Article

Intra- and Inter-Population Genetic Diversity of "Russello" and "Timilia" Landraces from Sicily: A Proxy towards the Identification of Favorable Alleles in Durum Wheat

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Abstract: Climate change and global population growth call for urgent recovery of genetic variation from underexploited or unexplored durum wheat (*Triticum turgidum* ssp. *durum*) landraces. Indeed, these untapped genetic resources can be a valuable source of favorable alleles for environmental adaptation and tolerance or resistance to (a)biotic stress. In southern Italy, in addition to the widespread modern and highly productive durum wheat cultivars, various landraces have been rediscovered and reused for their adaptation to sustainable and low-input cropping systems and for their peculiar qualitative characteristics. Sicily is a semiarid area rich in landraces, some of which are independently reproduced by many farmers. Among these, "Timilia" and "Russello" have been independently grown in various areas and are now cultivated, mostly under organic systems, for their hypothetical greater benefits and height, which give them a high level of competitiveness against weeds despite their low yield potential. So far, there is little information on the genetic variations of "Timilia" and "Russello" despite their putative origin from a common funder. This work aims to dissect the genetic variation patterns of two large germplasm collections of "Timilia" and "Russello" using SNP genotyping. The analysis of intra- and inter-population genetic variation and the identification of divergent loci between genetic groups showed that (i) there are two "Russello" genetic groups associated with different Sicilian geographical areas, which differ in important traits related to gluten quality and adaptation, and (ii) the individuals of "Timilia", although presenting wide genetic variation, have undergone a conservative selection, likely associated with their distinctive traits. This work paves the way for a deeper exploration of the wide genetic diversity in Sicilian landraces, which could be conveniently exploited in future breeding programs, and points out that intra-population genetic diversity should be taken into account when 'conservation varieties' are to be registered in national registers of crops.

Keywords: *Triticum turgidum* ssp. *durum*; landraces; divergent loci; genetic diversity; *Fst*; favorable alleles; adaptation; climate change

1. Introduction

Durum wheat (*Triticum turgidum* ssp. *durum*, 2n = 28) is a strategic crop for the Italian economy due to its multiple uses in the production of pasta and some traditional breads and ability to provide high yield and profits in marginal lands. In Italy, the first durum wheat breeding program was launched at the beginning of the 20th century with the

aim of developing varieties with higher yields, reduced height, early maturity, and, to a lesser extent, tolerance to pathogens. Breeding activities began through the selection of different pure lines within landrace populations cultivated in southern Italy and in other Mediterranean countries [1,2]. Subsequently, the introduction of the *Rht-B1* dwarfing gene from the Japanese cultivar "Norin 10" in the Italian germplasm during the 'green revolution' led to the release of new cultivars with increased yield and better technological quality of the grain [3].

The processes of domestication and breeding led to a reduction in genetic variation over time [4]. In the last century, a narrowing of the genetic basis of the Italian durum wheat germplasm was observed due to the repeated use of a few élite varieties as parents in breeding programs [5]. This is especially true for cultivars released after the year 2000 [6]. Meanwhile, old landraces were confined to niche areas, and their conservation was delegated to public research institutes and private farmers. The climate emergency and the increase in the world population have made it urgent to recover genetic variation from underexploited or unexplored landraces [7]. These untapped resources are known to be a valuable source of useful alleles for environmental adaptation and tolerance to (a)biotic stress [8,9]. Furthermore, consumers' demand for a range of healthy wheat-based food products is driving processing industries to diversify their products, including the use of ancient wheats, despite the fact that their supposed superior healthy properties have never been demonstrated [10–12].

In southern Italy, especially in Apulia and Sicily, it is still possible to find old landraces of durum wheat grown in situ, mainly on organic farms [6,13,14]. Indeed, many of them are no longer cultivated over large areas due to the spread of new, more productive cultivars. However, in recent years, thanks to their adaptation to sustainable and low-input cropping systems, several landraces have been rediscovered and reused.

In Sicily, durum wheat has historically represented, and still represents, one of the main crops. The different pedoclimatic areas where this cereal is grown generated a wide array of well-adapted indigenous landraces, such as "Timilia", "Russello", "Perciasacchi", "Bidi", "Ciciredda", "Faricello", "Francesa", "Gioia", "Martinella", "Paola", "Scorsonera", "Tripolino" and "Margherito" [14–17]. However, since their seeds are mainly produced, stored, and exchanged among farmers, it is extremely hard to keep each landrace pure. In order to preserve these local resources from genetic erosion, some of them have been registered in the Italian National Register of crop varieties as 'conservation varieties', introduced by Council Directive 98/95/EC and improved with Council Directive 08/63/CE. This is the case of durum wheat landraces "Russello" and "Timilia", released, respectively, in 2014 and 2018. These two landraces, in the last few years, have attracted the interest of both consumers and the scientific community due to their strong link with the territory and their valuable qualitative characteristics, mainly intended for specialty breads [18,19].

"Timilia" was one of the most widespread cereals in the Mediterranean area in the 18th and 19th centuries thanks to its lack of vernalization, in contrast with most of the other landraces. Indeed, it has also been found in Portugal, North Africa, France, and particularly in Seville, Spain [20]. Its synonym "Triminia" indicates the ability that this wheat has to complete its crop cycle in three months because it was normally sown in the late winter or early spring and harvested in June [13]. Its cultivation has been widespread for centuries throughout Sicily but has never been very intense, as its low crop cycle, tall size, and late spiking date are major constraints to its productivity. At the beginning of the 20th century, the breading activity of within-population selection led to the release of new pure lines such as "Timilia with white awns", "Timilia with black awns", and "Timilia SG3", which are more productive and have a better ability to adapt [15]. "Timilia" is still used to produce local and traditional breads that are much appreciated by consumers, such as 'Castelvetrano black bread' [18,21] and 'Monreale bread' [22,23] which have been labeled as traditional agri-food products.

Similarly, "Russello" was one of the most cultivated landraces in Sicily in the early 1900s, especially in the western areas of Agrigento, Caltanissetta, and Palermo. In the

Hyblaean area (the eastern districts of Ragusa, Syracuse, and Catania), "Russello" was often confused with the landrace "Ruscìa" [15,23]. Over time, different populations were referred to as "Russello" even though they have different morpho-physiological and agronomic traits [24].

Given the growing interest of farmers and consumers, many efforts were made to preserve and enhance Sicilian durum wheat landraces and their end-products, leading to the development of traceability methods based on morphological descriptors, storage protein composition, digestibility of starch, and concentration of secondary compounds [22–29]. In contrast, the genetic diversity of Sicilian landraces was poorly explored using molecular markers. Some studies analyzed the genetic variation of a few accessions of "Russello" and "Timilia" as pure lines, using amplified fragment length polymorphism (AFLP) and simple sequence repeat (SSR) markers [5,30–32]. Advances in high-throughput next-generation sequencing (NGS)-based genotyping offer the opportunity to generate a large panel of single nucleotide polymorphism (SNP) markers for use in genetic studies [33,34]. Using these new technologies, several studies on durum wheat were performed to dissect the patterns of genetic variation between landraces and old/modern varieties [6,14,35–38]. Unfortunately, no studies have ever been performed on large collections of Sicilian durum wheat landraces using SNP genotyping.

This work explored intra- and inter-population genetic variation of two large collections (including over 350 individuals) of "Russello" and "Timilia" with the aim of (i) identifying and removing duplicate individuals, (ii) defining patterns of genetic diversity within and between populations, (iii) estimating the pattern and extent of linkage disequilibrium and identifying haplotype blocks, and (iv) searching for divergent loci that may contribute to phenotypic differentiation.

2. Materials and Methods

2.1. Plant Material, Genotyping, and SNP Filtering

The durum wheat germplasm under investigation included 357 individuals collected from farmers' fields that belong to two Sicilian durum wheat landraces, namely "Russello" and "Timilia". The whole collection (RTC) consisted of 5 "Russello" populations (Pop-R) with 30 individuals each and 7 "Timilia" populations with black awns (Pop-T), 5 of which with 30 individuals, 1 with 29, and 1 with 28 individuals. The populations were sampled in different Sicilian geographical areas (Figure 1), advanced by three generations using the Single Seed Descendent (SSD) method, and conserved at the Research Centre for Cereal and Industrial Crops (CREA-CI), Foggia, Italy, up to the time of this study.
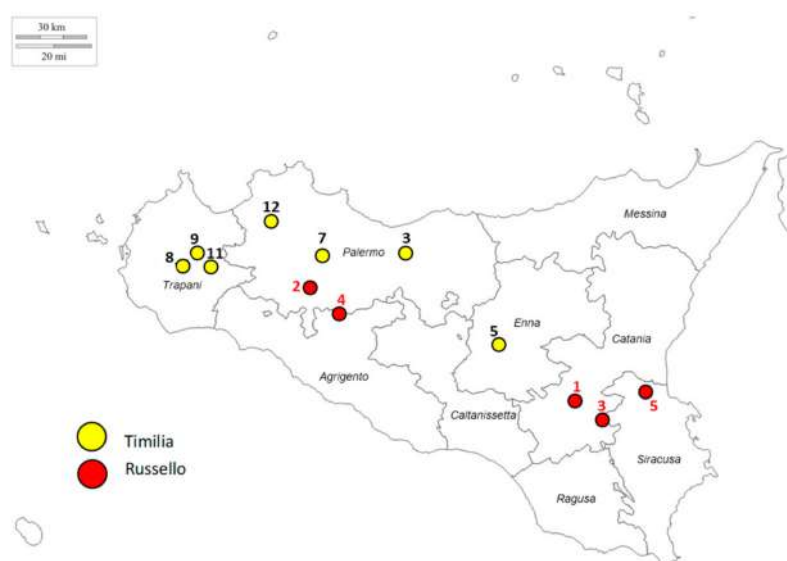

Figure 1. Map of Sicily showing the sampling locations and district boundaries.

