Development of a Microsatellite Map as a Prerequisite for Mapping QTL for Heterosis in Rapeseed (*Brassica napus* L.)

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Introduction

Microsatellites, called also simple sequence repeats (SSR), were discovered in 1989 by Weber & May and Litt & Luty in animals. They are characterised by high levels of polymorphisms due to variations in the number of tandem repeats, abundance and even distribution across the genome. These particularities make microsatellites DNA markers of great interest and determine their wide use in the genetic studies of humans, animals and plants. In plant breeding they find numerous applications, including marker assisted selection, gene tagging, genome mapping, study of genetic diversity, gene flow determination etc.
A prerequisite for many of these application is a SSR- linkage map of the organism under study. Currently we are developing a genetic map of rapeseed comprised up to now of 114 SSR markers, derived from 98 primer pairs. It will be further saturated with 100 additional SSR markers. This map will be used for the identification and characterisation of QTL, involved in the heterosis in rapeseed.

Materials and Methods

Plant Material

For the construction of the SSR map a segregating DH population was developed from microspores of a single F_1 plant from a cross between an inbred line of the winter rapeseed variety ‘Express’ and a resynthesized rapeseed ‘R53’ (**Fig. 1**). The F_1 hybrids of this cross had shown a high level of heterosis for seed yield in previous studies (Girke, 2002), making the derived DH population especially suitable for an analysis of heterosis at the QTL level.

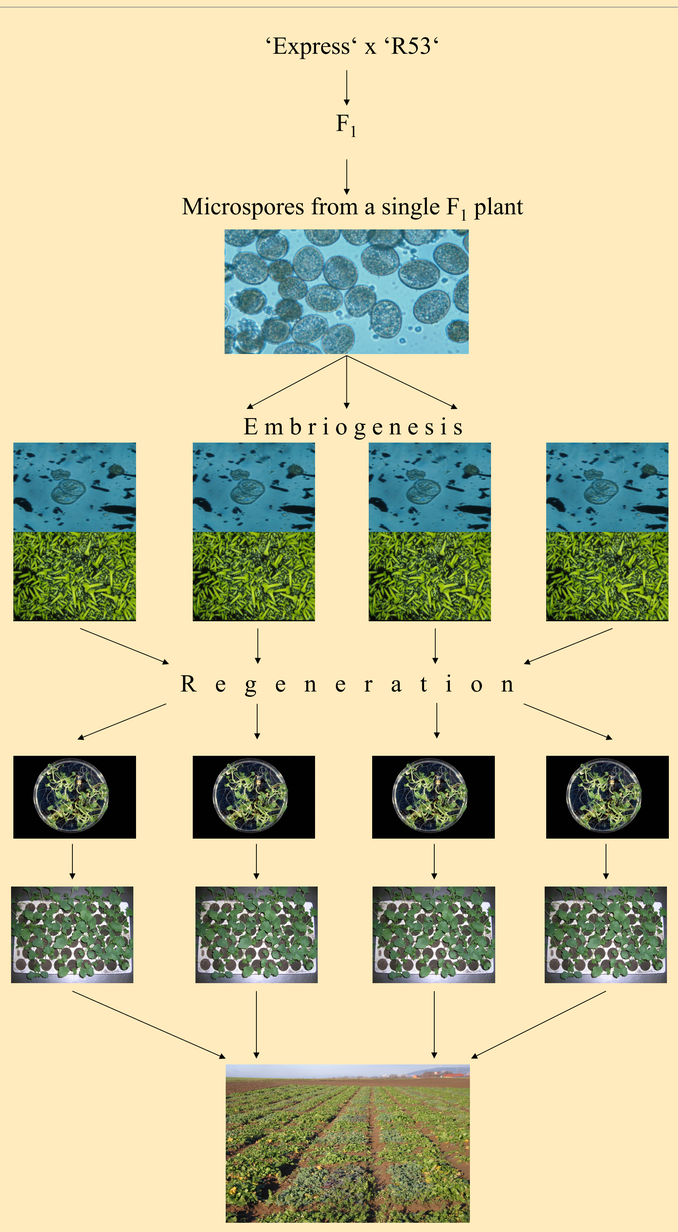

Fig 1: Development of a DH mapping population

Linkage analysis

Two-, three- and multipoint linkage analysis was performed using the Mapmaker 3.0 program. To identify linked markers a minimum LOD score threshold of 4.0 and and maximum recombination frequency of 0.4 were applied.

Results and Discussion

Marker Screening

For map development a total of 524 SSR primer pairs were available at Göttingen. To identify polymorphic markers all of the primer pairs were screened with the parental lines ‘Express’, ‘R53’ and ‘V8’ the male parent of a DH mapping population used in Gießen in a parallel project. From the 524 SSR primer pairs screened, 375 gave clearly scorable banding patterns. 217 from them showed polymorphisms resulting in 260 markers. 81 of the primer pairs showed also polymorphisms in the mapping population in Gießen, representing 87 shared markers - a good base for an alignment of the two maps (**Table 1**).

