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Research article

THE IDENTIFICATION OF QTLs ASSOCIATED WITH THE *in vitro* RESPONSE OF RYE (*Secale cereale* L.)

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Abstract: This study was conducted in order to identify quantitative trait loci (QTLs) for the *in vitro* culture response of winter rye (*Secale cereale* L.) immature embryos and immature inflorescences. A genetic linkage map comprising 67 SSRs, 9 ISSRs, 13 SAMPLs, 7 RAPDs, 2 SCARs and one EST marker was created based on the analyses of 102 recombinant inbred lines from the cross between lines L318 (which has a good response in tissue cultures) and L9 (which is unable to regenerate plants from somatic tissues and anthers). The map spans 979.2 cM, and the average distance between markers is 9.9 cM. Two characteristics were evaluated: callus induction (CI) and somatic embryogenesis ability (SE). They were expressed as the percentage of immature embryos/inflorescences producing callus (designated ECI/ICI) and the percentage of explants producing somatic embryos (ESE/ISE). All the analysed traits showed continuous variation in the mapping population but a non-normal frequency distribution. We identified nine putative QTLs controlling the tissue culture response of rye, explaining up to 41.6% of the total phenotypic variation: two QTLs for ECI – *eci-1*, *eci-2*; 4 for ESE – *ece-1*, *ese-2*, *ese-3*, *ese-4*; 2 for ICI – *ici-1*, *ici2*; and 1 for ISE – *ise-1*. They were detected on chromosomes 1R, 4R, 5R, 6R and 7R.

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Abbreviations used: CI - callus induction; ECI – percentage of immature embryos producing callus; ESE – percentage of immature embryos producing embryogenic callus; ICI – percentage of immature inflorescences producing callus; ISE – percentage of immature inflorescences producing embryogenic callus; GDDSC – genetically directed differential subtraction chain; MAS – marker assisted selection; QTL – quantitative trait locus; RIL – recombinant inbred line; SE - somatic embryogenesis ability; TCR – tissue culture response

Key words: Rye, *Secale cereale* L., Somatic embryogenesis, Tissue culture, Immature embryos, Immature inflorescences, Molecular marker, QTL, Genetic mapping

INTRODUCTION

Rye is an important crop in Central and Eastern Europe. Despite its many merits, the breeding progress of rye is slow in comparison with other cereals such as wheat or triticale. Attempts to improve rye by means of traditional breeding methods are highly limited because of its self-incompatibility. Biotechnological methods including selection of somaclonal variants or transformation could be very useful to achieve this end. However, such methods require highly efficient, reproducible methods of plant regeneration *in vitro*.

Rye is known as a recalcitrant species with regard to plant regeneration *in vitro*. Many authors reported on its regeneration from somatic tissues via organogenesis [1, 2] or embryogenesis [3-5]. However, the efficiency of the process was often very low. Therefore, detailed genetic studies are needed to identify the genes controlling the *in vitro* response. Our knowledge of the genetic mechanisms influencing the *in vitro* response of rye is rather poor and is based on analysis at the classical level. Rakoczy-Trojanowska and Malepszy [2, 5] found significant differences between the *in vitro* response of several inbred rye lines, both in the case of immature inflorescences and immature embryos. Similar differences between tested genotypes were demonstrated by Popelka and Altpeter [6], Krumbiegel-Schroeren *et al.* [7] and Flehinghaus-Roux *et al.* [8], respectively for immature embryos, immature inflorescences and anther cultures. Genetic analysis at the Mendelian level showed that the *in vitro* response of immature inflorescences is controlled by a polygenic system with different gene interactions, and that the ability to regenerate plants is a recessive trait [2]. In the case of immature embryos, embryogenic callus production and plant and root regeneration were determined by recessive genes, whereas the reduced ability to produce non-embryogenic callus most probably by dominant genes. The lack of response was proven to be controlled by at least two interacting genes [5].

With the recent advances in DNA-based molecular marker systems and the development of quantitative trait loci (QTL) mapping technology, it has become possible to resolve complex traits into several single Mendelian components, and to characterize their map position, gene action and phenotypic effect. [9]. There are several examples of identifying QTLs for the *in vitro* response in cereals, e.g. in rice [10], wheat [11], maize [12] and barley [13]. No such study has thusfar been performed for rye.

