

## Intrachromosomal mapping of seven biochemical loci in barley, *Hordeum vulgare*

C. J. Liu<sup>1</sup>, M. Heun<sup>2</sup>, M. D. Gale<sup>1</sup>

<sup>1</sup> Cambridge Laboratory, Colney Lane, Norwich NR4 7UJ, UK

<sup>2</sup> Dept of Crop Science, Molecular Marker Facility, NC State University, Raleigh NC 27695-7620, USA

Received: 10 February 1993 / Accepted: 1 March 1993

**Abstract.** Seven biochemical loci, *AmpA*, *Amy1*, *Amy2*, *Est-H5*, *Hor1*, *Hor2*, and *Wsp-H1*, have been intrachromosomally mapped in the barley genome using a previously published RFLP-based genetic map. In all cases, the map locations confirmed prior chromosome assignments and agreed closely with the map positions of their homoeoloci in hexaploid wheat.

**Key words:** Isozymes – Storage proteins – Barley – RFLP mapping

### Introduction

The number of available mapped genetic markers has increased rapidly in all the major crops in recent years. Most are DNA markers, but biochemical markers offer significant advantages in price and speed and are equally efficient in many marker-aided applications, such as the identification of aneuploids and the introgression and manipulation of alien chromosomes or chromosome segments into cultivated crops, where homoeoallelic variation can almost always be detected. Thus biochemical markers are unlikely to become obsolete in the near future. Indeed, the new RFLP maps facilitate the intrachromosomal mapping of biochemical loci, thus enhancing their value. In this study we report the incorporation of seven biochemical loci in the existing RFLP-based genetic map of barley (Heun et al. 1991).

### Materials and methods

Fifty-six out of the one-hundred and thirteen doubled haploids from the cross “Proctor” × “Nudinka” used by Heun et al.

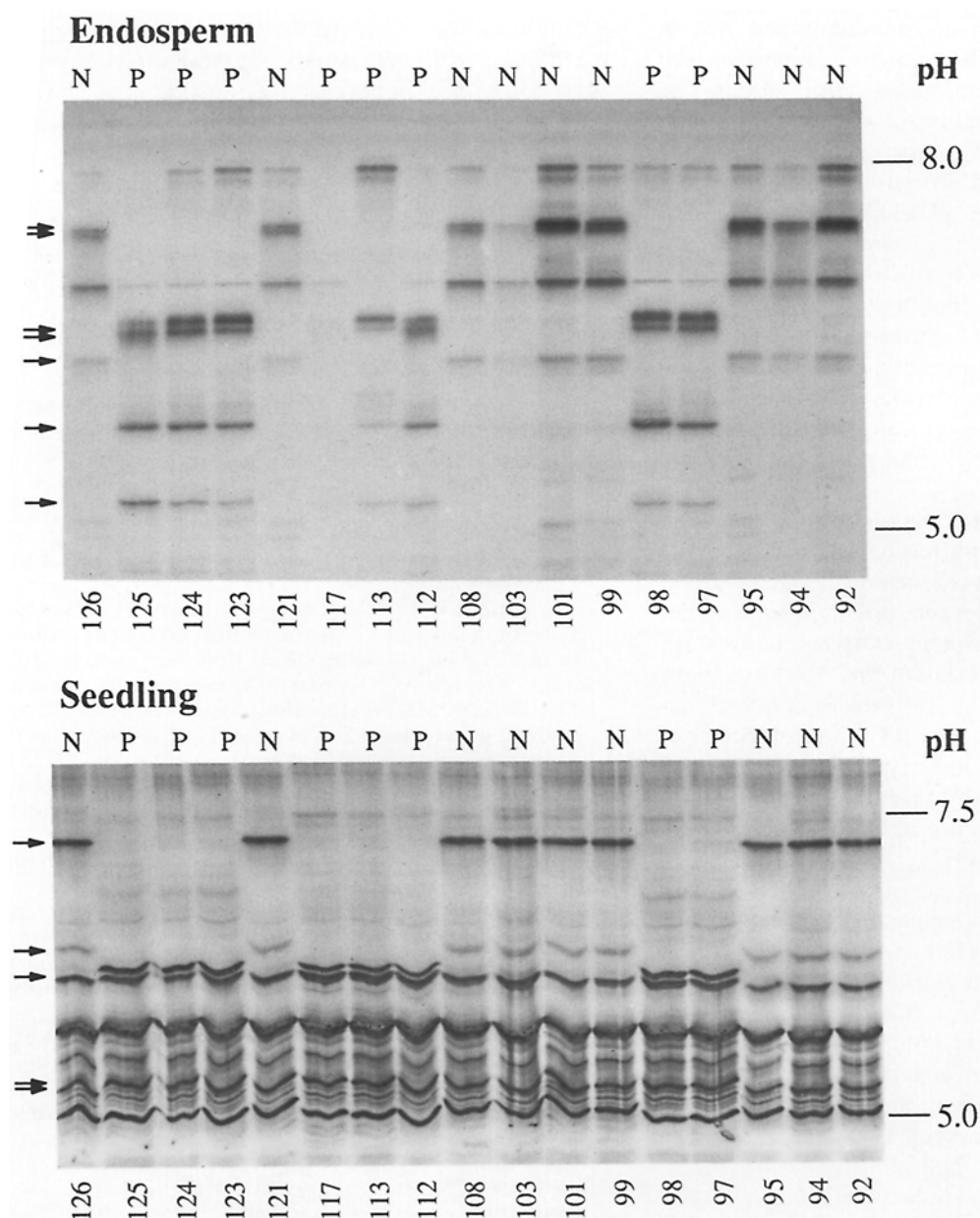
(1991) for the construction of an RFLP map were used in this study. The hordein-1 and hordein-2 storage proteins were analyzed according to Payne et al. (1980). The methods used were, for aminopeptidases that described by Koebner and Martin (1989), for water-soluble proteins that of Liu et al. (1989), and for  $\alpha$ -amylases and esterases that of Liu (1991). No polymorphisms were detected for  $\beta$ -amylases, dipeptidases, endopeptidases, glucosylphosphate isomerases, peroxidases, phosphoglucosyltransferases, shikimate dehydrogenases, subtilisin inhibitors, and trypsin inhibitors. The nomenclature for the *Est-5* and *Wsp-1* loci is that used for homoeoloci in hexaploid wheat, since the relationship between these genes and known barley loci is unresolved. The linkage analysis was performed using the Macintosh II version of MAPMAKER supplied by Du Pont, Wilmington, Del.

