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Gene mutations in rye causing embryo lethality in hybrids with wheat: allelism and chromosomal localisation

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Abstract

In crosses between hexaploid wheat and inbred lines of rye, a small number of rye genotypes produce seeds carrying undifferentiated, non-viable embryos. Hybrids between such lines and those not showing this phenotype were used as pollen donors in crosses with bread wheat in order to determine the genetic basis of disturbed embryo development. A single gene, designated *Eml-R1b*, is causing this character. Molecular markers associated with F_2 genotypes derived from a contrasting rye inbred progeny were used for a linkage study. Recombinant inbred lines of an F_5 population served as testers. *Eml-R1b* maps to chromosome arm 6RL, along with two co-segregating microsatellite loci, *Xgwm1103* and *Xgwm732*. Complementary interactions of deleterious genes in wheat and rye are discussed.

Additional key words: genetic mapping, genomic interaction, incompatibility, post-zygotic isolation, *Secale cereale*, *Triticum aestivum*.

Introduction

Intergeneric hybridization has been proven to be an effective means for introgressing genes into cultivated crops. Because of progamous and postgamous incompatibility, not all crosses result in viable hybrids. The genetic interaction between parental genomes can produce either seedling lethality, weakness, or sterility of the hybrid offspring. Crossing barriers are one of the means of speciation. Frequently, they are under simple genetic control. They do not affect the fitness of individuals within the species (Dobzhansky 1937, Müller 1942).

Recently, it has become apparent that species achieve post-zygotic reproductive isolation by a range of genes, acting at different stages of hybrid ontogenesis (Orr *et al.* 2004). Genes of rye have been shown to be expressed in primary, as well as in amphidiploid wheat-rye hybrids. Besides embryo lethality, hybrid dwarfness, hybrid weakness, necrosis/chlorosis, morphological abnormalities, and sterility are some of the consequences (*e.g.* Ren and Lelley 1988, 1989, Tomar and Singh 1998).

Earlier a set of 101 rye inbred lines originating from the Peterhof rye genetic stock collection of the Laboratory of Plant Genetics (St. Petersburg State University, Russia) and selected from the rye cultivars Vyatka, Steel, Heine, Petkus and Volkhova was used to pollinate bread wheat. Three closely related self-fertile lines (L2, L3 and L564) and one unrelated line (L535) gave rise to non-germinating hybrid seeds. The hybrid seeds of all other lines were viable. The non-germinating hybrid seeds obtained from crosses with both L2 and L535 had a normal endosperm, but hybrid embryos were either absent, or varied in size from small to normal, but without any indication of tissue differentiation (Tikhenko *et al.* 2005, 2008). In the present paper, we describe the identification and mapping of the gene(s) responsible for the failure of these wheat × rye hybrid embryos to develop, *i.e.* gene(s) determining embryo lethality.

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Abbreviations: CS - Chinese Spring; Eml - embryo lethality; L - inbred line; P421 - Priekulskaya 421; RFLP - restriction fragment length polymorphism; RILs - recombinant inbred lines; SSR - simple sequence repeats.
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Materials and methods

Two bread wheat (*Triticum aestivum* L.) cultivars were used as female parents, the spring wheat Chinese Spring (CS) and a derivative of the winter wheat Priekulskaya 421 (P421), into which the crossability genes from CS (*kr*) had been transferred by backcrossing. Crosses between pairs of the inbred rye (*Secale cereale* L.) lines L2, L6, L7 and L535, originating from the 'Peterhof' genetic collection (St. Petersburg, Russia), were used as pollen donors. L2 and L535 carry gene(s) which block hybrid embryo-development, while L6 and L7 produce viable hybrid embryos. For segregation analyses F_1 hybrids between L2/L6, L2/L7 and L535/L6 were used as pollen parents (Table 1). Allelism tests of gene(s) responsible for wheat-rye embryo lethality, detected in the unrelated lines L2 and L535, were carried out by pollinating CS and P421 with the F_1 hybrid L535 × L2 (Table 2).

For intrachromosomal mapping of gene(s) causing embryo lethality, CS was also pollinated by a set of 79 F_5 single seed descent derived recombinant inbred lines (RILs), originating from a single F_2 progeny of the cross L7 × L2. For trait mapping, the RFLP- and SSR-based genotype of this set of F_2 selections (Khlestkina *et al.* 2004) was employed. Frequency distributions of the F_2-ancestors for each marker in the two groups (normal versus lethal) of CS × F_5 (L7 × L2) rye hybrids were analysed, using classical χ^2- and χ^2-heterogeneity tests to reveal non-random associations, typical for linkage, and to check correspondence of observed and expected segregations. In each of the 75 cross combinations 30 to 72 hybrid seeds were examined.

