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Genetics of the α-Amylase/Trypsin inhibitor family in wheat and related species

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ABSTRACT

R. Sánchez-Monge, F. García-Olmedo, L. Gómez, J. Royo, P. Carbonero and G. Salcedo. Genetics of the α-Amylase/Trypsin inhibitor family in wheat and related species. *An. Aula Dei* 19 (1-2): 195-205.

Several members of an inhibitor family of heterologous α-amylases and of trypsin have been isolated from wheat, barley and rye endosperms. Comparisons of the amino acid sequences of these inhibitors together with the chromosomal locations of their structural genes in the three species, indicate that they are encoded by a disperse multi-gene family that must have originated both by translocations and intrachromosomal duplications events that took place in common ancestors of wheat, barley and probably rye. Homology among some members of the inhibitor family and various proteins of maize, ragi, castor bean and others, indicate that this protein family extends to other phylogenetically distant species.

INTRODUCTION

Protein inhibitors which are active against different types of hydrolytic enzymes are widely distributed in plants (for a review see García-Olmedo et al., 1987). Only a few of these inhibitors affects endogenous enzymes and may be specifically involved in plant metabolism. A majority of the plant inhibitors are active towards heterologous proteases or α-amylases from various organisms. Several lines of evidence indicate that these inhibitors play a protective role against the attacks of animal predators, insects, fungi, bacteria and virus. In this context, the following observations merit special mention: a) the levels of some proteinase inhibitors are increased in

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response to mechanical lesions caused by insects or by fungal infections (Ryan, 1984); b) the correlation between trypsin inhibitor levels and pests resistance in cowpea cultivars (Gatehouse et al., 1979); c) the expression of cowpea trypsin inhibitor in transgenic plants confers resistance to two of its major insect pests (Hilder et al., 1987).

Another field of practical interest concerning plant inhibitors that inhibit mammalian enzymes is their potential incidence on animal production and human nutrition, as well as their possible use in some clinical treatments (see Buonocore and Silano, 1986, and García-Olmedo et al., 1987, for reviews).

A cereal inhibitor family of heterologous α-amylases and of trypsin is among those that have been more actively studied. Since Kneen and Sandstedt (1943) described the first wheat α-amylase inhibitor, it has been shown that a substantial fraction of the albumins and globulins of wheat and barley endosperms is represented by a group of homologous inhibitors of α-amylases and trypsin (Buonocore et al., 1977; Shewry et al., 1984; Barber et al., 1986a; Sánchez-Monge et al., 1986a,b). Some members of this protein family can be selectively extracted by chloroform/methanol mixtures and have been designated CM-proteins (García-Olmedo and Carbonero, 1970; Rodríguez-Loperena et al., 1975; Salcedo et al., 1978, 1982).

The wheat α-amylase inhibitors have been classified into three classes: monomeric, dimeric and tetrameric (Deporte et al., 1976). The first type represents monomeric variants of about Mr 12 kDa, while the dimeric one can be dissociated into identical 12 kDa subunits. The chemical and inhibitory properties of these two inhibitor classes have been extensively studied (Buonocore et al., 1977; O'Connor and McGeeney, 1981; Maeda et al., 1982), and the complete amino acid sequences of one monomeric and two dimeric inhibitors have been estabished (Kashlan and Richardson, 1981; Maeda et al., 1983, 1985). Although the tetrameric type has been less actively investigated, Buonocore et al. (1985) have apparently isolated an inhibitor with Mr 48 kDa, which dissociates into unidentified 12-14 kDa subunits. Reconstitution experiments carried out in our laboratory (Sánchez-Monge et al., 1987; Gómez et al., 1989) have shown that the previously isolated proteins CM2, CM3 and CM16 form *T. turgidum*, and the same ones plus CM1 and CM17 from *T. aestivum* are the subunits of wheat tetrameric inhibitors.

