

Short communication

A CONSENSUS MAP OF CHROMOSOME 6R IN RYE (*Secale cereale* L.)

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Abstract: Four F₂ mapping populations derived from crosses between rye inbred lines DS2×RXL10, 541×Ot1-3, S120×S76 and 544×Ot0-20 were used to develop a consensus map of chromosome 6R. Thirteen marker loci that were polymorphic in more than one mapping population constituted the basis for the alignment of the four maps using the JoinMap v. 3.0 software package. The consensus map consists of 104 molecular marker loci including RFLPs, RAPDs, AFLPs, SSRs, ISSRs, SCARs, STSs and isozymes. The average distance between the marker loci is 1.3 cM, and the total map length is 135.5 cM. This consensus map may be used as a source of molecular markers for the rapid development of new maps of chromosome 6R in any mapping population.

Key words: Rye, Consensus map, Molecular markers

INTRODUCTION

Rye (*Secale cereale* L.) is one of the most important cereal crops for Central and Eastern Europe. Substantial progress has been made in improving rye yield over the last two decades, mainly due to the breeding of modern populational and hybrid cultivars. However, this progress has been less efficient than the work done with wheat varieties, meaning that rye breeding needs further study [1].

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Abbreviations used: AFLP – amplified fragment length polymorphism; cM – centimorgan; ISSR – inter-simple sequence repeat; LOD – logarithm of odds; QTL – quantitative trait locus; RAPD – random amplified polymorphic DNA; RFLP – restriction fragment length polymorphism; SCAR – sequence-characterized amplified region; SSR – simple sequence repeat; STS – sequence-tagged site

One of the possible ways of enhancing breeding efficiency is the application of molecular markers as selection tools. Over the past 15 years, a large number of molecular markers for rye have been elucidated by constructing molecular maps [2-13]. Maps based on molecular markers are also suitable tools for mapping the genes and QTLs underlying agronomic traits [14-19]. The low level of polymorphism of some PCR-based markers often hampers their application in a wider range of plant material, especially in marker-assisted selection within breeding populations. It seems that the construction of consensus genetic maps containing integrated linkage data from different mapping populations can increase the availability of polymorphic markers for future studies carried out on a variety of plant materials. Consensus linkage maps are being constructed for major crops like wheat [20] and barley [21, 22]. There are no reports on the development of consensus genetic maps of rye. The objective of this study was to develop a consensus linkage map of chromosome 6R using four genetically different mapping populations of rye.

MATERIALS AND METHODS

Segregation data from four F₂ mapping populations, each consisting of 94 individuals, was used to develop the consensus linkage map of chromosome 6R. The mapping populations were derived from crosses between advanced, homozygous and homogeneous inbred lines with known pedigree data [23]. The genetic maps of the DS2×RXL10 and 541×Ot1-3 populations had been constructed using MapMaker v. 3.0 software and published earlier [2, 13]. As a reference map for this study, we used the DS2×RXL10 linkage map of chromosome 6R developed by Devos *et al.* [2] using RFLP markers, and saturated by Masojć *et al.* [24] and Bednarek *et al.* [25] using RAPD and AFLP markers. The maps for two consecutive interline crosses (S120×S76 and 544×Ot0-20) were developed using RAPD (random amplified polymorphic DNA), ISSR (inter-simple sequence repeat), SCAR (sequence-characterized amplified region), STS (sequence-tagged site) and SSR (simple sequence repeat) markers. The methods used for the RAPD, ISSR, SCAR and STS analyses were described previously [13, 24, 26, 27]. Polymorphisms in the microsatellite markers (SSR-s) developed by Hackauf and Wehling [11] were analyzed using a modified version of the method given by Khlestkina *et al.* [12].

