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Molecular mapping, phenotypic expression and geographical distribution of genes determining anthocyanin pigmentation of coleoptiles in wheat (*Triticum aestivum* L.)

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Abstract Three major gene loci determining the anthocyanin pigmentation of coleoptiles were mapped on the short arms of chromosomes 7A, 7B and 7D, respectively. All three genes map about 15 to 20 cM distal from the centromere and, therefore, it may be concluded that they are members of a homoeologous series and should be designated *Rc-A1*, *Rc-B1* and *Rc-D1*, respectively. Further homoeologous loci exist in *Triticum durum*, *Triticum tauschii*, and most probably in *Secale cereale* and *Hordeum vulgare*. By analyzing a synthetic×cultivated wheat cross (ITMI mapping population) under different environmental conditions it was shown that the expression of the genes determining anthocyanin pigmentation of the coleoptiles varies. One additional locus was detected on chromosome 4BL. Beside the mapping data, results of a screening for red coleoptile color genes in 468 mainly European wheat varieties are presented.

Keywords Anthocyanin pigmentation · Coleoptile color · Genetic mapping · Homoeology · Microsatellites · *Triticum aestivum*

Introduction

Anthocyanin pigmentation of different parts of the plant is found in many species including the cereals. In wheat, major genes are described for the coloration of anthers, auricles, coleoptiles, straw or grains (McIntosh 1998). Although the importance of these color traits for plant breeding may be limited, it should be mentioned here that the anthocyanin pigmentation of coleoptiles or auricles was recommended by the UPOV (Union internationale pour la protection des obtentions vegetales i.e. International Union for the Protection of New Varieties of Plants) as 2 out of 26 traits can be used for determination of the homogeneity or distinctness of wheat varieties (UPOV 1994).

By analyzing cytogenetic stock collections the chromosomal location of genes responsible for anthocyanin pigmentation was determined. For coleoptile color three major genes were found to be located on the homoeologous group-7 chromosomes. One was described by E.R. Sears to be present on chromosome IX (7A) of the variety ‘Hope’ (Sears 1954). This location was confirmed by Gale and Flavell (1971), identifying in addition a second major gene for red coleoptile color on chromosome 7B of ‘Hope’ by analyzing ‘Chinese Spring/Hope’ substitution lines. The genes were designated *Rc1* and *Rc2*, respectively. A third red coleoptile gene on chromosome 7D (*Rc3*) was identified by Jha (1964) studying the synthetic wheat of McFadden and Sears (1946). Again, chromosome 7D was described to carry a gene for red coleoptile color by Sutka (1977) who investigated the Ukrainian winter wheat variety ‘Mironovskaya 808.’ Both *Rc3* and *Rc1* were mapped in relation to RFLP markers with a distance of 3 cM from the marker *Xpsr108* (Chao et al. 1989) and by using a QTL approach in the region of the marker *Xcdo17* (Nelson et al. 1995b), respectively.

The present study was initiated in order to map the three homoeologous group-7 red coleoptile color genes in wheat by using microsatellite markers, and to obtain more information about gene expression. In addition, we investigated the geographical distribution of these genes in 468 mainly European wheat varieties.

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Materials and methods

Plant materials

Two intrachromosomal substitution lines, ‘Chinese Spring/Hope 7A’ carrying *Rc1* and ‘Chinese Spring/Hope 7B’ carrying *Rc2*, were crossed with the non-colored spring wheat accessions

TRI15010 (population 1) and TRI2732 (population 2), respectively. The substitution lines were kindly supplied by A.J. Worland, JIC, Norwich. One single F_1 plant of each population was used to produce 103 and 153 F_2 plants for populations 1 and 2 respectively. For mapping *Rc3* on chromosome 7D an already existing mapping population ('Mironovskaya 808'×'Aibian 1') created in order to map the GA insensitive dwarfing gene *Rht10* on chromosome 4D (Börner et al. 1997) was used (population 3). Here 74 genotypes were analyzed. In addition 109 recombinant inbred lines (RILs) of the 'International Triticeae Mapping Initiative' (ITMI) population were evaluated phenotypically. The RILs were developed by single-seed descent to the F_8 or F_9 at Cornell University, Ithaca (Nelson et al. 1995a), and obtained by Dr. Philippe Leroy, INRA, Clermont-Ferrand, France. The RILs were analyzed together with their parents 'Opata 85' and 'W7984', a synthetic hexaploid wheat, as well as the progenitors of the synthetic wheat, the *Triticum tauschii* accession 'CIGM86.940' (DD) and the tetraploid wheat 'Altar 84' (AABB). For studying the geographical distribution of coleoptile anthocyanin pigmentation 468 mainly European wheat varieties obtained from different breeders have been analyzed. A list of these varieties was published by Khlestkina et al. (2001).