DNA was extracted from the leaf tissue of each sample according to [39]. DNA was genotyped using the Illumina iSelect 15K wheat SNP array (TraitGenetics GmbH, Gatersleben, Germany), which includes 13,006 markers distributed along the durum wheat genome. The physical location of each SNP was assigned based on the physical map of the Svevo durum wheat cultivar, available at https://www.interomics.eu/durum-wheat-genome (accessed on 10 May 2022) [4]. SNP markers with unknown chromosomal positions were arbitrarily assigned to chromosome 0.

For all downstream analyses, the following groups of individuals were considered: (i) the individual "Russello" and "Timilia" populations (Pop-R and Pop-T); (ii) two collections, grouping either the "Russello" (RC) or the "Timilia" (TC) populations; (iii) the entire collection, combining all the "Russello" and "Timilia" (RTC).

SNP markers with minor allele frequency (MAF) < 5%, minimum site count < 10%, and individuals with missing call rate > 10% were filtered out. Finally, each dataset was LD (linkage disequilibrium) pruned ($r^2 = 0.20$) using the SNP and Variation suite (SVS) v.8.4.0 (Golden Helix Inc., Bozeman, MT, USA).

The filtering procedure and LD pruning resulted in datasets of high-quality SNP markers that were used to calculate the pairwise identical-by-state (IBS) distance matrix among all individuals using PLINK (v. 1.90). Duplicated individuals were inferred for a percentage of shared alleles ≥0.99. Once duplications were found, only one individual (the one with the highest call rate) was used for downstream analyses. The R ggplot2 package [40] was used to generate bar charts showing the frequency distribution of the IBS values and the number of high-quality SNPs per chromosome.

2.2. Population Genetics and Diversity

Genetic structure was evaluated by MultiDimensional Scaling (MDS) on the basis of the IBS distance and by constructing a neighbor-joining tree with 1000 bootstrap replicates using Mega X [41]. FigTree v.1.4.3 was used for tree visualization.

GenAlEx 6.5 [42] was used to estimate the number of observed alleles (Na), Nei's genetic distance index (He), Shannon's index (I), the percentage of private alleles (PA), and to run the analysis of molecular variance (AMOVA) test.

SVS was used to estimate the pairwise fixation index (F_{ST}) between populations. Finally, the R ComplexHeatmap [43] and circlize [44] packages were used to obtain heatmaps based on pairwise F_{ST} values.

2.3. Divergent Loci and Putative Genes under Selection

The identification of divergent loci was performed by applying the Weir and Cockerham formula [45] implemented in SVS. F_{ST} values at individual loci were computed by pairwise comparison between groups in RC, TC, and RTC. The 95% confidence interval around the F_{ST} value was calculated using the percentile -t bootstrapping technique [46]. A significance threshold >0.25 was fixed. All divergent loci with $F_{ST} > 0.50$ were then used to construct the haplotype network using the minimum spanning method in PopART [47].

The LD Adjacent Pairs Analysis function in SVS was used to calculate the average decay of LD in the RC, TC, and RTC collections. The average LD decay distance was used to define the size of the region containing each divergent marker, thus including putative QTLs or genes under selection.

3. Results

3.1. IBS Analysis

Out of 13,006 SNPs spotted onto the 15K Infinium iSelect array, 4624 high-quality SNPs were retained after filtering, which were physically mapped onto the 14 chromosomes of the durum "Svevo" genome (Figure 2). A total of 1970 and 2654 SNPs were located on the genomes A and B, respectively, ranging from 178 (4B) to 525 (5B) (Figure S1).

Figure 2. Distribution of the 4624 high-quality SNP markers within 1 Mb windows for the 14 chromosomes of the durum wheat genome.

IBS matrices were used to identify duplicated samples by setting a percentage of shared alleles ≥ 0.99 as the threshold.

The frequency distribution of IBS values ranged between 0.5 and 1 for Pop-R1, Pop-R3, and Pop-R5. No duplicated samples were found in Pop-R1 and Pop-R5, whereas Pop-R3 included a single pair of duplicated samples (Table S1).

Estimates of pairwise IBS ranged from 0.4 to 1 in the case of Pop-R2 and Pop-R4. A total of 8 and 17 duplicated samples were found in Pop-R2 and Pop-R4, respectively (Tables S1 and S2).

The 7 populations of "Timilia" had a very similar IBS frequency distribution. Most pairwise IBS values fell within a range of 0.5 to 0.8. At least three duplicated samples were identified in each population. A total of 10 and 8 duplicated samples were detected in Pop-T8 and Pop-T11, respectively.

As for RC, the frequency distribution of the pairwise IBS estimates was bimodal (Figure S2A). Based on the threshold value, only one pair of duplicated samples was found (Tables S1 and S2).

In TC, most IBS values were between 0.6 to 0.8 (Figure S2B), and 31 pairs of duplicated samples were found. Finally, a total of 8 duplicated samples were found in RTC (Supplementary Table S1). The duplicated samples were discarded to avoid affecting the genetic diversity analysis (Tables S1 and S2).

3.2. Genetic Relationships and Differentiation

The genetic relationship of each population, cleaned of duplicate samples based on IBS values, was described by an MDS scatterplot and an NJ tree. Both approaches returned the same results. In RC, it was possible to clearly distinguish two groups: the first included the R1, R3, and R5 populations, and the second included R2 and R4 (Figure S3A,B). In contrast, the NJ and MDS did not indicate any clustering patterns for the populations of "Timilia" (Figure S3C,D).

MDS and NJ analyses performed on the entire collection (RTC) supported the genetic structure described above, as "Timilia", R1-3-5 and R2-4 were separated into three clusters (Figure 3A,B), except for three individuals belonging to R2 which were included in the cluster together with all those R1-3-5.
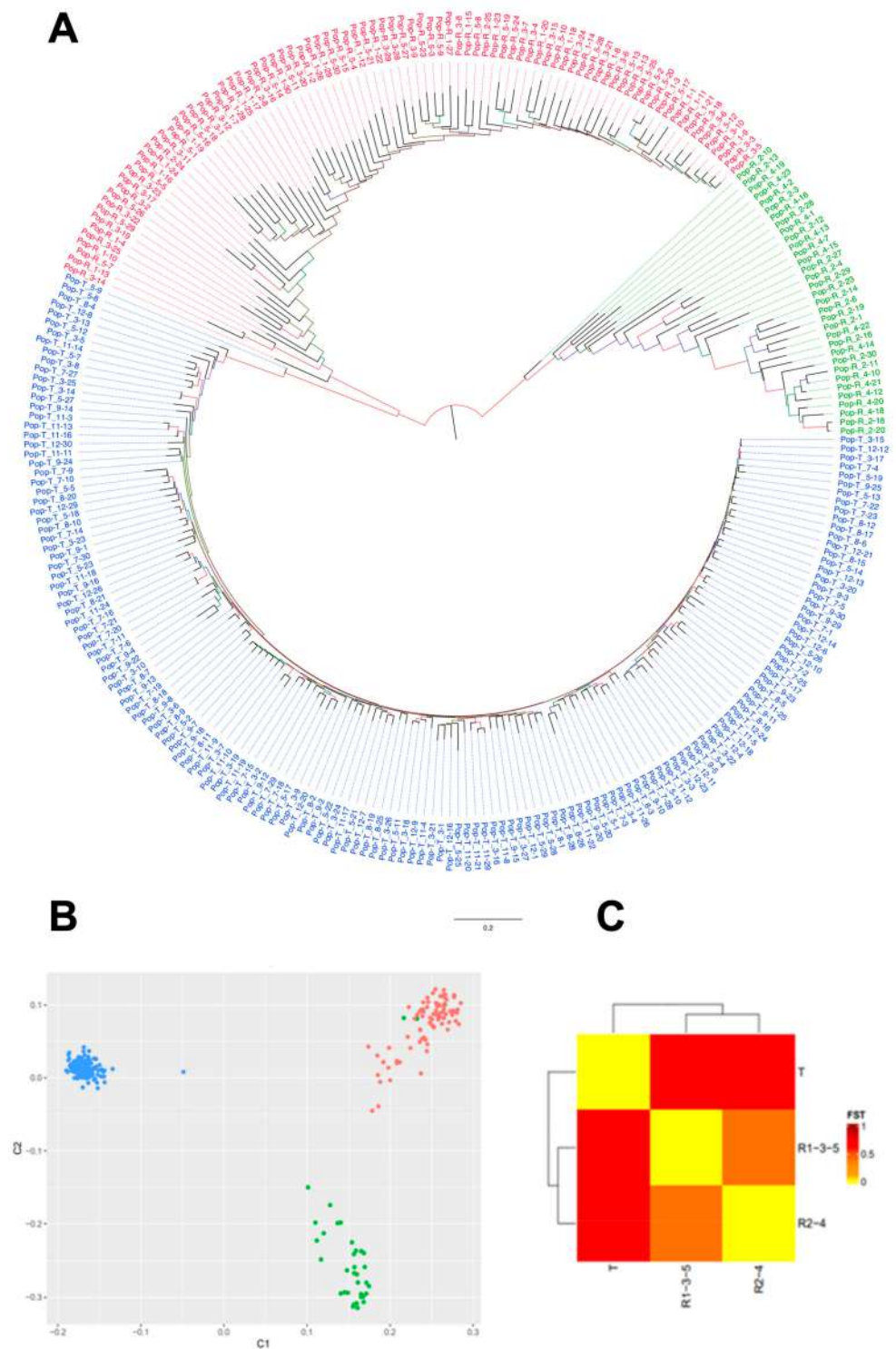
Figure 3. (**A**) Neighbor-joining tree; (**B**) MDS scatterplot based on the identity-by-state (IBS) matrix of the entire collection (RTC). Different colors in the NJ tree and in the MDS scatterplot distinguish the 3 RTC groups. TC (blue), R1-3-5 (red) and R2-4 (green); (**C**) Heatmap of pairwise F_{ST} values. Color scale ranges from yellow ($F_{ST} = 0$) to red ($F_{ST} = 1$).