The objectives of this study were to identify the QTLs of the *in vitro* response of rye immature embryos and immature inflorescences. For QTL analysis, we used a map constructed on the basis of the mapping population L318 x L9, predominantly composed of microsatellite-based markers.

MATERIALS AND METHODS

Plant material

The plant material used in the experiments consisted of 102 RILs (F_5 and F_6 generations) developed using single-seed descent selection from the cross between lines L318 and L9. L318 was previously characterized as a well-responding line for both immature inflorescences and immature embryos, while line L9 proved unable to form embryogenic callus and regenerate plants from either tissue [2, 5]. In the culture of immature embryos, the percentages of explants producing callus and explants producing embryogenic callus were respectively 100 and 60.2% for line L318, and 65.3 and 0.00% for line L9. In the immature inflorescence culture, the percentages of explants producing callus and explants producing embryogenic callus were respectively 100 and 60.16% for line L318, and 11.02 and 0.00% for line L9.

The donor plants for the tissue culture experiments were grown under standard field conditions. Each line was represented by 30 plants, and kernels were sown individually 12 cm apart. The plants for DNA extraction were grown in a greenhouse under 16 h light at 21ºC and 8 h dark at 17ºC.

Tissue culture procedure

The tissue culture protocols applied for the immature embryos and immature inflorescences were the same as described previously [2, 5] except for the type of auxin added to the medium for callus induction.

The explants were immature embryos 17-19 days after pollination and immature inflorescences 0.5-1.0 cm long. They were cultured on an induction medium (MS medium [14] with 20.0 g/dm^3 sucrose and 7.5 g/dm^3 Difco agar) supplemented with 3 mg/dm^3 dicamba. Each RIL was represented by 60 embryos and 25 inflorescences. The calli were subcultured every 4 weeks in the case of immature embryos and every 3 weeks in the case of immature inflorescences. After 3 subcultures, the explants were transferred to regeneration medium without growth regulators. For the genetic analysis of the tissue culture response (TCR) of the immature inflorescences, the data from 100 RILs were scored due to contamination during culture.

Analyses of tissue culture response

Callus induction (CI) and somatic embryogenesis ability (SE) were the two characteristics evaluated using the following parameters: percentage of immature embryos producing callus (ECI), percentage of immature embryos producing embryogenic callus (ESE), percentage of immature inflorescences producing callus (ICI) and percentage of immature inflorescences producing embryogenic callus (ISE).

The logarithmic and Bliss ($\arcsin\sqrt{x}$) [15] transformations were applied to improve the normality of distribution of the analyzed data as the computer program used for QTL identification requires a normal distribution.

Molecular marker analyses

The genomic DNA of the RILs and parental components was extracted from the leaves of 6-week old greenhouse-grown plants using the CTAB method [16]. The three groups of SSR primer pairs listed below were used to screen for polymorphism between the parental lines. Group I consisted of 85 primer pairs (pp), developed from rye genomic libraries: 41 pp (WRM) by Bolibok *et al.* [17]; 27 pp (SCM) by Saal and Wricke [18]; and 17 pp (RMS) by Lochow Petkus GmbH, Bergen, Germany, supplied as aliquots by Dr. V. Korzun. Group II consisted of 159 pp developed from EST sequences deposited in public data bases: 99 pp (SCM0) designed by Hackauf and Wehling [19]; and 60 pp (REMS) developed by Khlestkina *et al.* [20] and provided by Dr. V. Korzun. Group III consisted of 20 pp (WMS) used for wheat mapping and displaying polymorphism in rye [20, 21]. A total of 264 SSR primer pairs were used. The PCR reactions, electrophoresis and visualization were performed as described by Bolibok *et al.* [22].

ISSR and SAMPL analyses were done as described previously [22]. ISSR markers were designated ISSRx_n, where x is the primer number, and n is the product length. SAMPL markers were designated Mx Sy_n, where x is the AFLP primer number, y is the SAMPL primer number, and n is the product length.

RAPD amplicons were generated using seven Operon primers (D1, D12, D16, F17, I6, Q8, and S10). The protocols applied for PCR reactions, electrophoresis and gel recording were the same as for ISSR analysis. Additionally, we used one EST marker (a fragment of SERK gene cDNA, acc. No. DX383098) and 2 SCAR markers (OPB19_968, acc. No. AY615365; and OPN1_667, acc. No. AY587508).

