

Ethiopian durum wheat germplasm and hitchhiking mapping for drought QTL

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Nowadays, several wheat genetic maps are available, so it is possible to select several loci along a chromosome to assess their distribution of variability, and to find out if selective sweeps are present. In fact, if a locus is under selection pressure, its variability is reduced due to the fixation of single positive allele, and so are the loci near the selected ones due to their linkage. The neutral variants that are linked to the beneficial mutations are also affected by selective sweeps. Ethiopia is an important centre of diversification for different crops, including tetraploid wheat (*Triticum turgidum*), whose variation could provide useful breeding traits, including, environmental stability, drought and low temperature stress tolerance. We have analyzed a durum wheat collection of 234 genotypes from nine populations of Ethiopian regions with contrasting environments, with 22 SSR loci localized along chromosome 4 to identify the hitchhiking mapping of this chromosome and detect QTLs for drought tolerance.

INTRODUCTION

Ethiopia is an important centre of diversification for cultivated plants, including tetraploid wheat [*Triticum turgidum* ($2n = 4x = 28$)]¹. Here farmers grow it since time immemorial, under severe environmental conditions, including i) high elevation with low temperature every night during 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. 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 conditions². 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 traits⁵ and isozymes⁶. Microsatellites are abundant and highly polymorphic molecular markers that are scattered over the euchromatic part of the genome and are also particularly suited for population genetic studies and germplasm characterization⁷, since their detection is independent from the environmental conditions under which plants are grown, tissue analysed and developmental stage. Developing of genetic linkage maps⁸ using molecular markers are an important tool for several purposes such as the identification of the genetic determinants underlying the expression of agronomically important quantitative traits, facilitating marker-assisted breeding and map-based cloning⁹. In durum wheat, several genetic maps have been produced by analyzing recombinant lines segregating from intra- or intra-specific crosses⁹, making possible to come closer to genes controlling complex traits.

Alternative approaches¹⁰ include the use of natural populations, whose diversity, also in self-pollinated plants, is the result of rare crosses between lines, followed by some fine recombination events. Hitchhiking mapping is an approach that relies on population genetic principles to identify beneficial mutations from patterns of natural variation¹¹. The basic idea of hitchhiking mapping is that beneficial mutations increase in frequency until they become fixed in the population. Hereby, not only the selected site but also linked neutral variants are affected by such selective sweep¹². As a consequence of the spread of a beneficial mutation, levels of variability are strongly reduced in the genomic region flanking the selected site. Hence, hitchhiking mapping does not require the exact location of the selected site to be known; it is sufficient to analyze linked neutral markers¹³. This approach has gained widespread interest and studies have set out to survey genome wide levels of genetic variability to trace regions in the genome that may have been shaped by natural selection.

Selected loci will show a marked reduction in variation, as will occur to closely linked loci, as a result of hitchhiking effect^{14,11}. The extent of this latter is, however, variable, depending on mating system and sample size. The procedure is quite evident when dealing with simple characters. However, NUZHDIIN *et al.*¹⁵ have demonstrated that it can be utilized also for complex traits, since experimental selection modified frequency of the concerned alleles. Here, we report the results in relation to a durum wheat collection of 234 genotypes from nine populations of three Ethiopian regions, Tigray, Gonder and Shewa, with contrasting environments. In particular, 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. These populations were analysed by 22 SSR loci on chromosome 4 to identify the presence of selective sweeps due to selective pressure in the particular climatic conditions of Ethiopian territory, which is always from a natural laboratory for the study of the genetic variability and for the selection of genotypes with particular climatic adaptations. Chromosome 4 was chosen because of its involvement in drought tolerance as reported by some previous analyses¹⁶.

MATERIALS AND METHODS

DNA was extracted from 234 single plants of *T. durum* belonging to three populations for each of the three Ethiopian regions and amplified with 18 SSR primers (i.e. WMC516, BARC106, GPW2283, WMS610, GPW1010, GPW2140, GPW2138, GPW2279, WMS269, BARC343, WMC468, GPW2244, CFD257, WMS637, GPW2228, CFD88, GPW356, BARC78, BARC153) located on chromosome 4A (Table 1). SSR amplification was done with a sequence-specific forward primer containing a M13 tail at its 5' end, a sequence-specific reverse primer, and a universal fluorescent-labelled M13 primer. The thermocycling conditions are chosen such that during the first cycles, the forward primer with its M13 sequence is incorporated into the accumulating PCR products. Later, the temperature is lowered to facilitate the annealing of the universal M13 primer at 5' end of forward primer with complementary sequence. Thus, the fluorescence was incorporated on PCR product¹⁷. The fluorescent dye-labelled products of PCR were run on ABI-PRISM 3130xl genetic analyzer, and fragments analyzed with Gene Mapping software. Statistical analyses were performed by the softwares Arlequin ver 3.11¹⁸, GDA Ver 1.1¹⁹ and GenAlEx ver 6²⁰.