Phenotypic evaluation

F_2 seeds of populations 1 and 2, ten seeds of each of the 74 F_3 lines of population 3 and ten seeds of each of the 468 wheat varieties were placed together with the parents of the mapping populations on filter paper, moistened with distilled water and kept for 36 h at 4°C without light to synchronize germination. After that the temperature of the growth chamber was increased to 15°C and after 5-days growing at a photoperiod of 14 h light/10 h darkness the color of the coleoptiles was scored.

The parents and the 109 RILs of the ITMI population were divided into three replicates. Whereas one set was grown under the same conditions as mapping populations 1 to 3 (exp. 1), the second and the third replications were cultivated at 20°C under constant lighting (exp. 2), as described by Nelson et al. (1995b), and at 25°C and constant lighting (exp. 3), respectively. In addition, data for coleoptile color were obtained from a field experiment (exp. 4) grown in order to perform QTL mapping studies on agronomically important genes (Börner et al., unpublished).

Molecular-marker analysis

Leaf material was cut from five to 6-week-old F_2 seedlings of populations 1 and 2, which were transferred from the growth chamber to the green-house after scoring the coleoptile color. The fresh leaves were used for DNA extraction following a modified procedure of Plaschke et al. (1995). For population 3, DNA was already available (Börner et al. 1997). Because the *Rc* genes were known to be located on the homoeologous group-7 chromosomes, wheat microsatellite (WMS) markers known to map on chromosomes 7A (31), 7B (34) and 7D (26) were selected. WMS markers and the procedures of WMS analysis are as described by Röder et al. (1998). Unpublished primer sequences are available upon request. In order to determine polymorphic markers the parents of the mapping populations were screened. After identifying polymorphic markers, individual F_2 plants were genotyped.

The phenotypic data obtained from analyzing the RILs of the ITMI population were integrated into a framework map composed of more than 300 RFLP and ten microsatellite markers (Röder et al. 1998; Röder, unpublished data). Linkage maps were constructed with the MAPMAKER 2.0 computer program (Lander et al. 1987) and QTL-analysis was performed using the QGENE application (Nelson 1997).

Chromosomal arm positions of markers mapped in the centromere region were confirmed by employing di-telosomic lines of the variety 'Chinese Spring.'

In order to find out whether closly linked microsatellites could be used as diagnostic markers 57 red-colored varieties were selected randomly out of the 468 European varieties and screened together with 15 colorless controls. As microsatellite markers we used *Xgwm913* (7 A), *Xgwm263* (7B), *Xgwm1184* (7B) and *Xgwm111* (7D).

Results

Genetic mapping of *Rc* genes using F_2/F_3 populations

The phenotypic segregation data were obtained from scoring F_2 (population 1 and 2) or F_3 (population 3) plants. The F_2 segregation patterns observed gave clear indication for a monogenic inheritance of the target trait. In population 1, of the 103 plants 78 were red colored, whereas 25 were determined as having no coloration. The ratio tested by χ^2 fitted an expected 3:1 ratio for a monogenic dominant inheritance of the target trait (χ^2=0.03, P>0.80). Analyzing population 2, a segregation ratio of 33 red: 120 non-colored F_2 plants was detected, indicating again the presence of a single gene as tested by χ^2 (χ^2=0.96; P>0.30). However, the mode of inheritance of the red coleoptile color was recessive. Scoring the F_3 families of population 3, 17 (red): 31 (heterozygous): 25 (non-colored) plants were observed. For the expected segregation ratio of 1:2:1 a χ^2=3.42 was calculated (P>0.20).

