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Control of lectins in *Triticum aestivum* and *Aegilops umbellulata* by homoeologous group 1 chromosomes

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Summary. Each of the three genomes in hexaploid wheat controls the expression of a specific lectin in the embryo. The chromosomes which control their synthesis were determined using nullisomic-tetrasomic and inter-varietal chromosome substitution lines of 'Chinese Spring'. All three wheat lectins were shown to be controlled by the homoeologous group 1 chromosomes. Using ditelosomic lines of 'Chinese Spring' the lectin genes could be localized on the long arms of chromosomes 1A and 1D. Inter-specific addition and substitution lines of *Aegilops umbellulata* chromosomes to 'Chinese Spring' indicated that chromosome 1U, which is homoeologous to the group 1 chromosomes of wheat, controls lectin synthesis.

Key words: *Aegilops umbellulata* – Genetics – Lectin – *Triticum aestivum*

Introduction

Plant lectins (phytohemagglutinins) are a heterogeneous class of proteins (or glycoproteins) sharing in common their ability to recognize and bind specific sugars or sugar-containing molecules. They are widely distributed in the plant kingdom and in most – but not all – cases are typically found in seeds (Goldstein and Hayes 1978; Lis and Sharon 1981; Barondes 1981; Kauss 1981). A classical example of such a seed lectin is "wheat germ agglutinin" (WGA). Its biochemical, physico-chemical and biological properties have been determined (Allen et al. 1973; Rice and Etzler 1974; Nagata and Burger 1974; Wright 1980). It is a dimeric protein consisting of two subunits of molecular weight 18,000. It has a high isoelectric point and is characterized by unusually high contents of gly and cys, each exceeding 20% on a molar basis. WGA binds specifically to N-acetylglucosamine or oligomers of this sugar, a property which is exploited in its purification by affinity chromatography.

WGA has distinctive properties to lectins from dicotyledonous seeds (Goldstein and Hayes 1978; Mishkind et al. 1980) and has for a long time been considered as a unique lectin. However, the isolation and characterization of lectins from such other cereals as rye and barley (Peumans et al. 1982 g), *Brachypodium sylvaticum* (Peumans et al. 1982 d) and rice (Peumans et al. 1982 g) changed this view, since it became evident all these lectins belong to the same family of closely related proteins which have been highly conserved during evolution. Within the tribe of the Triticeae, lectins from all species analysed up to now are structurally so closely related that they are immunologically identical and can exchange their subunits without any loss of activity (Peumans et al. 1982 c). Because of the striking resemblances between all these lectins, they have all been classified as "cereal lectins".

In a detailed study of the origin of isolectins in polyploid wheats and triticales, it was shown that both in single and complex genomes, each individual genome directs the synthesis of its own lectin-polypeptide (Peumans et al. 1982 b). In hexaploid wheat for instance, three different lectin subunits are synthesized, one each being under the control of the A, B and D genomes. However, since in the living cell no distinction is made between identical and non-identical lectin subunits, both homomeric (composed of two identical subunits) and heteromeric (composed of two different subunits) active lectin dimers are assembled. In hexaploid wheat, for instance, this results in the formation of 6 different isolectins (3 homomeric and 3 heteromeric forms) (Peumans et al. 1982 b). All these isolectins can be separated by ion-exchange chromatography and are characterized by the salt concentration at which they elute from the column. To facilitate isolectin pattern analyses, ion-exchange chromatography is usually performed at low pH (3.8), under which condition only the homomeric isolectins are stable. The genome by which each isolectin in polyploid wheat is coded for, could be unequivocally determined by comparing (iso)lectin patterns in extracts from embryos of hexaploid, tetraploid and diploid wheats (Peumans et al. 1982 b).

The purpose of the present investigation was to determine the chromosomal location of the different lectin genes on the chromosomes of *Triticum aestivum* and *Aegilops umbellulata.*

Materials and methods

Plant material

a) Compensated nullisomic-tetrasomic lines of Triticum aestivum 'Chinese Spring' (CS). These nullisomic-tetrasomic lines of CS, originally developed by Sears (1966), were used to study the chromosomal control of wheat lectins. A compensated nullisomic-tetrasomic line (2n = 42) lacks one pair of chromosomes but contains two pairs of a homoeologous chromosome, which compensates for the activities of the missing pair.

The following nullisomic-tetrasomic lines were analysed:

CS N1A T1D (nullisomic 1A – tetrasomic 1D), N1A T1B, N1B T1A, N1B T1D, N1D T1A, N1D T1B, N2D T2B, N3A T3D, N5A T5D, N6A T6D, N7A T7D.

b) Ditelosomic lines of Triticum aestivum 'Chinese Spring'. Lectin genes were localized on chromosome arms using ditelosomic lines of CS, again developed by Sears (1954). Ditelosomic lines lack an arm of both chromosomes of a chromosome pair.