### Results and discussion

*AmpA*, *Amy1*, *Amy2*, *Est-H5*, *Hor1*, *Hor2* and *Wsp-H1* were mapped to barley chromosomes 1, 3, 5 and 6 respectively:

*AmpA*. One aminopeptidase locus was mapped on the short arm of barley chromosome 1 (7H), 18.9 cM distal to *Xcnl.CDO595* which is the most proximal short arm marker. It is likely, from the location, that this locus is *AmpA* as described by von Wettstein-Knowles (1989). This set of genes was located on the short arms of the homoeologous group 7 chromosomes in hexaploid wheat and on chromosome 1 (7H) in barley (Koebner and Martin 1989). In wheat the homoeolocus, *Amp-A3*, was mapped on 7AS with a maximum distance of 30 cM from the centromere (R. L. Harcourt, unpublished).

*Amy1* and *Amy2*. Two  $\alpha$ -amylase genes, *Amy2* and *Amy1*, have been located on barley chromosomes 1 (7H) and 6 (6H) respectively, and their homoeoloci have been located on homoeologous group 7 and 6 chromosomes in



**Fig. 1.** The esterase phenotypes of 17 DH lines from the cross of "Proctor"  $\times$  "Nudinka", showing the co-segregation of isozymes from both endosperm and young seedling extracts

wheat. As expected, *Amy1* mapped on the long arm of chromosome 6 (6H) (*Xcnl.WG282* – 4.9 cM – *Amy1* – 1.4 cM – *Xcnl.BCD269*) and *Amy2* on the long arm of chromosome 1 (7H) (co-segregating with *Xcnl.BCD351A*). The map position of the latter indicated that the locus *Xcnl.WG380B*, 24.7 cM from *Amy2*, should also be on the long arm of this chromosome, whereas *Xcnl.WG686* was taken as the most proximal long arm marker in the original barley RFLP map.

The map positions for both loci agree well with the positions of their homoeoloci in wheat.  $\alpha$ -*Amy-1* was mapped about 30 cM from the centromeres on the long

arms of the homoeologous group 6 chromosomes (Jia and Gale, unpublished), and  $\alpha$ -*Amy-2* was mapped near the centromeres on the long arms of the group 7 chromosomes (Chao et al. 1989).

*Est-H5*. As many as four different esterase loci, *Est1*, *Est2*, *Est4* and *Est10*, have been reported on the long arm of chromosome 3 (3H) in barley (von Wettstein-Knowles 1989), and three of them, *Est2*, *Est1* and *Est4*, map in a tight linkage block (Brown 1983). Isozymes over a wide range of isoelectric points were detected in both endosperm and young-seedling extracts. In this population

some 12 isozymes (seven in endosperm and five in seedling tissues) segregated, however all isozymes which distinguish the parents segregated together, as shown in Fig. 1. Clearly, it is possible that this complex, equivalent to the products of the wheat homoeoloci *Est-5* (Ainsworth et al. 1984) and *Est-6* (Jouve and Diaz 1990), involves the same isozymes as described by Kahler and Allard (1970). Thus the esterase isozymes from these two different tissue extracts can be treated as the products of a single, albeit probably complex, locus. Segregation analysis showed that *Est-H5* was the most distal locus on the long arm of this chromosome and extended the original map by 1.8 cM. One esterase locus, identified as *Est1*, which may be the same as that described by Brown (1983), has been mapped by Graner et al. (1991) on the same chromosome arm.