From the wheat microsatellites tested, 20 out of 31 (chromosome 7A; 65%), 23 out of 34 (chromosome 7B; 68%) and 11 out of 26 (chromosome 7D; 42%) were found to be polymorphic between the parents. Because the *Rc* genes were expected to map close to the centromere (McIntosh et al. 1998), polymorphic markers of that region were chosen for analyzing the F_2 individuals of the whole mapping populations.

In order to clarify the chromosomal arm location of the *Rc* loci the markers were tested by using DNA of the di-telosomic lines of ‚Chinese Spring' for the 7S and 7L chromosomes. Based on these data it could be concluded that the *Rc* loci are located on the short arms of chromosomes 7A, 7B and 7D. The constructed maps are presented in Fig. 1. Because the map positions of all three genes are highly comparable it may be concluded that they are members of a homoeologous series. Regarding the rules for the symbolization of genes in homoeologous sets we decided to designate the group-7 red coleoptile color genes as *Rc-A1*, *Rc-B1* and *Rc-D1*.

Identification of diagnostic markers

In order to test the most-closely linked microsatellite markers, if they are diagnostic for the trait, 57 red-colored varieties were tested with the selected markers *Xgwm913* (7A), *Xgwm263* (7B), *Xgwm1184* (7B) and *Xgwm111* (7D).

Only one and seven of them showed the expected 173-bp allele of *Xgwm913* (linked with *Rc-A1* in 'Hope') and the 192-bp allele of *Xgwm111* (linked with *Rc-D1* in 'Mironovskaya 808'), respectively. The 192-bp allele, however, was also found in two colorless varieties.

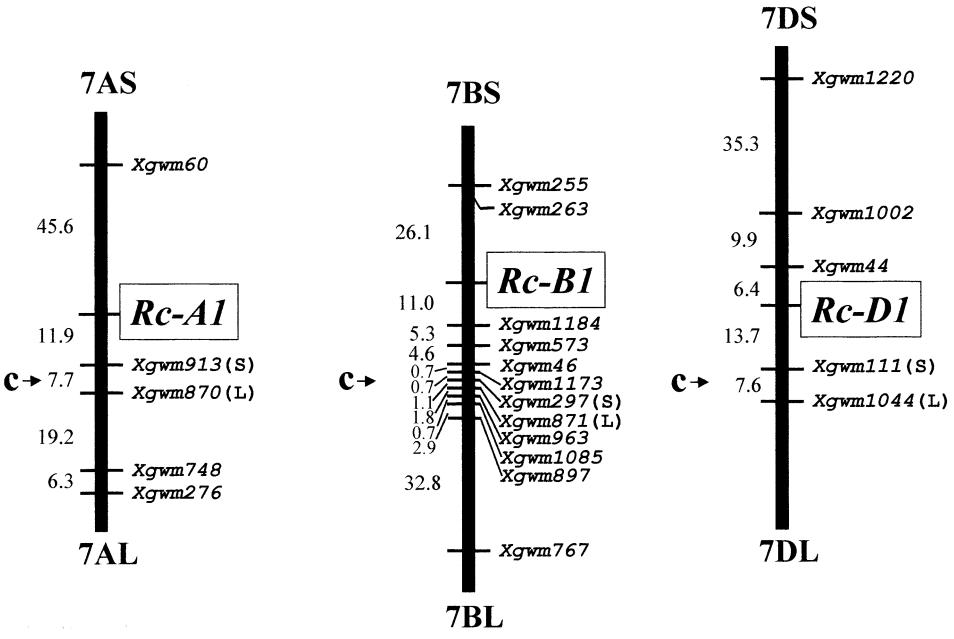

Fig. 1 Partial maps of chromosomes 7A, 7B and 7D showing the map positions of *Rc-A1*, *Rc-B1* and *Rc-D1*, respectively. Genetic distances are given in centimorgans (cM), *S*=short arm, *L*=long arm, *c*=centromere region, as confirmed by testing flanking markers with di-telosomic lines