In cultivated barley, we have recently demonstrated the existence of α-amylase inhibitors corresponding to the three classes, as well as that the previously characterized proteins CMa, Cμb and CMd are the subunits of the tetrameric inhibitor (Sánchez-Monge et al., 1986b). Also the complete sequence of a homodimeric α-amylase inhibitor 'BDAI-1' has been deduced from the nucleotide sequence of its corresponded cDNA clone (Lázaro et al., 1988).

Trypsin inhibitors that belong to the same protein family of the α-amylase inhibitors mentioned above have been isolated and characterized in barley (Mikola and Soulina, 1969; Odani et al., 1983; Lazaro et al., 1985; Barber et al., 1986b) and wheat (Boisen and Djurtoft, 1981). Recently the complete amino acid sequence of a trypsin inhibitor, and the N-terminal one of an α-amylase inhibitor from rye endosperm have been reported (Lyons et al., 1987).

We discuss in this paper the chromosomal organization of the multi-gene family that encodes the α-amylase/trypsin inhibitor family of cereal endosperms, as well as the evolutionary implications of the structural homologies among different members of this protein family.

RESULTS AND DISCUSSION

Two-dimensional electrophoretic maps of endosperm extracts from hexaploid wheat (*T. aestivum*, genomes AABBDD) and barley (*H. vulgare*, genomes HH), containing α-amylase and trypsin inhibitors are shown in Fig. 1. The fractionation by gel filtration under non-dissociating conditions of crude inhibor extracts, allow the classification of the proteins that appeared in the two dimensional maps of Fig. 1 as subunits of tetrameric, dimeric or monomeric α-amylase inhibitors, or monomeric trypsin inhibitors (Sánchez-Mong et al., 1986b, 1987; Barber et al., 1986b; Gomez et al., 1989).

Total or N-terminal amino acid sequences for different members of the inhibitor family isolated from wheat, barley and rye endosperms are presented in Fig. 2, and the percentages of homology calculated for all possible binary comparisons are summarized in Table 1. Evolutionary implications of the observed homologies are best understood if they are considered together with the chromosonal locations of the inhibitors structural genes in the three species, and will be discussed hereafter.

The distribution among chromosomes of the multi-gene family encoding this group of inhibitors has been studied through the analysis of wheat nulli-tetrasomic and ditelosomic lines (García-Olmedo and Carbonero, 1970; Aragoncillo et al., 1975; Fra-Mon et al., 1984; Sánchez-Monge et al., 1986a), wheat-barley addition lines (Salcedo et al., 1984; Hejgaard et al., 1984a) and wheat-rye addition lines (Hejgaard et al., 1984b and authors unpublished results). A summary of these observations, together with the "in vitro" activities of the corresponding proteins is presented in Table 2. It can be seen that loci encoding monomeric and dimeric α-amylase inhibitors as well as the rye trypsin inhibitor and the barley trypsin inhibitor CMe, are located in chromosome groups 3 and 6. The loci for subunits of tetrameric α-amylase inhibitors and the barley trypsin inhibitor CMc are in chromosomes of groups 4 and 7 of wheat, and 4 and 1 of barley. This finding further supports the proposed homology of barley chromosome 1 with chromosome group 7 of wheat (Hart et al., 1980; Powling et al., 1981). It is to be noted that in the case of wheat, which is allohexaploid, we have failed to identify triplicate genes (one in each of the three chromosomes of each chromosome group involved), which is in line with the idea of diploidization of redundant genetic information subsequent to the alloploid formation (García-Olmedo et al., 1978). The fact that among the genes involved the only ones associated with the A genome are those located in chromosome 4A, for which a B genome origin has been postulated (Rayburn and Gill, 1985), indicate that the distribution of this multi-gene family in wheat could be compatible with the hypothesis of a non-random diploidization (García-Olmedo, 1968; Carbonero and García-Olmedo, 1969; Galili and Feldman, 1985).