The following ditelosomic lines were analyzed:

CS DT 4Aα (ditelosomic: β-arm missing), DT 1AL (ditelosomic: short arms of 1A missing), DT 1BL, DT 1DL.

c) Inter-varietal and inter-specific chromosome substitution and addition lines of Triticum aestivum 'Chinese Spring'. In a substitution line, a pair of chromosomes of CS has been replaced by a homologous pair of chromosomes from another variety or species.

The following substitution lines were analysed:

CS (Hope 1B), CS 1B/1U, CS 1D/1U.

The 1U chromosome is derived from the diploid wheat *Aegilops umbellulata.*

An inter-specific addition line has an extra chromosome pair from a related species in addition to the CS chromosomes.

The following addition line was analysed:

CS addition 1U.

All the seed used in these studies were obtained from cytologically and genetically verified lines maintained at the Plant Breeding Institute, Cambridge.

Separation and identification of isolectins

Isolectin patterns of different lines, varieties and species, listed above, were analysed by ion-exchange chromatography, coupled to a detection (and quantitation) method of the lectins based on agglutination assays.

Fifty embryos (isolated from grain using a scalpel blade) were homogenised in 1 ml Na acetate buffer (50 mM Na-acetate pH 3.8, containing 100 mM NaCl) at room temperature and the extract centrifuged at 2,000 g for 5 min. The supernatant was removed, heated at 60 °C for 5 min and recentrifuged at 2,000 g for 5 min. The resulting clear supernatant was applied onto a small column (1 × 10 cm) of sulphopropyl-Sephadex equilibrated with the acetate buffer above and eluted with a 100 ml linear gradient of 0.1 to 0.7 M NaCl dissolved in the same buffer. Fractions of 1 ml were collected and the agglutination titer of each was determined. The agglutination titer was expressed as the highest dilution at which gradient fractions still agglutinated trypsin-treated red blood cells.

Agglutination was assayed in small glass tubes at a final volume of 0.1 ml, containing 10 µl of a 10% suspension of trypsin-treated red blood cells in phosphate buffered-saline (1.15 g/l Na_2HPO_4, 200 mg/l KH_2PO_4, 8 g/l NaCl, 120 mg/l $MgSO_4$, 200 mg/l KCl, 100 mg/l $CaCl_2$), different volumes of the gradient fraction and phosphate buffered-saline. Agglutination was assessed visually after 1 h.

Trypsin-treated red blood cells were prepared by incubating a 10% suspension of washed human erythrocytes (group A) in phosphate buffered-saline with 1 mg/ml trypsin for 1 h at 37 °C. After the incubation, the erythrocytes were thoroughly washed (at least five times) with phosphate buffered-saline.

Results

Nomenclature of cereal lectins

To facilitate the determination of different cereal lectins and isolectins, and avoid ambiguities, we have proposed a nomenclature in which the lectins are indicated by the abbreviation CL (for cereal lectin) followed by a subscript, indicating the genomes which code for the subunits (Peumans et al. 1982b). According to this nomenclature CL_{AA}, CL_{BB}, CL_{DD} and CL_{UU} refer to the homomeric lectins coded for by the A, B and D genome of *Triticum aestivum,* and the genome of *Aegilops umbellulata* respectively. Since in the experiments described below, no heteromeric lectin forms occur (because of the low pH during ion-exchange chromatography), we simply use the symbol CL followed by a subscript of only one character (CL_A, CL_B, CL_D and CL_U).

Choice of the method

In contrast to most seed lectins, cereal lectins are not distributed all over the seed, but are exclusively localized in the primary axes (Mishkind et al. 1980; Stinissen et al. 1981), in which they accumulate during embryogenesis (Peumans et al. 1982f). In addition, they are present in relatively small concentrations since they represent not more than 0.1–0.2% of the total protein content of the primary axes (200 ng/primary axis), which corresponds to about 0.0033% of total grain protein, in contrast to some legume seeds where lectins constitute up to 10% of total protein (Liener 1976). Since most of wheat lines analysed were available in limited amounts, a sensitive method had to be developed for the separation and detection of different isolectins. Fortunately, cereal lectins are able to agglutinate trypsin-treated red blood cells at concentrations as low as 20 ng/ml, which enabled us to detect (and quantify) different isolectins after ion-exchange chromatography of extracts from only 50 embryos (Peumans et al. 1982a).