The fixation index (F_{ST}) between pairs of populations was computed to assess their degree of differentiation (Figure 3C). An F_{ST} value < 0.05 (low differentiation) was observed among the R1, R3, and R5 populations, as well as between the R2 and R4 populations. On the other hand, large genetic differentiation ($F_{ST} > 0.40$) was observed by comparing

the two groups (i.e., R1-3-5 vs. R2-4). As previously suggested by AMOVA, F_{ST} values obtained by comparing the populations of "Timilia" in pairs were close to 0, thus indicating an almost absent genetic differentiation among the populations. In addition, a strong differentiation was found when comparing "Timilia" vs. R1-3-5 (F_{ST} = 0.59) and "Timilia" vs. R2-4 (F_{ST} = 0.60).

3.3. Genetic Diversity Analysis

Genetic diversity analysis was first performed by comparing the Pop-R and Pop-T populations with each other. Based on genetic relationships, the RC collection was also divided into two groups, RC1-3-5 and RC2-4. Then, for RTC, the three groups (RC1-3-5, RC2-4, and TC) were compared in pairs.

A large genetic variation was observed within all the "Russello" and "Timilia" populations (Table 1). Indeed, Shannon and Nei's indices changed slightly in Pop-R and Pop-T populations, with the highest values in Pop-T7 (I = 0.602, He = 0.355). As for "Russello", Pop-R2 and Pop-R4 showed the lowest average number of observed alleles (Na = 1.913 and 1.734, respectively). The same trend was observed for the frequency of private alleles (PA $\leq$ 0.012). As for "Timilia", the lowest values of Na (2.179), I (0.485), and He (0.304) were found in Pop-T8.

Table 1. Summary of genetic diversity and nucleotide variation indices calculated for each "Russello" and "Timilia" populations and for the 3 RTC groups, after the removal of duplicate samples based on IBS values. Na = number of average alleles, I = Shannon's index, PA = frequency of private alleles, He = Nei's index.

Population	#Individuals	Na	PA	I	He
Russello					
R1	28	2.167	0.064	0.422	0.255
R2	21	1.913	0.002	0.455	0.300
R3	27	2.304	0.187	0.458	0.273
R4	15	1.734	0.012	0.384	0.255
R5	29	2.139	0.066	0.407	0.244
Timilia					
T3	25	2.202	0.004	0.534	0.342
T5	24	2.313	0.009	0.559	0.355
T7	24	2.601	0.053	0.602	0.355
T8	21	2.179	0.006	0.485	0.304
T9	22	2.557	0.041	0.575	0.340
T11	21	2.350	0.02	0.564	0.352
T12	20	2.228	0.009	0.505	0.315
RT Collection					
TC	157	2.233	0.301	0.288	0.167
RC	120	2.597	0.665	0.515	0.319
RC1-3-5	84	2.481	0.265	0.419	0.246
RC2-4	36	1.871	0.048	0.396	0.258

The genetic diversity parameters estimated for RC and TC revealed that all the "Russello" (RC) had higher variability than "Timilia" (TC). In addition, the RC1-3-5 group was characterized by greater genetic diversity than RC2-4 and TC (Table 1).

Within RTC, RC1-3-5 and RC2-4 showed a higher Shannon's index (I = 0.419 and I = 0.396, respectively) and Nei's index (He = 0.246 and He = 0.258, respectively) than TC

(I = 0.288 and He = 0.167) (Table 1). Conversely, TC had a larger number of private alleles than the other two groups (Table 1).

AMOVA was used to describe population differentiation. The test revealed that there was greater genetic variation within (76%) than among populations (24%) in RC. In TC, 100% within-population variation was observed. As for RTC, within-population variation (54%) was slightly higher than among-populations variation (46%).

3.4. Divergent Loci and Haplotype Network

The genetic differentiation between the three groups mentioned above was further investigated by analyzing the F_{ST} index at individual loci, using $F_{ST} > 0.50$ as the threshold. A total of 1126, 1673, and 1968 divergent markers were detected in RC1-3-5 vs. R2-4, TC vs. R1-3-5, and TC vs. R2-4, respectively. Figure 4 shows the distribution of divergent loci along all chromosomes (A) and a Venn diagram (B) reporting the number of unique and common divergent markers among the three groups.
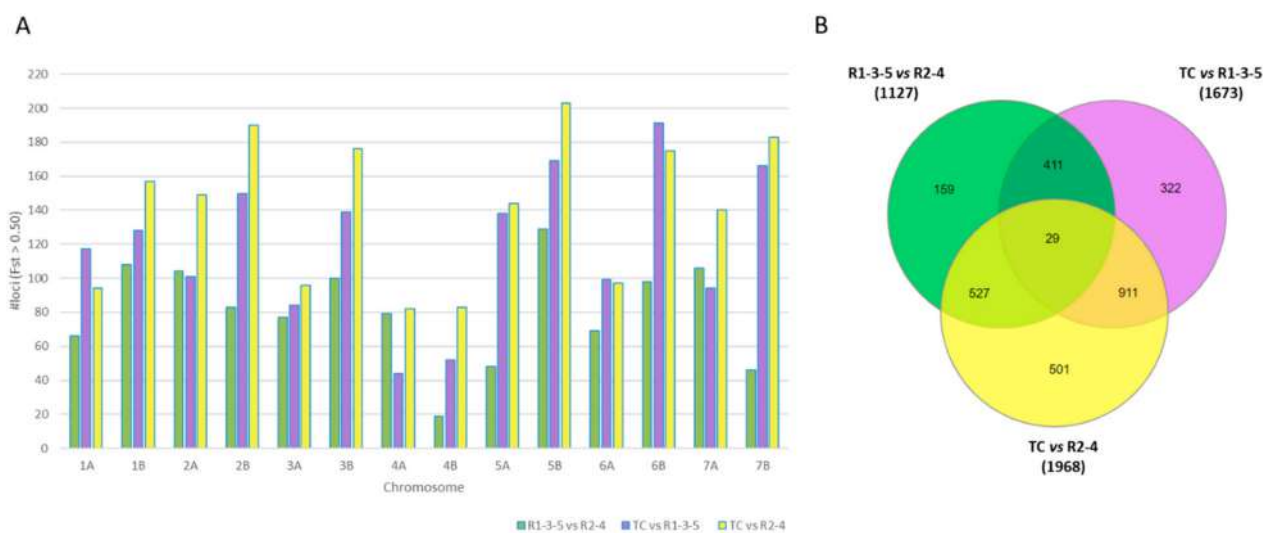

Figure 4. (**A**) Distribution of divergent loci ($F_{ST} > 0.50$) along the 14 chromosomes of durum wheat. (**B**) Venn diagram showing the number of divergent SNP markers detected in TC vs. R1-3-5, TC vs. R2-4 and R1-3-5 vs. R2-4.

Divergent loci were identified along all chromosomes, with the lowest values in the R1-3-5 vs. R2-4 comparison (Figure 3). When comparing TC vs. R1-3-5 and TC vs. R2-4, the chromosomes with the largest number (n) of divergent SNP markers were chromosomes 5B (n = 203) and 6B (n = 191). In contrast, chromosome 4A showed the smallest number of divergent SNPs in TC vs. R1-3-5 and TC vs. R2-4 (82 and 44, respectively). The largest and smallest number of divergent SNPs between the groups R1-3-5 and R2-4 were on chromosomes 5B (n = 129) and 4B (n = 19).

