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Genetic control of dipeptidase in the Triticeae

R. M. D. Koebner

Plant Breeding Institute, Maris Lane, Trumpington, Cambridge CB2 2LQ, UK

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Summary. Isoelectric focusing has been employed to elucidate the genetic control of a series of dipeptidase isozymes in wheat and its relatives. The phenotype of wheat shows four bands, three of which are shown by aneuploid analysis to be controlled by the loci *Dip-A1*, *Dip-B1* and *Dip-D1* on chromosome arms 6AL, 6BL and 6DL, respectively. Varietal polymorphism for *Dip-A1* and *Dip-B1* was observed. Different homoeoloci were found in barley, *Haynaldia villosa* and *Agropyron junceum*.

Key words: Isozymes – Dipeptidase – Wheat

Introduction

The genetics of bread wheat and its domesticated and wild relatives have been intensively investigated in recent years, but the Triticeae genomes are rather poorly characterised by genetic markers in comparison with other plant species such as maize and tomato. The importance of producing a comprehensive genetic map of such a major crop plant lies in its applications to both conventional plant breeding and to future possibilities of genetic engineering. In the former case, where readily scored loci can be shown to be linked to loci involved with quantitative or environmentally modulated traits, the markers can be used for indirect selection in segregating populations for characters which otherwise would require a degree of replication of both genotype and environment; whereas in the latter case, a detailed knowledge of the genetic map is required to select and characterise transformants. Some of the most useful markers control biochemical phenotypes, which can usually be scored on single individuals, often from individual grains, and are generally unaffected by environment. The present paper reports the genetic control of the isozyme dipeptidase, extending the observations of Golenberg (1986), by using a separation system which gives superior resolution. Although Golenberg proposes that the isozyme should be termed peptidase, in this report the term dipeptidase is preferred, as nothing is known of the function of this isozyme, except that it can be assayed with a dipeptide substrate.

Materials and methods

Plant material

The following plant materials were used:
Bread wheat (*Triticum aestivum*) cv Chinese Spring (CS), Holdfast, Bersée, Koga II, Cappelle-Desprez, Bezostaya I, Poros, Cheyenne, Timstein, Hope, Lutescens 62, Champlein, Ciano 67, Hobbit 'sib', Vilmorin 27; 'Synthetic' (the amphidiploid derived from the cross *T. dicoccum* × *Aegilops squarrosa;* McFadden and Sears 1946); *T. macha* and *T. spelta;* homoeologous group 6 chromosome intervarietal substitutions involving cv CS (Lutescens 62), CS (Cheyenne), CS (Cappelle-Desprez), CS (*T. spelta*) [CS (Cheyenne) produced by Morris, others by Law and Worland]; the nullisomic tetrasomic (NT) series, except nullisomics for 2A and 4A (Sears 1966), selfed progeny of monosomic 4A tetrasomic 4D and ditelosomics (DT) of group 6 (Sears and Sears 1978) in cv CS; complete (where available) or partial alien addition series in cv CS and the amphiploid between it and cereal rye (*Secale cereale*) cv Imperial (Driscoll and Sears 1971) and King II (Miller 1973), cultivated barley (*Hordeum vulgare*) cv Betzes (Islam et al. 1981), *S. montanum* (Miller 1973), *Haynaldia villosa* (genome V) (Sears, unpublished), *Hordeum chilense* (Miller et al. 1982b), *Aegilops umbellulata* (Kimber 1967), *Ae. Longissima* (Feldman 1975), *Ae. sharonensis* (Miller 1983), *Agropyron elongatum* (Dvorak and Knott 1974), *Ag. junceum* (genome J) (Alonso and Kimber 1980; Forster and Miller 1985); partial addition series and the amphiploid TAF 46 of *Ag. intermedium* to wheat cv Vilmorin 27 (Cauderon et al. 1978). Substitutions of chro-

mosome 6R of King II for wheat group 6 chromosomes in Holdfast (Miller 1973), 2M of *Ae. comosa* (Riley et al. 1966), 2U of *Ae. umbellulata* (Chapman et al. 1974) and 2R of cereal rye (Miller 1973) for 2A, and $4S^l$ of *Ae. sharonensis* for 4A (Miller et al. 1982 a) in CS.