Chromosomal control of lectin synthesis in 'Chinese Spring'

The chromosomes controlling lectin synthesis in embryos of CS wheat were determined using nullisomic-tetrasomic stocks. Euploid CS contains only 2 isolectins, namely CL_A and CL_D (Fig. 1a). This is not so unusual since as has been demonstrated previously,

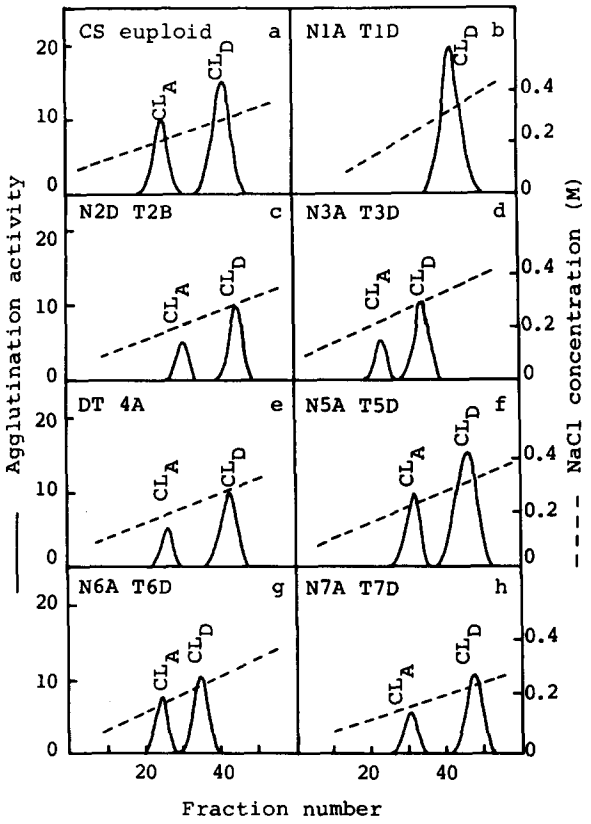

Fig. 1. Elution patterns of wheat embryo isolectins on SP-Sephadex: euploid 'Chinese Spring' (**a**); compensated null-isomic-tetrasomic lines of CS (**b–d, f–h**); ditelosomic line of CS (**e**). The agglutination activity (——) is expressed as the agglutination titer (highest dilution of the fractions at which agglutination of trypsin-treated red blood cells still occurs)

the absence of CL_B in hexaploid wheat varieties is common (Peumans et al. 1982 b).

Figure 1, b–h shows the elution patterns of the nullisomic A – tetrasomic D lines of CS for the seven chromosomes of wheat, except for chromosome 2, where nulli 2D – tetra 2B was used, and chromosome 4, where ditelosomic 4A had to be used. All of the elution profiles were similar to that obtained for euploid CS except for the line which is nullisomic for chromosome 1A. This line lacks the CL_A lectin, indicating that chromosome 1A controls the synthesis of CL_A. It should be mentioned here that, although the relative amounts of CL_A and CL_D in Fig. 1 are roughly the same, considerable differences of absolute lectin content occur with different analyses. However, most of the differences, if not all, are ascribed to differences in the size of the embryos from the grains. This is especially the case for certain genetic lines which contain poorly developed embryos.

Since, by deduction, it is likely that CL_D and CL_B are controlled by homoeologous chromosomes 1D and 1B, isolectin patterns were analysed in the six null-isomic-tetrasomic lines of homoeologous group 1 of CS. Nulli 1A – tetra 1D and nulli 1A – tetra 1B lines do not contain CL_A (Fig. 2 a, b), which confirms that CL_A synthesis is controlled by chromosome 1A. Similarly, the absence of CL_D in nulli 1D – tetra 1A and nulli 1D – tetra 1B shows that CL_D synthesis is under the control of chromosome 1D (Fig. 2 e, f).

Since CS does not contain CL_B, the chromosome controlling CL_B could not be determined in the above described experiments. Therefore, a substitution line of CS was used in which chromosome 1B was replaced by the homologous chromosome pair of the variety 'Hope'. As shown in Fig. 2 g, h, both 'Hope' and the substitution line contain an extra component. Thus, the genes controlling isolectin CL_B must be localized on chromosome 1B.

