to 23 SNPs per chromosome distant of 10cM and with a PIC > 0.3, were selected to perform the analysis in the present study. PIC values were calculated by determining the frequency of alleles per locus as Powell et al [85] (**Table S3**). Genotypes with > 10% of missing data out of the 286 SNPs were eliminated, and 366 genotypes were included in the rest of the analysis.

The genotypic data based on the 286 selected SNP markers were used to study the population structure of the Tunisian durum wheat accessions with the model-based clustering algorithm STRUCTURE 2.3.4 [58]. STRUCTURE program was run with K values between 1 and 20, by applying 10 independent runs for each value of K, 100,000 burnins and 100,000 Markov Chain Monte Carlo (MCMC) repetitions. The optimal number of genetic subpopulations was determined using the mean posterior probability (ln P(D)) value per cluster (K) and the delta-K method of ln P(D) under STRUCTURE harvester 0.6.9.4 [59]. Population structure was investigated at the optimal K as well as at K ranging from 13 to 20 in order to ascertain the genetic grouping according to landrace denomination. Individuals presenting a membership coefficient less than 0.7 to any genetic subpopulation were considered as admixed genotypes. To study

the phylogenetic relationships between accessions/landraces, an Unweighted Pair Group Method with Arithmetic Average (UPGMA) tree was created using Nei standard genetic distance [86]. A bootstrap analysis was performed with the program Populations 1.2.32 [87] and branch support values were estimated using 1000 bootstrap randomizations. A consensus tree was generated and viewed using TreeView 1.6.6 [88].

In addition, a pairwise F_{st} and gene flow (Nm) coefficients were calculated between the genetic subpopulations with GenAlEx 6.501 [89]. An analysis of molecular variance (AMOVA) was also carried out using GenAlEx 6.501 [89] in order to investigate the significance of genetic differentiation within and among genetic subpopulations (as defined by STRUCTURE). A Principal Components Analysis (PCAs) on the genotypic data was performed under GenAlEx 6.501 [89]. This multivariate analysis was completed to visualize the structure of the 366 durum wheat accessions sorted by genetic subpopulations (as defined by STRUCTURE), by landrace denomination [22] and by STB resistance class to Tun06 (R, I and S).

An analyses of variance (ANOVA) were carried out under R 3.3.2 [90] to determine the effect of genetic subpopulations and isolates on RAUDPC scores. Genetic subpopulations x isolates interaction were also investigated.

Abbreviations

Not applicable

Declarations

Ethics approval and consent to participate

The used plant material consist on *Triticum durum* landraces registered at the Tunisian gene bank (BNG) and provided by the co-author M.M. Experimental research and field studies conducted at the National Institute of Agronomy (INAT) and at the Regional Field Crops Research Center of Beja (CRRGC) including the collection of plant material complies with relevant institutional, national and international guidelines and legislation.

Consent for publication

Not applicable

Availability of data and materials

The data sets supporting the results of this article are included in this manuscript and its additional information files. Genotyping data used in this study are available from the corresponding author on reasonable request

Competing interests

The authors declare that they have no competing interests.

Funding

Field experimentssupported by the federated project untitled 'Identification of durum wheat resistant genotypes to biotic and drought stress and their valorization for sustainable agriculture' acronym RESIDUR, supported by IRESA under the Tunisian Ministry of Agriculture

Genotyping was supported by the International Treaty for Plant Genetic Resources for Food and Agriculture/FAO (FAO/ITPGRFA) and European Union

M.O. was supported by the PhD fellowship from the Ministry of Higher Education and Scientific Research - Tunisia.

Authors' contributions

M.O and B.B conducted the data collection, carried out the analyses and the interpretation of the results. M.O drafted the manuscript. S.F contributed to the data collection. M.M provided the studied durum wheat accessions from the BNG. S.M.U participated on organizing the genotyping with Illumina 90K bread wheat array. S.H contributed in the conception, design and coordination of the study. All authors read and approved the final manuscript.

Acknowledgments

We are thankful to Shiaoman Ciao from the USDA small grains genotyping laboratory in Fargo, ND for conducting SNP genotyping, and to Aouini Lamia for her help in editing the manuscript.