Joint consideration of the observed sequence homologies together with the inhibitory activities and the chromosomal locations of genes encoding these proteins, allows some evolutionary conclusions concerning the origin and dispersion of this multi-gene family to be drawn. There is a greater homology between a given protein from one genome and the appropriate one from a different genome than between that protein and any other encoded in the same genome. See for instance the high degree of homology (86%) between WTAI CM1 and WTAI CM2, encoded by genes located in the short arms of chromosomes 7D and 7B, respectively, and between these proteins and BTAI CMa (82-86% homology), whose genes is located in chromosome 1 of barley (equivalent to group 7 of wheat).

Barley trypsin inhibitor BTI CMc, whose gene is in the same chromosome as the α-amylase inhibitor BTAI CMa, has a higher homology to this inhibitor (78%) than to the other barley trypsin inhibitor BTI CMe (45%), whose gene is located in a different chromosome.

These observations clearly indicate that the dispersion of this multi-gene family must have ocurred before the evolutionary differenciation of the genomes of barley and those included in allohexaploid wheat.

The only reported α-amylase inhibitor from rye, designated RAI (Lyons et al., 1987), is 70-80% homologous to proteins WTAI CM1, WTAI CM2, and BTAI CMa, which are subunits of the tetrameric α-amylase inhibitors, so it is likely that RAI is also part of a tetrameric inhibitor from rye.

Proteins encoded by genes in chromosomes of group 4 of wheat and barley may be included into two groups. Homologies between WTAI CM16, WTAI CM17 and BTAI CMb (70-83%) and between WTAI CM3 and BTAI CMd (70%) are high, while the inter-group homologies are much lower (36-45%). This suggests two distinct loci associated with group 4 chromosomes whose origin may be an intrachromosomal duplication in a common ancestor of wheat and barley. In conclussion this disperse multi-gene family must have originated both by translocations and intrachromosomal duplications events that took place in common ancestors of wheat, barley and probably rye.

Although the most prominent members of this family in wheat and barley are those so far described, there is evidence for additional members of the family. Thus, Paz-Ares et al. (1986) obtained and sequenced barley cDNA clones which coded for proteins with 35-45% homology to the above-described ones. In Fig. 3, the amino acid sequence deduced from barley cDNA clone C23 is compared with that directly determined for barley trypsin inhibitor CMe. Also included in Fig. 3A are the amino acid sequences of a maize trypsin inhibitor and of a bifunctional α-amylase/trypsin inhibitor from ragi (*Eleusine coracana*). The homology between the last three proteins is fairly high (50-65%), indicating that this protein family extends to other species which are more phylogenetically distant than wheat, barley and rye. It should be also pointed out that there are weak but significant homologies between these proteins and reserve globulins of some dicotyledoneous plants and with certain non repetitive domains in prolamins (Kreis et al., 1985). In Fig. 3B, the homology between the B-region of two inhibitors of this family, a 2S globulin from castor bean and a γ-secalin of rye is shown.

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Table 1.- Binay comparisons (% homology) of wheat, barley and rye members of the α-amylase/trypsin inhibitor family. Homology was calculated for common segments of the sequences that appear in Fig. 2. Abreviations are as in Fig. 2.

PROTEINS		CM16	CM17	CMb	CM3	CMd	CM2	CM1	CMa	RAI	CMc	0.53	0.19	0.28	BDAI	CMe	RTI
WTAI	CM16		78	83	45	40	45	41	38	38	33	27	27	27	32	38	35
WTAI	CM17			70	37	36	37	37	35	29	33	26	26	30	30	37	33
BTAI	CMb				52	47	45	41	38	38	33	30	30	30	29	45	38
WTAI	CM3					70	50	39	43	41	38	41	41	30	26	46	48
BTAI	CMd						47	37	40	45	43	30	30	23	22	43	45
WTAI	CM2							86	86	80	72	38	34	37	39	54	41
WTAI	CM1								82	74	69	34	31	33	35	53	45
BTAI	CMa									74	78	31	28	20	32	50	41
RAI										64	25	25	29	41	41	41	
BTI	CMc											31	28	30	32	45	25
WDAI	0.53												94	58	45	28	20
WDAI	0.19													56	44	30	17
WMAI	0.28														63	25	17
BDAI																23	17
RTI																	76

Table 2.- Chromosomal locations of genes and inhibitory activities of the α-amylase/trypsin inhibitors from wheat, barley and rye.