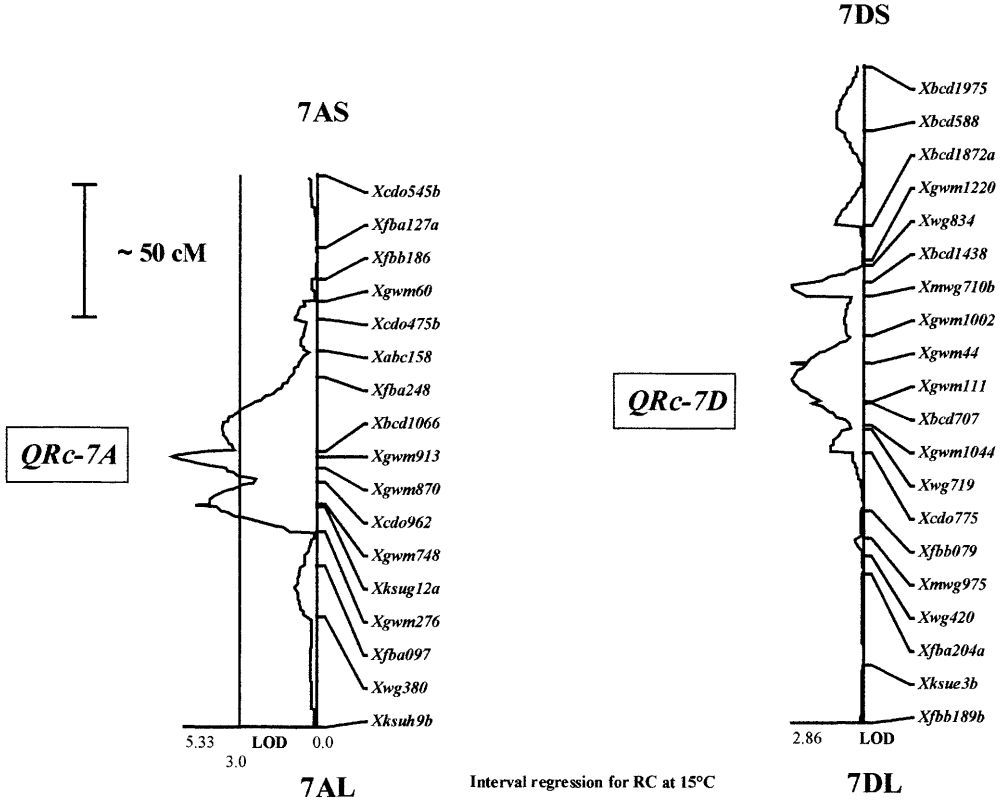

Fig. 2 Linkage maps of chromosomes 7A and 7D together with LOD-score plots showing the locations of *QRc-7A* and *QRc-7D*. The loci were detected by scoring the ITMI mapping population grown at 15°C

Phenotypic evaluation and mapping of *Rc* genes as QTLs in RILs

Different experiments were performed for mapping red coleoptile genes by analyzing the ITMI population. In exp. 1 and 2, beside the RILs, the parents and the progenitors of the synthetic wheat were scored. It was shown that the coleoptiles of 'Opata' were non-colored whereas the synthetic wheat 'W7984', as well as the two progenitors 'Altar 84' and '*Triticum tauschii*', did show an anthocyanin pigmentation. Therefore, it could be concluded that there are at least two genes present on the A or B and D genomes.

The results of the QTL analysis are given in Table 1. The maps of chromosomes 7A and 7D with LOD score plots of QTLs detected in exp. 1 are presented in Fig. 2. It is shown, that in all three growth-chamber experiments two loci on chromosomes 7AS and 7DS, respectively,

Table 1 Putative QTLs for the red coleoptile in the ITMI-population depending on the environmental conditions