A similar situation exists in wheat, where at least three different sets of esterase loci have been reported to be located on the long arms of the homoeologous group 3 chromosomes: one encodes coleoptile isozymes (Jaaska 1980), one encodes endosperm isozymes (Ainsworth et al. 1984) and the third encodes leaf esterases (Jouve and Diaz 1990). However, an effort to detect recombination between these loci among 60 F<sub>2</sub>s was unsuccessful (Liu 1991). The *Est-5* loci in hexaploid wheat are also, to-date, the most terminally mapped loci on homoeologous group 3 chromosomes, at least 80 cM from the centromeres (Devos et al. 1992).

*Hor1* and *Hor2*. Both loci were mapped on the short arm of barley chromosome 5 (1H) as expected. *Hor2* was 13.0 cM distal to *Mla12* while *Hor1* co-segregated with *Xcnl.BCD249* and *X22Epr8*, i.e. *Hor1* was located 3.9 cM proximal to *Mla12*. The results obtained in this study for *Hor1*, *Hor2* and *Mla12* were in good agreement with the previously reported linkage among these three loci (Shewry et al. 1980; Graner et al. 1991).

*Wsp-H1*. This locus mapped in between *Xcnl.CDO420B* and *Xcnl.CDO347* on the long arm of chromosome 1 (7H) (*Xcnl.CDO420B* – 22.1 cM – *Wsp-H1* – 10.8 cM – *Xcnl.CDO347*). However, the original map distance between *Xcnl.CDO420B* and *Xcnl.CDO347* (24.6 cM) was stretched by 8.3 cM when *Wsp-1* was inserted. One of the possible explanations for this discrepancy is that only a subset of the DH lines used to construct the original

RFLP maps was employed for WSP-1 analysis. Again, the location of *Wsp-H1* in barley agrees with the location of its homoeoloci in hexaploid wheat (Liu et al. 1989).

## References

- Ainsworth CC, Gale MD, Baird S (1984) The genetic control of grain esterases in hexaploid wheat. 1. Allelic variation. *Theor Appl Genet* 68:219–226
- Brown AHD (1983) Barley. In: Tanksley SD, Orton TJ (eds) *Isozymes in plant genetics and breeding*. Elsevier, Amsterdam, Part B, pp 57–77
- Chao S, Sharp PJ, Worland AJ, Warham EJ, Koebner RMD, Gale MD (1989) RFLP-based genetic maps of wheat homoeologous group 7 chromosomes. *Theor Appl Genet* 78:495–504
- Devos KM, Atkinson MD, Chinoy CN, Liu CJ, Gale MD (1992) RFLP-based genetic map of the homoeologous group 3 chromosomes of wheat and rye. *Theor Appl Genet* 83:931–939
- Graner A, Jahoor A, Schondelmaier J, Siedler H, Pillen K, Fischbeck G, Wenzel G, Herrmann RG (1991) Construction of an RFLP map of barley. *Theor Appl Genet* 83:250–256
- Heun M, Kennedy AE, Anderson JA, Lapitan NLV, Sorrells ME, Tanksley SD (1991) Construction of a restriction fragment length polymorphism map for barley (*Hordeum vulgare*). *Genome* 34:437–447
- Jaaska V (1980) Electrophoretic survey of seedling esterases in wheats in relation to their phylogeny. *Theor Appl Genet* 56:273–284
- Jouve N, Diaz F (1990) Genetic control of esterase-6 isozymes in hexaploid wheat and rye. *Euphytica* 47:165–169
- Kahler AL, Allard RW (1970) Genetics of isozyme variants in barley. I. Esterases. *Crop Sci* 10:444–449
- Koebner RMD, Martin PK (1989) Chromosomal control of the aminopeptidases of wheat and its close relatives. *Theor Appl Genet* 78:657–664
- Liu CJ (1991) Biochemical marker genes in hexaploid wheat, *Triticum aestivum*. PhD dissertation, Cambridge University, UK
- Liu CJ, Chao S, Gale MD (1989) *Wsp-1*, a set of genes controlling water-soluble proteins in wheat and related species. *Genet Res* 54:173–181
- Payne PI, Law CN, Mudd EE (1980) Control by homoeologous group 1 chromosomes of the high-molecular-weight subunits of glutenin, a major protein of wheat endosperm. *Theor Appl Genet* 58:113–120
- Shewry PR, Faulks AJ, Pickering RA, Jones IT, Finch RA, Mifflin BJ (1980) The genetic analysis of barley storage proteins. *Heredity* 44:383–389
- Wettstein-Knowles P von (1989) Barley (*Hordeum vulgare*) 2n=14. In: O'Brien SJ (ed) *Genetic maps*, 5th edn., pp 6.125–6.134