The PCR mixture (10 µl) contained a reaction buffer (70 mM Tris-HCl, pH 8.3, 16.6 mM ammonium sulphate, 2.5 mM MgCl₂), 0.5 pmol of the forward primer (with an M13 17-bp tail), 2.5 pmol of the reverse primer, 1.5 pmol BigDye modified M13 primer, 0.5 U hot-start Taq-polymerase (Viva Taq-polymerase from Novazym, Poznań, Poland), 140 µM of each deoxynucleotide, and 15-20 ng of template DNA. The reactions were carried out in an Eppendorf Mastercycler Gradient, according to the protocol: initial denaturation at 95°C for 15 min; five cycles of 30 s at 94°C, 1 min at 60°C (-1°C per cycle), and 1 min at 72°C; a further five cycles of 30 s at 94°C, 1 min at 68°C (-1°C per cycle), and 1 min at

72°C; followed by 28 cycles of 94°C for 30 s, 48°C for 45 s, and 72°C for 1 min. The reaction mixtures were kept at 72°C for 10 min for the final elongation step. The PCR products obtained from three separate reactions using different dye-modified M13 primers were mixed and separated with the addition of CEQ DNA Standard 400 in an automated CEQ8000 sequencer (Beckman-Coulter). The JoinMap v. 3.0 [28] was used to analyze the segregation data from all four mapping populations at a critical LOD value of 3.0. For markers with a known map location, the “fixed order” command was applied.

RESULTS

The reference DS2×RXL10 map developed using the JoinMap software (Fig. 1) consists of 17 RFLP, 11 RAPD, 10 AFLP, 2 SSR, 2 ISSR, 1 SCAR and 4 isozyme markers, giving a total of 47 markers distributed along the 6R chromosome and covering a distance of about 165 cM (Fig. 1). The second map for the 541×Ot1-3 cross consists of 20 marker loci: 9 RAPD, 6 SSR, 1 SCAR, 3 STS (transformed from RFLP markers) and 1 isozyme. This 143-cM long linkage map shares six common markers with the DS2×RXL10 reference map, namely *Xscm176* (SSR), *Xscm078* (SSR), *Amy1-1* (isozyme), *psr106* (RFLP/STS), *psr454L1030* (RFLP/STS) and *SCSz980L650B* (SCAR), distributed in a similar order.

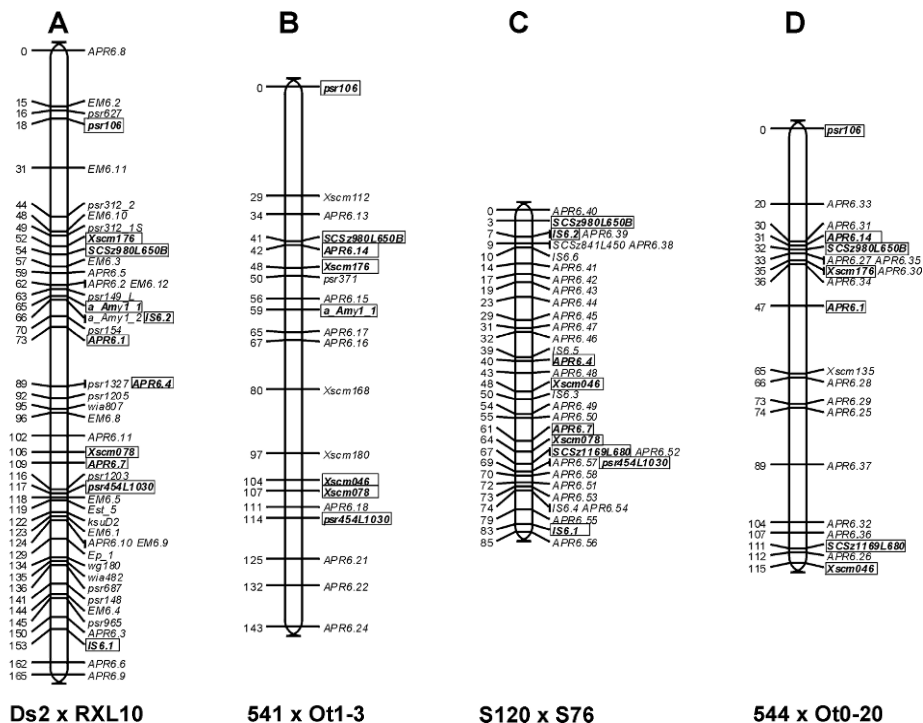


Fig. 1. The alignment of the four genetic maps of chromosome 6R using common molecular markers.

Tab. 1. Coding of the RAPD, ISSR and STS markers.