To differentiate between normal from abnormal hybrid embryos, each seed was allowed to germinate for 36 h at 24 °C, and immersed in a drop of 0.5 % (m/v) aqueous tetrazolium chloride solution. Living embryos respond to this solution by staining red, while dead ones remain colourless.

Table 1. Segregation for the number of differentiated and undifferentiated embryos in crosses between two bread wheat cultivars and intraspecific rye hybrids.

Parental forms wheat	rye hybrid	Embryos differ.	undiffer.	χ^2-test 1:1
CS	L2 × L6	63	74	0.73
		67	71	0.12
	L6 × L2	66	82	1.73
		343	319	0.88
	L2 × L7	91	90	0.00
	L7 × L2	66	76	0.70
P 421	L2 × L6	104	101	0.04
	L7 × L2	132	144	0.52
		84	87	0.51
CS	L6 × L535	130	149	1.29

Table 2. Test for allelism of *Eml* genes carried by rye lines L2 and L535

Parental forms wheat	rye L535 × L2	Number of seeds	Embryos differ.	undiffer.
P 421	plant 1	175	0	175
	plant 2	366	0	366
	plant 3	197	0	197
	plant 4	175	0	175
	plant 5	282	0	282
	plant 6	317	0	317
CS	plant 1	136	0	136
	plant 3	104	0	104
	plant 4	126	0	126

Results

Segregation analysis: For all cross combinations under investigation a 1:1 segregation for the presence of differentiated (normal) *vs.* undifferentiated (nonviable) embryos in hybrid grains was obtained according to expectation, with a total ratio of 1.146:1.193. Therefore, it was concluded that embryo lethality, in wheat rye crosses, is determined in rye by one gene having two alleles. Rye lines L2 (and by inference, also the genetically related lines L3 and L564), as well as the unrelated line L535, carry the allele preventing the normal development of a hybrid embryo. This gene was named by Tikhenko *et al.* (2005, 2008) *Eml* (*embryo lethality*). Because we cannot determine yet, whether the allele for embryo lethality is dominant or recessive, we designate the alleles determining the production of differentiated (normal or wild-type) and undifferentiated (lethal or mutant) embryos *Eml-R1a* and *Eml-R1b*, respectively, following the rules for gene symbolization (McIntosh *et al.* 2008).

Allelism tests: Since L2 and L535 are unrelated by descent, the question as to whether they both carry the same gene was tested by an analysis of segregation for embryo viability in crosses between P421 or CS and the L535 × L2 hybrid. As all 1 878 hybrid seeds obtained from these crosses contained undifferentiated, non-viable embryos (Table 2), it was concluded that L2 and L535 most probably carry the same embryo lethality gene.

Linkage test: The map location of *Eml* in rye was derived from its linkage to molecular marker loci, based on the segregation ratio at each marker locus separately, within both, the group of L7 × L2 RILs, which produced only non-viable embryos, and that, producing viable ones. The linkage test was based on the analysis of segregation ratio for markers in F_2 plants, divided in groups in accordance to *Eml*-genotypes of their progenies. Considering that segregation for a codominant marker M_1/M_2 absolutely linked to gene *A/a* in successive generations is based on F_1 constitution AM_1/aM_2, the segregation ratio is expressed as $(2^{k-1}-1)$ AM_1/AM_1: 2 AM_1/aM_2 : $(2^{k-1}-1)$ aM_2/aM_2, where k is the number of generations. Thus, for F_5 this ratio will be equal to 15 AM_1/AM_1:2AM_1/aM_2:15 aM_2/aM_2. Double homozygotes of both types originate from corresponding F_2 homozygotes (AM_1/AM_1 or aM_2/aM_2), or occur through segregation of F_2 - F_4 heterozygotes (AM_1/aM_2). The ratio of "pure" homozygotes and homozygotes resulting from segregation is expressed as 2^{k-2}: $(2^{k-2}- 1)$, this ratio in F_5 generation is equal to 8:7. In the case of absolute linkage, the same ratio is expected for F_2 plant progenitors, giving rise to homozygosity for the gene in F_5. If a marker is not linked to the gene of interest (*Eml*), the segregation ratio will be the same within all genotypic classes (*AA, Aa, aa*) and equal to monohybrid ratio 1:2:1. Thus, as test for linkage, one can use χ^2-test for heterogeneity between homozygous group or χ^2-test for correspondence to monohybrid ratio in both groups.

