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Integration of a barley (*Hordeum vulgare*) molecular linkage map with the position of genetic loci hosting 29 developmental mutants

C Pozzi[1], D di Pietro, G Halas, C Roig and F Salamini
Max-Planck-Institut für Züchtungsforschung, Carl-von-Linné -Weg, 10 50829 Cologne, Germany

The first step in positional gene cloning is the integration into available molecular maps of genetic loci for which mutant alleles exist. We report the placement of 29 barley developmental mutants on a restriction fragment length polymorphism–amplified fragment length polymorphism (RFLP–AFLP) map. The mapping procedure used homozygous mutant F_2 plants in an iterative process: once a mutant linked AFLP was found, primer combinations were successively selected to generate AFLP fragments more tightly linked to the mutant locus. The mutants considered were *adp*, *als*, *aur-a1*, *aur-a2*, *br1*, *br2*, *bra-d7*, *cul3*, *cul5*, *cul15*, *cul16*, *den6*, *den8*, *dub1*, *hex-v3*, *hex-v4*, *int-c5*, *K*, *li*, *lig-a2*, *lk2*, *lk5*, *sld1*, *sld4*, *tr*, *trd*, *unc*, *uc2* and *uz*. The 29 mutant loci were linked to the closest molecular markers by distances ranging from 0 to 23 cM, with an average value of 3.8 cM away. Since the efficiency of the mapping procedure is a function of the density of molecular markers, the RFLP–AFLP map of Castiglioni *et al* was further integrated with new AFLPs using 87 doubled haploid lines derived from the barley cross Igri × Danilo. A total of 819 mapped AFLP marker loci are now available in the combined map.
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Introduction

Positional or map-based cloning techniques are widely used to identify genes defined by the existence of mutant alleles. In Arabidopsis, the information generated by the Genome Initiative has demonstrated the value of this approach to discover new gene functions (Lukowitz *et al*, 2000). In crop species, progress in the enrichment of molecular maps, as well as the availability of PCR-based markers and extensive collections of ESTs that facilitate SNP scanning, also favor the adoption of the positional cloning approach (Richmond and Somerville, 2000). Barley has a genome size of 5×10^9 bp but, in spite of this size, positional cloning is feasible (Büschges *et al*, 1997; Lahaye *et al*, 1998; Schwarz *et al*, 1999). The first step in positional cloning is the localization, on the available molecular map, of the genetic loci defined by mutant alleles. We have developed a procedure for mapping barley mutants and DNA probes based on amplified fragment length polymorphism (AFLP), in combination with restriction fragment length polymorphism (RFLP) (Castiglioni *et al*, 1998). The strategy makes use of the Proctor × Nudinka (RFLP) map assembled by Heun *et al* (1991) and has proved to be reliable and efficient (Castiglioni *et al*, 1998; Pozzi *et al*, 2000; Schmitz *et al*, 2000; Müller *et al*, 2001). The positioning of mutant loci and expressed genes on the same linkage map allows the integration of map-based cloning procedures with the choice of candidate genes. This approach has been successfully applied to the mapping of quantitative trait loci (QTLs) for disease resistance in *Capsicum* and in wheat (Faris *et al*, 1999), while in maize a candidate gene involved in flavone synthesis could be associated with resistance to corn earworm (Byrne *et al*, 1996). Further examples of the candidate gene approach for the cloning of monogenic traits in plants are reported in Molinero-Rosales *et al* (1999) and Müller *et al* (1995).

In this paper, we report on the enrichment of a barley molecular map by the addition of 368 AFLP loci and on the integration into this map of 29 genetic loci for which developmental mutants are described.

The mutations investigated in this paper can be described in the light of an interpretation of the morphology of the barley plant as a succession of growth units (phytomers; Bossinger *et al*, 1992). For the 29 mutants considered, growth units of a specific region of the plant, which assume characteristics proper to phytomers of other regions, are evident for *uniculm*, *third outer glume*, *densinodosum*, *intermedium*, *branched* and *awned palea*. Mutants characterized by the presence of modified organs on the unit of growth are *liguleless*, *bracteatum*, *triple awned lemma* and *awned palea*. Mutants with additional phytomeres are represented by *densinodosum* and *branched*.

