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The *Q* locus of Iranian and European spelt wheat

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Abstract A dominant allele at the *Q* locus on chromosome 5A is believed to be the principal factor responsible for free-threshing, square-head spikes with a non-fragile rachis in bread wheat, *Triticum aestivum* ssp. *aestivum*. The spelt syndrome, resulting in pyramidal spikes with a brittle rachis and hulled grain in *T. aestivum*, is believed to be principally caused by the *q* allele. Chromosome 5A of European and Iranian spelt was substituted for 5A of bread wheat and the lines were characterized with molecular markers. The substitution of bread wheat chromosome 5A by 5A of European spelt resulted in weakly hulled, pyramidal spikes with a non-brittle rachis, whereas and the substitution of 5A by 5A of Iranian spelt did not alter spike morphology at all. It is concluded that the expression of the spelt syndrome depends, to a large extent, on the interactions of *q* with genes controlling glume tenacity and rachis fragility on other chromosomes. The genetic basis for the spelt syndrome and the apparent presence of the *Q* allele in Iranian spelt are discussed.

Key words *Triticum* · Hexaploid · Mapping · RFLP · RSL · Spelt

Introduction

Hexaploid wheat, *Triticum aestivum* L. (genomes AABBDD), originated from the hybridization of tetraploid emmer wheat, *Triticum turgidum* L. (genomes AABB) with goatgrass *Aegilops tauschii* Coss. (Kihara 1944; McFadden and Sears 1946). McFadden and Sears (1946) pointed out that synthetic hexaploid wheats produced by the hybridization of *T. turgidum* with *Ae. tauschii* strongly resembled spelt, *T. aestivum* ssp. *spelta* (L.) Thell. Spelt is characterized by a narrow, pyramidal spike with adhering glumes and brittle rachis. These characters appear primitive in comparison to the free-threshing forms of *T. aestivum*, such as bread wheat (*T. aestivum* ssp. *aestivum*). McFadden and Sears (1946) therefore concluded that the free-threshing forms of *T. aestivum* evolved from spelt. At the time of McFadden and Sears's studies, spelt wheat was known to come only from Europe, where it has been cultivated by the Germanic people since Roman times. The lack of evidence for the existence of spelt in Asia was an apparent contradiction, since *T. aestivum* had to originate in Asia, the home of *Ae. tauschii*. The discovery of spelt in Iran (Kuckuck and Schiemann 1957; Kuckuck 1959) and other locations in Asia resolved this dilemma.

The morphological characteristics of spelt, such as spike shape, hulled grain and brittle rachis, are principally controlled by a recessive allele at the *Q* locus on chromosome 5A. Muramatsu (1963) suggested that the *Q* allele is a hypermorph of *q*. An increase in the *q* dose to five in the sporophyte resulted in a squarehead spike morphology. The *Q* locus is located distally in the long arm of chromosome 5A (Liu and Tsunewaki 1991; Gill et al. 1996; Kato et al. 1998). The free-threshing forms of hexaploid wheat have the dominant *Q* allele whereas European spelt, other hulled forms of hexaploid wheat and hulled tetraploid wheats have been shown to have the *q* allele (Mac Key 1954; Mac Key 1966; Muramatsu 1986). The critical position of Asian spelt in the scheme of the evolution of hexaploid wheat brings up a relevant concern of whether the various forms of Asian spelt have *Q* or *q* alleles. Kuckuck (1964) investigated segregation in crosses between European spelt, Iranian spelt, and *T. aestivum* ssp. *macha* (Dekapr. & Menabde) Mac Key (further *T. macha*), which is a Transcaucasian hulled wheat with a brittle rachis and dense spikes, and bread wheat. He concluded that Iranian spelt shares the *q* allele with European spelt and *T. macha* and provided evidence for the existence of several alleles at the *Q* locus of different strengths to suppress the spelt syndrome and different dominance relationships. The late E. R. Sears (un-

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published) substituted chromosome 5A from European spelt and Iranian spelt for the *T. aestivum* ssp. *aestivum* cv Chinese Spring (henceforth CS) chromosome 5A. While a disomic substitution (DS) line involving the European spelt (henceforth DS 5AEurope) had a speltoid spike morphology, suggesting the presence of *q*, the DS line involving Iranian spelt (henceforth DS 5AIran) had the typical square-head morphology of bread wheat. Since the spelt chromosome 5A could have potentially recombined with 5A of Chinese Spring during the substitution process, the late E. R. Sears requested that we characterize the lines with molecular markers. This characterization is reported here.

Materials and methods

The late E. R. Sears acquired European spelt (Sears' accession P78–81–1) from E. S. McFadden and Iranian spelt (H. Kuckuck's accession 407a) from H. Kuckuck. To develop DS lines, Sears crossed the two spelt accessions with CS monosomic 5A (female) and recurrently backcrossed the monosomic F_1 to CS monosomic 5A, always using the CS monosomic as a female. Seven backcrosses were made to develop each DS line.

