Assessment of the genetic variation in Ethiopian germplasm of durum wheat from region with contrasting environment and water stress

Mondini L, Farina A, Santangelo E, Pagnotta M.A, Porceddu E
University of Tuscia, Via S. De Lellis, 01100 Viterbo (Italy)

ABSTRACT

Ethiopian durum wheat germplasm provide useful breeding traits, including disease resistance, environmental stability, drought and low temperature stress tolerance. We analyzed a collection of 234 durum wheat genotypes belonging to nine populations from 3 Ethiopian regions (Tigray, Gonder and Shewa) that are distinguished for their climatic conditions. This gene pool was analysed using 28 SSRs markers randomly chosen one for each chromosome arm in order to define: a) population structure, b) genetic variation, c) relationships between and within populations, d) rare or unique genotypes. The results of this study allow us to get information on the genetic structure of the analyzed populations by evaluating the percent of polymorphism (P), number of alleles (A), number of polymorphic allele per locus (Ap), the expected (He) and the observed heterozigosity (Ho). Our results provide information for the identification of more favorable genotypes to tolerate adverse conditions, contributing to safeguard and to evaluate the available germplasm to assist wheat breeders in developing of new promising genotypes.

INTRODUCTION

The regions of the Mediterranean are characterized by the diffused presence of water stress that strongly limits the productions, both in quantitative and qualitative terms, suggesting the necessity to find, and select, genotypes more tolerant to such stress. In particular, Ethiopia includes zones that are suitable for this type of analysis since it is an important centre of diversification for cultivated plants, including tetraploid wheat [*Triticum turgidum* (2n = 4x = 28)][1]; moreover Ethiopian territories vary both in geologic and environmental terms and range from very wet zones to dry ones. This allows a wide genetic pool to be assessed with regard to its adaptation to very different climatic conditions. Farmers in the region have grown wheat since time immemorial, under severe environmental conditions, including i) high elevation with low temperature every night at heading time and seed-setting, which cause pollen sterility and cross pollination; ii) monsoon type rainfall, which causes plants to grown in very low humidity cracked soils. Although the National Durum Wheat Improvement Programme released a substantial number of modern, high yielding durum wheat varieties, modern cultivars are grown in less than 20% of the durum wheat cultivation area, due to difficulties in developing a modern seed market[2], and in farmers' poor purchasing power. Landraces are thus still largely cultivated. One important characteristic of these landraces is their relevant variation for various qualitative and quantitative traits, with the result of a good adaptability to changing environmental conditions. These factors make the Ethiopian durum wheat germplasm extremely interesting for genetic studies and as a source of genes and gene complexes[3,4]. Several studies have been conducted on the Ethiopian durum wheat germplasm, most of them concerned with the variation for morphological traits[2,5]. Molecular markers are particularly suited for population genetic studies and in germplasm characterization[6], since their detection is independent from the environmental conditions under which plants are grown, tissue analysed and developmental stage. In the present work, we analyzed a collection of 234 genotypes belonging to nine populations of durum wheat [*Triticum turgidum* ssp. *durum* (2n = 4x = 28)] from three Ethiopian regions (Tigray, Gonder and Shewa) that are distinguished for the climatic conditions and for the annual precipitations. This gene pool was analysed by 28 SSRs markers randomly chosen one for each chromosome arm.

MATERIALS AND METHODS

DNA was extracted from 234 single plants of *T. durum* belonging to three populations for each of the three Ethiopian regions: Tigray (T), Gonder (G) and Shewa (S). Tigray is a plateau more than 3,000 m asl with less than 900 mm annual rainfall and brown-black soils. Gonder altitude ranges from 1750 to 2050 m asl, with an annual rainfall of 1200 mm in May-Sep and clay soil. Shewa altitude ranges from 1700 to 2500 m asl, it is dry in Oct-Mar and its annual rainfall is about 1000 mm, the soil is highly clay. DNA was amplified with 28 SSR primers randomly chosen one for each chromosome arm (Table 1). Amplifications, using annealing temperature specific for each primer, were run on ABI 3130xl sequencer. Statistical Analyses were performed by utilizing the softwares Arlequin[7]ver3.11, GDA[8] Ver1.1 and GenAlEx[9] ver 6.

RESULTS AND DISCUSSION

The 28 SSR markers have been used to evaluate the percent of polymorphism (P), number of alleles (A), number of polymorphic allele per locus (Ap), the expected (He) and the observed heterozygosity (Ho) (Table 2).

Table 1. Primers utilized for linkage disequilibrium analysis on 9 populations of durum wheat from 3 Ethiopian regions.

Primer	Chrom.loc	Primer	Chrom.loc
Wms24	1AS	CFA2091	4BS
Barc213	1AL	Wms375	4BL
Barc8	1BS	Wms205	5AS
Wms124	1BL	Wms291	5AL
Wms177	2AS	Wms159	5BS
Wms170	2AL	Wms371	5BL
CFA2278	2BS	Wms459	6AS
Wms 120	2BL	Wms256	6AL
Wmc532	3AS	Wms518	6BS
Wms5	3AL	Wms219	6BL
Wms493	3BS	CFA2049	7AS
Wms181	3BL	Wms260	7AL
Wmc516	4AS	Wms46	7BS
Wms610	4AL	Wms302	7BL

These results underline the great diversity existing in Ethiopian germplasm, which has populations not clearly different one from the other. The level of polymorphism is very high and quite homogeneous among the populations (Table 3). The higher level of diversity was found within population (about 74.6%) while about 5.6% was attributed to differences between regions. The great variation within population is probably also due to the high level of out-crossing, as result of cold temperatures during seed setting. The level of expected heterozygosity was, on average 51%, ranging from 42% to 58% in different populations, while the observed heterozigosity amounted, on average, to 15%.