References

1. Mackey J: **Wheat: its concept, evolution and taxonomy** In: Royo C, Nachit MM, Di Fonzo N, Araus JL, Pfeiffer WH, Slafer GA, editors. **Durum wheat breeding: current approaches and future strategies**. In.: New York: Food Products Press; 2005.
2. Oliveira HR, Campana MG, Jones H, Hunt HV, Leigh F, Redhouse DI, Lister DL, Jones MK: **Tetraploid wheat landraces in the Mediterranean basin: taxonomy, evolution and genetic diversity**. *Plos one* 2012, **7**(5):e37063.
3. Kabbaj H, Sall AT, Al-Abdallat A, Geleta M, Amri A, Filali-Maltouf A, Belkadi B, Ortiz R, Bassi FM: **Genetic Diversity within a Global Panel of Durum Wheat (*Triticum durum*) Landraces and Modern Germplasm Reveals the History of Alleles Exchange**. *Frontiers in Plant Science* 2017, **8**(1277).
4. Baloch FS, Alsaleh A, Shahid MQ, Çiftçi V, de Miera LES, Aasim M, Nadeem MA, Aktaş H, Özkan H, Hatipoğlu R: **A whole genome DArTseq and SNP analysis for genetic diversity assessment in durum wheat from central fertile crescent**. *PloS one* 2017, **12**(1):e0167821.

5. Martínez-Moreno F, Solís I, Noguerro D, Blanco A, Özberk İ, Nsarellah N, Elias E, Mylonas I, Soriano JM: **Durum wheat in the Mediterranean Rim: Historical evolution and genetic resources.** *Genetic Resources and Crop Evolution* 2020.
6. Xynias IN, Mylonas I, Korpetis EG, Ninou E, Tsaballa A, Avdikos ID, Mavromatis AG: **Durum wheat breeding in the Mediterranean region: Current status and future prospects.** *Agronomy* 2020, **10**(3):432.
7. De Ron AM, Bebeli PJ, Negri V, Vaz Patto MC, Revilla P: **Warm season grain legume landraces from the south of Europe for germplasm conservation and genetic improvement.** *Frontiers in plant science* 2018, **9**:1524.
8. Balfourier F, Bouchet S, Robert S, De Oliveira R, Rimbart H, Kitt J, Choulet F, International Wheat Genome Sequencing Consortium, BreadWheat Consortium, Paux E: **Worldwide phylogeography and history of wheat genetic diversity.** *Science Advances* 2019, **5**:eaav0536.
9. Moragues M, Moralejo M, Sorrells ME, Royo C: **Dispersal of durum wheat [*Triticum turgidum* L. ssp. *turgidum* convar. *durum* (Desf.) MacKey] landraces across the Mediterranean basin assessed by AFLPs and microsatellites.** *Genetic resources and crop evolution* 2007, **54**(5):1133-1144.
10. Haudry A, Cenci A, Ravel C, Bataillon T, Brunel D, Poncet C, Hochu I, Poirier S, Santoni S, Glémin S *et al*: **Grinding up wheat: a massive loss of nucleotide diversity since domestication.** *Mol Biol Evol* 2007, **24**.
11. Soriano JM, Villegas D, Aranzana MJ, García del Moral LF, Royo C: **Genetic Structure of Modern Durum Wheat Cultivars and Mediterranean Landraces Matches with Their Agronomic Performance.** *PLOS ONE* 2016, **11**(8):e0160983.
12. Essid MY: **History of Mediterranean food.** In: *MediTERRA 2012 (english)*. Presses de Sciences Po; 2012: 51-69.
13. Robbana C, Kehel Z, Naceur B, Sansaloni C, Bassi F, Amri A: **Genome-wide genetic diversity and population structure of Tunisian durum wheat landraces based on DArTseq technology.** *International journal of molecular sciences* 2019, **20**(6):1352.
14. Boeuf F: **Le Blé en Tunisie: La plante. Le milieu physico-chimique:** Société anonyme de l'impr. rapide de Tunis; 1932.
15. Vavilov NI: **The origin, variation, immunity and breeding of cultivated plants**, vol. 72: LWW; 1951.
16. Ren J, Sun D, Chen L, You FM, Wang J, Peng Y, Nevo E, Sun D, Luo M-C, Peng J: **Genetic diversity revealed by single nucleotide polymorphism markers in a worldwide germplasm collection of durum wheat.** *International journal of molecular sciences* 2013, **14**(4):7061-7088.
17. Deghaï's M, Kouki M, Gharbi M, El Felah M: **Les variétés de céréales cultivées en Tunisie.** *Ministère de L'Agriculture et des Ressources Hydrauliques Tunis, Tunisie* 2007.
18. Sourour A, Chahine K, Youssef T, Olfa S-A, Hajer S-A: **Phenotypic diversity of Tunisian durum wheat landraces.** *African Crop Science Journal* 2010, **18**(1).
19. Amine S, Sourour A, Olfa S-A, Cyrine R, da Silva JAT, Hajer S-A: **Morphological diversity of some qualitative traits in Tetraploid wheat landrace populations collected in the south of Tunisia.**