Mapping population	Marker code	Primer sequence	Marker length (bp)	
544×Ot0-20	<i>APR6.25</i>	CTCGAGGTAA	390	
	<i>APR6.26</i>	ACCTCAGCGT	550	
	<i>APR6.27</i>	GTAGCCGTCT	700	
	<i>APR6.28</i>	AGGAGACTGG	260	
	<i>APR6.29</i>	CCGTTCCAAA	900	
	<i>APR6.30</i>	AACCCACCCG	650	
	<i>APR6.31</i>	GCGCGATAAG	600	
	<i>APR6.32</i>	CGTGATAAGG	670	
	<i>APR6.33</i>	CCCAAGTCAT	1300	
	<i>APR6.34</i>	ATGTGTGTGG	530	
	<i>APR6.35</i>	GGAGTGTGTA	1000	
	<i>APR6.36</i>	GGAGTGTGTA	750	
	<i>APR6.37</i>	CTGTAGCCGG	750	
	<i>APR6.38</i>	ACCCCCACG	620	
	<i>APR6.39</i>	CTGAGACGGA	480	
	<i>APR6.40</i>	ACCTCCAGCG	1030	
	<i>APR6.41</i>	CCGCCTAGTC	900	
	<i>APR6.42</i>	CCTCTGCCCA	650	
	<i>APR6.43</i>	CGCCCCCAGC	430	
	<i>APR6.44</i>	CAAGCGGATC	950	
	<i>APR6.45</i>	CAGCCTACAG	600	
	<i>APR6.46</i>	GTCCGTTGGG	650	
	<i>APR6.47</i>	GACAAAGAGG	550	
	<i>APR6.48</i>	AAGCCTCGTC	1700	
	<i>APR6.49</i>	CAGTCGCGTG	1400	
	S120×S76	<i>APR6.50</i>	TGTCCAGCTT	710
		<i>APR6.51</i>	GCCATAAGCG	920
		<i>APR6.52</i>	ACGAAGCTAA	500
		<i>APR6.53</i>	CCACGCTATA	600
<i>APR6.54</i>		ACATGTTCCGG	1700	
<i>APR6.55</i>		CCTCTCGCAA	410	
<i>APR6.56</i>		CGGCCACGT	690	
<i>APR6.57</i>		TGCAGTTAGT	730	
<i>APR6.58</i>		GGGTAACGCA	1100	
		<i>IS6.3</i>	(GA) ₉ A	890
	<i>IS5.4</i>	(GA) ₉ T	400	
	<i>IS6.5</i>	(AC) ₈ T	610	
	<i>IS6.6</i>	(TC) ₈ (Y)G	630	
All populations	<i>SCSz980L650B</i>	<i>F*</i> ACGATCGCTCCGATAAATAG <i>R*</i> ACGATCGCTCATAGAATCCA	650	
S120×S76	<i>SCSz1169L680</i>	<i>F*</i> AAACTGAGCCCAGAGACGAA <i>R*</i> AAACTGAGCCGGCATATCAT	680	
544×Ot0-20				
DS2×RXL10	<i>psr106</i>	<i>F*</i> AGCGGAGTTCATCTCATTCAA <i>R*</i> CGTCGTCAAGAACATCGAGA	350	
541×Ot1-3				
544×Ot0-20				
541×Ot1-3	<i>psr371</i>	<i>F*</i> CTCGTGAAGAGCCAGGTGAT <i>R*</i> CACGGTGGAGAAGATGATGA	700	
DS2×RXL10				
541×Ot1-3	<i>psr454L1030</i>	<i>F*</i> TGTCCCCTCTCTATCGTG <i>R*</i> ATGCAGCGATCACCACAT	1030	
S120×S76				

*F – forward primer, R – reverse primer.

