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Intra-specific genetic structure of durum wheat based on SSR and SNP markers

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Introduction

Triticum turgidum sb. *durum* is a tetraploid specie (2n=28, genomes AABB) and with about 21.0 million hectares under cultivation (about 8% of the total wheat cultivated area), durum wheat ranks eighth among all cereals. Nowadays several technologies are available to increase the abundance of DNA markers and to contribute in developing high resolution genetic maps suitable for genetic analysis and to perform marker assistance selection to breeding activities. The main objective of this study consists in increase the number of available Simple Sequence Repeat (SSR) and Single Nucleotide Polymorphism (SNP) markers on wheat array, to determine their chromosomal locations mapping them in durum wheat genome and to evaluate the segregation of the different markers in relation to their chromosome and genome position (i.e. A versus B genome). In present work, the choice of markers to be use for designing genetic maps fall on SSR markers, being highly dispersed in the genomes.

Materials and methods

A genetic linkage map of tetraploid wheat (*Triticum turgidum* L. ssp. *durum* (Desf.) Husn.) is under construction starting from an intraspecific cross between two Recombinant Inbred Lines (RILs): one deriving from the cross of *Jennah Khetifa* x *ChamI* (J.K x C), the other from the cross [*Omrabi* x *Dicoccoides*] x *Omrabi* ([O x D] x O). The choice of these lines is due to the fact that both show contrasting morpho-physiological characters. After cross the resulted progeny has been multiplied by single seed descent procedures and DNA extracted from young leaves of the 192 F8 RILs.

Initially, a screening of the different markers was performed in order to test potential polymorphisms between the parents and in order to select just the ones resulting polymorphic. PCR reactions have been performed in a final volume of 10µl containing 10ng of DNA, 0.3µM primer, 100µM dNTP, 10mM of PCR reaction buffer and 1U of Taq polymerase. The amplification regime was 94°C for 2min, followed by 36 cycles of 96°C for 1 min, an annealing step with a temperature ranging from 50° to 65°C in accord with the Tm°C (melting temperature) of the different primers for 40sec and 72°C for 2min followed by a final extension of 72°C for 10 min. For every forward primer of all SSR markers it has been added a M13 tail of 20bp complementary to an additional primer labeled with one of the following fluorochrome: FAM, VIC, PET or NED. PCR amplicons were resolved in an ABI 3130xl sequencer machine. The SSR fragments considered, showed a length falling in a range of 150–350 bp.