An AFLP-Based Procedure for the Efficient Mapping of Mutations and DNA Probes in Barley

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ABSTRACT

A strategy based upon AFLP markers for high-efficiency mapping of morphological mutations and DNA probes to linkage groups in barley is presented. First, 511 AFLP markers were placed on the linkage map derived from the cross Proctor × Nudinka. Second, loci controlling phenotypic traits were assigned to linkage groups by AFLP analysis, using F_2 populations consisting of 30–50 mutant plants derived from crosses of the type "mutant × Proctor" and "mutant × Nudinka." To map DNA probes, 67 different wild-type barley lines were selected to generate F_2 populations by crossing with Proctor and Nudinka. F_2 plants that were polymorphic for a given RFLP fragment were classified into genotypic classes. Linkage of the RFLP polymorphism to 1 of the 511 AFLP loci was indicated by cosegregation. The use of the strategy is exemplified by the mapping of the mutation *branched-5* to chromosome *2* and of the DNA probes *Bkn2* and *BM-7* to chromosomes *5* and *1*, respectively. Map expansion and marker order in map regions with dense clustering of markers represented a particular problem. A discussion considering the effect of noncanonical recombinant products on these two parameters is provided.

MORE than 1000 molecular markers, predominantly RFLPs, are mapped onto barley chromosomes (Graner *et al.* 1991; Heun *et al.* 1991; Kleinhofs *et al.* 1993; Kasha and Kleinhofs 1994). Recently, the amplified fragment length polymorphism (AFLP) procedure (Vos *et al.* 1995) has provided a convenient and reliable tool with which to generate markers to further facilitate map construction (Becker *et al.* 1995; Qi *et al.* 1997; Waugh *et al.* 1997). The AFLP method is a PCR-based technique that avoids the laborious steps involved in restriction fragment length polymorphism (RFLP) mapping. Like RFLPs, the majority of AFLP fragments define unique loci in the barley genome (Vos *et al.* 1995; Qi and Lindhout 1997; Waugh *et al.* 1997). Here we report the use of AFLP markers to efficiently map mutations and DNA probes to barley linkage groups. AFLP analysis has a very high diversity index (Russell *et al.* 1997), resulting in a limited number of primer combinations required to screen a whole genome. In this respect, the method for integrating genetic and molecular maps presented in this article is novel. To implement this procedure it was necessary to (1) place a sufficient number of AFLP markers on a barley linkage map constructed from a cross of two specific barley lines, (2) obtain F_2 populations in which barley mutations segregated in crosses with the mapping parents, and (3) generate a set of F_2 populations segregating at specific RFLP loci that can be mapped on the basis of their linkage with AFLP polymorphisms.

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MATERIALS AND METHODS

Plant material: The 113 doubled haploid barley lines (DH lines) used for mapping originated from a cross between the lines Proctor and Nudinka (Heun *et al.* 1991). Seeds were provided, together with the parental lines, by M. Heun in 1991, and were maintained at the Max-Planck-Institut für Züchtungsforschung (MPIZ; Köln, Germany). The 67 barley lines used in crosses with Proctor and Nudinka for mapping of DNA probes were selected from a collection of 5842 accessions obtained from the plant germplasm bank in Braunschweig (Germany). Their origins, gene bank numbers, and MPIZ collection numbers are reported in Table 1. Crosses between each of these lines and the varieties Proctor and Nudinka were done at the MPIZ. Seed from F_2 progeny of individual F_1 plants was harvested separately and stored at 4°. F_3 seed was harvested from single F_2 plants from the cross *v.h.* elses (G397 in Table 1) × Nudinka for mapping of the *Bkn2* gene, and from the cross *v.h.* isthmos (G392 in Table 1) × Nudinka to map the *BM-7* gene.

A set of barley mutants (Table 2) was crossed with Proctor and Nudinka to generate F_2 populations. These were stored as such or grown in the field, where wild-type (WT) and mutant (M) plants were selected and stored as F_3 seed families. The segregating populations of the mutants listed in Table 2, together with the genetic materials reported in Table 1, are available to those interested in using our procedure.