- International Journal of Plant Breeding* 2011, **5**(1):67-70.
20. Slim A, Piarulli L, Chennaoui Kourda H, Rouaissi M, Robbana C, Chaabane R, Pignone D, Montemurro C, Mangini G: **Genetic structure analysis of a collection of Tunisian durum wheat Germplasm.** *International journal of molecular sciences* 2019, **20**(13):3362.
 21. Ouaja M, Aouini L, Bahri B, Ferjaoui S, Medini M, Marcel TC, Hamza S: **Identification of valuable sources of resistance to *Zymoseptoria tritici* in the Tunisian durum wheat landraces.** *European Journal of Plant Pathology* 2020, **156**(2):647-661.
 22. Ouaja M, Bahri BA, Aouini L, Ferjaoui S, Medini M, Marcel TC, Hamza S: **Morphological characterization and genetic diversity analysis of Tunisian durum wheat (*Triticum turgidum* var. *durum*) accessions.** *BMC Genomic Data* 2021, **22**(1):1-17.
 23. Ayadi S, Karmous C, Hammami Z, Tamani N, Trifa Y, Esposito S, Rezgui S: **Genetic variability of Nitrogen Use Efficiency components in Tunisian improved genotypes and landraces of durum wheat.** *Agriculture Science Research Journal* 2012, **2**(11):591-601.
 24. Chamekh Z, Karmous C, Ayadi S, Sahli A, Hammami Z, Fraj MB, Benaissa N, Trifa Y, Slim-Amara H: **Stability analysis of yield component traits in 25 durum wheat (*Triticum durum* Desf.) genotypes under contrasting irrigation water salinity.** *Agricultural Water Management* 2015, **152**:1-6.
 25. Babay E, Mnasri SR, Mzid R, Hanana M: **Quality selection and genetic diversity of Tunisian durum wheat varieties using SSR markers.** *Bioscience Journal* 2019, **35**(4).
 26. Bouacha OD, Rezgui S: **Spaghetti quality: Comparison between landraces and high yielding Tunisian durum wheat varieties.** *Journal of New Sciences* 2019, **64**(7):4056-4060.
 27. Yacoubi I, Nigro D, Sayar R, Masmoudi K, Seo YW, Brini F, Giove S, Mangini G, Giancaspro A, Marcotuli I: **New insight into the North-African durum wheat biodiversity: phenotypic variations for adaptive and agronomic traits.** *Genetic Resources and Crop Evolution* 2020, **67**(2):445-455.
 28. Huhn MR: **Tetraploid Tunisian wheat germplasm as a new source of Fusarium head blight resistance:** North Dakota State University; 2008.
 29. Ferjaoui S, Sbei A, Aouadi N, Hamza S: **Monogenic inheritance of resistance to septoria tritici blotch in durum wheat 'Agili'.** *Int J Plant Breed* 2011, **5**:17-20.
 30. Krma SB, Slim A, Gelisse S, Kouki H, Nadaud I, Sourdille P, Yahyoui A, M'Barek SB, Suffert F, Marcel TC: **Life story of Tunisian durum wheat landraces revealed by their genetic and phenotypic diversity.** *bioRxiv* 2020.
 31. Sahri A, Chentoufi L, Arbaoui M, Ardisson M, Belqadi L, Birouk A, Roumet P, Muller M-H: **Towards a comprehensive characterization of durum wheat landraces in Moroccan traditional agrosystems: analysing genetic diversity in the light of geography, farmers' taxonomy and tetraploid wheat domestication history.** *BMC Evolutionary Biology* 2014, **14**(1):1-18.
 32. Soriano JM, Villegas D, Aranzana MJ, del Moral LFG, Royo C: **Genetic structure of modern durum wheat cultivars and Mediterranean landraces matches with their agronomic performance.** *PloS one* 2016, **11**(8):e0160983.