Protein grouped by loci*		Chromosome** (or chromosome arm)	Inhibitory activity	References
WDAI	0.53	3BS		a,b,c
WDAI	0.19	3DS	α-amylase	a,b,c
BDAI		?H	dimeric	d
BTI	CMe	3H		e,f
RTI		3R	trypsin	g
WMAI	0.28	6DS	α-amylase monomeric	b,c
WTAI	CM16	4AS		a,b
WTAI	CM17	4DS		a,b
BTAI	CMb	4H		f
WTAI	CM3	4AS	α-amylase	a,b
WTAI	CMd	4H	tetrameric	f
WTAI	CM2	7BS		a,b,h
WTAI	CM1	7DS		a,b,h
BTAI	CMa	1H		f
RAI		?R		i
BTI	CMc	1H	trypsin	f

* Abreviations as in Fig. 2.

** Hexaploid wheat (*T. aestivum*): genomes AABBDD; barley (*H. vulgare*): genomes HH, and rye (*S. cereale*): genomes RR. Barley chromosome 1 is homologous to chromosome group 7 of wheat.

References: a) Aragoncillo et al., 1975; b) Fra-Mon et al., 1984; c) Sánchez-Monge et al., 1986a; d) Lázaro et al., 1988; e) Hejgaard et al., 1984a) f) Salcedo et al., 1984; g) Hejgaard et al., 1984b; h) García-Olmedo and Carbonero, 1970; i) Lyons et al., 1987.