We crossed the two DS lines with Chinese Spring and the F_1 progenies were crossed as males with monotelosomic 5AL (mt5AL). Monosomic F_1 plants were selected and self-pollinated. Disomics were selected in the F_2 generation. Each of these recombinant substitution lines (RSLs) breed true for a recombined (or a parental) 5A chromosome substituted in the nearly isogenic background of CS. The population derived from DS 5AEurope comprised 107 RSLs while that derived from DS 5AIran comprised 87 RSLs.

To assess restriction fragment length polymorphism (RFLP) at loci flanking the *Q* locus, nuclear DNAs were isolated from individual plants (Dvořák et al. 1988) of DS lines, the spelt accessions and CS. Southern blots of DNAs of DS 5AIran and CS were hybridized according to Luo et al. (1998) with 30 probes selected from the pool of probes used to construct the map of chromosome $5A^m$ in *Triticum monococcum* (Dubcovsky et al. 1996). Polymorphisms detected in the survey were used to construct the map of chromosome 5A. The *Q* locus was mapped relative to polymorphic markers in the population of RSLs derived from DS 5AEurope. The computer program Mapmaker/EXP 3.0 (Lander et al. 1987; Lincoln et al. 1992) and the Kosambi function (Kosambi 1943) were employed in map construction. A LOD threshold of three was used. 5A loci showing free recombination in the investigated populations were placed on a map on the basis of their position on the *T. monococcum* $5A^m$ map (Dubcovsky et al. 1996) and the wheat 5A map (Gill et al. 1996).

F_1 plants from crosses DS 5AEurope and DS 5AIran with CS were self-pollinated and F_2 progeny were grown in the field. The spike morphology of the F_2 plants was compared with that of the parental DS lines. The fit between observed and expected segregation ratio was tested by χ^2.

Results

Both the Iranian spelt and European spelt which the late E. R. Sears used as sources of chromosome 5A in the development of DS lines are hulled and have lax spikes (Table 1, Fig. 1). Both forms have adhering glumes and a fragile rachis but they differ in rachis disarticulation. Under pressure the rachis of European spelt breaks, usually at the spikelet base (barrel disarticulation). This disarticulation is typical of *Ae. tauschii*. The rachis of the Iranian spelt breaks under pressure so that the rachis internode remains attached to the bottom of the spikelet base (wedge disarticulation). This disarticulation is typical of emmer and einkorn wheats. The spikes of DS 5AEurope have a pyramidal (speltoid) spike morphology (Fig. 1). The glumes adhere to seeds less tenaciously than do those of the parental spelt and the rachis is not at all brittle (Table 1). The spikes of DS 5AIran are indistinguishable from those of CS (Table 1, Fig. 1).

Fig. 1 Spike morphology of the 5A disomic substitution lines and their parents

Table 1 Spike characteristics of European and Iranian spelt, disomic substitution lines with chromosome 5A of spelt substituted in the Chinese Spring genetic background, and Chinese Spring

Line	Spike	Rachis	Rachis disarticulation	Glume
Chinese Spring	Square-head	Non-brittle	None	Non-adhering
Spelt Iran	Lax, speltoid	Brittle	Wedge	Adhering
DS 5AIran	Square-head	Non-brittle	None	Non-adhering
Spelt Europe	Lax, speltoid	Brittle	Barrel	Adhering
DS 5AEurope	Lax, speltoid	Non-brittle	None	Weakly adhering