33. Ortiz R, Trethowan R, Ferrara GO, Iwanaga M, Dodds JH, Crouch JH, Crossa J, Braun H-J: **High yield potential, shuttle breeding, genetic diversity, and a new international wheat improvement strategy.** *Euphytica* 2007, **157**(3):365-384.
34. Bonjean AP, Angus WJ: **The World Wheat Book: A history of wheat breeding:** Lavoisier Publishing; 2001.
35. O'Driscoll A, Kildea S, Doohan F, Spink J, Mullins E: **The wheat–Septoria conflict: a new front opening up?** *Trends in Plant Science* 2014, **19**(9):602-610.
36. Dean R, Van Kan JAL, Pretorius ZA, Hammond-Kosack KE, Di Pietro A, Spanu PD, Rudd JJ, Dickman M, Kahmann R, Ellis J *et al.*: **The Top 10 fungal pathogens in molecular plant pathology.** *Molecular Plant Pathology* 2012, **13**(4):414-430.
37. Stukenbrock EH, Banke S, Javan-Nikkhah M, McDonald BA: **Origin and domestication of the fungal wheat pathogen *Mycosphaerella graminicola* via sympatric speciation.** *Molecular biology and evolution* 2007, **24**(2):398-411.
38. Stukenbrock EH, McDonald BA: **The origins of plant pathogens in agro-ecosystems.** *Annu Rev Phytopathol* 2008, **46**:75-100.
39. Torriani SFF, Stukenbrock EH, Brunner PC, McDonald BA, Croll D: **Evidence for extensive recent intron transposition in closely related fungi.** 2011, **21**:2017-2022.
40. McDonald MC, McDonald BA, Solomon PS: **Recent advances in the *Zymoseptoria tritici*–wheat interaction: insights from pathogenomics.** *Frontiers in plant science* 2015, **6**.
41. Stukenbrock EH, Bataillon T, Dutheil JY, Hansen TT, Li R, Zala M: **The making of a new pathogen: insights from comparative population genomics of the domesticated wheat pathogen *Mycosphaerella graminicola* and its wild sister species.** *Genome Res* 2011, **21**.
42. McDonald BA, Mundt CC: **How knowledge of pathogen population biology informs management of *Septoria tritici* blotch.** *Phytopathology* 2016, **106**(9):948-955.
43. Kema G, Annone JG, Sayoud R, Van Silfhout CH, Van Ginkel M, De Bree J: **Genetic variation for virulence and resistance in the wheat-*Mycosphaerella graminicola* pathosystem I. Interactions between pathogen isolates and host cultivars.** *Phytopathology* 1996, **86**(2):200-212.
44. Kema G, Sayoud R, Annone J, Van Silfhout C: **Genetic variation for virulence and resistance in the wheat-*Mycosphaerella graminicola* pathosystem. II: analysis of interactions between pathogen isolates and host cultivars.** *Phytopathology* 1996, **86**(2):213-220.
45. Kema GH, van Silfhout CH: **Genetic variation for virulence and resistance in the wheat-*Mycosphaerella graminicola* pathosystem III. Comparative seedling and adult plant experiments.** *Phytopathology* 1997, **87**(3):266-272.
46. Ghaffary SMT, Robert O, Laurent V, Lonnet P, Margalé E, van der Lee TA, Visser RG, Kema GH: **Genetic analysis of resistance to septoria tritici blotch in the French winter wheat cultivars Balance and Apache.** *Theoretical and Applied Genetics* 2011, **123**(5):741-754.
47. Brown JK, Chartrain L, Lasserre-Zuber P, Saintenac C: **Genetics of resistance to *Zymoseptoria tritici* and applications to wheat breeding.** *Fungal Genet Biol* 2015, **79**:33-41.