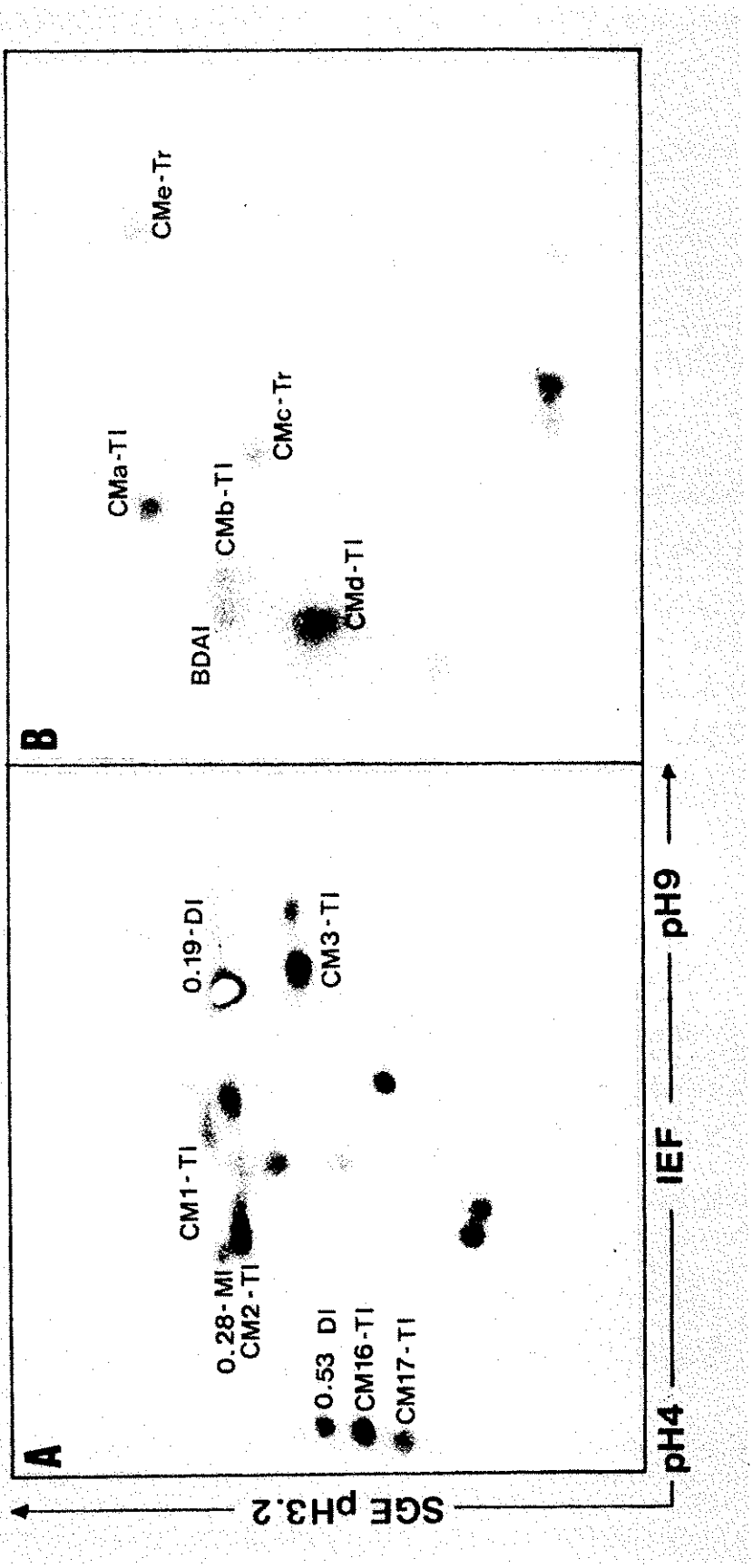

Figure 1.- Two-dimensional protein maps (electrofocusing, IEF, pH 4-9 x starh gel electrophoresis, SGE, pH 3.2) of crude 70% ethanol extracts from: A) hexaploid wheat (*T. aestivum* cv. Chinese Spring) and B) barley (*H. vulgare* cv. Bomi) endosperms. Proteins whose amino acid sequences appear in Fig. 2 are labeled MI, DI and TI = components of monomeric, dimeric and tetrameric α-amylase inhibitors, respectively; Tr = Trypsin inhibitor. BDAI = barley dimeric α-amylase inhibitor.