LOCUS	cM	LOCUS	cM
WMC 516	38	WMC 468	88
BARC 106	38	GPW 2244	91
GPW 2283	46	CFD 257	92
WMS 610	51	WMS 637	94
GPW 1010	54	GPW 2228	97
GPW 2140	56	CFD 88	108
GPW 2138	71	GPW 356	121
GPW 2279	74	BARC 78	139
WMS 269	76	BARC 153	195

Table 1. SSR markers with their position along chromosome 4A of durum wheat.

RESULTS

The hitchhiking maps, for each of the three populations of Tigray (Figure 1A), Shewa (Figure 1B) and Gonder (Figure 1C), were build up on the base of the variability detected at each of the 18 loci (y axis) and their position on chromosome 4 (x axis). Some points of the hitchhiking maps are consistent among the populations within region, whereas others are not. Populations from Shewa (Fig. 1B) showed a very uniform pattern, except at the proximal part of chromosome and around locus WMS637; all of them had a high reduction in variation around loci GPW2140 and GPW2279 (at about 70cM) and around locus WMS637 (at about 94cM). The three populations from Tigray showed limited variation on regions labelled by markers GPW2140, GPW2279, GPW2244 (about 56, 74 and 91 cM). Two populations (T7814 and T7755) had limited variation also close to locus CFD257 (about 94 cM). Conversely, a very high variation was detected at loci BARC106, CPW2283, WMS610, GPW1010, WMS269, GPW356 and BARC78. Populations from Gonder were each other less consistent, but all of them had limited variation

around markers GPW2140 and GPW2279. Population G7831 had very low variation also around locus WMS637. On overall, the results indicated the presence of two main selective sweeps, at about 56-76 cM and 91 cM. The first sweep was present across regions and populations; whereas the second one was present in Shewa populations, and on a lower extend in the ones from Tigray, and in population G7831 from the Gonder region.

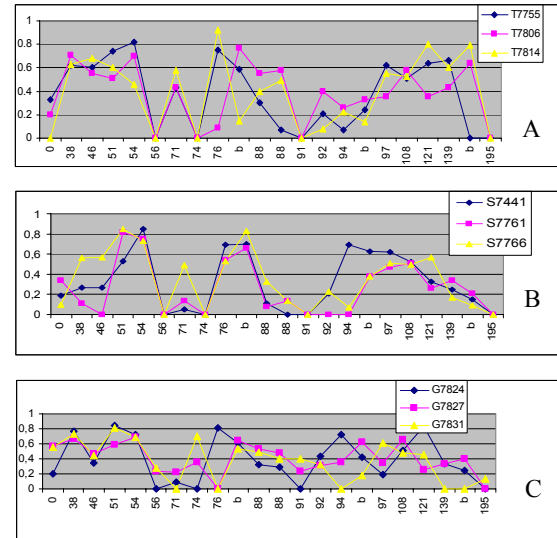


Figure 1. Relative variability estimates in regions Tigray (A), Shewa (B) and Gonder (C) affected by a selective sweep. Location of microsatellite loci in cM (see Table 1 for locus name) on 4A (x axis) their respective variability (y axis).

DISCUSSION

The presence of selective sweeps increases the correlation among alleles at loci close to the selected site, producing large chromosome regions uniform over populations¹⁴. The selective sweep located at about 70 cM on the short arm of chromosome 4 is one of these. The fact that the reduction in variation is localized close to the position previously identified as QTL for drought tolerance¹⁶ enforces the idea that genes involved in drought tolerance may be located there. Demonstration that, as reported by NUZHIDIN *et al.*¹⁵, the hitchhiking map obtained looks at the decrease of variation due to selective sweeps could be effectively used to identify regions where important traits are coded. However, the other main selective sweep, localized at 90 cM on the 4AL, could be due to drought too. In addition, it could also be due to other environmental factors, such as cold resistance or biotic stress, which are common in some particular population. In fact, the situation is not always consistent in all the populations of the same region as the case of Gonder. Moreover, this selective sweep is wider in populations S7761 and S7766, indicating a stronger selection, which decrease the variation in a wider area of the chromosome from about 90 to about 95 cM. Hence, the drought

tolerance could account for the selective sweep, located at 90 cM, but the involvement of other environmental factors, such as cold tolerance cannot be ruled out. The involvement of biotic stress variation such as rust and/or mildew resistance is not so foreseeable because the high altitude of the growth environment usually prevents the growth of those pathogens.

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