Table 1: Screening of 375 SSR primer pairs – number of polymorphic primer pairs and putative markers

No of	In individual populations		Shared between populations
	A Express x R53	B Express x V8	A & B
Polym. Primer pairs	217	119	81
Markers	260	134	87

The SSR map

A preliminary genetic map, derived from the segregation data of 114 SSR markers in a DH population of 96 lines is presented in **Fig. 2**. Seven markers could not be linked to any of the other markers. The length of the map is 986,8 cM and it is comprised of 19 linkage groups which is in accordance with the known number of chromosomes in rapeseed.

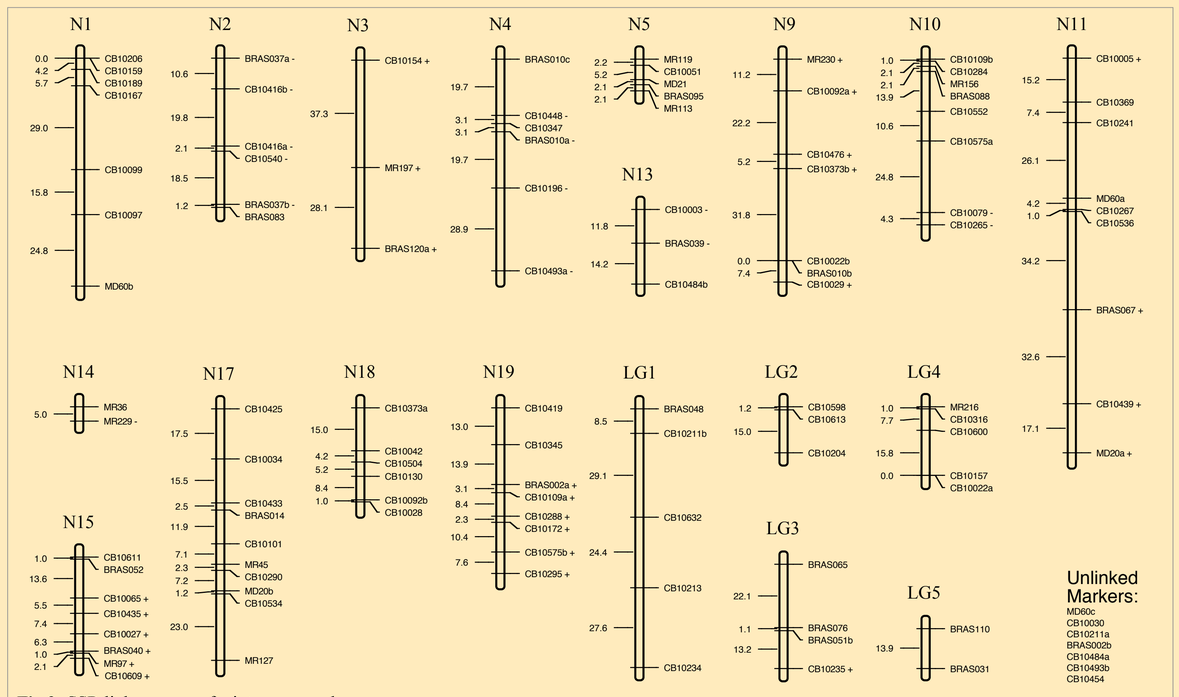

Fig 2: SSR linkage map of winter rapeseed

Distances between markers are in cM, calculated from recombination frequencies acording to the Kosambi mapping function. „+“ and „–“ indicate markers showing statistically significant deviation ($p \leq 0,05$) from the expected 1 : 1 segregation in favour of „Express“ and „R 53“ alleles, respectively

Disturbed Segregation

Out of the 114 markers analysed 39 (34%) showed a significant ($p \leq 0.05$) deviation from the segregation ratio expected in a DH population. From the disturbed segregations 25 markers are in favour of the alleles from ‘Express’ and 14 of the alleles inherited from ‘R53’.
The markers showing skewed segregations are not randomly distributed throughout the genome. They are clustered in distinctive groups. Such clusters are found on 8 linkage groups (N2, N3, N4, N9, N11, N13, N15, N19). Disturbed segregations are a common feature of microspore derived DH populations (Fiosset et al. 1993, Uzunova et al. 1995). In most cases such disturbances are not observed in the corresponding F2 or back cross generations, indicating that they are a result of selection interfering with in vitro androgenesis and/or plant regeneration. The favourable alleles of five of these factors were inherited from ‘Express’, while the other three originate from ‘R53’.

References:

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Uzunova M., W. Ecke, K. Weissleder and G. Röbbelen (1995) Mapping the genome of rapeseed (*Brassica napus* L.). I. Construction of an RFLP linkage map and localization of QTLs for seed glucosinolate content. Theor Appl Genet 90: 194-204
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