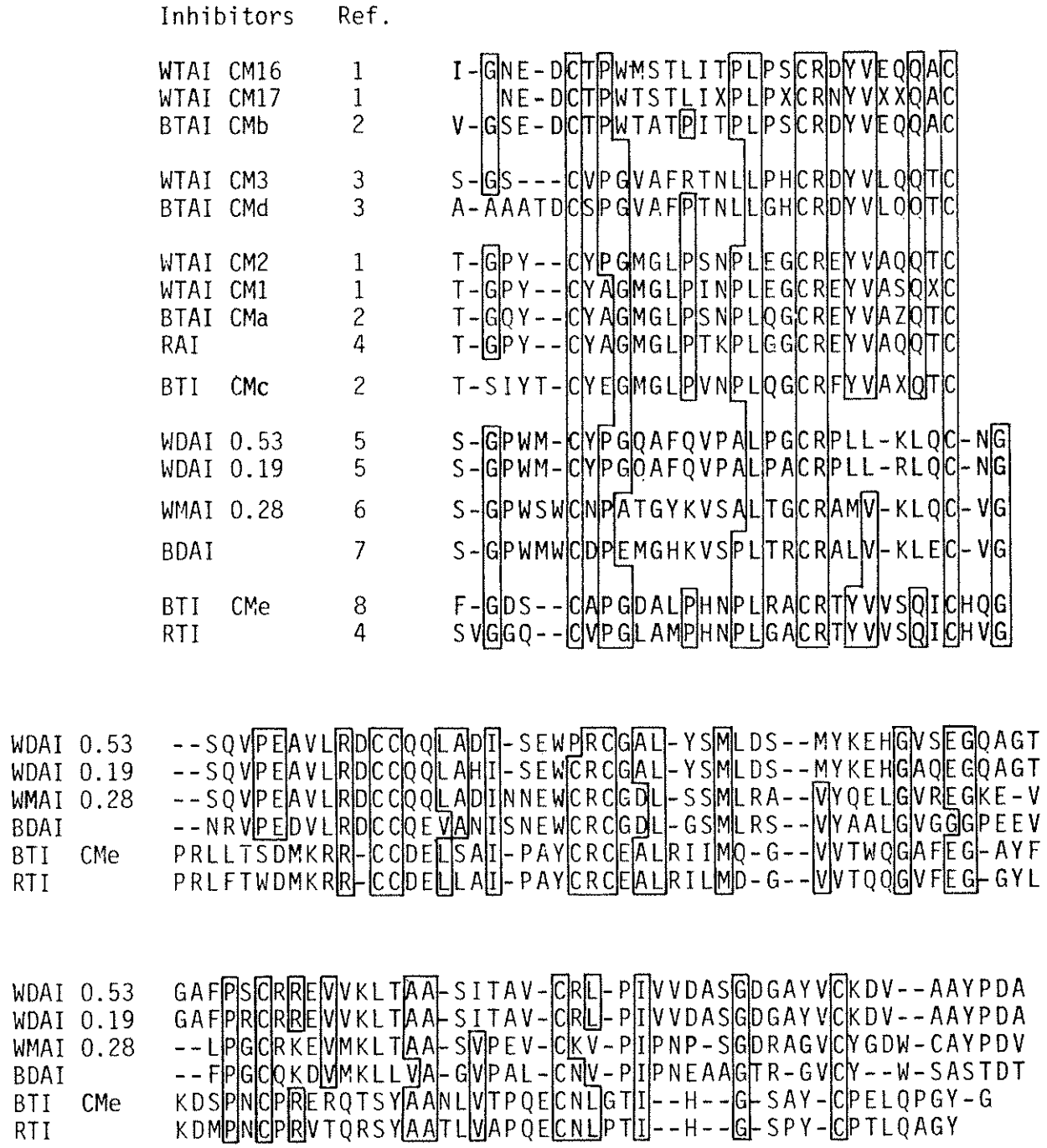

Figure 2.- Alignment of amino acid sequences of members of the α-amylase/trypsin inhibitors family from wheat, barley and rye endosperm. Gaps introduced for the alignment are indicate (-). Conserved positions are boxed. W = wheat; B = barley and R = rye. TAI, DAI and MAI = subunits of tetrameric, dimeric and monomeric α-amylase inhibitor, respectively. TI = trypsin inhibitor. References: (1) Barber et al., 1986b; (2) Barber et al., 1986a; (3) Shewry et al., 1984; (4) Lyons et al., 1987; (5) Maeda et al., 1985; (6) Kashlan and Richardson, 1981; (7) Lazaro et al., 1988; (8) Odani et al., 1983.