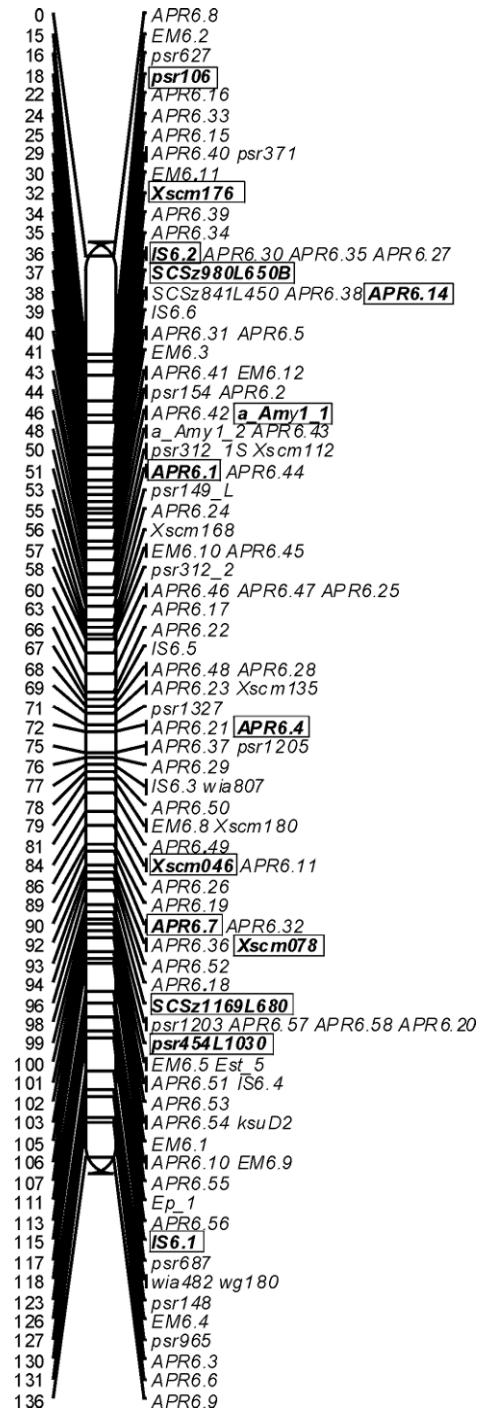


Fig. 2. A consensus map of chromosome 6R developed using the JoinMap v. 3.0 software and four sets of F_2 segregation data.

The third map, of the S120×S76 F₂ intercross, spans a distance of 85 cM, and contains 35 marker loci: 23 RAPD, 6 ISSR, 2 SSR, 3 SCAR and 1 STS. It shares three common sequence-specific markers with the reference map (*SCSz980L650B*, *psr454L1030*, *Xscm078*) and four common markers (*Xscm046*, *Xscm078*, *SCSz980L650B* and *psr454L1030*) with the 541×Ot1-3 map.

The fourth map developed on the 544×Ot0-20 F₂ intercross has a total of 21 marker loci: 15 RAPD, 3 SSR, 2 SCAR and 1 STS. The length of the chromosome 6R map exceeds 110 cM. This linkage group has one SCAR marker present in all four maps (*SCSz980L650B*). The next SCAR marker (*SCSz1169L680*) is also present in the S120×S76 map, and two SSR markers (*Xscm176* and *Xscm046*) are shared with the 541×Ot1-3 map. The only STS marker located on this map (*psr106*) is also mapped in the DS2×RXL10 and 541×Ot1-3 populations.

In total, the newly developed genetic maps of the S120×S76 and 544×Ot0-20 intercrosses contain 34 new RAPD and 4 ISSR loci (Tab. 1), expanding the database of molecular markers from chromosome 6R. These markers, after conversion into SCAR markers, should be very useful for further genomic studies and marker-assisted selection in rye. Besides the sequence-specific markers common for the different maps, 4 RAPD and 2 ISSR loci were simultaneously mapped on at least two studied populations. This set of markers mapped on chromosome 6R using different mapping populations allowed us to construct the consensus map of chromosome 6R (Fig. 2).

The consensus map contains 104 marker loci: 15 RFLP, 56 RAPD, 10 AFLP, 6 ISSR, 7 SSR, 3 SCAR, 3 STS (2 of them mapped as RFLP markers on the reference map) and 4 isozyme. As these markers are distributed across a distance of over 135 cM, the average space between loci on this map is less than 1.3 cM, and there is only one significant gap of 15 cM on the distal region of the short arm. Of the 14 common loci, 7, 6 and 1 were mapped in 2, 3 and 4 populations, respectively. The 104 known markers actually located on 6R should be sufficient to build at least a primary low-density map of this chromosome (10-20 loci) on any newly developed mapping population assuming low transferability of the markers.

DISCUSSION

The first series of genetic maps of rye chromosomes was based mainly on the restriction fragment length polymorphism (RFLP) technique [2-4, 6, 10]. In the last decade, the sequence-specific PCR markers (SSR, STS, SCAR) for the rye genome became more available for map construction. Microsatellites seem to especially be useful for this purpose [11, 12].