The barley mutant *branched-5* (*brc-5*) was isolated from the Braunschweig seed collection (see above). This line is also homozygous for the dominant allele *K* at the *Hooded* locus. The mutant was crossed to Nudinka and Proctor, to generate

TABLE 1

List of 67 WT lines of barley used in the crosses with Nudinka and Proctor

G No.[a]	Genotype	BR No.[b]	× N[c]	× P[d]	Origin[e]
G382	Wild *agriocrithon*	BR06016	8	8	TUM
G383	ucnw016	BR02831	8	8	GBR
G384	sp11 085–50	BR04183	8	8	IRL
G385	*vulg. coeleste* kleine nacktgerste	BR05175	7	4	DEU
G386	*vulg. hybernum* vikayarvi	BR05192	5	8	FIN
G387	*vulg. hybernum* tystofte korsby	BR05198	2	6	DNK
G388	*vulg. parallelum* montafon	BR05219	9	7	AUT
G389	*vulg. parallelum* sechszeilige	BR05236	8	8	DEU
G390	*vulg. hybernum* arrecife	BR05258	4	5	CRY
G391	*vulg. nigroibericum* otello	BR05268	8	7	ITA
G392	*vulg. hybernum* isthmos	BR05273	17[f]	8	BAL
G393	*vulg. hybernum* poliarnyj 14	BR05288	8	8	BAL
G394	*vulg. rikotense* brant	BR05291	19[f]	8	CAN
G395	*vulg. hybernum* oberbrucker	BR05302	14[f]	8	DEU
G396	*vulg. hybernum* estanzuela	BR05359	18[f]	8	URY
G397	*vulg. hybernum* elses	BR05363	16[f]	8	AFR
G398	*vulg. subviolaceum* abessinien	BR05366	8	8	ETH
G399	*vulg. hybernum* marokkanische	BR05367	7	8	MAR
G400	*vulg. trifurcatum* aegypten	BR05368	6	7	ETH
G401	*vulg. hybernum* algerian	BR05372	11	8	DZA
G402	*vulg. hybernum parallelum* samsun	BR05375	7	7	VAS
G403	*vulg. parallelum* libanon	BR05381	10[f]	7	VAS
G404	*vulg. himalayense* tibet	BR05383	6	6	TIB
G406	*vulg. horsfordianum* weihenstephan	BR05439	16[f]	7	CHN
G407	dist. *nudiforcatum* erfurt	BR05534	19	6	DDR
G408	dist. *nutans* kenia	BR05619	21[f]	6	SKA
G409	dist. *nutans* spratt archer	BR05691	21[f]	7	GBR
G410	dist. *nutans* sarah	BR05718	21[f]	8	BNL
G411	dist. *nutans* loosdorfer	BR05767	15[f]	8	AUT
G412	dist. *nutans* proskowetz gerste	BR05811	25[f]	7	CS
G413	dist. *nutans* triumf	BR05815	29[f]	7	CS/PL
G414	dist. *nutans* carbonera	BR05838	23[f]	8	ESP
G415	dist. *nutans* martonvasari	BR05858	23[f]	8	BAL
G418	dist.*medicum* anatolien	BR05896	16[f]	8	TUR
G419	dist. *nigricans* mandschurei	BR05948	22[f]	8	MAS
G420	dist. *erectum* hokudai no. 1	BR05949	27[f]	8	OAS
G421	dist. *nutans* australische fruche	BR05969	7	7	AUT
G422	*intermedium gymnanomalum*	BR05983	8	8	BEL
G423	*deficiens steudelii* abessinien	BR05995	8	7	ETH
G424	*vulg. hybernum* aegyptische	BR010621	25[f]	8	ETH
G425	*vulg. hybernum* lyallpur	BR010701	9	8	PAK
G426	*vulg.* wisconsin H42 (linie)	BR010789	8	8	USA
G427	ucnwc72a	BR011929	11[f]	1	PAK
G428	fap1 ooo8a	BR012396	16[f]	8	CHE
G429	dist. *nutans* bannerts	BR010708	18[f]	8	DUE
G431	fap1 2158 B	BR013150	14[f]	6	EAK
G432	fap 1 2158 H	BR013156	19[f]	8	CYP
G433	fap1 2158 L	BR013158	15[f]	8	AFG
G434	*deficiens erythraeum* foa II	BR015670	8	8	ETH
G435	*vulg. dundar-beyi* nippon	BR017711	6	7	OAS
G436	*intermedium horlani* arlington	BR017715	8	8	USA
G437	ucnw c177	BR018705	8	8	IND
G438	npc 0006	BR019389	3	6	PAK
G439	siglah	BR026054	8	4	YEM
G440	siglah	BR026085	6	8	YEM
G441	dist. *glabrierectum* sanalta	BR038255	11[f]	8	CAN
G442	*deficiens deficiens* fehlgerste	BR038322	8	5	NAF