Condition	Marker	Chromosome	LOD-IM[a]	F-SMA[b]	Rsq-SMA
Growth chamber 15°C	*Xgwm913*	7A	5.33	33.09	0.3306
	Xbcd707	7D	2.86	19.27	0.1502
Growth chamber 20°C	*Xgwm913*	7A	7.14	44.65	0.3999
	Xbcd707	7D	1.90	9.81	0.0840
Growth chamber 25°C	*Xgwm748*	7A	6.32	36.40	0.3487
	Xbcd707	7D	2.99	14.61	0.1192
Field data	*Xcdo1401*	4B	2.10	12.06	0.1057
	XFba097	7A	2.35	11.84	0.1826

[a] LOD determined by interval mapping (significance threshold 3.0)
[b] F-value determined by single-marker analysis (significance threshold 15.0)

Table 2 Number of varieties classified according to coleoptile color (++ dark-red colored; + red colored; – not colored; +/–, ++/– segregating)

Country	++	+	++/–; +/–	–	Total
France	–	28 (38%)	17 (23%)	28 (38%)	73
Germany	1 (1%)	20 (28%)	11 (15%)	39 (55%)	71
Austria	2 (4%)	13 (25%)	2 (4%)	34 (67%)	51
Greece	11 (28%)	2 (5%)	1 (2%)	26 (65%)	40
UK	–	24 (62%)	6 (15%)	9 (23%)	39
Bulgaria	3 (9%)	–	5 (14%)	27 (77%)	35
Italy	–	7 (22%)	1 (3%)	24 (75%)	32
Yugoslavia	–	4 (17%)	3 (12%)	17 (71%)	24
Spain	1 (5%)	1 (5%)	2 (9%)	18 (82%)	22
Turkey	1 (6%)	–	1 (6%)	15 (88%)	17
Ukraine	3 (19%)	1 (6%)	4 (25%)	8 (50%)	16
Netherlands	1 (7%)	4 (27%)	3 (20%)	7 (47%)	15
Switzerland	–	–	1	8	9
Belgium	–	1	3	1	5
Sweden	–	1	–	3	4
Portugal	–	–	–	3	3
Romania	1	–	–	1	2
Russia	–	–	2	–	2
USA	1	1	–	–	2
Finland	–	–	–	1	1
Poland	1	–	–	–	1
China	–	–	–	1	1
Argentina	–	–	–	1	1
Mexico	–	–	–	1	1
Japan	–	–	–	1	1
Total	26 (6%)	107 (23%)	62 (13%)	273 (58%)	468

were detected, suggesting that 'Altar 84' is carrying a red-coleoptile color gene on 7A. The QTLs, designated according to the rules of McIntosh et al. (1998), were mapped within intervals, highly comparable to the regions where the major genes in the F_2/F_3 mapping studies were detected. The LOD scores varied between the experiments and were only significant for *QRc-7A*.

Analyzing the field data (exp. 4) only one QTL was detected on the homoeologous group-7 chromosomes (*QRc-7A*). A second locus (*QRc-4B*) appeared to be associated with the RFLP marker *Xcdo1401* located close to the centromere on the long arm of chromosome 4B (Röder et al. 1998). Both loci did not reach the LOD 3.0 threshold.

Geographical distribution of *Rc* genes

The results of the coleoptile color screening are summarized in Table 2. Most of the tested varieties, about 60% (273), were found having non-colored coleoptiles, whereas in 23% (107) and 6% (26) of the wheat genotypes red and dark-red colored coleoptiles, respectively, were detected. Sixty two varieties (13%) were heterozygous. The highest percentage of varieties with red-colored coleoptiles was found in the United Kingdom (62%), followed by France (38%) and Germany (28%). High frequencies of heterozygous varieties were discovered in Ukraine (25%) and France (23%). A list with the results for all tested varieties is presented by Khlestkina et al. (2001).

Discussion

By using molecular marker techniques it became possible to map several sets of genes precisely, suggesting that they belong to homoeologous series within wheat. Examples are given for loci determining photoperiod response (*Ppd*), vernalization response (*Vrn*), red grain color (*R*) or sphaerococcoid spike morphology (*S*) (Flintham and Gale 1995; Börner et al. 1998; Salina et al. 2000). By performing comparative mapping studies within the Triticeae the homoeologous series of gene loci could often be extended to further species such as rye and barley (Korzun et al. 1997; Börner, 1999).