Correspondence: F Salamini, Max-Planck-Institut für Züchtungsforschung, Carl-von-Linné-Weg, 10, 50829 Cologne, Germany.
E-mail: salamini@mpiz-koeln.mpg.de
[1]*Current address: Fondazione Parco Tecnologico Padano-CERSA, c/o DiProVe, University of Milano, 20133 Milano, Italy.*
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Materials and methods

Plant material

A total of 87 doubled haploid barley lines (DH lines) were used to map 368 new AFLPs, which supplement

the 511 already mapped by Castiglioni *et al* (1998). The 87 lines originated from a cross between the lines Igri and Danilo (courtesy of A Jahoor, Technische Universität München, Germany). Developmental mutants were from the collection maintained at the Max-Planck-Institut für Züchtungsforschung (MPIZ), Cologne, Germany. This includes mutant stocks obtained from the Barley Genetic Stock Center (BGS), Fort Collins, Colorado (at present Aberdeen, Idaho); the Istituto per la Cerealicoltura (FIOR), Fiorenzuola, Italy; and from Udda Lundqvist (UD), Svalöf Collection, Svalöv, Sweden. The origins of mutants are listed in Castiglioni *et al* (1998). Mapping populations of the mutants listed in Table 1 were grown in the field and phenotypes reclassified in greenhouse tests. Wild-type (WT) and mutant (M) plants were selected and stored as F_3-seed families. Data concerning the sizes of the mapping populations are reported in Table 1. Current mutant symbols for the loci described are reported in column 3 of Table 1 according to the recommendations of Lundqvist *et al* (1997), Franckowiak (1997), Franckowiak *et al* (1997) and Franckowiak and Lundqvist (2002). In the following, the term 'revised nomenclature' refers to the gene symbols given in the four papers cited above. In Table 1, the symbols used by Gustafsson *et al* (1969) are also reported, where applicable.

DNA techniques

Seedlings were grown in the greenhouse and harvested at the four-leaf stage for DNA extraction. DNA was extracted using the QIAGEN (Hilden, Germany) kit adapted to 96-well microtiter plates. The original AFLP procedure described by Zabeau and Vos (1993) was carried out with the minor modifications specified by Castiglioni *et al* (1998).

Construction of the molecular linkage map using DH lines of Igri × Danilo

The mapping data for the Proctor × Nudinka population were integrated with a new set of AFLP bands mapped in an Igri × Danilo cross. *Eco*RI and *Mse*I AFLP primers were combined in the same 72 primer combinations used to develop the Proctor × Nudinka AFLP-RFLP map (Castiglioni *et al*, 1998). Each AFLP fragment was identified by the number of its primer combination and by an additional digit that refers to the figure stored as 'Visual catalogue of AFLP bands polymorphic between the barley lines Igri and Danilo', at the web site *http://www.diprove.unimi.it/*. A list of the AFLP bands mapped on the Igri × Danilo molecular linkage map is given at the same web site. In the 87 DH lines, polymorphic bands were scored as 0 or 1 for the absence or presence, respectively, and the data were tested for conformity to a 1:1 segregation ratio using a χ^2 test ($P = 0.05$). Only bands whose segregation ratios did not deviate significantly from 1:1 were analyzed using the MAPMAKER program (Lander *et al*, 1987; DOS version 3.0). Recombination data were at first analyzed at a LOD value of 10.0, allowing the definition of 28 preliminary linkage groups. Of those groups, 11 were not positioned into the Nudinka × Proctor linkage groups since no bridge bands were shared by the Igri × Danilo and Nudinka × Proctor populations. On the remaining 17 preliminary linkage groups, a three-point analysis was carried out and the ORDER routine of MAPMAKER was used to find the most probable order of their markers. An additional analysis at LOD 3.0 was performed on each group to confirm marker order and allow the integration of previously unmapped markers into preliminary linkage groups. Finally, the ORDER procedure was used to allow placement of markers previously not considered in the analysis and the TRY command allowed the placing of makers discarded by three-point analysis. Polymorphisms represented by segregating AFLP bands were assigned to chromosomes and chromosomal subgroups based on bridge AFLP loci that segregated both in the Igri × Danilo and Proctor × Nudinka populations. Identity of AFLP bands produced in the two populations by the same primer combination was assumed based on their electrophoretic mobility and relative intensity in gels (see also the Results and discussion).