(*continued*)

TABLE 1

(Continued)

G No.[a]	Genotype	BR No.[b]	× N[c]	× P[d]	Origin[e]
G443	dist. *nutans* agio	BR038414	29	8	NLD
G444	dist. *nutans* pfaelzer land	BR038451	11	—	DEU
G445	dist. *nutans* szekacs linie II	BR038651	4[f]	—	HUN
G449	dist. *nutans* maiamana	BR038846	13[f]	8	TKM
G451	*hexastichon hybernum* abarik	BR040419	—	3	TKM
G452	*hexastichon hybernum* chilean	BR041427	8	8	USA
G453	MPI 2	BR044619	5	7	DEU
G417	dist. *nutans* swannek	BR05894	6	7	AFR
G416	dist. *nutans* saratov	BR05865	27[f]	7	SVN
G430	fap 1 0266C	BR012482	22[f]	7	AFG

[a] Number in the stock collection at MPIZ, Köln; to be used to request seeds.
[b] Accession number of the Braunschweig seed collection.
[c] Number of available F_2 progenies from the cross with Nudinka. Each progeny consists at present of 100 to 300 F_2 seeds.
[d] Number of the available F_2 progenies from the cross with Proctor.
[e] Origins: TUM, Technical University München; BAL, Balkan; SAM, South American; AFR, Africa; VAS, Western Asia; MAS, Middle Asia; OAS, Eastern Asia; NAF, Northern Africa; all other origins are according to the ISO α-3 code (available at Gatersleben Seed Collection Web site).
[f] F_2 progenies obtained partly in the field and partly in the greenhouse.

F_2 populations. The 45 F_2 M plants from the cross with Nudinka and the15 F_2 M plants from the cross with Proctor, used in mapping, were selected in the field and F_3 seed was harvested. DNA was extracted from a pool of 20 F_3 seeds for each F_2 plant.

DNA techniques: Seeds of the barley lines were planted in the greenhouse and seedlings were harvested at the four-leaf stage for DNA extraction (Saghai-Maroof *et al.* 1984; or the "QIAtip 100" protocol of QIAGEN, Hilden, Germany).

The original AFLP procedure as described by Zabeau and Vos (1993) and Vos *et al.* (1995) was followed using the minor modifications of Becker *et al.* (1995). The selection of biotinylated fragments was avoided in the mapping experiments for *BM-7* and *Bkn2*. Adapters and the *Mse*I and *Eco*RI primers used were as follows: *Mse*I adapters, GACGATGAGTCCTGAG and TACTCAGGACTCAT; *Mse*I universal primer (MU), GATGAGTCCTGAGTA; *Mse*I +1 primer (M01), MU+A; *Mse*I +3 primers, M32, MU+AAC; M33, MU+AAG; M34, MU+AAT; M36, MU+ACC; M38, MU+ACT; M40, MU+AGC; M43, MU+ATA; M44, MU+ATC; M46, MU+ATT; *Eco*RI adapters, CTCGTAGACTGCGTACC and CATCTGACGCATGGTTAA; *Eco*RI universal primer (EU), GACTGCGTACCAATTC; *Eco*RI +1 adapters, EU+A; *Eco*RI +3 primers: E34, EU+AAT; E35, EU+ACA; E36, EU+ACC; E37, EU+ACG; E40, EU+AGC; E41, EU+AGG; E42, EU+AGT; and E43, EU+ATA. All sequences are given in the 5′ to 3′ direction. All PCR reactions were carried out in a UNO-Thermoblock (Biometra, Göttingen, Germany). Amplified fragments were separated on 4.5% polyacrylamide gels, at 58 W for 1 hr in 0.5× TBE. A dephosphorylated and γ-^{33}P-labeled 1-kb ladder (GIBCO BRL, Gaithersburg, MD) was used as size marker.