In the present paper a set of homoeologous genes determining the coloration of coleoptiles in wheat was mapped about 15 to 20 cM distal from the centromere on the short arms of the homoeologous group-7 chromosomes of hexaploid wheat. The mapping data confirm the former results of gene localization presented by Chao et al. (1989) or Nelson et al. (1995b). By analyzing the ITMI mapping population it could be suggested that the A genome of *Triticum durum* and the D genome of *Aegilops tauschii* are carrying homoeologous loci.

A gene determining a red coleoptile is also known in rye, designated anthocyaninless (*an* or *an1*) syn. viridis (*vi*). Using a translocation tester set, *an* was located on chromosome 7R (De Vries and Sybenga 1984). Based on linkage data to other morphological genes, Malyshev et al. (2001) concluded that *an1* is located close to the centromere on the short arm of chromosome 7R and, therefore, most probably was homoeologous to the group-7 wheat genes. Interestingly, in barley a gene designated *ant1* (*anthocyanin-less 1*) is described to be located near the centromere on chromosome 7HS influencing the anthocyanin pigmentation of stem, auricles, awns or lemma (Franckowiak 1997; Lundqvist et al. 1997). Coleoptile coloration was not reported.

The possibility to use microsatellites as diagnostic markers was demonstrated by Worland et al. (1998). Based on the close linkage of 0.6 cM between the commercially important dwarfing gene *Rht8* and the marker *Xgwm261*, the distribution of *Rht8* in various wheat varieties was determined by analysis of a diagnostic allele of *Xgwm261*. In the present paper only a few varieties having the red-coleoptile color phenotype were found to carry the alleles linked to these genes in 'Hope' (one variety) or 'Mironovskaya 808' (seven varieties). Therefore, one should conclude that a marker distance of about 10 cM to the gene of interest is too large for using a marker as diagnostic. Furthermore, the extreme low frequency of the *Rc* gene linked 'Hope' alleles observed with molecular markers gives some indication that the red coleoptile alleles (or even genes) in the European varieties may not have the same origin as those of the US variety 'Hope.'

The coleoptile color screening within 468 wheat varieties has shown that *Rc* genes are present in most of the European wheat collections. Interestingly, the frequency of varieties having red-colored coleoptiles was lower in Southern and Eastern Europe compared to Western European countries. In 1973 a coleoptile color screening was performed by Zeven (1973) including about 900 wheat varieties (436 from Europe). In this study 80% of varieties with non-colored coleoptiles were observed in comparison to 60% in the present investigation.

By analyzing the ITMI population in different environments it became clear that the phenotypic expression of red coleoptile color genes varies, and that, beside the three major genes on the homoeologous group-7 chromosomes, further loci may exist. It is demonstrated that *QRc-7D* was not significant (LOD <3) in the growth-chamber experiments and even not detectable in the field experiment, although the presence of a red-coleoptile color gene on the D genome was determined by testing the *Ae. tauschii* progenitor of synthetic wheat. The low LOD score of *QRc-7D* may be the reason why Nelson (1995b) described only the 7A QTL, analyzing the same mapping population. The detection of *QRc-4B* is in accordance with Sutka (1977) who described a supressor for coleoptile color to be located on chromosome 4B.

The phenotypic variability of this trait is generally known and was already reported in the Twenties (Bolsunov 1928). Beside the temperature, light intensity and duration was described to influence anthocyanin pigmentation (Ausemus et al. 1967). An explanation for the altered expression of the red coleoptile color genes may be the very complex biosynthetic pathway of the anthocyanins. Gale and Flavell (1971) postulated that at least eight anthocyanins are responsible for the anthocyanin pigmentation of wheat coleoptiles. Therefore, the suitability of color traits for determining the homogeneity of wheat varieties should be reconsidered.

There may be some potential for using the homoeologous group-7 coleoptile color genes as morphological markers. A gene determining adult plant resistance to septoria tritici blotch (*Mycosphaerella graminicola*), temporarily designated *AST*, was detected on chromosome 7D. *AST* maps 6.1 cM proximal to *Rc-D1* (Ellerbrook and Worland 2001).

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