Integration of mutant loci into the molecular map

Linkage between mutants and markers was established based on DNA data from F_3-seed families representing homozygous recessive F_2 plants, as explained in Pozzi *et al* (2000). The size of the mapping populations is reported in Table 1, together with the number of mapping populations produced. When linkage between an AFLP band and a given mutant phenotype was established, the map position of the polymorphism was assigned based on the map of Castiglioni *et al* (1998), supplemented by the new Igri × Danilo data. Segregation data were analyzed with the MAPMAKER program with LOD score value 3.0 and a maximum distance of 50 cM. The mutant locus was represented by a virtual marker in complete linkage to the mutant phenotype in F_2 plants. Coupling configurations – the presence of the AFLP band in the WT – were privileged because they allow the unambiguous classification of recombinants and, thus, a ready detection of putative cases of linkage. In case of the absence of recombination between a mutant and a specific AFLP loci, and in order to verify the actual existence in the F_2 population of the concerned AFLP polymorphism, five to 10 WT F_2 plants were also analyzed.

Results and discussion

The Igri × Danilo map

The 87 DH lines derived from the Igri × Danilo cross were analyzed with 72 AFLP primer combinations. Of 7668 readable bands (106.5 per primer combination), 520 were polymorphic and segregated in a ratio not significantly different from 1:1. Each primer combination yielded, on average, 6.9 polymorphic AFLP bands. In total, 72 AFLP markers were selected as bridges because they represented bands that migrated at identical positions on AFLP gels in both the Igri × Danilo and Proctor × Nudinka populations. Bridge bands were identified for 17 out of the 28 preliminary linkage groups and used to assign them to the seven linkage groups of the Proctor × Nudinka population (Heun *et al*, 1991). The final Igri × Danilo map included 368 AFLP markers out of the 520 newly detected. The reduction was a consequence of the presence of markers (1) placed on the 11 preliminary linkage groups that could not be assigned to a precise chromosomal location and (2)

Table 1 Positions of 29 developmental mutant loci in the Proctor × Nudinka AFLP map (Linkage and linkage subgroups are assigned to each mutant locus.)