Map construction

The segregation data was analyzed using MAPMAKER/EXP 3.0 [23]. Linkage groups were separated using the "group" command at LOD > 4.0 and a recombination fraction equal to 0.30. Recombination fractions were converted to cM with the Kosambi mapping function [24]. For chromosomal identification of linkage groups, a set of previously localized SSR markers was used. The order of markers within the linkage groups was determined using the "compare" command. For groups with more than 8 markers, first a subset of the 6 most informative markers was chosen according to the instructions in the MAPMAKER/EXP 3.0 manual. The order of the chosen markers was determined using the "compare" command, and the remaining markers were added using the "try" command. The obtained map orders were verified using the "ripple" command. The Chi square test, $P < 0.05$, was applied to compare the observed segregations of markers with the expected ones.

QTL analysis

For QTL detection, phenotypic data normalized by Bliss transformation was analyzed using MAPMAKER/QTL, version 1.1b [25], using a LOD score of 2.0 or greater as a criterion of significance.

RESULTS

***In vitro* response in RILs**

An induction of callus formation from both immature embryos and immature inflorescences was observed in all the RILs. The rate of response varied between lines, ranging from 7.9% (line LR37) to 95.9% (line LR28) with a 72.6% average for immature embryos, and 10.5% (line LR54) to 100.0% (15 of the 100 RILs) with a 68.3% average for immature inflorescences.

Tab. 1. The original and transformed values of ECI, ESE, ICI and ISE.

Trait	Transfor-mation	Mean value	Standard error	Skew	Kurtosis	Quartile ratio
ECI	Nt	72.66	20.48	-0,87	0.18	1.11
	Log	1.81	0.40	-8.37	76.36	0.34
	B	59.92	14.50	-0.79	1.33	1.08
ESE	Nt	4.81	8.46	2.91	11.26	0.71
	Log	-0.66	1.43	0.21	-1.80	1.51
	B	8.04	10.28	1.21	1.02	1.19
ICI	Nt	68.32	23.11	-0.41	-0.61	1.10
	Log	1.80	0.19	-1.55	2.94	0.85
	B	59.2	17.95	0.25	-0.55	0.89
ISE	Nt	17.56	18.33	1.24	0.80	0.91
	Log	0.48	1.39	-1.09	-0.55	0.53
	B	20.78	15.64	0.35	-0.55	0.99

Nt – non-transformed data, Log – logarithmic transformation, B – Bliss transformation

The immature embryos of 49 of the RILs (48.0%) produced embryogenic callus. The value of ESE varied across the mapping population, ranging from 0.8 (RL102) to 53.9 (RL34), with a mean of 4.8. In the case of immature inflorescences, an induction of embryogenic callus was observed for the majority of the RILs (77%). The percentage of EC-producing explants ranged from 2.3 (LR60) to 72.7% (LR94), with an average of 17.6%.

All the analyzed traits showed continuous variation but non-normal frequency distribution. The comparison between the normalized data and the original records showed that the Bliss transformation resulted in normal (or almost normal) frequency distribution in all cases (Tab. 1). Therefore, we decided to apply this transformation for QTL analysis.

Linkage map

For the SSRs, 72 pp (27.3%) differentiated the parental lines. The efficiency of detecting polymorphisms between the parental lines was comparable for all the groups of pp: 25.9%, 27.7% and 30.0% respectively for the pp from groups I, II and III.

Of the 47 ISSR primers, 28 (59.6%) successfully amplified PCR products and 8 (17.0%) detected polymorphisms between the parental lines, namely: ISSR_3 [5'-$(GA)_9$T-3'], ISSR_17 [5'-$(AG)_8$T-3'], ISSR_32 [5'$(CT)_8$G-3'], ISSR_42 [5'C$(AC)_4(AG)_4$A-3'], ISSR_43 [5'G$(AG)_7(AT)_2$A-3'], ISSR_44 [5'-$(CCT)_5$A-3'], and ISSR_45 [5'$(CCT)_5$G-3']. They generated one or two well scorable polymorphic bands/loci, on average 1.6 bands per assay, and several monomorphic products. In total, 13 ISSR markers were produced by 8 ISSR primers.

One hundred twenty six SAMPL loci were produced using six AFLP/SAMPL primer combinations (M1S29, M3S38, M5S38, M1S39, M3S39, M5S39), and 71.0% of them were polymorphic. Seventeen polymorphic bands were selected for the segregation analysis.