All the divergent loci were then used to construct the haplotype network shown in Figure 5, further confirming the genetic relationships among "Russello" and "Timilia" individuals (Figure 3). Indeed, most individuals of "Timilia" were arranged into a large haplotype group, which exhibited high genetic conservation. The remaining two groups (R1-3-5 and R2-4) showed greater haplotype variability. The analysis indicated that R1-3-5 appears to be closer to the "Timilia" haplotype than R-2-4. The latter had a lower and more distant number of haplotypes.
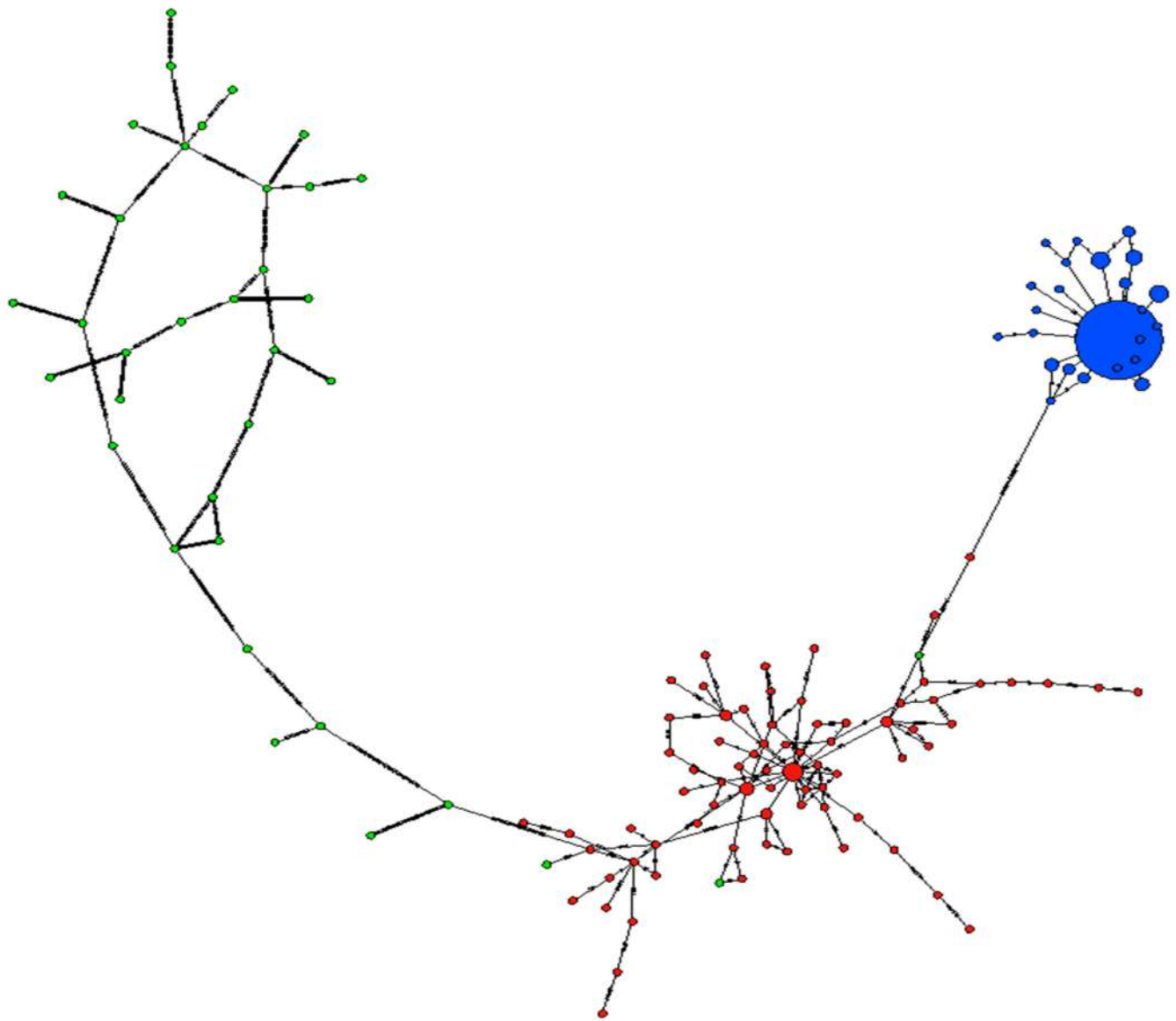
Figure 5. Minimum spanning networks using the three groups: TC (blue), R1-3-5 (red), and R2-4 (green). Each circle represents a haplotype; the circle sizes correspond to abundances. Bars indicate the number of mutations between haplotypes.

3.5. Patterns of Linkage Disequilibrium and Candidate Genes Associated with Divergent Loci

In order to identify the genes putatively associated with differences among the three groups, the decay of linkage disequilibrium within each group was estimated (Figure S4).

Average genome-wide LD decay at which r^2 fell to 0.20 was ∼11.5 Mb, 24 Mb, and 11 Mb for RC, TC, and RTC, respectively. In addition, the LD decay was also calculated considering TC+R1-3-5 (∼10 Mb) and TC+R2-4 (∼14 Mb); these LD decay values were then used as confidence intervals around each divergent SNP marker ($F_{ST} > 0.80$).

Candidate genes in the confidence interval around each divergent SNP, returned from the comparison among the three groups, suggested a high level of divergence between populations, mostly in genomic regions where the genes controlling wheat grain quality traits are (Table 2).

Table 2. Major genes detected in the flanking region of divergent SNPs exceeding the significant threshold of $F_{ST} > 0.80$. SNP name, chromosome, and position on "Svevo" genomes, distance (Mb) between divergent SNP and genes, transcript ID, and pairwise comparison are reported.

SNP Marker	Gene	Transcript ID	Distance (~Mb)	Comparison
tplb0025b13_1721	*MEI2-4 (OML4)*	TRITD1Av1G001750	0	R1-3-5 vs. R2-4 - TC vs. R2-4
tplb0025b13_1721; TA001286-0611-w	*Gamma-gliadin*	TRITD1Av1G002070	0.50; 0	R1-3-5 vs. R2-4; TC vs. R1-3-5 - TC vs. R2-4
tplb0025b13_1721; Excalibur_c35919_107	*glutenin (LMW)*	TRITD1Av1G002310	0.33; 0.01	R1-3-5 vs. R2-4; TC vs. R1-3-5 - TC vs. R2-4
Excalibur_c77035_156	*WCOR15*	TRITD1Av1G021590	0.55	R1-3-5 vs. R2-4
IAAV8147	PP2A regulatory subunit TAP46	TRITD1Av1G125250	0.18	TC vs. R1-3-5
RAC875_c6338_2719	*TELO2*	TRITD1Av1G127160	0.68	TC vs. R1-3-5
RAC875_c30138_595; IACX2941	*Gamma-gliadin*	TRITD1Bv1G001870	0.27; 0.07	TC vs. R1-3-5; TC vs. R2-4
BS00004903_51	*Anthocyanin 5-aromatic acyltransferase*	TRITD1Bv1G004660	0.38	TC vs. R1-3-5
BS00021975_51; CAP8_rep_c7343_88	*glutenin (HMW)*	TRITD1Bv1G177800	0.18; 0	TC vs. R1-3-5: R1-3-5 vs. R2-4 - TC vs. R2-4
wsnp_Ex_c19556_28530231	*Ppd-A1*	TRITD2Av1G019250	0.27	TC vs. R1-3-5; TC vs. R2-4
wsnp_CAP11_rep_c8768_3788007	*FD-GOGAT*	TRITD2Av1G036960	0	TC vs. R1-3-5 - TC vs. R2-4
wsnp_Ex_c12219_19526749	*SUS3*	TRITD2Av1G053920	3.9	R1-3-5 vs. R2-4; TC vs. R1-3-5
Kukri_c44442_274	*Lpx*	TRITD2Av1G054540	0.62	R1-3-5 vs. R2-4; TC vs. R1-3-5
wsnp_be471201A_Ta_1_1	*Anthocyanin 3'-O-beta-glucosyltransferase*	TRITD2Av1G252600	1.2	TC vs. R2-4
wsnp_be471201A_Ta_1_1	*Anthocyanin 5-aromatic acyltransferase*	TRITD2Av1G253600	1.12	R1-3-5 vs. R2-4; TC vs. R2-4
RAC875_c110838_165; Ku_c13700_1196	*Ppo-A1*	TRITD2Av1G261300	0.62	TC vs. R1-3-5; TC vs. R2-4
RAC875_c110838_165; Ku_c13700_1196	*Ppo-A2*	TRITD2Av1G261390	0.4	TC vs. R1-3-5; TC vs. R2-4
RAC875_c110838_165; Ku_c13700_1196	*starch synthase*	TRITD2Av1G261450	0.14	TC vs. R1-3-5; TC vs. R2-4
RAC875_c35438_474	*Beta-amylase*	TRITD2Bv1G043100	1.2	TC vs. R1-3-5; TC vs. R2-4
BS00011630_51	*Ppo-B2*	TRITD2Bv1G224170	0.46	TC vs. R1-3-5; TC vs. R2-4
RAC875_c19534_68	*9-cis-epoxycarotenoid dioxygenase*	TRITD3Av1G020620	0.46	R1-3-5 vs. R2-4
BS00024548_51	*LEA*	TRITD3Av1G260240	0	R1-3-5 vs. R2-4
BS00036352_51	Carotenoid cleavage dioxygenase	TRITD3Bv1G162510	1.9	TC vs. R2-4
BobWhite_c1196_297	*Anthocyanin 3'-O-beta-glucosyltransferase*	TRITD3Bv1G163770	0	TC vs. R2-4
Jagger_rep_c10288_53	*Rht-A1*	TRITD4Av1G194130	7.5	TC vs. R2-4
Jagger_rep_c10288_53	*NRT1/PTR*	TRITD4Av1G198820	0.53	TC vs. R2-4
Tdurum_contig82378_264	*Rht-B1*	TRITD4Bv1G012280	4.5	R1-3-5 vs. R2-4 - TC vs. R2-4