Mutant											*Population size*[i]	
Symbol and name[a]	*Synonyms in*		*Mutant source*[b]		*Cross*[d]							
	Gustafsson et al (1969)	*Recommended nomenclature*		*Map position*[c]	*N*	*P*	*Closest marker*[e]	*Distance*[f] *(cM)*	*Coupling/ repulsion*[g]	*Linked markers*[h] *(no.)*	*N*	*P*
adp, awned palea	—	*adp*	BGS	Chr. 3H/27	+	−	E3634-8	5.8	c	4	41	
als, absent lower laterals	—	*als*	BGS	Chr. 3H/28	−	+	E4234-11	0.0	c	2		50
aur-a1, auricleless a1	*aur*	*lig*	UD	Chr. 2H/20 and 21	+	−	E4034-3	4.1	c	5	39	
aur-a2, auricleless a2	*aur*	*lig*	UD	Chr. 2H/21	+	−	E3633-1	1.6	r	3	42	
br1, brachytic	—	*brh1*	BGS	Chr. 1H/1	+	+	E4134-8	5.0	c	5	15	24
br-2, brachytic dwarf	—	*brh2*	BGS	Chr. 4H/38–40	+	+	E4140-7	0.0	c	15	34	29
bra-d7, bracteatum d7	*bra*	*trd*	UD	Chr. 1H/gap	+	−	E3634-7	4.1	c	—	40	
cul15, uniculm 15	*cul*	*cul4*	UD	Chr. 3H/32	+	+	E4143-4	0.0	c	8	19	16
cul16, uniculm 16	*cul*	*cul4*	UD	Chr. 3H/31 and 32	+	−	E4144-5	8.0	c	9	36	
cul3, uniculm 3	*cul*	*cul4*	UD	Chr. 3H/31 and 32	+	−	E4038-1	5.5	c	5	20	
cul5, uniculm 5	*cul*	*cul4*	UD	Chr. 3H/32 and 33	+	−	E4244-3	6.9	c	5	30	
den6, densinodosum 6	*den*	*mnd6*	UD	Chr. 5H/65	−	+	E3743-3	0.0	c	4		16
den8, densinodosum 8	*den*	*mnd6*	UD	Chr. 5H/65	−	+	E3640-1	3.9	c	6		28
dub-1, double seed 1	—	*dub-1*	UD	Chr. 5H/66 and 67	+	+	E4038-4	0.0	c	11	38	23
hex-v3, hexastichon v3	*hex-v*	*vrs1*	UD	Chr. 2H/19–21	+	−	E4343-7	3.5	r	7	40	
hex-v4, hexastichon v4	*hex-v*	*vrs1*	UD	Chr. 2H/19 and 20	+	−	E3438-3	10.8	c	5	27	
int c.5, intermedium c5	*int*	*int-c*	UD	Chr. 4H/8	+	+	E4143-5	3.5	r	9	31	30
K Hooded	*K*	*Kap*	BGS	chr 4H/36 and 37	+	−	E4140-1	5.1	c	3	72[j]	
li, liguleless	*lig*	*lig*	BGS	Chr. 2H/22 and 23	+	+	E3544-9	6.8	c	5	35	19
lig-a2, liguleless a2	*lig*	*lig*	UD	Chr. 2H/21	+	−	E4244-8	5.0	c	6	29	
lk2, short awn	—	*lks2*	BGS	Chr. 7H/6	+	+	E4138-3	3.6	c	8	29	29
lk5, short awn	—	*lks5*	BGS	Chr. 4H/38	+	−	E4143-5	0.0	c	7	56	
sld1, slender dwarf 1	—	*sld1*	FIOR	Chr. 3H/27	+	−	E3634-8	1.2	c	6	44	
sld4, slender dwarf 4	—	*sld4*	FIOR	Chr. 7H/6	+	−	E4134-2	0.0	c	4	14	
tr, triple awned lemma	—	*trp*	BGS	Chr. 2H/22 and 23	−	+	E3644-13	0.0	c	5		30
trd, third outer glume	—	*trd*	BGS	Chr. 1H/52	+	−	E3634-7	2.5	c	2	57	
uc2, uniculm 2	—	*cul2*	BGS	Chr. 6H/54	+	−	E4343-10	9.2	c	5	31	
unc[k]*, uniculm*	—	—	MPIZ	Chr. 6H/54	+	−	E4034-2	10.0	c	4	37	
uz, semibrachytic	—	*uzu*	BGS	Chr. 3H/27	+	−	E3733-6	10.1	c	5	24	

[a]Nomenclature as in Castiglioni *et al* (1998). The recommended nomenclature refers to Franckowiak and Lundqvist (2002). [b]BGS, MPIZ, FIOR, UD: the abbreviations are explained in Materials and methods. [c]Both linkage and linkage subgroups are reported. Chromosome number refers to syntenic linkage groups of *Triticeae* with H for *Hordeum*. Numbering formerly adopted for barley was Chromosome 1 = 7H; Chromosome 2 = 2H; Chromosome 3 = 3H; Chromosome 4 = 4H; Chromosome 5 = 1H; Chromosome 6 = 6H; Chromosome 7 = 5H. Species-Specific structural rearrangements of the 7 *Triticae* chromosomes are described by Dunford *et al* (1995). [d]Crosses reported are: mutant × Nudinka (N) and mutant × Proctor (P). + and − indicate, respectively, the presence and absence of the closest AFLP marker in Nudinka and Proctor. [e]The marker more closely linked to the mutant has been chosen as described in the text. [f]The distance calculated by MAPMAKER is expressed in cM. [g]Coupling: presence of an AFLP band in the WT and absence in the mutant. [h]Number of markers found linked to the mutant. When both P and N populations were considered, the number given includes markers from both crosses. A list of linked markers can be found at the www.diprove.unimi.it web site, Table 3. [i]The entry refers to the numbers of F_2 recessive plants in the mapping populations. [j]A total of 41 dominants F_3 Hooded lines (*KK*) and 31 recessive (*kk*) lines. [k]Symbol assigned in this paper; see text.