For linkage analysis data for 42 codominant markers, segregating in F_2 of L7 × L2, were used. Only segregation for two microsatellite markers located on chromosome 6R showed the expected distortion (Table 3). The segregation was tested in F_2 for progenitors of two groups of F_5 plants – homozygotes for allele *Eml-R1b* and homo- and heterozygotes for allele *Eml-R1a* combined. Pooling was done to avoid mistakes in classification of homo- and heterozygotes for allele *Eml-R1a*. The ratio of F_5 plants in these two groups (33 vs. 42) corresponds to expected monohybrid proportion 15:17 ($\chi^2 = 0.25$). The markers in Table 3 are listed in accordance to their order on the linkage map (Fig. 1). Data for co-segregating markers *Xgwm1103* and *Xgwm732* are shown separately, because some F_2 plants were genotyped only for one of both markers. The direction of observed distortion corresponds to linkage phase of gene *Eml* and marker alleles. Low frequency of homozygotes for marker allele from L7 is combined with high frequency of homozygotes for marker allele from L2 in *Eml-R1b/Eml-R1b* group. The opposite situation is observed in *Eml-R1a/* group. But only for the latter group segregation differs significantly from expected 1:2:1 ratio. At the same time, segregation ratios in these two groups are significantly heterogeneous ($P < 0.001$). Thus, we can conclude that gene *Eml-R1* is located in the region of the two co-segregating microsatellite loci *Xgwm1103* and *Xgwm732* (Table 3, Fig. 1).

Table 3. Test for linkage of *Eml-R1* with molecular marker loci on chromosome 6R. *, *** significant at $P < 0.05$ and $P < 0.001$, respectively.

Plant genotypes in F_5	Marker	Plant genotypes for marker in F_2			Number of F_5 plants	χ^2 1:2:1 (heterogeneity)
		L2	F1	L7		
Eml-R1b/Eml-R1b	*Xpsr160*	9	15	7		0.48
Eml-R1a/-		7	20	15		3.15
Σ		16	35	22	73	1.30 (2.27)
Eml-R1b/Eml-R1b	*Xpsr915*	8	17	6		0.54
Eml-R1a/-		9	24	8		1.24
Σ		17	41	14	69	1.64 (0.16)
Eml-R1b/Eml-R1b	*Xgwm1103*	7	16	1		5.66
Eml-R1a/-		1	22	13		9.78*
Σ		8	38	14	60	5.47 (13.93***)
Eml-R1b/Eml-R1b	*Xgwm732*	8	14	1		5.34
Eml-R1a/-		1	22	13		9.78*
Σ		9	36	14	59	3.78 (15.49***)
Eml-R1b/Eml-R1b	*Xpsr1203*	11	11	10		3.18
Eml-R1a/-		5	25	11		3.73
Σ		16	36	21	73	0.72 (6.87*)
Eml-R1b/Eml-R1b	*Xpsr687a*	12	9	9		5.40
Eml-R1a/-		6	25	9		2.95
Σ		18	34	18	70	0.06 (8.29*)

Discussion

Crosses between bread wheat and rye are only successful, if the wheat parent carries the recessive allele(s) at the crossability genes, designated *kr* (Riley and Chapman 1967, Krolow 1970, Lange and Riley 1973, Sitch *et al.* 1985). In opposite case hybrid seeds may be formed, but with low frequency only (Müntzing 1939, Oettler 1983). In addition to the *kr* gene complex, other genetic systems also may cause reproductive barriers between wheat and rye at different developmental stages of the zygote. Among ~100 rye inbred lines, used in crosses with wheat carrying the recessive crossability alleles, four (L2, L3, L564, genetically related, and L535) were detected to be carriers of mutations causing embryo lethality after hybridization (Tikhenko *et al.* 2005).