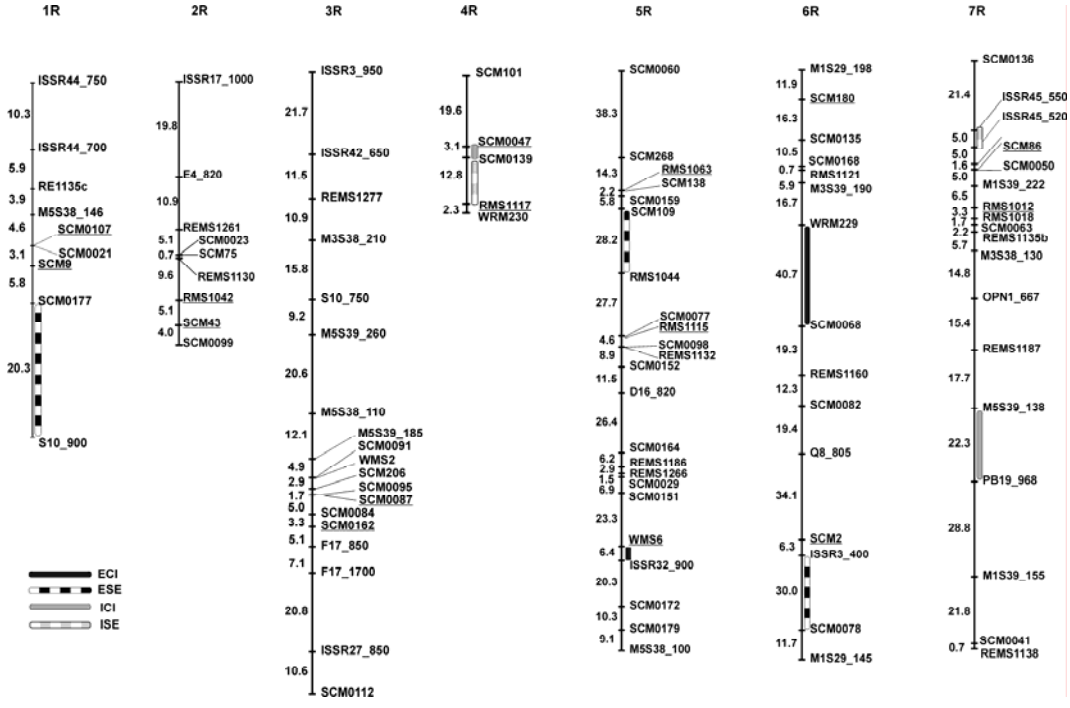

Fig. 1. A linkage map of *Secale cereale* L. based on the RIL population of a cross between L318 and L9. The names of the markers are shown on the right, and the distances between them on the left. The vertical bars indicate map intervals where the QTLs for TCR were mapped. Markers used as anchors are underlined.

The linkage map constructed for the identification of QTLs controlling the somatic embryogenesis of rye, spanning 979.2 cM, contains 99 molecular markers: 67 SSRs, 9 ISSRs, 13 SAMPLs, 7 RAPDs, 1 EST and 2 SCARs distributed in 7 linkage groups identified by means of previously anchored markers as chromosomes 1R-7R (Fig. 1).
The number of markers mapped differed between chromosomes, and ranged from 5 for 4R to 23 for 5R, with a mean distance between the markers of 9.9 cM.
These 99 markers covered all the chromosomes, but they were not evenly dispersed through the genome. The biggest gaps were found on chromosomes 5R and 6R, and the highly clustered markers on chromosomes 2R, 3R and 7R.
Markers characterized by distorted segregation were found on all the chromosomes, with the highest rate of such markers on chromosomes 5R and 7R (56.5% and 52.6%, respectively) and a mean value of 48.5% for the all chromosomes.

QTLs for *in vitro* response

Nine putative QTLs controlling the tissue culture response of rye were identified: two QTLs for ECI (*eci-1*, *eci-2*), 4 for ESE (*ece-1*, *ese-2*, *ese-3*, *ese-4*), 2 for ICI (*ici-1*, *ici2*) and 1 for ISE (*ise-1*). They were detected on chromosomes 1R, 4R, 5R, 6R and 7R (Tab. 2).
Two QTLs related to callus formation from immature embryos – *eci-1*(5R) and *eci-2* (6R) – were comparable with respect to their effects on the ECI (ca. 31)

Tab. 2. Characteristics of the QTLs controlling the rye tissue culture response.