Determination of chromosome arms carrying CL_A and CL_D genes

The chromosome arms carrying CL_A and CL_D genes in CS were determined using lines of CS ditelosomic for the long arm of either 1A or 1D. Since isolectin

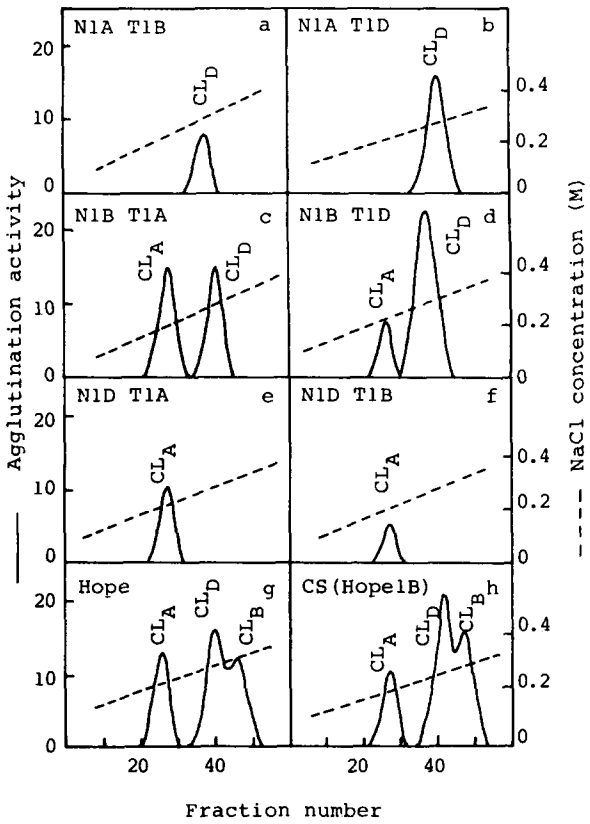

Fig. 2. Elution patterns of wheat embryo isolectins on SP-Sephadex: nullisomic-tetrasomic lines of 'Chinese Spring' (**a–f**); 'Hope' (**g**); CS ('Hope' 1B) (**h**). The agglutination activity (——) is expresed as described in the legend of Fig. 1

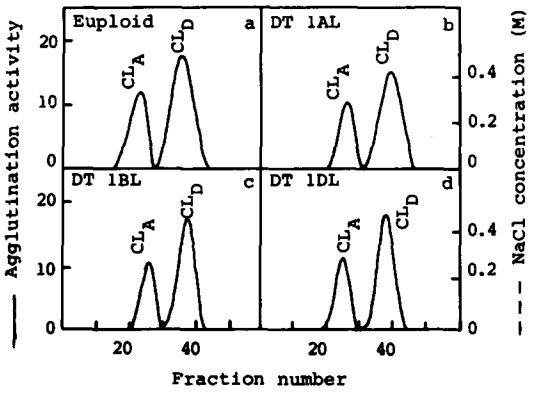

Fig. 3. Elution patterns of wheat embryo isolectins on SP-Sephadex: 'Chinese Spring' (**a**); ditelocentric lines (short arms missing) of CS (**b–d**). The agglutination activity (——) is expressed as described in the legend of Fig. 1

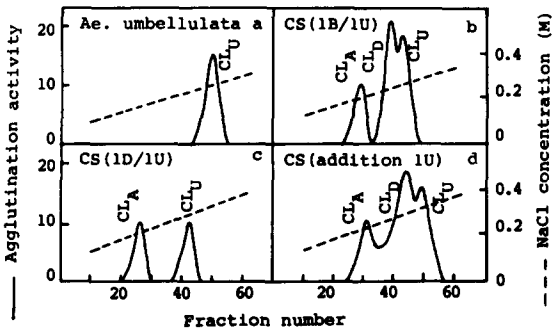

Fig. 4. Elution patterns of wheat and *Aegilops umbellulata* isolectins on SP-Sephadex: *Ae. umbellulata* (**a**); CS (*Ae. umbellulata* 1U/1B) (**b**); CS (*Ae. umbellulata* 1U/1D) (**c**); a line with 1U added to CS (**d**). The agglutination activity (——) is expressed as described in Fig. 1

patterns of extracts of euploid and ditelosomic lines were the same (Fig. 3), it could be concluded that the genes controlling CL_A and CL_D synthesis are carried by the long arms of chromosome 1A and 1D respectively.

Identification of chromosomes carrying lectin genes in a species related to hexaploid wheat

To find out whether the lectins of related species are controlled by chromosomes which are homoeologous to group 1 chromosomes of wheat, alien substitution and addition lines of CS were checked for the presence of the appropriate lectins. Addition of chromosome 1U of *Aegilops umbellulata* to CS results in the expression of CL_U in this addition line of CS (Fig. 4 d). Similarly, CS in which 1B was substituted by 1U, also synthesizes CL_U (together with CL_A and CL_D) (Fig. 4 b). It is evident, therefore, that CL_U synthesis is controlled by the 1U chromosome.