Table 2 Data concerning the Igri × Danilo molecular linkage map

	Markers	*No. of preliminary linkage groups*	*Total length (cM)*
Chromosome 1H	21	2	116
Chromosome 2H	65	3	511
Chromosome 3H	26	2	295
Chromosome 4H	34	3	293
Chromosome 5H	69	2	586
Chromosome 6H	72	2	488
Chromosome 7H	81	3	620

placed off-end by the ORDER command or that could not be positioned by three-point analysis or by the TRY routine. The reduction in the number of usable markers also applied to bridge bands (from 72 to 61), mainly because of discrepancies in their new map positions as compared with those derived from the Proctor × Nudinka map. This could be explained by the fact that while generating a large number of polymorphisms per gel, the AFLP procedure produces a small fraction of bands with similar mobilities in gels and which map to different linkage groups. The final robustness of the new map, as obtained here for the Igri × Danilo population, relies on the linkages of bridge bands with loci segregating only in the Igri × Danilo cross. Eventually, 16 bridge markers were allocated to Chromosome 7H; 11 to Chromosome 2H; five to Chromosome 3H; eight to Chromosome 4H; six each to Chromosomes 1H and 6H and nine to Chromosome 5H. The order of, and the relative distance between, bridge markers in the 17 chromosomal regions mapped in the cross Igri × Danilo were in agreement with the data of Castiglioni *et al* (1998).

Data concerning the number of AFLP markers mapped on the Igri × Danilo linkage groups, the number of preliminary linkage groups assigned to each chromosome and the map length are reported in Table 2. A significant map expansion in comparison to the previous RFLP–AFLP was observed, particularly in marker-dense regions (ie on Chromosomes 7H and 4H). Grouping of markers in specific regions of the seven linkage groups described in Castiglioni *et al* (1998) identified, in that map, linkage subgroups. The same nomenclature was adopted to indicate linkage subgroups in the Igri × Danilo map, as described in the cited web site. A graphic representation and statistics of the Igri × Danilo linkage map are obtainable at the web site *http://www.diprove.unimi.it* (PDF files and Table).

Mapping of 29 barley developmental mutants

A procedure that allows rapid mapping to barley linkage groups of mutant alleles of genetically characterized loci is described in Castiglioni *et al* (1998). The procedure uses homozygous recessive F_2 plants segregating in crosses between the mutants and the two WT lines Proctor and Nudinka, the parents of the population used to create the reference AFLP map. This procedure was used to map the loci *branched* and *calcaroides* (Castiglioni *et al*, 1998; Pozzi *et al*, 2000) and it is extended in this paper to a group of developmental mutants. The mapping approach was iterative: once an AFLP polymorphism revealed linkage to a genetic locus, other primer combinations were selected to generate AFLP fragments which, based on the Proctor × Nudinka and Igri × Danilo AFLP maps, were predicted to be in closer linkage to the mutant. The mapping data obtained are reported in Table 1. For each mutant, its map position in the linkage subgroups of Castiglioni *et al* (1998) or in an interval spanning linkage subgroups, or in regions poor in polymorphic markers (the latter situation applied to two cases, mutations *bra-d7* and *unc*) is listed.