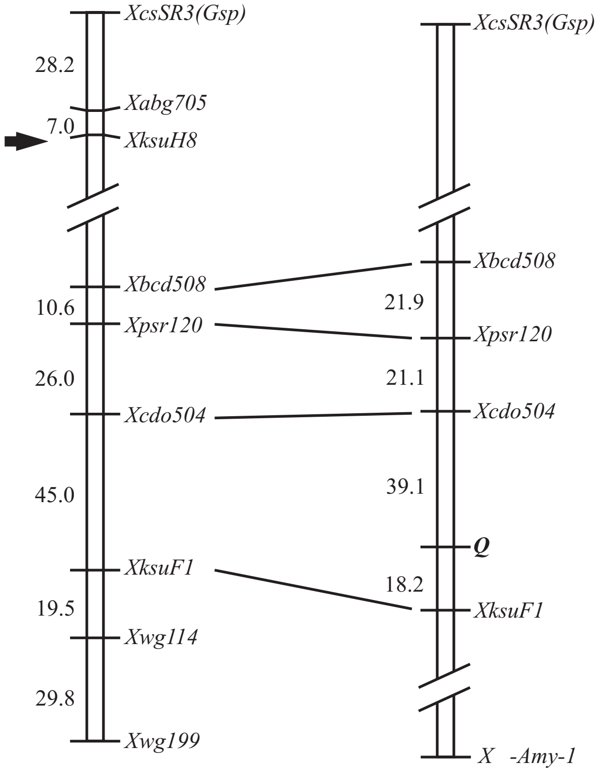

Fig. 2 Linkage maps of chromosome 5A. The map on the left is based on a population of 87 RSLs derived from recombination of the Iranian spelt chromosome 5A substituted in Chinese Spring with Chinese Spring 5A. The map on the right is based on a population of 107 RSLs derived from recombination of the European spelt chromosome 5A substituted in Chinese Spring with Chinese Spring 5A. The distances between markers are in centimorgans (cM). The approximate position of the centromere is indicated by an *arrow*. The position of all markers showing linkage was based on LOD >3.0

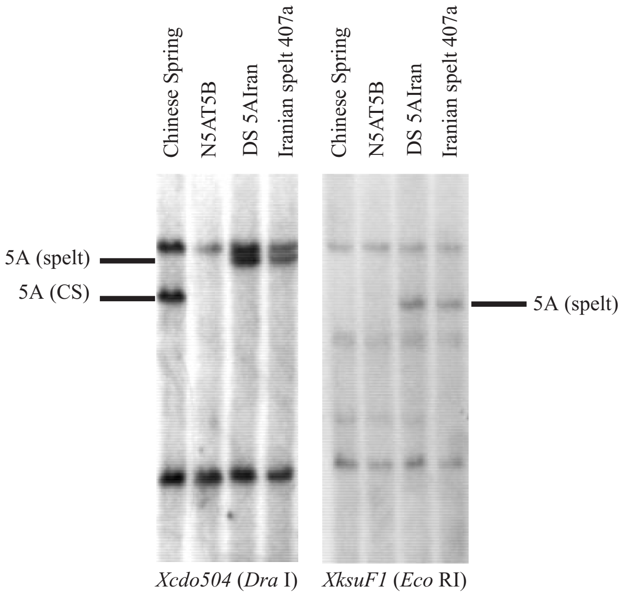

Fig. 3 Southern blots of genomic DNAs of Chinese Spring, Chinese Spring nullisomic 5A-tetrasomic 5B (*N5AT5B*), the disomic substitution line (*DS*) of chromosome 5A of Iranian spelt in Chinese Spring, and Iranian spelt (accession 407a) hybridized with CDO504 (*Dra*I) and KSUF1 (*Eco*RI). *Xcdo504* and *XksuF1* flank the *Q* gene on the 5A map (Fig. 2). Note that in both profiles, the CS 5A-chromosome DNA fragment (chromosome location indicated by absence in N5AT5B) is absent in the DS line and is replaced by a fragment shared by the DS line with the Iranian spelt

The morphology of spikes of F_2 plants from crosses of the DS lines with CS was compared with that of the parental DS lines and spelt. The spike morphology of 78 F_2 plants from the cross CS × DS 5AIran was identical to that of CS. The population of F_2 plants derived from the cross CS × DS 5AEurope segregated 33 speltoid : 52 intermediate : 17 square-head plants, conforming to a 1 : 2 : 1 segregation ratio ($P = 0.08$).

Polymorphism between chromosome 5A in the Iranian spelt and CS was investigated in 300 probe-enzyme combinations, involving 30 loci on chromosome 5A. Ten of the thirty loci were polymorphic (31 probe-enzyme combinations). Nine of them were used to construct a map of chromosome 5A employing an RSL population derived from DS 5AIran (Fig. 2). The nine clones were then used to search for polymorphism between chromosome 5A in European spelt and Chinese Spring. Five of these nine loci were polymorphic. In addition, locus *Xβ-Amy-1* was also polymorphic. A map of the chromosome was constructed using an RSL population derived from DS 5AEurope (Fig. 2) and the *Q* locus was mapped relative to molecular markers. The *Q* locus is in the interval *Xcdo504–XksuF1*, 39.1 cM distal to *Xcdo504* and 18.2 cM proximal to *XksuF1* (Fig. 2).

To determine whether the chromosome region including *Q* was from spelt or Chinese Spring in DS 5AIran, RFLPs among Iranian spelt 407a, DS 5AIran and Chinese Spring were compared. At each of the nine polymorphic loci, the DS line had an allele identical to that of the Iranian spelt and different from that of Chinese Spring. This is illustrated in Fig. 3 for loci flanking the *Q* locus, *Xcdo504* and *XksuF1*.

Discussion

The *Q* locus was mapped to the *Xcdo504–XksuF1* interval in the distal region of the long arm of chromosome 5A. This location of *Q* is consistent with its location on a map of chromosome 5A reported by Kato et al. (1998). On that map, *Q* was proximal to *Xpsr370*. The *Xpsr370* locus is distal to *XksuF1* (Dubcovsky et al. 1996).