Electrophoresis

The isozymes of dipeptidase were separated by flat bed isoelectric focusing on 0.25 mm thick gels containing the ampholytes Servalyt 3–4 and Isolyte 3–10 in the ratio 2:1. The position at which the samples were loaded had little effect on the gel pattern; however, best results were usually obtained by loading about 1–2 cm from the anode. The samples were obtained by incubating the crushed embryo half of a dry grain for 1–2 h in 50 µl of distilled water at room temperature, and were loaded by soaking a 0.5 × 1.0 cm paper wick (Pharmacia) in the briefly centrifuged sample. Electrode solutions were 1 *M* NaOH (cathode) and 1 *M* H_3PO_4 (anode). Gels of 12 cm width length were run at 1 W/cm, with a maximum voltage of 3,000 V. They were prefocused for 500 Vh, the samples were left on the gel for 1,000 Vh, and the run was ended at 4,000 Vh. Staining was achieved by agarose overlay. A 1% solution of low-melting point agarose was made in 0.2 *M* Tris HCl pH 8, to which was added 0.3 mg/ml L-leucyl-L-alanine, 0.1 mg/ml $MnCl_2 \cdot 4H_2O$, 0.1 mg/ml peroxidase, 0.015 u/ml 1-amino acid oxidase, type 1 (Sigma) and 0.4 mg/ml 3-amino-9-carbazole. The last item was first dissolved in a small volume of *N, N* dimethyl formamide. Sufficient solution was made to give a 2.5 mm thick overlay. Staining took 1–2 h at room temperature, after which the overlays were fixed in 7% acetic acid and photographed.

Results and discussion

The phenotype of euploid CS shows four distinct bands (labelled 1–4), focusing in the pH range 4.8–5.2 (Fig. 1, lanes 1, a). All the NTs, except those in group 6 show this same phenotype. Within the group 6 NTs and DTs, the removal of 6A or its long arm results in the loss of the most anodal band, band 1 (lanes 2–4, b). Similarly the removal of 6BL and 6DL results in the loss of bands 3 and 2, respectively (lanes 5–6, c; 7–9, d). This result confirms the observations of Golenberg (1986) who was able to show chromosomal control of dipeptidase by 6AS and 6BS, and it also shows that the isozyme behaves as a monomer. The superior resolution achieved by using isoelectric focusing in preference to starch gel electrophoresis has enabled the identification of a homoeolocus on 6DS, Dip-D1, thereby completing the Dip-1 homoeoallelic series in wheat. The most cathodal band, band 4, is neither removed in any NT, nor in a sample of 10 progeny from mono 4A tetra 4D, some of which may be expected to be nullisomic for 4A, nor in any of the 2A and 4A substitution lines tested. This indicates that the genetic control of this band is not simple, i.e. it is a compound product of genes present on more than one chromosome, or it may be under cytoplasmic control.

Analysis of the Dip-1 phenotype of a range of varieties has shown some polymorphism at the *Dip-1* locus. Thus the phenotype of the varieties Cappelle-Desprez, Lutescens 62, Holdfast, Vilmorin 27, Bersée, Koga II, Bezostaya I, Poros, Hobbit 'sib', Timstein, Hope, Champlein and Ciano 67 and of *T. macha,* while still consisting of four bands, does not possess band 3 (controlled by *Dip-B1*); instead the third band in these varieties (band 3 a) focuses at a higher isoelectric point (pI) than band 3. The phenotype of the substitution of Cappelle-Desprez 6B for CS 6B shows that Cappelle-Desprez 6B is responsible for this band difference, as this line has the identical phenotype to Cappelle-Desprez (Fig. 2, lanes 3, 5), whereas the phenotypes of CS (Cappelle-Desprez 6A) and CS (Cappelle-Desprez 6D) are indistinguishable from that of CS (lanes 2, 4, 1, respectively). A similar result was obtained for chromosome 6B of Lutescens 62 (not shown). In keeping with the rules for gene nomenclature (MacIntosh 1983), the CS 6B allele is designated *Dip-Bla,* and the Cappelle-Desprez allele *Dip-Blb.* The phenotypes of Cheyenne and Lutescens 62 differ from CS by possessing both band 3 a, and by the absence of band 1, controlled by *Dip-A1a* (Fig. 3, lane 3). The Dip-1 phenotype of the lines carrying Cheyenne 6A (or Lutescens 6A) substituted for 6A of CS also lacks band 1 (Fig. 3, lane 2), whereas this band is present in both CS (Cheyenne 6B) and CS (Cheyenne 6D) (not shown). This indicates that the Cheyenne allele (*Dip-A1b*) is either silent or codes for a product which overlaps with one of the other *Dip-1* products. As CS (Cheyenne 6A) lacks band 3 a, the *Dip-A1b* product cannot overlap the *Dip-Blb* product; however, it may produce a band which focuses at the same pI as either band 2 or band 4. The phenotype of *T. spelta,* which possesses both *Dip-A1b* and *Dip-Blb,* shows a further polymorphism, in that band 4, which could not be assigned to a particular chromosome in the CS aneuploids is replaced by an unfocused smear (Fig. 4, lane 2). This effect is highly reproducible, and is also seen in the substitution of *T. spelta* 6B into CS (Fig. 4, lane 3), but not in CS (*T. spelta* 6A) or CS (*T. spelta* 6D) (not shown). While the smear is therefore assignable to *T. spelta* chromosome 6B, it is unclear why the invariant band 4 is missing when its chromosomal control in CS has been shown to be complex. It is possible that *T. spelta* 6B contains a suppressor of the genes controlling the invariant band; there is some evidence for gene suppression in the Triticeae (Galili and Feldman 1984), but this hypothesis is still speculative.