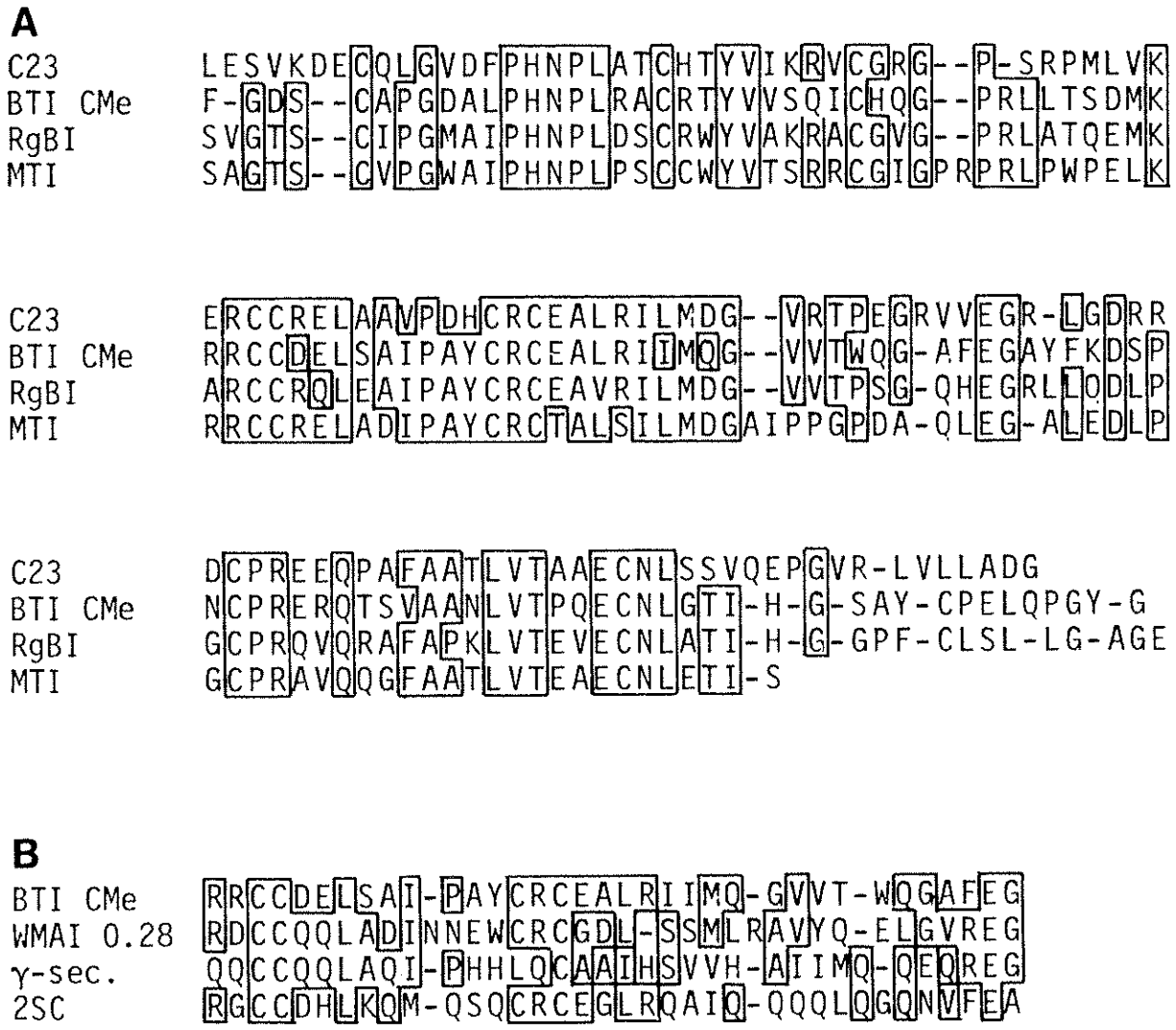

Figura 3.- Alignment of the following amino acid sequences. A) C23 = sequence deduced from barley cDNA clon pUP23 (Paz-Ares et al., 1986); BTI CMe = barley trypsin inhibitor CMe (Odani et al., 1983); RgBI = Ragi (*Eleusine coracana*) bifunctional α-amylase/trypsin inhibitor (Campos and Richardson, 1983); MTI = maize trypsin inhibitor (Mahoney et al., 1984). B) B-regions as defined by Kreis et al. (1985) of: BTI CMe = barley trypsin inhibitor; WMAI 0.28 = wheat monomeric α-amylase inhibitor 0.28 (Kashlan and Richardson, 1981); γ-sec. = γ-secalin from rye (Kreis et al., 1985); 2SC = 2S storage globulin from castor bean (Sharief and Li, 1982). Gaps introduced for the alignment are indicate (-). Conserved positions are bosed.