At each of the nine loci in which Iranian spelt differed from CS, DS 5AIran had the same allele as the parental Iranian spelt 407a, indicating that an intact chromosome 5A of spelt was substituted. To replace the *q* allele by the *Q* allele during the chromosome substitution procedure, a disomic plant would have to have been erroneously selected instead of a monosomic plant in one of the backcross generations and, simultaneously, a double-crossover within the *Xcdo504—XksuF1* interval would have had to have occurred. This seems unlikely.

Since the spike morphology of DS 5AIran is identical to that of Chinese Spring and no segregation was observed in the F_2 from the cross CS × DS 5AIran, the parental Iranian spelt seems to possess the *Q* allele on chromosome 5A, in spite of the fact that it has adhering glumes and a brittle rachis. This finding is in a stark contrast to the results reported by Kukuck (1964) who crossed Iranian spelt 407a with European spelt and observed no segregation of the speltoid syndrome in F_2 progeny derived from four F_1 plants. He also crossed accession 407a with *T. macha*, which is a Transcaucasian hulled wheat with a brittle rachis and the *q* allele (Kuckuck 1964; Mac Key 1966). Of 403 F_2 plants, 401 were hulled a with brittle rachis and two were hulled with a tough rachis. Both lines of evidence led Kuckuck (1964) to conclude that Iranian spelt, including accession 407a, has the *q* allele.

Adhering glumes, in addition to the *Q* locus, are controlled by the *Tg* loci on chromosomes in homoeologous group 2 (Kerber and Rowland 1974). Likewise, brittle rachis is controlled by loci in several homoeologous groups (Cao et al. 1997; M.C. Luo, R. S. Kota, and J. Dvořák, unpublished). Kuckuck observed plants in which the association between hulled habit and rachis brittleness was broken. He concluded that the *Q* factor is not a Mendelian locus but a region of chromosome 5A and the exceptional plants originate by recombination within the region or by mutation. Compared to the parental European spelt, the hulled phenotype is expressed weakly in DS 5AEurope and rachis brittleness is entirely absent. This finding clearly shows that the spelt syndrome in European spelt depends upon loci on other chromosomes in addition to 5A. The same must be true for Iranian spelt. In crosses of 407a with bread wheat, plants were observed with recombination between the hulled phenotype and the rachis brittleness (Kuckuck 1964). Kuckuck concluded that the traits are controlled by loci in coupling on chromosome 5A 14.1% apart. The absence of association between the hull phenotype and rachis brittleness in the comparison of European spelt and DS 5AEurope casts serious doubt on the existence of this linkage. Duplicate or multiplicate gene segregation was probably mistaken for linkage.

It is possible that the *Q* locus is regulatory and modifies the expression of structural genes on other chromosomes. This hypothesis could explain why the expression of *Q* depends on the genetic background (Kuckuck 1964; Mac Key 1954; Mac Key 1966) and why the expression of *Q* was apparently much weaker in the DS 5AEurope line than in the parental European spelt. These interactions could also account for the different dominance relations between *q* and *Q* in different genetic backgrounds observed by Kuckuck (1964). Similar changes in dominance were observed here. While Kuckuck reported that *Q* is completely dominant to *q* in crosses of spelt with bread wheat, the two alleles appeared incompletely dominant in the cross DS 5AEurope × CS.

If the full spelt syndrome results from interaction between *q* and structural loci on other chromosomes, as the comparison of DS 5AEurope with the parental European spelt suggests and as previously concluded by Mac Key (1966), the absence of segregation in tests for allelism among different hulled wheats reported by Kuckuck could potentially reflect monomorphism at other loci, not in *Q*. Since the expression of the syndrome may depend on as many as four or more loci, large populations need to be employed. Since allelism studies did not involve molecular markers, it was impossible to determine whether rare phenotypes, such as those in crosses of Iranian spelt 407a × *T. macha* reported by Kuckuck (see above), were irrelevant anomalies or segregants critical for the test of hypotheses.

Two hypotheses can be offered to account for the apparent presence of *Q* in DS 5AIran. (1) It is possible that accession 407a was a segregant out of a cross of spelt with bread wheat. Kuckuck (1964) reported that accession 407a was exceptionally heterogeneous, containing plants of bread wheat, *speltoiforme* plants (spelt-like free-threshing plants with non-brittle rachis), in addition to spelt. Hybridization among these types should be expected in such a population. In such a case, the 407a line may have *Q* and the spelt syndrome may have been controlled by other loci. Work aimed at the identification of these loci by co-segregation with molecular markers is in progress. A less-likely hypothesis (2) is that the 407a population contains a *q* allele which does not affect spike morphology in the CS genetic background but does in the Iranian spelt background. This allele would have to be different from the *q* allele of European spelt.

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