48. Arraiano LS, Brown JK: **Sources of resistance and susceptibility to *Septoria tritici* blotch of wheat.** *Molecular Plant Pathology* 2016.
49. Zhong Z: **A small secreted protein in *Zymoseptoria tritici* is responsible for avirulence on wheat cultivars carrying the *Stb6* resistance gene.** *New Phytol* 2017, **214**.
50. Kema GH, Verstappen EC, Waalwijk C: **Avirulence in the wheat *Septoria tritici* leaf blotch fungus *Mycosphaerella graminicola* is controlled by a single locus.** *Molecular Plant-Microbe Interactions* 2000, **13**(12):1375-1379.
51. Kema GH, Gohari AM, Aouini L, Gibriel HA, Ware SB, van Den Bosch F, Manning-Smith R, Alonso-Chavez V, Helps J, M'Barek SB: **Stress and sexual reproduction affect the dynamics of the wheat pathogen effector *AvrStb6* and strobilurin resistance.** *Nature genetics* 2018, **50**(3):375.
52. Fakhfakh M, Rezgui S, Jarrahi T, Zouani R, Bousalmi A, Sgatni K, Yahyaoui A: **Mapping distribution of *Septoria* allowed targeting management options of the disease.** In: *8th International Symposium on Mycosphaerella and Stagonospora Diseases of Cereals, Mexico City: 2011*; 2011: 10-14.
53. Berraies S, Ammar K, Salah Gharbi M, Yahyaoui A, Rezgui S: **Quantitative inheritance of resistance to *Septoria tritici* blotch in durum wheat in Tunisia.** *Chilean journal of agricultural research* 2014, **74**(1):35-40.
54. Gharbi M, Deghais M, Ben Amar F: **Breeding for resistance to *Septoria tritici* in durum wheat.** In: *Proceedings of Durum Wheat Conference, Zaragoza 2000: 2000*; 2000: 397-401.
55. Gharbi M, El Felah M: **Article de synthèse Les céréales en Tunisie: plus d'un siècle de recherche variétale.** *ANNALES DE L'INRATT* 2013, **86**:45.
56. Ferjaoui S, M'Barek S, Bahri B, Slimane R, Hamza S: **Identification of resistance sources to *septoria tritici* blotch in old Tunisian durum wheat germplasm appliad for the analysis of the *Zymoseptoria tritici*-durum wheat interaction** *Journal of Plant Pathology* 2015, **97**(3):471-481.
57. Aouini L: **Durum wheat and *septoria tritici* blotch: genes and prospects for breeding.** Wageningen University; 2018.
58. Pritchard JK, Stephens M, Donnelly P: **Inference of population structure using multilocus genotype data.** 2000, **155**:945-959.
59. Earl DA: **STRUCTURE HARVESTER: a website and program for visualizing STRUCTURE output and implementing the Evanno method.** *Conservation genetics resources* 2012, **4**(2):359-361.
60. Wang S, Wong D, Forrest K, Allen A, Chao S, Huang BE, Maccaferri M, Salvi S, Milner SG, Cattivelli L: **Characterization of polyploid wheat genomic diversity using a high-density 90 000 single nucleotide polymorphism array.** *Plant biotechnology journal* 2014, **12**(6):787-796.
61. Cíván P, Ivaničová Z, Brown TA: **Reticulated origin of domesticated emmer wheat supports a dynamic model for the emergence of agriculture in the fertile crescent.** *PLoS One* 2013, **8**(11):e81955.
62. Poets AM, Fang Z, Clegg MT, Morrell PL: **Barley landraces are characterized by geographically heterogeneous genomic origins.** *Genome biology* 2015, **16**(1):1-11.