Table 2. *Cont.*

SNP Marker	Gene	Transcript ID	Distance (~Mb)	Comparison
Tdurum_contig82378_264	*Lpx1*	TRITD4Bv1G010710	7.8	R1-3-5 vs. R2-4 - TC vs. R2-4
Tdurum_contig82378_264	*Teosinte branched 1 protein*	TRITD4Bv1G012050	4.5	R1-3-5 vs. R2-4 - TC vs. R2-4
wsnp_Ex_c6117_10704945	*Histone H3*	TRITD5Av1G041920	6	TC vs. R1-3-5
wsnp_Ra_c10053_16636851	*ASN*	TRITD5Av1G043240	6	TC vs. R1-3-5
BS00076246_51	*Lpx*	TRITD5Av1G200190	1.7	TC vs. R1-3-5; TC vs. R2-4
GENE-3601_145	*Vrn-A1*	TRITD5Av1G204680	10	TC vs. R2-4
BS00066456_51	*15-cis-zeta-carotene isomerase*	TRITD5Bv1G013720	5.7	TC vs. R1-3-5; TC vs. R2-4
GENE-3383_710	*9-cis-epoxycarotenoid dioxygenase*	TRITD5Bv1G192230	3.4	R1-3-5 vs. R2-4
wsnp_Ex_c5155_9140608	*Lpx*	TRITD5Bv1G195300	1.7	TC vs. R2-4
Excalibur_c24051_502	*TdHMA3-B1*	TRITD5Bv1G197370	1	TC vs. R1-3-5
Excalibur_c24051_502	*Vrn-B1*	TRITD5Bv1G200510	10	TC vs. R2-4
BS00071573_51	*Terpene cyclase/mutase*	TRITD6Av1G000890	0.08	TC vs. R1-3-5; TC vs. R2-4
BS00022660_51	*Terpene synthase*	TRITD6Av1G008120	0.04	TC vs. R2-4
IACX5772	*Alpha-gliadin*	TRITD6Av1G009340	1.6	TC vs. R2-4
IACX203	*Rht-14*	TRITD6Av1G140910	24	TC vs. R2-4
IACX203	*Nitrilase*	TRITD6Av1G150150	0.17	TC vs. R2-4
BS00065082_51	*Nrt*	TRITD6Av1G196930	0.56	TC vs. R1-3-5 - TC vs. R2-4
CAP7_c7415_267	*Nir*	TRITD6Av1G199660	0	TC vs. R2-4
BS00109708_51	*Alpha-gliadin*	TRITD6Bv1G014640	0.27	TC vs. R1-3-5
GENE-3945_245	*Anthocyanin 3′-O-beta-glucosyltransferase*	TRITD6Bv1G159080	1.2	R1-3-5 vs. R2-4; TC vs. R1-3-5
BS00109912_51; RFL_Contig3621_1157	*Terpene cyclase/mutase*	TRITD7Av1G000490	0.79	R1-3-5 vs. R2-4; TC vs. R2-4
BS00109912_51; RFL_Contig3621_1157	*Terpene synthase*	TRITD7Av1G000650	0.84	R1-3-5 vs. R2-4; TC vs. R2-4
Excalibur_c25891_1402	*SUS1*	TRITD7Av1G009720	0.75	TC vs. R1-3-5; TC vs. R2-4
RAC875_c63822_185	*Anthocyanin 5-aromatic acyltransferase*	TRITD7Av1G013740	0.94	R1-3-5 vs. R2-4; TC vs. R2-4
BS00062724_51	*GBSSI*	TRITD7Av1G018110	0.82	TC vs. R1-3-5
Excalibur_c96483_102	*Beta-carotene isomerase*	TRITD7Av1G226310	0.24	TC vs. R1-3-5
Jagger_c5275_99	*MADAGL17*	TRITD7Bv1G002790	0.56	TC vs. R2-4
Tdurum_contig85266_280	*GW2-B1*	TRITD7Bv1G002890	0	R1-3-5 vs. R2-4

The differences between "Timilia" and the two "Russello "groups are mostly related to genes encoding storage proteins (i.e., gliadins and glutenins) and genes responsible for grain color, such as those related to the browning reaction (i.e., polyphenol oxidase enzymes), and those belonging to the biosynthetic pathways of carotenoids and anthocyanins. However, comparisons did not always return the same loci but rather different genes on different chromosomes, as shown below.

Divergent loci between the two RC groups were associated with qualitative traits such as *γ-gliadin* and *glutenin* on chromosome 1A and *sucrose synthase* (SUS3) on chromosome 2A; *terpene synthase*, *terpene cyclase/mutase*, *9-cis-epoxycarotenoid dioxygenase* and *carotenoid isomerase* on chromosomes 3A, 3B, 5A, 6A and 7A, which are involved in the primary and part of the secondary metabolism, and starch biosynthesis on chromosome 3A.

Comparisons of TC vs. R1-3-5 and TC vs. R2-4 returned some common genes, such as those encoding the polyphenol oxidase enzymes *Ppo-A1*, *Ppo-A2,* and *Ppo-B2* on home-ologous chromosome 2 and the lipoxygenase enzyme on chromosome 5A. The analysis also revealed genes involved in starch metabolism (*starch synthase*, α and β *amylase* and *maltase*, *sucrose synthase*) on chromosomes 1B, 2A, 2B, 5A, and 7A; the gene that codes for the *15-cis-zeta-carotene-isomerase* involved in the biosynthesis of carotenoids and the gene that codes for a high molecular weight *glutenin* and *gliadin* on chromosome 1A, 1B, and 5B.

In the TC vs. R1-3-5 comparison, we identified genes such as: α, *γ-gliadin,* and *glutenin* (*hmw*) located on chromosomes 1A, 1B, and 6B; genes involved in starch metabolism, such as α and β *amylase* on chromosomes 2B and 5A, *starch synthase* on chromosomes 1B and 2B, *granule-bound starch synthase* (*GBSSI*) on chromosome 7A, and genes for maltose transport on chromosomes 2B, 4B and 6B. In addition, *lipoxygenase* genes were also detected on chromosome 2A.

In the TC vs. R2-4 comparison, we identified the genes that influence the quality of durum wheat, such as: α, *γ-gliadin,* and glutenin (*hmw*) on chromosomes 1A, 1B, and 6A; α and β *amylase* on chromosomes 2B, 3A, 4B, 6B, and 7A; genes involved in the biosynthesis of carotenoids (*9-cis-epoxycarotenoid dioxygenase*, *carotenoid oxugenase,* and *carotenoid isomerase*) on chromosomes 2B and 7A; a *lipoxygenase* (lpx) gene on chromosome 5B; genes encoding *anthocyanin 5-aromatic acyltransferase* and *anthocyanin 5,3-O-glucosyltransferase* on chromosomes 1B, 2A, 3A, 4B, 6A, and 6B.

Regions that include genes known to be related to the most renowned morpho-agronomic traits important during the durum wheat breeding process were identified mainly in "Timilia" vs. "Russello" (both R1-3-5 and R2-4) comparison.

Genes associated with other important traits associated with durum wheat artificial selection were detected, such as those controlling grain size and weight, i.e., *MEI2-LIKE PROTEIN 4* (*OML4*) and *GW2-B1*, which included the divergent markers tplb0025b13_1721 (chr.1A) and Tdurum_contig85266_280 (chr.7B), respectively. In this latter region (chr.7B), a cluster of *Agamous MADS-box transcription factor MADAGL17* was also found in TC vs. R2-4. Genes involved in the nitrogen metabolism were in regions harboring divergent SNP markers. In detail, the markers wsnp_CAP11_rep_c8768_3788007 and CAP7_c7415_267 fell into the sequence of *glutamate synthase* (*FD-GOGAT*) and *nitrite reductase* (*Nir*) genes on chromosomes 2A and 6A, respectively, the *asparagine synthetase* (*ASN*) and *histone H3* were in a region with divergent SNPs spanning ~6 Mb on chr. 5A. *Nitrate reductase* (*NR*) and *nitrilase* were in a region with divergent loci on chromosome 6A.

Divergent loci were in LD in the regions including genes involved in nitrogen metabolism (nitrilase and nitrate transporter) as well as the *Lipoxigenase 1* (*Lpx 1*) and genes controlling plant height (*Rht*), such as *Rht-A1*, *Rht-B1,* and *Rht-14* on chromosomes 4A, 4B, and 6A, respectively. Other SNP markers were proximal to *Ppd-A1*, *Vrn-A1,* and *Vrn-B1*. The latter gene was also adjacent to the gene TdHMA3-B1, responsible for cadmium accumulation and considered a signature of selection from wild emmer to durum wheat. Finally, divergent genes involved in response to abiotic stresses such as the cold-responsive *WCOR15* and the late embryogenesis abundant (LEA) hydroxyproline-rich glycoprotein were detected on chromosomes 1A and 3A, respectively. Furthermore, genes implicated in the

signaling pathway of the rapamycin target (TOR) such as *PP2A regulatory subunit TAP46*, *Telo2 interacting protein2*, *Kinase family protein*, *NAC*, *Phenylalanine–tRNA ligase beta subunit* were detected.

4. Discussion

In the current scenario of severe climate change, it is urgent to broaden the genetic basis of élite varieties through the introduction of favorable alleles conferring tolerance to (a)biotic stress. Landraces and old cultivars represent a precious resource for traits linked to adaptation to different pedoclimatic conditions [14,48]. Presently, thanks to the efforts of farmers and scientists, several wheat landraces and old cultivars have been collected and conserved on-farm and/or in ex situ genebanks [49,50]. However, within-population genetic variability is rarely studied by comparing a large number of individuals.

Within this context, our work aimed to assess the intra- and inter-population genetic diversity of two large germplasm collections of the landraces "Timilia" and "Russello" and to identify the distinguishing SNP loci.

The AMOVA and the assessment of the percentage of private alleles indicated very low genetic distance among seven populations of "Timilia" and moderately high intra-population variability. The five "Russello" populations were instead clearly separated into two distinct groups, respectively, R1-3-5 and R2-4. Inter-population variability was greater in the R1-3-5 populations than in R2-4. This is in accordance with the identification of several cases of duplicated individuals in R2 and R4. The identification of two "Russello" populations was in accordance with their geographical origin, as the R1-3-5 populations were collected from the Hyblaean mountains in south-eastern Sicily, which are characterized by different pedoclimatic conditions compared to central-western Sicily, where the R2-4 populations were sampled. The existence of two different "Russello" groups could also be explained by the fact that with the name "Russello", farmers have indicated different durum wheat populations over time, even with different morpho-agronomic traits [24,51]. Our work evidenced the existence of two groups of "Russello" in Sicily and revealed how these remained distinct over time, probably due to geographical isolation, and have adapted to the different pedoclimatic conditions of the two cultivation areas [26].