Trait	QTL	Chromo-some	Marker interval (length [cM])	QTL position	LOD score	Weight	Variance explained [%]
ECI	*eci-1*	5R	WMS6–S32_900 (6.8)	0.0	3.59	-31.309	20.8
	eci-2	6R	WRM229–SCM0068 (55.8)	4.0	3.23	-31.223	22.1
					4.81[a]		26.2[a]
ESE	*ese-1*	1R	SCM0177–S10_900 (24.3)	18.0	3.78	16.929	28.4
	ese-2	4R	SCM0139– RMS1117 (14.4)	6.0	2.33	18.211	21.5
	ese-3	5R	SCM109–RMS1044 (35.7)	10.0	2.55	17.453	24.2
	ese-4	6R	S_400–SCM0078 (38.4)	28.0	3.64	10.581	41.6
					7.69[a]		69.1[a]
ICI	*ici-1*	4R	SCM0047–SCM0139 (3.2)	0.0	2.32	8.029	11.4
	ici-2	7R	M539_138–PB19_968 (27.1)	22.0	3.67	11.008	20.6
					4.98[a]		24.4[a]
ISE	*ise-2*	7R	S45_550–S45_520 (5.3)	0.0	2.40	-9.203	10.8

a – values for the model considering the detected QTLs together

and LOD values (3.59 and 3.23); moreover, each QTL explained ca. 20% of the total phenotypic variation. The alleles of line L9 decreased the ECI values in these loci.

Three out of the four loci revealed by QTL analysis of ESE, located on chromosomes 1R, 4R and 5R, were characterized by moderate effects (from 16.9 to 18.2%) and explained a similar proportion of the phenotypic variance (from 21.5 to 28.4%). The fourth locus influencing ESE, located on chromosome 6R, accounted for 41.6% of the total phenotypic variation. However, its effect was smaller (10.6%). In all these loci (*ese-1* through *ese-4*), alleles contributed by L9 increased the trait value.

The QTLs identified for the *in vitro* response of immature inflorescences generally exhibited lower effects and explained a smaller percentage of the phenotypic variance. QTL *ici-1*, which accounts for 11.4% of the phenotypic variance, had an effect of 8.029. The second locus influencing callus induction in the culture of immature inflorescences, *ici-2*, located on chromosome 7R, explains 20.6% of the total phenotypic variance, and the L9 allele increases the value of the trait by 11.0.

The only QTL for ISE identified in this study was located on chromosome 7R. However, it was mapped in a different map interval than *ici-2*. This locus accounts for only 10.08% of the total phenotypic variance and has an effect of 9.2%, with the allele from the parent L9 decreasing the value of the trait.

DISCUSSION

QTLs for tissue culture response have been mapped for numerous plant species, both dicots and monocots, including major cereals such as rice, wheat, maize and barley [9]. The one existing paper on the molecular analysis of the *in vitro* response of rye describes only very preliminary results concerning anther culture [26].

In this paper, we present the results of a QTL analysis of rye immature embryo and immature inflorescencence TCR. The QTL analysis was conducted using a mapping population constructed especially for this purpose. The parents were found to differ widely in their tissue culture response during earlier studies [2, 5]. The polymorphism at the molecular level also turned out to be sufficient for constructing a genetic linkage map of the cross, as ca. 30% of the used SSR markers segregated in the RILs. This allowed the construction of a molecular map containing 99 markers and spanning 979.2 cM of the rye genome. In our opinion, these parameters along with the average density of the constructed map (9.9 cM) make it a useful tool for preliminary studies aimed at determining approximate QTL locations and for similar approaches. Maps of similar density were used successfully for identifying QTLs connected with TCR in sunflower [27], maize [12] and barley [13]. The value of the map is increased by the population type (RILs, rather rarely used in rye genetics, mainly due to the occurrence of inbreeding depression in this crop, which makes it difficult to

obtain such a population) and by the presence of 70 transferable PCR-based markers – 67 SSRs, 2 SCARs and 1 EST. This is a relatively high number, particularly when compared to the numbers for other currently available rye maps – 56 [28] and 45 [20]. Both these qualities enable the population and its map to be used for repeated phenotypic measurements and subsequent QTL analyses, comparing the localizations of the identified QTLs, and for integration with other genetic maps of rye.