For most mutants, F_2 plants from the cross mutant × Nudinka proved to be sufficiently informative. In some cases, however, it was necessary to reinforce the map position by using F_2 plants from the Proctor cross (Table 1). In the case of the mutant *bra-d7*, the linked AFLP marker could not be localized on the Proctor × Nudinka map, but the data available for the Igri × Danilo population made it possible to map it off-end on Chromosome 1H.

The AFLP marker in closest linkage with the mutant locus, as determined in coupling configuration on the largest mapping population analyzed, is indicated in Table 1 as the closest marker. The recombination distance between the closest marker and the next mutant locus ranged from 0 to 23 cM, with an average of 3.8 cM. In about one-fourth of cases, the iterative procedure was performed until no recombinants were found between marker and mutant. In Table 1, the number of markers within a linkage subgroup and linked to a given mutant is also reported. In few cases, only two markers were found linked to the mutant because of the absence of polymorphisms for other markers of the region. The number of individuals included in the mapping populations was kept low to allow a rapid screening by AFLP: mapping populations ranged in size from 16 to 72 F_3 individuals (Table 1).

In some cases, our data provide molecular markers for mutants previously assigned to chromosomes by linkage to other classical markers, thus contributing linkage subgroups mapping. The mutants *adp*, *bra-d7*, *cul3*, *cul5*, *cul15*, *cul16*, *den6*, *den8*, *dub-1*, *sld4* and *unc* were here assigned to precise positions in specific linkage groups for the first time.

The mutant *awned palea* (*adp*) was described by Ahokas (1977), and was mapped here for the first time to Chromosome 3H, linkage subgroup 27 (Figure 1).

The *absent lower laterals* (*als*) locus (Kasha and Walker, 1960; Tsuchiya and Haus, 1971) is here precisely mapped on Chromosome 3H, linkage subgroups 28 and 29. For this locus, the revised nomenclature uses the same symbol. The mutant was induced with γ-rays in the variety Montcalm (Franckowiak, 1997).

The mutations *auricleless* (*aur-a1* and *aur-a2*) and *liguleless* (*lig-a2* and *li*), which affect ligule–auricle development, all mapped on Chromosome 2H, in an interval spanning sublinkage groups 21–23. The allele *li (lig)*, obtained from BGS, a spontaneous mutant that is allelic to the Swedish *aur* mutants (Tsuchiya, 1973a), is confirmed to map to Chromosome 2H as reported by Takahashi *et al* (1953). The mapping data confirm allelism tests conducted by U Lundqvist (personal communication) in that *aur-a1* and *aur-a2* are allelic and both map on Chromosome 2H, linkage subgroups 20 and 21. The *lig-a2* mutant is not allelic to *li*, according to U Lundqvist (personal communication), but both mutants comap with

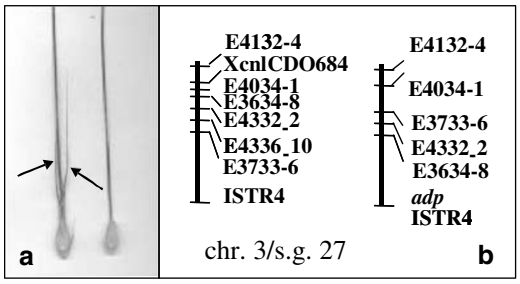

Figure 1 Mapping of *awned palea* (*adp*), one of the 29 recessive Mendelian loci integrated into the molecular marker map described here. (**a**) Mutant (left) and WT (right) florets of barley. The mutant is characterized by the presence of an awn not only on the lemma but also on the palea (indicated by the arrows). Because the palea ontogenetically derives from the fusion of two bracts (Williams, 1975), there are actually two awns on this organ. (**b**) Map position of the mutation *adp* on Chromosome 3H, linkage subgroup 27 of the Proctor × Nudinka map. Left: Proctor × Nudinka map. Right: mapping in the cross *adp* × Nudinka.

aur mutations. The recommended nomenclature proposes also for *lig-a2* the symbol *lig*.