Table 2. AMoVa on 9 populations from 3 Ethiopian regions analyzed with 28 SSR markers localized in each chromosome arm.

Source of variation	d.f	SS	Variance component	%of variance
Among regions	2	390.37	0.54**	5.55
Among pop. within	6	669.91	1.92***	19.87
Within populations	459	3254.05	7.19***	74.58
Total	467	4314.83	9.64	

About 5 alleles per locus have been found in each population. Even if marker WMS 610 (chrom. 4AL) has detected 55 different alleles, marker WMS 302 has detected 62 alleles, marker WMS 291 have detected only 3 alleles each. These alleles are not equally present in the populations as shown by the high level of expected heterozigosity for the 5 alleles present with an equal frequency.

Table 3. Polymorphism (P), alleles (A), expected (He) and observed (Ho) heterozygosity in 9 population of durum wheat from Ethiopia.

Population	N	P	A	He	Ho
S7441	29	0.89	5.11	0.47	0.13
S7761	28	0.89	4.86	0.42	0.12
S7766	28	1.00	5.64	0.48	0.15
T7755	30	0.96	5.18	0.52	0.16
T7806	23	0.96	5.79	0.56	0.18
T7814	26	0.93	5.25	0.54	0.17
G7824	23	0.96	5.75	0.58	0.15
G7827	24	0.96	5.21	0.50	0.15
G7831	23	0.89	5.29	0.51	0.16
Mean	26	0.94	5.34	0.51	0.15

Furthermore, the presence and frequency of rare or unique alleles, helps define particular genetic configurations linked to extreme conditions and in the possible location of a more favorable genetic equipment to tolerate such conditions. The likelihood relations among the 9 Ethiopian populations (Figure 1) indicate that the material collected from the Gonder region is genetically more distant than the materials from the Shewa and Tigray regions between which intermixing has occurred.

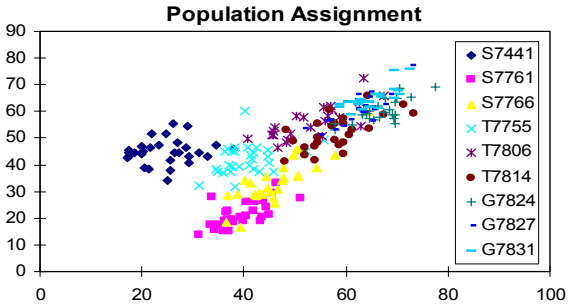
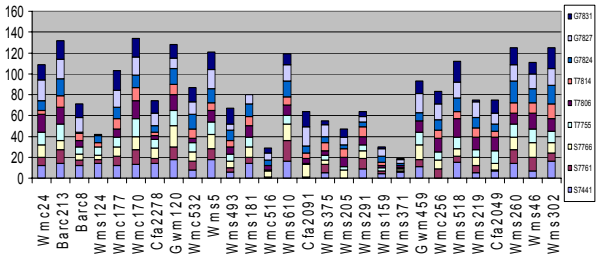


Figure 1. Likelihood relations among the 9 Ethiopian populations.

In spite of being located on the different arms of the 14 durum wheat chromosomes, the 28 analyzed loci were non-randomly associated as shown in Fig. 2, which shows the number of loci per population in linkage disequilibrium with the locus indicated. Each bar height represents the number of loci in linkage with the considered locus. Different colours in the bars correspond to different populations. Locus WMC170 on chromosome 2AL had widest linkage, being linked to 13 loci in population S7441, 14 loci in S7761, 13 in S7766, 17 in T7755, 17 loci in T7806, 13 in T7814, 12 loci in G7824, 17 loci in G7827, and 18 loci in population G7831. Conversely locus WMS371 on chromosome 5BL had the lower LD values; it was linked on average only with 2.1 loci per population (ranging from 0 in T7814 to 6 loci in S7441). The LD among 21 loci was the maximum number of loci linked to locus WMS260 in population G7824. Populations from Gonder were the ones with the highest number of loci in LD; among them population G7827 had more than 12 loci in LD per locus. On the other hand, the population T7814 from Tigray had the lowest number of loci in LD, 7 loci in LD per locus. Populations with the highest number of loci (3) with no LD were T7814 (Tigray) and S7761 (Shewa). Only WMS124 in population T7806; WMS124 and WMS181 in population G7831; WMC516, WMS371 and GWM459 in population T7814;

WMC516, WMS205 and CFA2091 in population S7761; WMS205 and WMC256 in population S7441; WMS159 in population S7766 were randomly associated. The number of haplotypes ranged from 46 in populations T7806, G7824 and G7831, to 60 in population T7755. Haplotypes were peculiar for one population, as a result of the high variation present in this germplasm.

Figure 2. Linkage disequilibrium for each of the 28 loci analysed and for each population of the three regions Tigray, Shewa and Gonder.



The high number of loci in linkage disequilibrium, even if located on different chromosome/arms, demonstrate that the loci are associated irrespective of their physical location. As reported also by Goldstein and Weale[10] and by Evans and Cardon[11] the situation detected in one population may not be transposed to other populations since the LD can differ markedly among populations. Moreover, the linkage disequilibrium can extend over much larger genomic regions than expected. In any event, linkage, relatedness, population stratification, and genetic drift are all important forces generating and conserving LD[12]. Linkage disequilibrium has become important in the context of gene mapping; comprehension of the pattern of association between alleles at different loci is useful for considering the underlying genealogy of the chromosomes[13]. A high level of LD indicates a reduction in population effective size (Ne) with a consequent population bottleneck, founder effect or genetic drift[14,15]. Hence, in the present case, the populations from Gonder had a lower Ne compared with the other populations examined. On the other hand, population T7814 from Tigray should have the higher effective population size (Ne) and the lower drift.

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