The RFLP analysis was performed essentially as described by Gebhardt *et al.* (1989). The restriction enzymes *Taq*I, *Msp*I, *Mse*I, *Rsa*I, and *Alu*I (Boehringer Mannheim, Mannheim, Germany) were used to digest the DNA samples. A total of 7 μg of DNA was loaded per lane on 4.5% polyacrylamide gels and run at 40 W for 6 hr, electroblotted onto Hybond-N filters at 20 A for 1 hr, and probed with [α-^{32}P]dCTP randomly labeled probes (Feinberg and Vogelstein 1984).

The inverse sequence-tagged repeat (ISTR)-based technique was performed as described in Rohde (1996). Forward and backward primers, designed to reveal polymorphisms connected with *copia*-like elements, were labeled with γ-^{33}P and used in standard PCR reactions incorporating an annealing step at 45° for 30 sec. PCR products were separated on 4% polyacrylamide gels.

Scoring and mapping: The E and M AFLP primers were combined in all 72 possible combinations [16 were used earlier by Becker *et al.* (1995)]. Each mapped AFLP fragment can be identified by the number of its primer combination and an additional digit that refers to the figure stored under "A visual catalog of AFLP bands polymorphic between the barley lines Proctor and Nudinka," at the Web site http://www.mpiz-koeln.mpg.de/salamini/salamini.html (for example, the AFLP marker e3432-7 corresponds in the figure to band 7 obtained with the primer combination E34-M32).

In the 113 DH lines, polymorphic bands were scored as 0 or 1 for absence or presence, respectively, and were tested against the expected 1:1 segregation ratio using a chi-squared test ($P = 0.05$). Only AFLP data segregating 1:1 were added to the datafile of Becker *et al.* (1995) and analyzed using MAPMAKER (Lander *et al.* 1987; UNIX version /EXP3.0b) and JoinMap (Stam 1993; PC/MS-DOS 1.4 version) programs. All the AFLP-mapped bands are reported in Table 3, with the corresponding subgroup assignment. Allelic state of AFLP bands in autoradiograms was controlled independently twice. Furthermore, singletons (or doubletons; see discussion) were identified by computer analysis, and the existence of the concerned polymorphisms was checked again in the autoradiograms.

Data analysis with MAPMAKER was performed with and without the ERROR DETECTION option. RFLP loci mapped in the original Proctor × Nudinka cross (Heun *et al.* 1991) were chosen as backbone markers, by virtue of their order reliability supported by data from other mapping populations. The backbone RFLPs are indicated, in Figure 1, to the left of each chromosome, where the number in parentheses refers to the mapping population from which they are derived [their

TABLE 2

Populations of the barley mutants crossed with Nudinka and Proctor

			Populations from crosses					
			With Nudinka			With Proctor		
				No. of F_2 selected plants (F_3 seeds)			No. of F_2 selected plants (F_3 seeds)	
	Mutant symbol and name	Mutant source	F_2[a]	WT	M	F_2[a]	WT	M
aa[c]	Abnormal awn	MPIZ		5	37		5	18
acr1[c]	Accordion rachis1[d]	UD				6		
acr2[c]	[d]	UD	6			6		
acr3[c]	[d]	UD		8	28		5	13
acr4[c]	[d]	UD				6		
acr8[c]	[d]	UD				4		
adp	Awned palea	BGS		5	13 + 19		5	11
als	Absent lower laterals	BGS		0	19		5	51
brh1	Brachityc1							
	*br1**	FIOR		5	23		5	37
	*br**	BGS		5	15		4	27
	*ari-1**	FIOR		5	23	6		
brh2	Brachityc2	BGS		5	35	6		
bra c-1	Brachityc1	BGS		5	15		5	51
bra-d7	Bracteatum	UD[b]		5	47	6		
cul2	Uniculm							
	*uc-2**	BGS		5	32		5	44
cul3	Uniculm3[d]	UD		5	19			
cul5	Uniculm5[d]	UD		5	31 + 28		5	30
cul15	Uniculm15[d]	UD		5	21		5	16
cul16	Uniculm16[d]	UD		5	36	6		
den3	Densinodosum3[d]	UD		3	18			
den6	Densinodosum6[d]	UD		5	42			
den7	Densinodosum7[d]	UD		40	37		40	26
den8	Densinodosum8[d]	UD		5	28	5		
dub-1	Double seed1[d]	UD		4	27	6		
dub-2	Double seed2	UD		4	46		5	43
dub-3	Double seed3[d]	UD		5	29		5	38
extra floret-1	[d]	UD		5	46	6		
extra floret-2	[d]	UD		5	31	6		
extra glumes		UD		47	12			
hex-v3	Exastichon[d]	UD		5	42			
hex-v4	Exastichon[d]	UD		5	27			
int-a1	Intermedium	UD		5	32			
int-b3		UD		5	26		5	32
int-c5		UD		5	27		5	31
int-e20		UD		5	33		5	23
int-f19		UD		5	21			
int-h42		UD		5	37		5	41
int-i39		UD		5	23	6		
isp 25	Irregular spike	UD		5	41	6		
lax-a01	Laxatum	UD		5	22 + 70			
lax-a4	Laxatum a4	UD		5	23			
lax-a8	Laxatum a8	UD			21			
lbi	Long weak basal internode	BGS		5	26		5	31
lc	Lax spike	BGS		5	19 + 36	6		
Lc	Lax spike	BGS	4			6		
leo[c]	Leonessa dwarf	FIOR		5	47		5	15
lep-e1	Macrolepis	UD		5	45	6		
lig	Liguleless1			5	63			
	*aur-a1**	UD		19 + 5	42 + 38			
	*aur-a2**	UD		5	42		5	43