Our results based on SNP markers agree with those by [23], who pointed out that all the "Timilia" individuals they sampled in different areas showed a single electrophoretic profile (HMW-GS, Bx6 + By8) of glutenins. In contrast, the same authors found two profiles for "Russello" (HMW-GS, Bx13 + By16 and Bx6 + By8), thus suggesting that the "Russello" grown between the two districts of Palermo and Agrigento has distinct characteristics compared with that grown in the Hyblaean area, where it is also referred to as "Ruscìa" [23,51]. The differentiation between the two "Russello" groups was also supported by the identification of divergent loci associated with genes encoding storage proteins. This difference may also be due to selection driven by contrasting temperatures during the grain filling stage in these areas [52].

Finally, our findings are also corroborated by the previous work by [6], in which single individuals randomly taken from each of the five "Russello" populations analyzed in this study were compared with a large collection of Italian landraces, ancient, and modern cultivars. Taranto [6] reported that individuals belonging to the R1-3-5 populations grouped with "Ruscìa", while the individuals taken from the R2-4 populations were more similar to the ancient variety "Tangarog", from which "Russello" could have originated [2].

The study of LD decay within the populations of "Timilia" and "Russello" further validated the genetic structure of the groups. Indeed, the slower decay of LD in TC confirmed the presence of larger haplotype blocks, probably due to the conservative selection made by farmers [6]. Indeed, although "Timilia" seeds have been exchanged among farmers even outside Sicily, as demonstrated by [6], it has maintained its genetic integrity for its exclusive use in spring sowing.

The haplotype and the F_{ST} analysis clearly show that although "Timilia" and "Russello" are two Sicilian landraces, they are genetically different. Furthermore, the R2 and R4

populations are the most variable and distant from "Timilia", probably because they have been crossed with other cultivars, either other ancient Sicilian varieties or not, or erroneously referred to as "Russello" [24]. The analysis of the divergent loci supports and corroborates the genetic differences between the two "Russello" groups and between "Russello" and "Timilia".

It is known that these two landraces differ in the traits related to the quality of the grain. The comparison between TC and R1-3-5/R2-4 allowed identifying key genes that influence the composition of gluten, in accordance with the distinct electrophoretic profiles identified in several studies [3,23,25,26].

Another important aspect emerging from the present analysis is related to the genes encoding for the polyphenol oxidase (PPO) enzymes, which are involved in the oxidation of phenolic compounds, giving rise to the browning reactions of the kernels. Taranto [53] demonstrated that the PPO activity was positively correlated with the brown index of wholemeal flour and dough, and both were very high in "Timilia" (giving a typical brown color to kernels) compared with both "Russello" and modern varieties [54,55]. Significant differences in phenolic compounds were found between "Timilia" and other old and modern varieties analyzed by Lo Bianco [56], who suggested these molecules as markers for the traceability of "Timilia". Although PPO genes located on homeologous chromosome 2 have been extensively studied for browning and discoloration of wheat flour [55,57–61], they may also be involved in adaptation mechanisms, like peroxidase and lipoxygenase genes [6–64]; they could therefore be involved in conferring unique characteristics to "Timilia", which is particularly suitable for hot-arid Mediterranean climates [28,65]. This is further corroborated by its late sowing (in the late winter), which forces it to withstand the late spring heat spells and drought in the area during the grain filling stage. This result is also consistent with the fact that "Timilia" showed a higher level of expressions of genes involved in response to attacks by fungi, herbivores, and pathogens, as well as wounds and other abiotic stresses than "Russello" [28]. All these results suggested that "Timilia" should be explored more carefully as a potential source of favorable alleles to counteract the direct and indirect effects of climate change and that a preservation plan is needed to avoid its genetic contamination. In this context, PPOs could play a key role both in "Timilia" traceability and in the study of traits related to the adaptation mechanisms of the plant.

Divergent loci have also been identified as linked to other qualitative characteristics of the flours, in particular to the biosynthesis of starch and carotenoids concentration in the grain. Again, the divergence in the genes associated with the biosynthesis of carotenoids agreed with previous results by [3,14,66], showing a higher content of carotenoids in "Russello" grain than in "Timilia". Several genes related to starch metabolism seem to differentiate "Russello" from "Timila". This finding agrees with the work by [28], who reported the quantitative label-free comparison of the metabolic protein fraction in "Russello", "Timilia", and the modern variety "Simeto". Their results suggest that "Russello" and "Timilia" differ in genes related to starch compositions, such as *SUS1* and *SUS3*, which also affect grain size and grain yield. In our work, we found divergent SNP loci associated with *SUS1, SUS3,* and other key genes controlling grain size and weight; these findings explain the genetic distance between "Russello" and "Timilia", as evidenced in previous studies [14,25,66,67]. Indeed, in all these studies, "Timilia" showed a thousand kernel weight (TKW) lower than "Russello" (on average 35–45 g vs. 45–55 g, respectively).

Another aspect that affects the grain yield and the physiology of the plant is the role of source and sink mechanisms of nitrogen transport and use. Here we found that several divergent SNP loci were associated with genes involved in nitrogen metabolism and plant adaptations.

Finally, due to the use of the durum wheat genome (cv. Svevo) as the reference for the identification of candidate genes responsible for phenotypic differentiation, some genes fall into regions harboring *Rht* genes. However, the "Russello" and "Timilia" landraces are known to be ancient and tall grains and therefore do not contain the semi-dwarfing *Rht* gene, as on the contrary observed in Polish wheat by [68]. Those regions are rich in nitrogen

metabolism-related genes and lipoxygenases, which played a key role in the domestication process of tetraploid wheat [6,55].

The combined effect of these genes should explain the genetic basis that differentiates the two Sicilian landraces and deserve further study.

5. Conclusions

Landraces and obsolete cultivars are an important reservoir of genes/alleles to be used in durum wheat breeding to counteract the effects of climate change. The recovery and characterization of landraces are the first steps towards promoting and restoring the conservation and sustainable use of agricultural biodiversity, as well as the protection of marginal areas.

In this work, large populations of two Sicilian landraces, namely "Russello" and "Timilia", were analyzed using approximately 5000 high-quality SNP markers with the aim of revealing intra- and inter-populations genetic diversity and identifying divergent loci between genetic groups.

Our findings demonstrated a wide variability within and between populations, evidenced by the fact that there are two different types of "Russello" scattered throughout Sicily, which differ in important traits associated with the adaptation and quality of gluten. On the other hand, the accessions of "Timilia", although cultivated throughout Sicily, showed a great genetic distance from the two Russello groups, maintaining its genetic integrity probably due to its exclusive destination for spring sowing. For this reason, farmers managed these landraces separately, retaining some distinctive characteristics (i.e., polyphenol oxidases, carotenoids, TKW).

This work paves the way for a greater exploration of the broad genetic diversity that characterizes Sicilian landraces and points out that intra-population genetic diversity should be taken into account when 'conservation variety' are to be registered in National Registers of crops.

Supplementary Materials: The following supporting information can be downloaded at https://www.mdpi.com/article/10.3390/agronomy12061326/s1. Figure S1: Bar chart showing the number of high-quality SNPs per chromosome. Figure S2: Distribution of identity-by-state (IBS) allele sharing values amongst (A) "Russello" (RC), "Timilia" (TC), and C) "Russello" + "Timilia" (RTC) populations determined by the analysis of 4624 unlinked single nucleotide polymorphisms. Figure S3: Genetic diversity assessment of "Russello" and "Timilia" populations using 4624 high-quality SNP markers. (A–C) Neighbor-joining tree based on the genetic distances for "Russello" and "Timilia" individuals, respectively. (B–D) Multidimensional scaling (MDS) plot representing relationships between the" Russello" and "Timilia" individuals, respectively. Individuals are colored according to different populations. Figure S4: Intra-chromosomal LD decay distance (kb) evaluated for each "Russello" (RC) and "Timilia" (TC) population, and for the whole population (RTC). LD decay was also calculated considering two other collections composed of TC+R1-3-5 and TC+R2-4. Red lines indicate the r2 threshold at 0.20. The intersection point between the decay LD curve and the LD threshold was indicated by "+". Table S1: Initial of individuals for the "Russello" and "Timilia" populations, duplicated individuals (IBS > 0.99), discarded individuals, and the final number of individuals used in downstream analysis. Table S2: List of pairs of individuals with identity-by-state (IBS) allele-sharing estimates >0.99. Stars indicate the individuals retained. Table S3: Divergent SNP loci detected by pairwise comparisons between the populations R1-3-5, R2-4, and TC using F_{ST} (>0.50). SNP name, physical position on "Svevo" (chromosome and bp), and F_{ST} values were reported.