In our study, sequence-specific markers were applied to establish maps for all four analyzed mapping populations, but above all they allowed for the integration of the segregation data into one consensus map of chromosome 6R. However, the number of markers with a known sequence is still low in rye,

therefore applying such PCR-based techniques as RAPD, AFLP and ISSR for developing saturated maps is essential.

To the best of our knowledge, the presented consensus map of chromosome 6R is currently the most saturated among the published maps of individual rye chromosomes. The order of RFLP and sequence-specific PCR-based markers on the consensus map is generally consistent with formerly published linkage groups [11, 13].

We noticed some disagreements in the order of closely linked markers between the individual maps. The same was observed in consensus maps of others species, like grapevine [29], wheat [20], barley [21] and rye [6]. A local order inconsistency could be partially attributed to the sampling bias, mainly in small mapping populations, which is rather not a concern in our study. Another reason is the discrepancy in the recombination rate in different populations, while the most probable cause is imprecision of the mapping process due to a deficiency in the mapping program. Barley map researchers [21 and references therein] found that Joinmap v. 3.0 reproducibly generated erroneous results with high-density data sets. However, the most commonly observed discrepancies in marker order were found over short intervals, which should not be of great importance in making plant selections [20].

Chromosome 6R contains genes controlling important agronomic traits in rye such as: aluminium tolerance [16], alfa-amylase activity [18], and restoration of male fertility [15]. Markers identified on the consensus map can be useful for future map-based cloning of important genes from chromosome 6R and for marker-assisted selection in breeding new rye varieties.

REFERENCES

1. Geiger, H.H. Strategies of hybrid rye breeding. **Votr. Pflanzzüchtg.** 71 (2007) 1-5.
2. Devos, K.M., Atkinson, M.D., Chinoy, C.N., Francis, H.A., Harcourt, R.L., Koebner, R.M.D., Liu, C.J., Masojć, P., Xie, D.X. and Gale, M.D. Chromosomal rearrangements in the rye genome relative to that of wheat. **Theor. Appl. Genet.** 85 (1993) 673-680.
3. Philipp, U., Wehling, P. and Wricke, G. A linkage map of rye. **Theor. Appl. Genet.** 88 (1994) 243-248.
4. Loarce, Y., Hueros, G. and Ferrer, E. A molecular linkage map of rye. **Theor. Appl. Genet.** 93 (1996) 1112-1118.
5. Korzun, V., Malyshev, S., Kartel, N., Westermann, T., Weber, W.E. and Börner, A. A genetic linkage map of rye (*Secale cereale* L.). **Theor. Appl. Genet.** 96 (1998) 203-208.
6. Börner, A. and Korzun, V. A consensus linkage map of rye (*Secale cereale* L.) including 374 RFLPs, 24 isozymes and 15 gene loci. **Theor. Appl. Genet.** 97 (1998) 1279-1288.