In most cases, the locations of the SSR markers on the L318 x L9 map were in agreement with previously published data [18, 28, 31, Korzun, pers. comm.], with three exceptions: the SSRs REMS1187, REMS1266 and REMS1261. The first two markers were assigned in this study to chromosome 5R, the third to chromosome 2R. Khlestkina *et al.* [20] mapped REMS1187 on chromosome 7R, RMS1024 on chromosome 4R, and REMS1261 on chromosome 1R.

One of the major factors affecting the map quality was the relatively high number of markers with deviating segregation. Segregation distortion is a common phenomenon in rye, even in F_2 populations [18, 29-32], and is believed to be the result of selection at the gametic or zygotic stage. It can be expected that inbreeding depression causing certain lines to be lost during the development of the population resulted in a shift in the allele proportions in the remaining lines resulting in distorted segregation. The relatively high ratio of distorted markers on chromosome 7R is in accordance with results of Hackauf and Wehlig [28], who reported that 67.8% of markers deviated significantly from expectations and attributed this to the existence of a locus governing zygotic selection on that chromosome.

Another weakness of the constructed map is the small number of markers mapped on chromosome 4R, which may indicate a low level of polymorphism between parents in this particular region. To address this problem, additional analyses including markers reported to be located on chromosome 4R are needed. Unfortunately, such markers are not abundant at this time, as rye is a relatively poorly researched crop and most of the available transferable molecular markers with determined chromosome locations have already been used in this study.

The QTL analysis was performed on the basis of two parameters – the percentage of explants producing callus and the percentage of explants producing embryogenic callus, respectively reflecting an ability for callus induction and somatic embryogenesis. Both have been commonly used in studies aimed at mapping genes for TCR [27, 33, 34], and proved to be a good predictors for the final yield of regenerated plants [12, 33].

The skewed distributions observed for all the characteristics analyzed in this study were also reported in many other papers dealing with mapping of the tissue culture response [13, 34, 35], and could be expected, as rye is generally a recalcitrant plant with respect to tissue culture response. The Bliss transformation, often applied in similar studies [10, 35, 36], improved the normality of distribution.

Phenotypic measurements of the *in vitro* response of immature embryos expressed as the percentage of explants producing callus and the percentage of explants producing somatic embryos turned out to be a good basis for QTL analysis, as a total of 6 loci influencing the traits could be identified. The relatively large effects of the loci and the proportion of total phenotypic variation explained by the loci (up to 41.6%) may suggest that the major loci influencing *in vitro* response of immature rye embryos in the studied population were detected. The results of QTL identification for the *in vitro* response of immature inflorescences are less satisfactory. Only 2 loci for ICI and 1 for ISE were detected with small to moderate effects. The proportions of the total phenotypic variance explained by the loci were also lower than in the case of QTLs influencing the tissue culture response of immature embryos. However, their map location could be useful for marker-assisted selection (MAS).

The obtained results are in partial agreement with the outcome of an earlier study focusing on the *in vitro* response of rye. Using disomic Chinese Spring-Imperial wheat-rye addition lines, Lazar *et al.* [37] demonstrated that chromosomes 6R and 7R bear factors promoting the positive *in vitro* response of rye immature embryos or factors alleviating the negative influence of wheat genes. In our study, two of the identified QTLs influencing the response of immature embryos were detected on chromosome 6R: *eci-2* and *ese-4*. Moreover, regions of chromosomes 6R and 5R were indicated as the localizations of QTLs controlling the response of rye immature embryos identified in the mapping population Ot 1-3 x 541 examined previously in our department [Boczkowska, personal communication]. In the analysis of L318 x L9, two QTLs were identified on chromosome 5: *eci-1* and *ese-3*. Unfortunately, it is impossible at this point to determine if the QTLs were identified in the same chromosome regions due to a lack of common markers on the linkage maps.

It was demonstrated that molecular markers linked to a QTL influencing the *in vitro* response can be used to determine the value of a genotype [35] or in the map-based cloning and isolation of genes for plant regenerability [37]. Although the QTL localizations determined in this study need to be verified, perhaps by comparing them with the results of the currently ongoing Genetically Directed Differential Subtraction Chain (GDDSC) analysis of the DNA pools from RILs contrasting in TCR [38] and their effects evaluated, in our opinion, the first step toward identifying the genes governing the *in vitro* response in rye has been made.