63. Papa R, Gepts P: **Asymmetry of gene flow and differential geographical structure of molecular diversity in wild and domesticated common bean (*Phaseolus vulgaris* L.) from Mesoamerica.** *Theoretical and Applied Genetics* 2003, **106**(2):239-250.
64. Bitocchi E, Rau D, Benazzo A, Bellucci E, Goretti D, Biagetti E, Panziera A, Laido G, Rodriguez M, Gioia T: **High level of nonsynonymous changes in common bean suggests that selection under domestication increased functional diversity at target traits.** *Frontiers in plant science* 2017, **7**:2005.
65. Bitocchi E, Rau D, Bellucci E, Rodriguez M, Murgia ML, Gioia T, Santo D, Nanni L, Attene G, Papa R: **Beans (*Phaseolus* spp.) as a model for understanding crop evolution.** *Frontiers in plant science* 2017, **8**:722.
66. Di Vittori V, Bellucci E, Bitocchi E, Rau D, Rodriguez M, Murgia ML, Nanni L, Attene G, Papa R: **Domestication and crop history.** In: *The common bean genome*. Springer; 2017: 21-55.
67. Deghais M, El Faleh M, Gharbi M, Zarcouna T, Chakroun M: **Les acquis de l'amélioration génétique des céréales en Tunisie.** In: *Annales de l'INRAT: 1999*, 1999: 3-30.
68. Sahri A, Chentoufi L, Arbaoui M, Ardisson M, Belqadi L, Birouk A, Roumet P, Muller M-H: **Towards a comprehensive characterization of durum wheat landraces in Moroccan traditional agrosystems: analysing genetic diversity in the light of geography, farmers' taxonomy and tetraploid wheat domestication history.** *BMC Evolutionary Biology* 2014, **14**(1):264.
69. Fabricant F: **Rome's glory is now Tunisia's.** *New York Times* 1998.
70. Latiri K, Lhomme J-P, Annabi M, Setter TL: **Wheat production in Tunisia: progress, inter-annual variability and relation to rainfall.** *European Journal of Agronomy* 2010, **33**(1):33-42.
71. Moragues M, Zarco-Hernández J, Moralejo MA, Royo C: **Genetic diversity of glutenin protein subunits composition in durum wheat landraces [*Triticum turgidum* ssp. *turgidum* convar. *durum* (Desf.) MacKey] from the Mediterranean basin.** *Genetic Resources and Crop Evolution* 2006, **53**(5):993-1002.
72. Boukef S, McDonald BA, Yahyaoui A, Rezgui S, Brunner PC: **Frequency of mutations associated with fungicide resistance and population structure of *Mycosphaerella graminicola* in Tunisia.** *European Journal of Plant Pathology* 2012, **132**(1):111-122.
73. Hassine M, Siah A, Hellin P, Cadalen T, Halama P, Hilbert J-L, Hamada W, Baraket M, Yahyaoui A, Legrève A: **Sexual reproduction of *Zymoseptoria tritici* on durum wheat in Tunisia revealed by presence of airborne inoculum, fruiting bodies and high levels of genetic diversity.** *Fungal biology* 2019, **123**(10):763-772.
74. Suffert F, Delestre G, Gélisse S: **Sexual reproduction in the fungal foliar pathogen *Zymoseptoria tritici* is driven by antagonistic density dependence mechanisms.** *Microbial ecology* 2019, **77**(1):110-123.
75. Chedli RBH, M'Barek SB, Yahyaoui A, Kehel Z, Rezgui S: **Occurrence of *Septoria tritici* blotch (*Zymoseptoria tritici*) disease on durum wheat, triticale, and bread wheat in Northern Tunisia.** *Chilean journal of agricultural research* 2018, **78**(4):559-568.
76. Morais D, Gélisse S, Laval V, Sache I, Suffert F: **Inferring the origin of primary inoculum of *Zymoseptoria tritici* from differential adaptation of resident and immigrant populations to wheat cultivars.** *European Journal of Plant Pathology* 2016, **145**(2):393-404.