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References

1. Bozzini, A.; Scarascia-Mugnozza, G.T. Relative frequency of chlorophyll to morphological and sterility mutations induced in durum wheat by radiations and chemicals. *Mutat. Res.-Fundam. Mol. Mech. Mutagen.* **1970**, *9*, 589–597. [CrossRef]
2. D'Amato, F. The progress of Italian wheat production in the first half of the 20th century: The contribution of breeders. *Agric. Medit.* **1989**, *119*, 157–174.
3. De Vita, P.; Nicosia, O.L.D.; Nigro, F.; Platani, C.; Riefolo, C.; Di Fonzo, N.; Cattivelli, L. Breeding progress in morpho-physiological, agronomical and qualitative traits of durum wheat cultivars released in Italy during the 20th century. *Eur. J. Agron.* **2007**, *26*, 39–53. [CrossRef]
4. Maccaferri, M.; Harris, N.S.; Twardziok, S.O.; Pasam, R.K.; Gundlach, H.; Spannagl, M.; Ormanbekova, D.; Lux, T.; Prade, V.M.; Milner, S.G.; et al. Durum wheat genome highlights past domestication signatures and future improvement targets. *Nat. Genet.* **2019**, *51*, 885–895. [CrossRef]
5. Laidò, G.; Mangini, G.; Taranto, F.; Gadaleta, A.; Blanco, A.; Cattivelli, L.; Marone, D.; Mastrangelo, A.M.; Papa, R.; De Vita, P. Genetic diversity and population structure of tetraploid wheats (*Triticum turgidum* L.) estimated by SSR, DArT and pedigree data. *PLoS ONE* **2013**, *8*, e67280.
6. Taranto, F.; D'Agostino, N.; Rodriguez, M.; Pavan, S.; Minervini, A.P.; Pecchioni, N.; Papa, R.; De Vita, P. Whole genome scan reveals molecular signatures of divergence and selection related to important traits in durum wheat germplasm. *Front. Genet.* **2020**, *11*, 217. [CrossRef] [PubMed]
7. Vita, P.D.; Taranto, F. Durum wheat (*Triticum turgidum* ssp. *durum*) breeding to meet the challenge of climate change. In *Advances in Plant Breeding Strategies: Cereals*; Springer Pub.: Berlin/Heidelberg, Germany, 2019; pp. 471–524.
8. Sharma, S.; Schulthess, A.W.; Bassi, F.M.; Badaeva, E.D.; Neumann, K.; Graner, A.; Özkan, H.; Werner, P.; Knüpffer, H.; Kilian, B. Introducing beneficial alleles from plant genetic resources into the wheat germplasm. *Biology* **2021**, *10*, 982. [CrossRef]
9. Lopes, M.S.; El-Basyoni, I.; Baenziger, P.S.; Singh, S.; Royo, C.; Ozbek, K.; Aktas, H.; Ozer, E.; Ozdemir, F.; Manickavelu, A.; et al. Exploiting genetic diversity from landraces in wheat breeding for adaptation to climate change. *J. Exp. Bot.* **2015**, *66*, 3477–3486. [CrossRef]
10. Migliorini, P.; Spagnolo, S.; Torri, L.; Arnoulet, M.; Lazzerini, G.; Ceccarelli, S. Agronomic and quality characteristics of old, modern and mixture wheat varieties and landraces for organic bread chain in diverse environments of northern Italy. *Eur. J. Agron.* **2016**, *79*, 131–141. [CrossRef]
11. Giunta, F.; Bassu, S.; Mefleh, M.; Motzo, R. Is the technological quality of old durum wheat cultivars superior to that of modern ones when exposed to moderately high temperatures during grain filling? *Foods* **2020**, *9*, 778. [CrossRef]
12. Salantur, A.; Karaoğlu, C. Macro-Microelements in Wheat Landraces and Their Use in Breeding. In *Wheat Landraces*; Springer Pub.: Berlin/Heidelberg, Germany, 2021; pp. 83–91.
13. Mangini, G.; Nigro, D.; Margiotta, B.; De Vita, P.; Gadaleta, A.; Simeone, R.; Blanco, A. Exploring SNP diversity in wheat landraces germplasm and setting of a molecular barcode for fingerprinting. *Cereal Res. Communic.* **2018**, *46*, 377–387. [CrossRef]
14. Fiore, M.C.; Mercati, F.; Spina, A.; Blangiforti, S.; Venora, G.; Dell'Acqua, M.; Lupini, A.; Preiti, G.; Monti, M.; Pè, M.E.; et al. High-Throughput Genotype, Morphology, and Quality Traits Evaluation for the Assessment of Genetic Diversity of Wheat Landraces from Sicily. *Plants* **2019**, *8*, 116. [CrossRef] [PubMed]
15. De Cillis, U. *I Frumenti Siciliani*; Tipografia Zuccarello and Izzi: Catania, Italy, 1942; ISBN 88-7751-229-6.
16. Spina, A.; Blangiforti, S.; Cambrea, M.; Gallo, G.; Licciardello, S. Valorizzazione di germoplasma locale di frumento duro per la produzione di pani tipici. In Proceedings of the VIII Convegno Nazionale "La Biodiversità-una Risorsa per Sistemi Multifunzionali", Lecce, Italy, 21–23 April 2008.
17. Sciacca, F.; Cambrea, M.; Licciardello, S.; Pesce, A.; Romano, E.; Spina, A.; Virza, N.; Palumbo, M. Evolution of durum wheat: From Sicilian landraces to improved varieties. *Options Méditerranéennes Ser. A* **2014**, *110*, 139–145.
18. Giancaspro, A.; Colasuonno, P.; Zito, A.; Blanco, A.; Pasqualone, A.; Gadaleta, A. Varietal traceability of bread 'Pane Nero di Castelvetrano' by denaturing high pressure liquid chromatography analysis of single nucleotide polymorphisms. *Food Control* **2016**, *59*, 809–817. [CrossRef]
19. Spina, A.; Dinelli, G.; Palumbo, M.; Whittaker, A.; Cambrea, M.; Negri, L.; Bosi, S. Evaluation of standard physico-chemical and rheological parameters in predicting bread-making quality of durum wheat (*Triticum turgidum* L. ssp. *durum* [Desf.] Husn.). *Intern. J. Food Sci. Techn.* **2021**, *56*, 3278–3288. [CrossRef]
20. Kornicke, F.; Werner, H. *Handbuch des Getreidebaues: Die Arten and Varietaten des Getreides*; Die sorten und der Anbau des Gertréîdes: Berlin, Germany, 1885.
21. Sciacca, F.; Blanco, C.; Spina, A.; Virzì, N.; Palumbo, M. Biochemical and molecular characterization of durum wheat Sicilian landraces (*Triticum durum* Desf.). *Italus Hortus* **2006**, *13*, 503–507.
22. Melini, V.; Melini, F.; Acquistucci, R. Nutritional characterization of an Italian traditional bread from ancient grains: The case study of the durum wheat bread "Pane di Monreale". *Eur. Food Res. Technol.* **2020**, *247*, 193–200. [CrossRef]

23. Visioli, G.; Bonas, U.; Dal Cortivo, C.; Pasini, G.; Marmiroli, N.; Mosca, G.; Vamerali, T. Variations in yield and gluten proteins in durum wheat varieties under late-season foliar vs. soil application of nitrogen fertilizer in a northern Mediterranean environment. *J. Sci. Food Agric.* **2018**, *98*, 2360–2369. [CrossRef]

24. Lombardo, G.M. La scelta della varietà ai fini della valorizzazione della qualità della granicoltura iblea. In Proceedings of the Conference "Il Futuro Della Granicoltura Iblea", Modica, Italy, 11–17 December 1994.

25. De Santis, M.A.; Giuliani, M.M.; Giuzio, L.; De Vita, P.; Lovegrove, A.; Shewry, P.R.; Flagella, Z. Differences in gluten protein composition between old and modern durum wheat genotypes in relation to 20th century breeding in Italy. *Eur. J. Agron.* **2017**, *87*, 19–29. [CrossRef]

26. Di Francesco, A.; Saletti, R.; Cunsolo, V.; Svensson, B.; Muccilli, V.; De Vita, P.; Foti, S. Dataset of the metabolic and CM-like protein fractions in old and modern wheat Italian genotypes. *Data Brief* **2019**, *27*, 104730. [CrossRef]

27. Di Francesco, A.; Saletti, R.; Cunsolo, V.; Svensson, B.; Muccilli, V.; De Vita, P.; Foti, S. Qualitative proteomic comparison of metabolic and CM-like protein fractions in old and modern wheat Italian genotypes by a shotgun approach. *J. Proteom.* **2020**, *211*, 103530. [CrossRef] [PubMed]

28. Di Francesco, A.; Saletti, R.; Cunsolo, V.; Svensson, B.; Muccilli, V.; De Vita, P.; Foti, S. Quantitative label-free comparison of the metabolic protein fraction in old and modern italian wheat genotypes by a shotgun approach. *Molecules* **2021**, *26*, 2596. [CrossRef] [PubMed]

29. Pandolfo, A.; Messina, B.; Russo, G. Evaluation of Glycemic Index of Six Different Samples of Commercial and Experimental Pasta Differing in Wheat Varieties and Production Processes. *Foods* **2021**, *10*, 2221. [CrossRef] [PubMed]

30. Blangiforti, S.; Gallo, G.; Porfiri, O. Morpho-agronomic and molecular assessment of a collection of Sicilian wheats [*Triticum aestivum* L.; *Triticum durum* Desf.]. *Italus Hortus* **2006**, *13*, 364–369.