(*continued*)

TABLE 2

(Continued)

			Populations from crosses					
			With Nudinka			With Proctor		
				No. of F_2 selected plants (F_3 seeds)			No. of F_2 selected plants (F_3 seeds)	
	Mutant symbol and name	Mutant source	F_2[a]	WT	M	F_2[a]	WT	M
	*aur-a3**	UD		5	34		5	62
	*li**	BGS		5	31 + 42		5	40 + 53 + 14
lig a	Liguleless							
	*lig-a2**	UD		5	63 + 31		6	30
	*lig-a3**	UD		5	51		5	45
	lig-b5	UD					5	38
lin	Lesser internode number	BGS	5			6		
lks	Short awn							
	*lk2**	BGS		5	29	6		
lnt	Lower No. of tillers	BGS		5	30			
mnd	Many noded dwarf	FIOR		5	38			
oss-1	Opposite spikelets1[d]	UD		6	43	6		
oss-2	Opposite spikelets2[d]	UD		5	22		5	41
oss-3	Opposite spikelets3[d]	UD		5	38	6		
rac-1	Long basal rachis internode[d]	UD		4	36			
rac-3	[d]	UD	6			6		
sid	Single internoded dwarf	BGS	7			1		
sld1	Slender1							
	*dw1**	FIOR		5	47		5	36
sld2	Slender2	FIOR		5	50			
sld4[c]	Slender4	FIOR				6		
	*dw 4**	FIOR		5	18	6		
sld5[c]	Slender 5							
	*ch5**	FIOR		5	17		5	11
tar20	Triaristatum[d]	UD		5	46 + 29		5	40
tr	Triple awned palea	UD		5	40		5	18
trd	Third outer glume							
	*trd**	BGS		6 + 39	40 + 37		39	40
	*bra-c1**	UD		5	16		4	52
u	Unbranched style	BGS						21 + 30
unc[c]	Uniculm	MPIZ		4	37		5	40
uz	Semibrachitic	BGS		5	24			
vin3		FIOR		5	21		6	38
vine		FIOR		5	16		5	28
viv1	Viviparoides[d]	UD		5	16		5	28
viv3	[d]	UD	11			11		
viv4	[d]	UD		5	35	6		
viv6	[d]	UD		5	34	6		
viv7	[d]	UD		5	21	6		
vrs1	Six row spike							
	V[d*]	BGS			20			
	*v**	MPIZ			20 + 30 + 36		5	26
	V*	MPIZ			12			

[a] Mutants were obtained from Max-Planck-Institut (MPIZ), Cologne, Germany; Barley Genetics Stock Center (BGS), Fort Collins, Colorado; Fiorenzuola (FIOR), Instituto per la Cerealicoltura, Fiorenzuola, Italy; and Udda Lundgvist (UD), Svälov, Sweden. Whenever possible, the symbols given to mutants in Barley Genetics Newsletter, Vol. 26, or in Sogaard and Wettstein-Knowles (1987), are here adopted. *, Synonyms of the same mutant. In the collection are also present *msg* (male sterile) mutants nos. 1, 2, 4–14, 16–19, 22–30, 32, 33, 35–48, and 50, as F_2 seed populations for both crosses (1–30 plants, depending on the cross).