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REFERENCES

1. Rybczyński, J.J. *In vitro* culture of *Secale cereale* L. explants – callus formation and organ differentiation. **Acta Soc. Bot. Pol.** 49 (1980) 155-166.
2. Rakoczy-Trojanowska, M. and Malepszy, S. Genetic factors influencing regeneration ability in rye (*Secale cereale* L.). I. Immature inflorescences. **Theor. Appl. Genet.** 86 (1993) 406-410.
3. Zimny, J. and Lörz, H. High frequency of somatic embryogenesis and plant regeneration of rye (*Secale cereale* L.). **Plant Breed.** 102 (1989) 89-100.
4. Linacero, R. and Vazquez, A.M. Somatic embryogenesis from immature inflorescences of rye. **Plant Science** 72 (1990) 253-258.
5. Rakoczy-Trojanowska, M. and Malepszy, S. Genetic factors influencing the regeneration ability of rye (*Secale cereale* L.). II. Immature embryos. **Euphytica** 83 (1995) 233-239.
6. Popelka, J.C. and Altpeter, F. Interactions between genotypes and culture media components for improved *in vitro* response of rye (*Secale cereale* L.) inbred lines. **Plant Cell Rep.** 20 (2001) 575-582.
7. Krumbiegel-Schroeren, G., Schroeren, V. and Binding, H. Embroid formation and plant regeneration from callus of *Secale cereale*. **Z. Pflanzenzüchtg.** 92 (1984) 89-94.
8. Flehinghaus-Roux, T., Deimling, S. and Geiger, H.H. Anther culture ability in *Secale cereale* L. **Plant Breed.** 114 (1995) 259-261.
9. Bolibok, H. and Rakoczy-Trojanowska, M. Genetic mapping of QTLs for tissue-culture response in plants. **Euphytica** 149 (2006) 73-83.
10. Taguchi-Shiobara, F., Lin, S.Y., Tanno, K., Komatsuda, T., Yano, M., Sasaki, T. and Oka, S. Mapping quantitative trait loci associated with regeneration ability of seed callus in rice, *Oryza sativa* L. **Theor. Appl. Genet.** 95 (1997) 828-833.
11. Ben Amer, I.M., Korzun, V., Worland, A.J. and Börner, A. Genetic mapping of QTL controlling tissue-culture response on chromosome 2B of wheat (*Triticum aestivum* L.) in relation to major genes and RFLP markers. **Theor. Appl. Genet.** 94 (1997) 1047-1052.
12. Murigneux, A., Bentollila, S., Hardy, T., Baud, S., Guitton, C., Jullien, H., Ben Tahar S., Freyssinet, G. and Beckert, M. Genotypic variation of quantitative trait loci controlling *in vitro* androgenesis in maize. **Genome** 37 (1994) 970-976.
13. Mano, Y. and Komatsuda, T. Identification of QTLs controlling tissue-culture traits in barley (*Hordeum vulgare* L.). **Theor. Appl. Genet.** 105 (2002) 708-715.
14. Murashige, F. And Skoog, F. A revised medium for rapid growth and bioassays with tobacco tissue culture. **Physiol. Plant** 15 (1962) 473-497
15. Bliss, C.I. The Transformation of Percentages for Use in the Analysis of Variance. **Ohio J. Sci.** 38 (1938) 9-12.