Two *brachytic* mutants *br* and *br2* (Franckowiak, 1995) were previously studied: *br1* maps on Chromosome 7H, and is now assigned to sublinkage group 1 of the same Chromosome. The mutant arose spontaneously in the variety Himalaya. The second mutant, *br2*, maps between sublinkage groups 38 and 40 on Chromosome 4H, confirming the chromosomal assignment given by Tsuchiya (1980). *br2* was induced by X-ray treatment in the variety Svanhals (Tsuchiya, 1962). The recommended nomenclature for these mutants is *brh*. The semibrachytic mutant *uz*, obtained from BGS, is a spontaneous mutation present in several Japanese varieties. The mutant was previously assigned to Chromosome 3H (Takahashi and Yamamoto, 1951; Tsuchiya, 1980). We mapped this mutant to sublinkage group 27 of Chromosome 3H. The symbol for the mutant locus in the recommended nomenclature is *uzu*.

The *third outer glume* (*trd*) mutants, also referred to as *bracteatum* (*bra*; Gustafsson *et al*, 1969), have been frequently investigated. The bract-like structures that in the mutant cover a triplet of spikelets, are homologous to a normal leaf. Our data support the possibility that *bra-d7* (which maps on Chromosome 1H south of sublinkage group 52), a mutant obtained from the Svalöf collection, and *trd* (which maps in the same chromosomal region and was obtained from the BGS) could be allelic. Tsuchiya (1974) previously proposed that *bra-c1* and *trd* were alleles. The mapping data support the assignment by Konzak (1953) and Søgaard and von Wettstein-Knowles (1987) to Chromosome 1H. *bra-d7* was obtained from Dr U Lundqvist (Svalöf collection) and *trd* is the symbol used in the recommended nomenclature.

The *uniculm* mutants (*uc* in Søgaard and von Wettstein-Knowles, 1987) are characterized by a single culm and the absence of lateral tillers. The mutants we have considered include a group obtained from Svalöf induced with X-rays and represented by *cul3*, *cul5*, *cul15* and *cul16*, which all map on Chromosome 3H, sublinkage groups 31–33. The second group includes the mutant *uc2* obtained from BGS (Shands, 1963) and *unc* isolated at MPIZ. Both mutants map on Chromosome 6H close to sublinkage group 54 in a region already identified for *uc2* by Robertson *et al* (1965). In the recommended nomenclature the two different loci hosting *uniculm* mutants are designated *cul2* and *cul4* (Table 1).

The two *densinodosum* mutants *den6* and *den8* originated from the Svalöf collection, but allelism tests were not available. Similar mutations (*multinoded branched, mnb*) were described by Walker *et al* (1963) and (*multinoded dwarf, mnd*) by Prasad and Tripathi (1985), following the first description by Gustafsson (1947). Both *den6* and *den8* are now mapped on Chromosome 5H, sublinkage group 65. The recommended nomenclature uses the symbol *mnd6* for this locus.

The mutant *double seed 1* (*dub-1*) was obtained from the Svalöf collection and is here mapped to Chromosome 5H, linkage subgroups 66 and 67.

The two six-row ear character is controlled in barley by several independent gene loci, including the *v* locus on Chromosome 2H (Nilan, 1964) – with the six-rowed allele *v* being inherited recessively – and the allelic series for the fertility of the lateral florets on Chromosome 4H (Gymer, 1977). A group of *intermedium* genes has been described that control the same character (Lundqvist, 1981; Lundqvist and Lundqvist, 1998): the *int-c5* locus is assigned to Chromosome 4H (Woodward, 1949; Gymer, 1977). Based on our data, this locus maps to the same chromosome, linkage subgroup 38. Most of the induced recessive mutants at the *v* locus are designated by the symbol *hex-v* (Gustafsson *et al*, 1969; Søgaard and von Wettstein-Knowles, 1987). We have mapped two such alleles obtained from the Svalöf collection, *hex-v3* and *hex-v4*, and they are, as expected, located on Chromosome 2H, linkage subgroups 19, 20. This locus is now indicated by the symbol *vrs1*.