Discussion

Isolectin pattern analyses of euploid and compensated nullisomic-tetrasomic stocks of CS and some addition and substitution lines of the same variety enabled the genes controlling lectin synthesis to be located on the homoeologous group 1 chromosomes of the A, B and D genomes of *Triticum aestivum* and the U genome of *Aegilops umbellulata.* In addition, similar experiments with ditelosomic lines of CS indicate that at least the genes controlling CL_A and CL_D synthesis are carried by the long arms of their respective chromosomes.

In wheat, therefore, the lectin genes occur on the same chromosomes as the genes coding for ω- and γ-gliadins (Payne et al. 1982) and on the same chromosome arms as the genes controlling the synthesis of the high-molecular-weight subunits of glutenin (Payne et al. 1980 b). Gliadin and glutenin are the major reserve proteins of the endosperm and they play an important role in the bread-making process (Payne et al. 1980 a). Other proteins which have been shown to be under control of the long arm of the group 1 chromosomes are the lipopurothionins, a class of small, toxic endosperm-specific proteins (Fernandez de Calaya et al. 1976). So it can be concluded that group 1 chromosomes of wheat controls several groups of seed-specific proteins. Some of these proteins are exclusively expressed in the endosperm (glutenin, gliadins and lipopurothionins), whereas others (lectins) are exclusively synthesized in the primary axes. Since typical storage proteins such as gliadins and glutenins represent a very heterogeneous group of proteins, whereas lectins and purothionins have been highly conserved during the evolution and divergence of the different cereal genomes, the group 1 chromosomes apparently carry in an evolutionary sense very stable genes together with families of highly variable genes.

The localisation of lectin genes on chromosome 1 of *Aegilops umbellulata,* which is known to be genetically similar to the group 1 chromosomes of *Triticum aestivum* (since it carries the genes controlling the synthesis of prolamins (gliadins) and high-molecular-weight glutenin subunits (Brown et al. 1979; Lawrence and Shepherd 1981) establishes further the genetic similarity between 1U and its homoeologous chromosomes in wheat.

Euploid CS lacks CL_B, which implies that it does not synthesize a CL_B lectin product or at least not a functional one. The occurrence of CL_B mutants is not in fact exceptional, since most hexaploid wheat varieties do not contain the CL_B isolectin either (Peumans et al. 1982 b). Attempts to detect a $CL_{\overline{B}}$ mutant in tetraploid wheats (containing A and B genome), were

unsuccessful (although about 20 lines from different origins were analyzed), which indicates that the mutation might have arisen after the formation of hexaploid wheat. Further work is in progress to determine the geographical distribution of hexaploid lines and species related to wheat which contain $CL_{\overline{B}}$ mutants.

Isolectin pattern analyses in compensated null-isomic-tetrasomic lines of CS not only enabled us to localize the lectin genes, but also provided the opportunity to follow the gene dosage effect on the expression of lectin in the embryos. Indeed, in euploid CS the absolute amount of CL_A is only half of that of CL_D. However, when chromosome 1A is present in double dosage, the amount of CL_A equals that of CL_D. Similarly, doubling the dosage of chromosome 1D results in about a 4-fold greater synthesis of CL_D than CL_A. Since in these experiments the amount of lectin controlled by a chromosome at normal dosage can be used as an internal standard, the relative amounts of CL_A and CL_D shown in Fig. 2c–d indicate that the dosages of either CL_A or CL_D genes, directly determine the amount of the corresponding lectin synthesized in the primary axes. Gene dosage effects can also explain the relative amounts of CL_A and CL_D in euploid CS, since it could be possible that the observed differences in agglutination activity (between CL_A and CL_D) reflect differences in gene copy number within complex loci. However, an alternative explanation could be the operation of regulatory genes located elsewhere in the genome. Further work will be required to investigate this problem.

Since lectins are gene products which can be identified relatively easily, their genes can be useful as marker genes in cytogenetical work and for breeding purposes. Chromosomes of homoeologous group 1 are important chromosomes from a breeders viewpoint since over one half of the major reserve-protein genes are located on these chromosomes. The availability of varieties with and without CL_B lectin provides the necessary allelic variation to study the segregation from a plant heterozygous for this factor and establish a more precise mapping of the CL_B lectin on chromosome 1B. Genetic crosses have been made which will enable the lectin genes on chromosome 1B to be mapped to the genes coding for the high-molecular-weight subunits of glutenin and the gliadin genes.

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