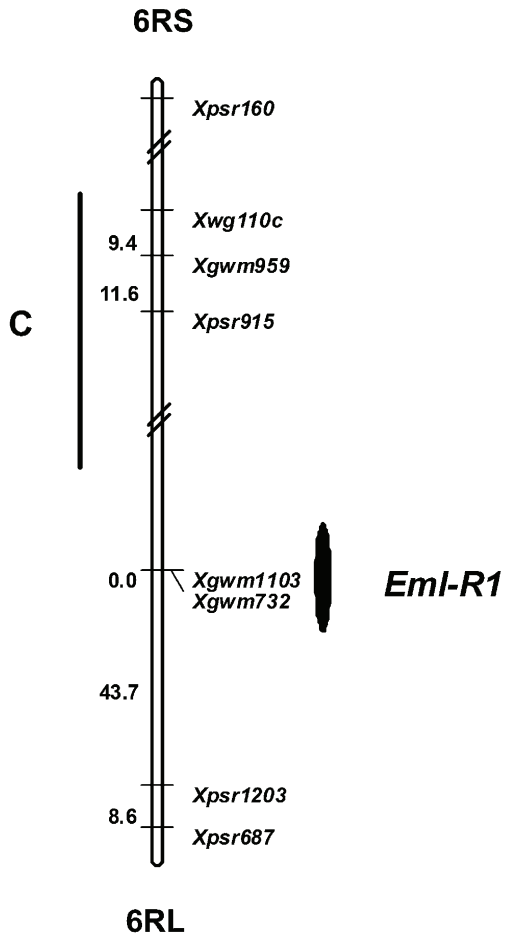

Fig. 1. Estimated map location of *Eml.R1* on the chromosome 6RL map produced by Khlestkina *et al.* 2004.

The genetic analysis presented here revealed a monogenic inheritance for embryo lethality, as well as allelism between the genetically different lines L2 and L535. There appear to be at least two functionally different alleles at this gene locus (*Eml*) – one carried by lines L2 (L3, L564) and L535 (*Eml-R1b*) and producing abnormal embryos, and the other (*Eml-R1a*) present in the majority of rye genotypes, producing normal embryos after fertilization.

To ascertain, whether the alleles of *Eml-R1* act in dominant/recessive fashion, it would be necessary to obtain the heterozygous condition, which could be achieved in a cross between *e.g.* L2 (*Eml-R1b*) and a wheat-rye addition line carrying chromosome 6R but with allele *Eml-R1a*. This hybrid would carry two copies of chromosome 6R and would be heterozygous for *Eml-R1*. If the hybrid grains obtained have normal embryos, *Eml-R1a,* the wild type allele, is dominant, whereas in the opposite case, if hybrid grains have abnormal embryos, the allele *Eml-R1b* is dominant.

The expression of the embryo lethality character in a wheat-rye hybrid, but not in rye itself, reflects a genomic interaction between wheat and rye genes. According to this suggestion, the *Eml* allele of rye interact with the corresponding gene(s) of wheat. Negative interaction causes embryo lethality. In the simplest case there may be only two genes involved – *Eml-R1* in rye and another in wheat. In accordance to this suggestion allele *Eml-R1b* complements the corresponding incompatible gene in wheat, but allele *Eml-R1a* does not.

A more intricate situation can arise when an epistatic interaction is involved, following the Dobzhansky-Müller model (Orr 1996). This type of interaction is the reason for hybrid incompatibility in many plants (Bomblies and Weigel 2007). Incompatibility can be both intra- as well as interspecific. It was believed that alleles for compatibility and incompatibility did not differ for effect on their own genomic background (Orr 1996). Recently, an alternative hypothesis has been proposed, according to which polymorphism for "speciation" genes evolved and was maintained in populations, because these genes were associated with some adaptive significance, and so the isolating role of these genes is just an accidental by-product of adaptation (Wu 2001).

The phenomenon of hybrid necrosis has also been considered as a pleiotropic effect of genes evolved to defend against pathogen attack (Bomblies and Weigel 2007), so the adaptive value of compatibility/ incompatibility genes probably extends to other well-studied manifestations of hybrid failure. In wheat, several complementary dominant genes have been implicated in various forms of hybrid incompatibility. These include the hybrid necrosis genes *Ne1*, *Ne2* (Tsunewaki 1960, Hermsen 1963), two types of hybrid chlorosis (*Ch1*, *Ch2*; Hermsen 1966 and *Cs1*, *Cs2*; Kawahara 1993), and hybrid dwarfness (*D1*, *D2*, *D3*, *D4*; McMillan 1937, Hermsen 1967, Worland and Law 1980). The expression of chlorosis in certain wheat x rye hybrids led Tomar and Singh (1998) to propose the existence of the gene *Clr1* in rye, while the two dominant complementary hybrid necrosis rye genes *Ner1* and *Ner2* are well established

(Scoles 1985, Ren and Lelley 1988). The dominant alleles of these two genes complement each other within the species, as well as in species hybrids.

The assumption that the embryo lethality described here is a product of interaction between two complementary genes should be further tested from crosses between each of the nullisomic-tetrasomic or ditelosomic lines of CS developed by Sears (Sears 1966, Sears and Sears 1978) and L2 (L3, L564) or L535. If correct, this strategy would identify just one chromosome as containing a complementary gene, as, only when lines lacking this chromosome (arm) are used as the female parent, will the hybrid embryos develop normally.

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