Hooded (*K*) is a dominant mutant introduced into western countries from the Himalayas (Harlan, 1931; Stebbins and Yagil, 1966; Badr *et al*, 2000). The mutation is caused by a tandem direct duplication on 305bp in the fourth intron of homeobox gene *Bkn*3, resulting in the ectopic overexpression of BKN3 at the lemma–awn transition region: a local acquisition of meristematic activity is followed by the formation on the lemma of extra floral structures (Müller *et al*, 1995). The locus was assigned to Chromosome 4H and now it has been associated to linkage subgroup 37 of the same linkage group. The recommended symbol is *Kap*.

The *short awn* (*lk*) mutants constitute a family of loci that map to different chromosomes (Tsuchiya, 1973b). *lk5* (Litzenberger and Green, 1951) was confirmed in this work to map on Chromosome 4H (Tsuchiya, 1980) linkage subgroup 38, while *lk2* (Takahashi *et al*, 1953) lies on Chromosome 7H, linkage subgroup 6. The two loci now have the recommended symbols *lks5* and *lks2*, respectively. The mutant *slender dwarf* (*sld1*) was induced by EMS treatment (Konishi, 1975; Szarejko and Maluszinski, 1984) and the gene was localized on Chromosome 3H near the mutant locus *uzu* (Konishi *et al*, 1984). We have now refined this map position by assigning the locus to linkage subgroup 27. The symbol for *sld1* remains unchanged in the recommended nomenclature. *sld4* obtained from FIOR was mapped here, for the first time, to Chromosome 7H, linkage subgroup 6.

The mutant *triple awned lemma* (*tr*; Martini and Harlan, 1942) was here mapped to Chromosome 2H, linkage

subgroups 22-23, in accordance with Immer and Henderson (1943). The recommended symbol for the mutant is *trp*.

The mapping activity reported is based on the use of AFLP markers, which allow one to score several polymorphic loci in a single gel lane (Zabeau and Vos, 1993). When the goal is the construction of a molecular map of general value in genetic or plant breeding studies, the assumption has to be made that cosegregating AFLP bands produced by amplifying DNAs of different genotypes derive from identical DNA sequences. This seems to be the case for barley (Waugh *et al*, 1997). Qi *et al* (1998) and Qi and Lindhout (1996), for example, have produced an integrated AFLP map of barley linkage groups starting from four different segregating populations, using bridge AFLP bands common to the populations considered and migrating at the same positions in AFLP gels. A recent study based on AFLP band sequencing indicates that, even in interspecific comparisons within the genus *Hordeum*, AFLP product size and band intensity are reliable indicators of band DNA homology (El Rabey *et al*, 2002). It is therefore envisionable that AFLP polymorphisms mapped in one population serve as chromosome-specific markers, if they also segregate in a second one. Some exceptions to this generalization have, however, been noted: in this paper, the map positions of bridge markers agreed in general with published maps. Discrepancies noted could be due to coincidental comigration of AFLP fragments derived from distinct genomic positions. Duplications of parts of the genome, as well as the existence in barley of a large genome fraction made up of repetitive sequences (Barakan *et al*, 1997), can account for the phenomenon. This complication, in any case, did not interfere with the incorporation into the Proctor × Nudinka map of AFLP loci from the Igri × Danilo cross. Although the two populations have no parents in common, and even if the parental lines themselves are not closely related, 61 common AFLP markers (17% of the total mapped) were available for the alignment of the two maps. The linkage map obtained was in good agreement with previously published maps (Becker *et al*, 1995; Castiglioni *et al*, 1998; Qi *et al*, 1998 and references therein). This successful integration makes now available a total of 819 AFLP marker loci useful for future mutant mapping and cloning in barley.

The major contribution of this paper is the mapping data for 29 Mendelian mutants. This allows to integrate these loci into a molecular map with an average density of about one AFLP marker every 3 cM. The average distance between the mapped genetic loci and the nearest AFLP marker is 3.8 cM, but frequently no recombination events were detected. The small number of F_2 plants analyzed may have contributed to reduce the probability of finding recombinants, but local maker density frequently made also possible to select markers comapping with mutants.

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