77. Olson MV: **When less is more: gene loss as an engine of evolutionary change.** *American journal of human genetics* 1999, **64**(1):18.
78. Gepts P, Papa R: **Evolution during domestication.** *e LS* 2001.
79. Rahman S, Islam S, Yu Z, She M, Nevo E, Ma W: **Current progress in understanding and recovering the wheat genes lost in evolution and domestication.** *International journal of molecular sciences* 2020, **21**(16):5836.
80. Roussel V, Koenig J, Beckert M, Balfourier F: **Molecular diversity in French bread wheat accessions related to temporal trends and breeding programmes.** *Theoretical and Applied Genetics* 2004, **108**(5):920-930.
81. Thomas M, Dawson JC, Goldringer I, Bonneuil C: **Seed exchanges, a key to analyze crop diversity dynamics in farmer-led on-farm conservation.** *Genetic Resources and Crop Evolution* 2011, **58**(3):321-338.
82. Chakraborty R, Weiss KM: **Admixture as a tool for finding linked genes and detecting that difference from allelic association between loci.** *Proceedings of the National Academy of Sciences* 1988, **85**(23):9119-9123.
83. Medini M, Hamza S: **Pathotype and molecular characterisation of *Mycosphaerella graminicola* isolates collected from Tunisia, Algeria and Canada.** *Journal of Plant Pathology* 2008, **90**(1):65-73.
84. Zadoks JC, Chang TT, Konzak CF: **A decimal code for the growth stages of cereals.** *Weed research* 1974, **14**(6):415-421.
85. Powell W, Morgante M, Andre C, Hanafey M, Vogel J, Tingey S, Rafalski A: **The comparison of RFLP, RAPD, AFLP and SSR (microsatellite) markers for germplasm analysis.** *Molecular breeding* 1996, **2**(3):225-238.
86. Nei M: **Genetic distance between populations.** *The American Naturalist* 1972, **106**(949):283-292.
87. Langella O: **Populations a free population genetic software. Version 1.2. 31.** In.; 2002.
88. Page RD: **Tree View: An application to display phylogenetic trees on personal computers.** *Bioinformatics* 1996, **12**(4):357-358.
89. Peakall R, Smouse PE: **GENALEX 6: genetic analysis in Excel. Population genetic software for teaching and research.** *Molecular ecology notes* 2006, **6**(1):288-295.
90. Team RC: **R: A language and environment for statistical computing.** 2013.

Tables

Table 1. Pairwise F_{st} values (under the diagonal) and gene flow (Nm) (above the diagonal) between the genetic subpopulations at $K=3^*$

	GS1	GS2	GS3
GS1	-	0.408	0.078
GS2	0.380	-	0.424
GS3	0.762	0.371	-

*Admixed genotypes were not included in this analysis

Table 2. Analysis of molecular variance (AMOVA) among and within the genetic subpopulation at K=3*

Source	Df	SS	MS	Est. Var.	%
Among subpopulations	2	33000.954	16500.477	199.754	61%
Within subpopulations	283	35911.928	126.897	126.897	39%
Total*	285	68912.881		326.652	100%

*Admixed genotypes were not included in this analysis

Table 3. Analyses of variance (ANOVA) on based on RAUDPC scores.

	Df	SS	MS	F value	Pr (>F)	
Genetic subpopulations	2	128937	64468	110.509	<2e-16	***
Isolates	1	751	751	1.287	0.257	
Genetic subpopulations x Isolates	2	765	383	0.656	0.520	
Residuals*	497	289938	583			

*Admixed genotypes were not included in this analysis

Figures

Figure 1

Genetic structure analysis of 366 Tunisian durum wheat accessions genotyped with 286 SNP markers: (a) Plot of mean posterior probability ($\ln P(D)$) values per cluster (K) ; (b) delta-K analysis of $\ln P(D)$, for K ranging from 1 to 20 ; (c) Membership coefficient bar plot displaying genetic structure at $K=3$ from STRUCTURE. Each genotype is represented by a vertical line.

Figure 2

Principal component analysis plot of 366 Tunisian durum wheat accessions belonging to 13 landraces genotyped with 286 SNPs color-coded by (a) genetic structure (GS1, GS2, GS3 and admixed genotypes) at K=3; (b) landraces denomination; and (c) resistance class to *Z. tritici* isolate « Tun06 ».

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [additionalfiles.xlsx](#)