16. Murray, M.G., and Thompson, W.F. Rapid isolation of high molecular weight plant DNA. **Nucleic Acid Res.** 8 (1980) 4321-4325.
17. Bolibok, H., Rakoczy-Trojanowska, M., Wyrzykowska, M., Radecka, M. and Orczyk, W. Identification of microsatellite markers in the rye genome. **Cell. Mol. Biol. Lett.** 11 (2006) 291-298.
18. Saal, B. and Wricke, G. Development of simple sequence repeat markers in rye (*Secale cereale* L.). **Genome** 42 (1999) 964-972.
19. Hackauf, B. and Wehling, P. Identification of microsatellite polymorphisms in expressed portion of the rye genome. **Plant Breed.** 121 (2002) 17-25.
20. 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.** 19 (2004) 725-732.
21. Röder, M.S., Korzun, V., Wendehake, K., Plaschke, J., Tixier, M.H., Leroy, P. and Ganal, M.W. A microsatellite map of wheat. **Mol. Gen. Genet.** 246 (1998) 327-333.
22. Bolibok, H., Rakoczy-Trojanowska, M., Hromada, A. and Pietrzykowski, R. The efficiency of different PCR-based marker system in assessing genetic diversity among winter rye (*Secale cereale* L.) inbred lines. **Euphytica** 146 (2005) 109-115.
23. Lander, E.S., Green, P., Abrahamson, J., Barlow, A., Daly, M.J., Lincoln, S.E. and Newberg, L. MAPMAKER: an interactive computer package for constructing primary genetic linkage maps of experimental and natural populations. **Genomics** 1 (1987) 174-181.
24. Kosambi, D.D. The estimation of map distances from recombination values. **Ann. Eugen.** 12 (1944) 172-175.
25. Lincoln, S., Daly, M. and Lander, E. Mapping genes controlling quantitative traits with MAPMAKER/QTL 1.1. Whitehead Institute Technical Report 2nd edn. (1992).
26. Grosse, B.A., Deimling, S. and Geiger, H.H. Mapping of genes for anther culture ability in rye by molecular markers. **Vortr. Pflanzenzuechtg.** 35 (1996) 282-283.
27. Flores Berrios, E., Sarrafi, A., Fabre, F., Alibert, G. and Gentzbittel, L. Genotypic variation and chromosomal location of QTLs for somatic embryogenesis revealed by epidermal layers culture of recombinant inbred lines in the sunflower (*Helianthus annuus* L.). **Theor. Appl. Genet.** 101 (2000b) 1307-1312.
28. Hackauf, B. and Wehling, P. Development of microsatellite markers in rye: map construction. **Plant Breed Seed Sci.** 48 (2003) 143-151.
29. Philipp, U., Wehling, P. and Wricke, G. A linkage map of rye. **Theor. Appl. Genet.** 88 (1994) 243-248.
30. Senft, P. and Wricke, G. An extended genetic map of rye (*Secale cereale* L.). **Plant Breed.** 115 (1996) 508-510.

31. 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.
32. Ma, X.F., Wanous, M.K., Houchins, K., Rodriguez Milla, M.A., Goicoechea, P.G., Wang, Z., Xie, M. and Gustafson, J.P. Molecular linkage mapping in rye (*Secale cereale* L). **Theor. Appl. Genet.** 102 (2001) 517-523.
33. He, P., Sheng, L., Lu, C., Chen, Y. and Zhu, L. Analysis of quantitative trait loci which contribute to anther culturability in rice (*Oryza sativa* L.). **Mol. Breed.** 4 (1998) 165-172.
34. Manninen, O.M. Associations between anther-culture response and molecular markers on chromosomes 2H, 3H and 4H of barley (*Hordeum vulgare* L.). **Theor. Appl. Genet.** 100 (2000) 57-62.
35. Torp, A.M., Hansen, A.L. and Andersen, S.B. Chromosomal regions associated with green plant regeneration in wheat (*Triticum aestivum* L.) anther culture. **Euphytica** 119 (2001) 377-387.
36. Kwon, Y.S., Kim, K.M., Eun, M.Y. and Sohn, J.K. QTL mapping and associated marker selection for the efficacy of green plant regeneration in anther culture of rice. **Plant Breed.** 12 (2002) 10-16.
37. Lazar, M.D., Chen, T.H.H., Scoles, G.J. and Kartha, K.K. Immature embryo and anther culture of chromosome addition lines of rye in Chinese Spring wheat. **Plant Sci.** 51 (1987) 77-81.
38. Nishimura, A., Ashikari, M., Lin, S., Takashi, T., Angeles, E.R., Yamamoto, T. and Matsuoka, M. Isolation of a rice regeneration quantitative trait loci gene and its application to transformation systems. **Proc. Natl. Acad. Sci. USA** 102 (2005) 11940-11944.
39. Hromada, A., Bolibok, H. and Rakoczy-Trojanowska, M. Application of the GDDSC for the isolation of winter rye (*Secale cereale* L.) genome regions connected with *in vitro* reaction of immature embryos. **Vortr. Pflanzenzuechtg,** in press.