Alien variation can be demonstrated for *Dip-1* by observing the phenotypes of the wheat-alien amphiploids and addition lines. Brown (1983) has already noted the presence of a *Dip-1* locus on barley 6H (the locus is referred to as *Dip 1,* on barley chromosome 6). The present work confirms this result, as the phenotype of the 6H addition line is equivalent to an additive pattern of those of CS and Betzes barley (Fig. 5, lanes 3,

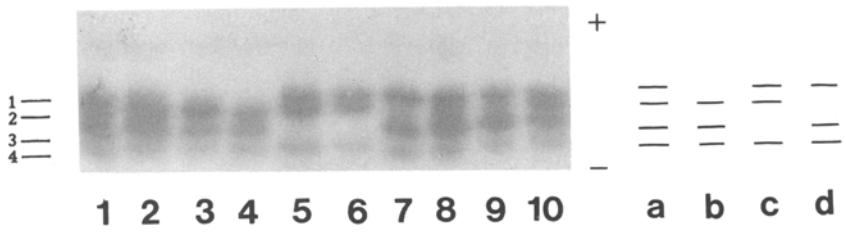

Fig. 1. Dipeptidase IEF phenotypes of Chinese Spring (CS) and homoeologous group 6 aneuploids. *1* CS, *2* Nulli-tetra (NT) 6AB, *3* NT 6AD, *4* Ditelosomic (DT) 6AS, *5* NT 6BA, *6* DT 6BS, *7* NT 6DA, *8* NT 6DB, *9* DT 6DS, *10* DT 6DL. *a–d:* interpretation of the patterns of euploid CS and homoeologous group 6 aneuploids. *a* euploid CS, *b* lacking 6AL, *c* lacking 6BL, *d* lacking 6DL

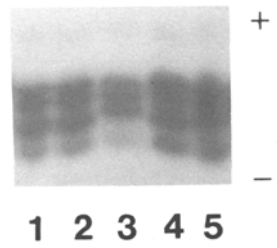

Fig. 2. Dipeptidase IEF phenotypes of CS and CS/Cappelle-Desprez intervarietal substitutions of homoelogous group 6. *1* CS, *2* CS/Cappelle-Desprez 6A, *3* CS/Cappelle-Desprez 6B, *4* CS/Cappelle-Desprez 6D, *5* Cappelle-Desprez

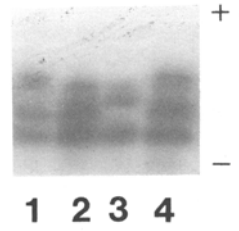

Fig. 3. Dipeptidase IEF phenotypes of Dip-A1 variants. *1* CS, *2* CS/Cheyenne 6A, *3* Cheyenne, *4* CS

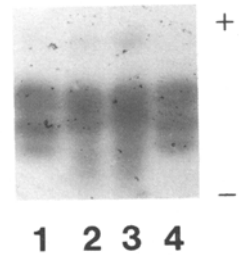

Fig. 4. Effect of *Triticum spelta* 6B on dipeptidase IEF Phenotype. *1* CS NT 7AB (euploid phenotype). *2 T. spelta*, *3* CS/*T. spelta* 6B, *4* CS NT 5DB (euploid phenotype)

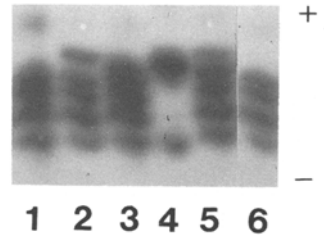

Fig. 5. Dipeptidase IEF phenotype of alien variants. *1* Euploid wheat, *2* Addition 6Ha, *3* Addition 6H, *4* Betzes barley, *5* CS × *Agropyron junceum* amphiploid, *6* CS

4), while the other five addition lines all have a CS phenotype. Thus the *Dip-1* locus is equivalent to *Dip-H1*. An additional band to the four wheat bands is also expressed by the 6Ha addition of *Haynaldia villosa* (*Dip-V1*) (Fig. 5, lane 2), and by the amphiploid (lane 5) and certain putative group 6 addition lines of *Agropyron junceum to wheat* (*Dip-J1*). Both these novel bands focus at a higher pI than band 1, but they are clearly distinguishable from one another. However, the isozymes of rye cv Imperial and King II, *Secale montanum*, *Ae. umbellulata*, *Ae. longissima*, *Ae. sharonensis*, *Hordeum chilense*, *Agropyron elongatum* and *Ag. intermedium* apparently have similar pIs to those of wheat and thus cannot be assigned to a particular chromosome using the present technique.

While a substantial number of isozyme loci have now been assigned to wheat chromosomes by the zymogram technique, the majority thus far reported lack polymorphism, which restricts their use as markers in wheat breeding. It is unclear whether this reflects merely a limited search or whether there is indeed a lack of variation in bread wheat, resulting from the narrowness of the wheat genetic base or from a high degree of gene conservation in wheat. Variation in other inbreeding cereals (e.g. barley) is not uncommon and it would be of benefit if study of biochemical markers in wheat were routinely extended beyond CS and its aneuploids.

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