31. Figliuolo, G.; Mazzeo, M.; Greco, I. Temporal variation of diversity in Italian durum wheat germplasm. *Gen. Res. Crop Evol.* **2007**, *54*, 615–626. [CrossRef]

32. Marzario, S.; Logozzo, G.; David, J.L.; Zeuli, P.S.; Gioia, T. Molecular genotyping (SSR) and agronomic phenotyping for utilization of durum wheat (*Triticum durum* Desf.) ex situ collection from Southern Italy: A combined approach including pedigreed varieties. *Genes* **2018**, *9*, 465. [CrossRef]

33. Taranto, F.; Nicolia, A.; Pavan, S.; De Vita, P.; D'Agostino, N. Biotechnological and digital revolution for climate-smart plant breeding. *Agronomy* **2018**, *8*, 277. [CrossRef]

34. Pavan, S.; Delvento, C.; Ricciardi, L.; Lotti, C.; Ciani, E.; D'Agostino, N. Recommendations for choosing the genotyping method and best practices for quality control in crop genome-wide association studies. *Front. Genet.* **2020**, *11*, 447. [CrossRef]

35. Kabbaj, H.; Sall, A.T.; Al-Abdallat, A.; Geleta, M.; Amri, A.; Filali-Maltouf, A.; Belkadi, B.; Ortiz, R.; Bassi, F.M. Genetic diversity within a global panel of durum wheat (*Triticum durum*) landraces and modern germplasm reveals the history of alleles exchange. *Front. Plant Sci.* **2017**, *8*, 1277. [CrossRef]

36. Rufo, R.; Alvaro, F.; Royo, C.; Soriano, J.M. From landraces to improved cultivars: Assessment of genetic diversity and population structure of Mediterranean wheat using SNP markers. *PLoS ONE* **2019**, *14*, e0219867. [CrossRef]

37. Ganugi, P.; Palchetti, E.; Gori, M.; Calamai, A.; Burridge, A.; Biricolti, S.; Benedettelli, S.; Masoni, A. Molecular Diversity within a Mediterranean and European Panel of Tetraploid Wheat (*T. turgidum* subsp.) Landraces and Modern Germplasm Inferred Using a High-Density SNP Array. *Agronomy* **2021**, *11*, 414. [CrossRef]

38. Soriano, J.M.; Colasuonno, P.; Marcotuli, I.; Gadaleta, A. Meta-QTL analysis and identification of candidate genes for quality, abiotic and biotic stress in durum wheat. *Sci. Rep.* **2021**, *11*, 11877. [CrossRef] [PubMed]

39. Mangini, G.; Taranto, F.; Delvecchio, L.N.; Pasqualone, A.; Blanco, A. Development and validation of a new Ppo-A1 marker useful for marker-assisted selection in tetraploid wheats. *Molec. Breed.* **2014**, *34*, 385–392. [CrossRef]

40. Wickham, H. Getting started with qplot. In *Ggplot2*; Springer Pub.: New York, NY, USA, 2009; pp. 9–26.

41. Kumar, S.; Stecher, G.; Li, M.; Knyaz, C.; Tamura, K. MEGA X: Molecular evolutionary genetics analysis across computing platforms. *Molec. Biol. Evol.* **2018**, *35*, 1547. [CrossRef] [PubMed]

42. Smouse, R.P.P.; Peakall, R. GenAlEx 6.5: Genetic analysis in Excel. Population genetic software for teaching and research—An update. *Bioinformatics* **2012**, *28*, 2537–2539.

43. Gu, Z.; Eils, R.; Schlesner, M. Complex heatmaps reveal patterns and correlations in multidimensional genomic data. *Bioinformatics* **2016**, *32*, 2847–2849. [CrossRef]

44. Gu, Z.; Gu, L.; Eils, R.; Schlesner, M.; Brors, B. Circlize implements and enhances circular visualization in R. *Bioinformatics* **2014**, *30*, 2811–2812. [CrossRef]

45. Weir, B.S.; Cockerham, C.C. Estimating F-statistics for the analysis of population structure. *Evolution* **1984**, *38*, 1358–1370.

46. Leviyang, S.; Hamilton, M.B. Properties of Weir and Cockerham's Fst estimators and associated bootstrap confidence intervals. *Theor. Popul. Biol.* **2011**, *79*, 39–52. [CrossRef]

47. Leigh, J.W.; Bryant, D. POPART: Full-feature software for haplotype network construction. *Methods Ecol. Evol.* **2015**, *6*, 1110–1116. [CrossRef]

48. Carranza-Gallego, G.; Guzmán, G.I.; Garcia-Ruíz, R.; Gonzalez de Molina, M.; Aguilera, E. Addressing the role of landraces in the sustainability of Mediterranean agroecosystems. *Sustainability* **2019**, *11*, 6029. [CrossRef]

49. Mascher, M.; Schreiber, M.; Scholz, U.; Graner, A.; Reif, J.C.; Stein, N. Genebank genomics bridges the gap between the conservation of crop diversity and plant breeding. *Nat. Genet.* **2019**, *51*, 1076–1081. [CrossRef] [PubMed]

50.	Raggi, L.; Pacicco, L.C.; Caproni, L.; Álvarez-Muñiz, C.; Annamaa, K.; Barata, A.M.; Negri, V. Analysis of landrace cultivation in Europe: A means to support in situ conservation of crop diversity. *Biol. Conserv.* **2022**, *267*, 109460. [CrossRef]

51.	Porceddu, E.; Vannella, S.; Perrino, P. Character analysis and numerical classification in a wheat collection from Sicily. *Die Kult.* **1981**, *29*, 251–266. [CrossRef]

52.	Laino, P.; Shelton, D.; Finnie, C.; Leonardis, A.M.D.; Mastrangelo, A.M.; Svensson, B.; Lafiandra, D.; Masci, S. Comparative proteome analysis of metabolic proteins from seeds of durum wheat (cv. Svevo) subjected to heat stress. *Proteomics* **2010**, *10*, 2359–2368. [CrossRef]

53.	Taranto, F.; Delvecchio, L.N.; Mangini, G.; Del Faro, L.; Blanco, A.; Pasqualone, A. Molecular and physico-chemical evaluation of enzymatic browning of whole meal and dough in a collection of tetraploid wheats. *J. Cereal Sci.* **2012**, *55*, 405–414. [CrossRef]

54.	Taranto, F.; Mangini, G.; Pasqualone, A.; Gadaleta, A.; Blanco, A. Mapping and allelic variations of Ppo-B1 and Ppo-B2 gene-related polyphenol oxidase activity in durum wheat. *Mol. Breed.* **2015**, *35*, 80. [CrossRef]

55.	Taranto, F.; Mangini, G.; Miazzi, M.M.; Stevanato, P.; De Vita, P. Polyphenol oxidase genes as integral part of the evolutionary history of domesticated tetraploid wheat. *Genomics* **2021**, *113*, 2989–3001. [CrossRef]

56.	Lo Bianco, M.; Siracusa, L.; Dattilo, S.; Venora, G.; Ruberto, G. Phenolic fingerprint of Sicilian modern varieties and old durum wheat landraces: A tool to assess with diversity. *Cereal Chem.* **2017**, *94*, 1045–1051.

57.	Morris, C.F. Determinants of wheat noodle color. *J. Sci. Food Agric.* **2018**, *98*, 5171–5180. [CrossRef]

58.	Siah, S.; Quail, K.J. Factors affecting Asian wheat noodle color and time-dependent discoloration—A review. *Cereal Chem.* **2018**, *95*, 189–205. [CrossRef]

59.	Liu, S.; Sun, Y.; Obadi, M.; Jiang, Y.; Chen, Z.; Jiang, S.; Xu, B. Effects of vacuum mixing and mixing time on the processing quality of noodle dough with high oat flour content. *J. Cereal Sci.* **2020**, *91*, 102885. [CrossRef]

60.	Zhai, S.; He, Z.; Wen, W.; Liu, J.; Jin, H.; Yan, J.; Zhang, P.; Zhang, Y.; Wan, Y.; Xia, X. Genetic architecture of polyphenol oxidase activity in wheat flour by genome-wide association study. *Crop Sci.* **2020**, *60*, 1281–1293. [CrossRef]

61.	Ali, I.; Iqbal, A.; Ullah, S.; Muhammad, I.; Yuan, P.; Zhao, Q.; Yang, M.; Zhang, H.; Huang, M.; Liang, H.; et al. Effects of Biochar Amendment and Nitrogen Fertilizer on RVA Profile and Rice Grain Quality Attributes. *Foods* **2022**, *11*, 625. [CrossRef]

62.	Taranto, F.; Pasqualone, A.; Mangini, G.; Tripodi, P.; Miazzi, M.M.; Pavan, S.; Montemurro, C. Polyphenol oxidases in crops: Biochemical, physiological and genetic aspects. *Int. J. Mol. Sci.* **2017**, *18*, 377. [CrossRef]

63.	Zhang, J.; Sun, X. Recent advances in polyphenol oxidase-mediated plant stress responses. *Phytochem* **2021**, *181*, 112588. [CrossRef]

64.	Singh, S.; Kaur, I.; Kariyat, R. The multifunctional roles of polyphenols in plant-herbivore interactions. *Int. J. Mol. Sci.* **2021**, *22*, 1442. [CrossRef]

65.	Maucieri, C.; Caruso, C.; Bona, S.; Borin, M.; Barbera, A.C.; Cavallaro, V. Influence of salinity and osmotic stress on germination process in an old Sicilian landrace and a modern cultivar of *Triticum durum* Desf. *Cereal Res. Commun.* **2018**, *46*, 253–262. [CrossRef]

66.	Ruisi, P.; Ingraffia, R.; Urso, V.; Giambalvo, D.; Alfonzo, A.; Corona, O.; Frenda, A.S. Influence of grain quality, semolinas and baker's yeast on bread made from old landraces and modern genotypes of Sicilian durum wheat. *Food Res. Int.* **2021**, *140*, 110029. [CrossRef]

67.	Ficco, D.; Beleggia, R.; Pecorella, I.; Giovanniello, V.; Frenda, A.S.; Vita, P.D. Relationship between Seed Morphological Traits and Ash and Mineral Distribution along the Kernel Using Debranning in Durum Wheats from Different Geographic Sites. *Foods* **2020**, *9*, 1523. [CrossRef]

68.	Chai, S.; Yao, Q.; Zhang, X.; Xiao, X.; Fan, X.; Zeng, J.; Sha, L.; Kang, H.; Zhang, H.; Li, J. The semi-dwarfing gene Rht-dp from dwarf polish wheat (*Triticum polonicum* L.) is the "Green Revolution" gene Rht-B1b. *BMC Genom.* **2021**, *22*, 63. [CrossRef] [PubMed]