7. Korzun, V., Malyshev, S., Voylokov, A.V. and Börner, A. A genetic map of rye (*Secale cereale* L.) combining RFLP, isozyme, protein, microsatellite and gene loci. **Theor. Appl. Genet.** 102 (2001) 709-717.
8. Saal, B. and Wricke, G. Clustering of amplified fragment length polymorphism markers in a linkage map of rye (*Secale cereale* L.). **Plant Breed.** 121 (2002) 117-123.
9. Senft, P. and Wricke, G. An extended genetic map of rye (*Secale cereale* L.). **Plant Breed.** 115 (1996) 508-510.
10. Ma, X-F, Wanous, M.K., Houchins, K., Rodriguez Milla, M.A., Goicoechea, P.G. and Wang Z., Molecular linkage mapping in rye (*Secale cereale* L.). **Theor. Appl. Genet.** 102 (2001) 517-523.
11. Hackauf, B. and Wehling, P. Development of microsatellite markers in rye: map construction. **Plant Breed. Seed Sci.** 48 (2003) 143-151.
12. Khlestkina, E.K., Than, M.H.M., Pestsova, E.G., Röder, M.S., Malyshev, S.V., Korzun, V. and Börner A. Mapping of 99 new microsatellite-derived loci in rye (*Secale cereale* L.) including 39 expressed sequence tags. **Theor. Appl. Genet.** 109 (2004) 725-732.
13. Milczarski, P., Banek-Tabor, A., Lebiecka, K., Stojałowski, S., Myśków, B. and Masojć P., New genetic map of rye composed of PCR-based molecular markers and its alignment with the reference map of the DS2 × RXL10 intercross. **J. Appl. Genet.** 48 (2007) 11-24.
14. Börner, A., Korzun, V., Voylokov, A.V., Worland, A.J. and Weber, W.E. Genetic mapping of quantitative trait loci in rye (*Secale cereale* L.). **Euphytica** 116 (2000) 203-209.
15. Miedaner, T., Glass, C., Dreyer, F., Wilde, P., Wortmann, H. and Geiger H. H. Mapping of genes for male-fertility restoration in 'Pampa' CMS winter rye (*Secale cereale* L.). **Theor. Appl. Genet.** 101 (2000) 1226-1233.
16. Miftahudin, Scoles, G.J. and Gustafson, J.P. AFLP markers tightly linked to the aluminium-tolerance gene *Alt3* in rye (*Secale cereale* L.) **Theor. Appl. Genet.** 104 (2002) 626-631.
17. Milczarski, P. and Masojć, P. Interval mapping of genes controlling growth of rye plants. **Plant Breed. Seed Sci.** 48 (2003) 135-142.
18. Masojć, P. and Milczarski P. Mapping QTLs for alpha-amylase activity in rye grain. **J. Appl. Genet.** 46 (2005) 115-123.
19. Masojć, P., Banek-Tabor, A., Milczarski, P. and Twardowska, M. QTLs for resistance to preharvest sprouting in rye (*Secale cereale* L.) **J. Appl. Genet.** 48 (2007) 211-217.
20. Somers, D.J., Isaac, P. and Edwards K. A high-density microsatellite consensus map for bread wheat (*Triticum aestivum* L.) **Theor. Appl. Genet.** 109 (2004) 1105-1114.
21. Wenzl, P., Li, H., Carling, J., Zhou, M., Raman, H., Paul, E., Hearnden, P., Maier, Ch., Xia, L., Caig, V., Ovesna, J., Cakir, M., Poulsen, D., Wang, J., Raman, R., Smith, K.P., Muehlbauer, G.J., Chalmers, K.J., Kleinhofs, A., Huttner, E. and Kilian, A. A high density consensus map of barley linking

- DarT markers to SSR, RFLP and STS loci and agricultural traits. **BMC Genomics** 7 (2006) 206.
22. Hearnden, P.R., Eckermann, P.J., McMichael, G.L., Hayden, M.J., Eglinton, J.K. and Chalmers K.J. A genetic map of 1,000 SSR and DArT markers in a wide barley cross. **Theor. Appl. Genet.** 115 (2007) 383-391.
 23. Myśków, B., Masojć, P., Banek-Tabor, A. and Szołkowski A. Genetic diversity of inbred rye lines evaluated by RAPD analysis. **J. Appl. Genet.** 42 (2001) 1-14.
 24. Masojć, P., Myśków, B. and Milczarski P. Extending a RFLP-based genetic map of rye using random amplified polymorphic DNA (RAPD) and isozyme markers. **Theor. Appl. Genet.** 102 (2001) 1273-1279.
 25. Bednarek, P.T., Masojć, P., Lewandowska, R. and Myśków, B. Saturating rye genetic map with amplified fragment length polymorphism (AFLP) and random amplified polymorphic DNA (RAPD) markers. **J. Appl. Genet.** 44 (2003) 21-33.
 26. Stojalowski, S., Milczarski, P. and Masojć, P. Usefulness of ISSR markers for identification of inbred lines and mapping of rye genome. **Biul. Inst. Hod. Aklim. Rośl.** 231 (2004) 237-246.
 27. Stojalowski, S., Jaciubek, M., Masojć, P. Rye SCAR markers for male fertility restoration in the P cytoplasm are also applicable to marker-assisted selection in the C cytoplasm. **J. Appl. Genet.** 46 (2005) 371-373.
 28. Van Ooijen, J.W., Voorrips, R.E., **JoinMap[®] v. 3.0, Software for the calculation of genetic linkage maps**. Plant Research International B.V. Wageningen, the Netherlands, 2001.
 29. Doligez, A., Adam-Blondon, A.F., Cipriani, G., Di Gaspero, G., Laucou, V., Merdinoglu, D., Meredith, C.P., Riaz, S., Roux, C. and This, P. An itegrated SSR map of grapevine based on five mapping populations **Theor. Appl. Genet.** 113 (2006) 369-382.