[b] In mutants received from UD, letters indicate genes and numbers of alleles.

[c] Number or symbols assigned in this article.

[d] Allelism test not available.

TABLE 3

List of AFLP polymorphisms revealed by the 72 primer combinations

PC[a]	BN[b]	Chr[c]	Position[d]	PC	BN	Chr	Position	PC	BN	Chr	Position	PC	BN	Chr	Position	PC	BN	Chr	Position
e3432	1	5	47		8	1	7		2	7	63		6	2	22		6	5	48
	2	7	62		12	1	6		3	3	30	e4133	1	2	19		7	4	35
	3	4	45		13	2	17		4	3	29		2	7	66		8	7	60
	5	6	54	e3632	1	2	20		5	2	21		3	7	67		9	1	20
	6	1	8		2	7	62	e3738	1	4	42		4	3	25		10	4	34
e3433	2	1	7		3	3	30		2	2	15		5	4	39		11	2	16
	3	6	54		4	7	62		3	3	25		6	1	1		12	2	21
	4	3	33		5	7	67		4	6	54		7	5	49	e4244	1	7	64
	5	1	10		6	5	48		5	2	15	e4134	1	7	65		2	1	6
	6	6	54		7	6	56		6	3	27		2	1	7		3	3	32
	7	2	18		8	1	1		7	2	22		4	2	17		4	4	41
	8	7	59		9	3	25		8	3	28		7	6	57		5	7	64
e3434	1	1	4	e3633	1	2	21		9	2	24		8	1	1		8	2	21
	2	5	47		4	6	56		10	1	9		9	7	62		10	7	59
	3	1	1		5	4	38	e3740	1	4	35	e4136	1	6	56		12	4	37
	5	5	49		6	1	11		3	5	51		2	5	51		13	6	58
	6	6	57		8	3	26		4	1	8		3	4	45		14	3	26
	7	7	68		9	1	7		5	4	37		6	6	56	e4246	1	3	25
	8	2	19		10	7	60	e3743	2	3	26	e4138	1	4	36		2	4	42
e3436	1	2	19		11	5	47		3	7	65		2	1	6		3	3	28
	2	1	7	e3634	1	6	53		4	3	30		3	1	6		4	4	41
	3	6	55		2	2	17		5	4	36	e4140	1	4	37		5	3	25
e3438	1	2	24		3	4	37		7	7	63		2	6	53		6	2	16
	2	2	20		5	5	52		8	4	37		3	3	26		9	4	38
	3	2	20		8	3	27		9	5	49		4	5	51		12	3	25
	4	5	51		9	4	37	e3744	1	1	12		5	7	60		13	2	23
	6	4	45	e3636	1	6	54		2	3	28		6	4	41		14	7	68
	7	5	48		2	2	17		3	4	44		7	4	39		15	2	22
e3440	1	1	6		3	7	63		4	7	63		8	2	17	e4332	1	4	37
	2	4	37		4	6	56		5	4	37	e4143	1	4	36		5	3	26
	3	1	10		5	7	63	e3746	1	3	28		2	1	6		2	3	27
	4	1	6		6	7	63		2	1	6		4	3	32	e4333	1	4	39
	5	1	7		7	1	11		3	4	43		5	4	38		3	3	32
e3443	1	7	62		8	6	54		4	3	32		6	6	53		4	4	34
	2	2	20	e3638	1	4	42		6	3	31		7	7	59		5	3	31
	3	2	20		2	7	62		7	2	23		8	1	2		6	2	24
	4	7	67		4	2	21	e4032	1	2	16		9	4	44		7	7	64
	6	7	66		5	5	52		2	4	42	e4144	1	5	46		8	1	2
	7	1	13		7	2	18		3	1	8		2	2	18		10	7	63
	8	4	45		8	7	65		5	4	38		3	3	29		12	4	43
e3444	1	1	8		10	3	32		6	6	57		4	2	16		13	3	25
	2	7	63		11	1	13		7	4	39		5	3	32	e4334	1	1	2
	3	1	1	e3640	1	7	65		8	4	38	e4146	1	1	6		2	7	60
	4	6	57		2	4	37		9	4	40		2	2	18		4	7	61
e3446	1	7	66		3	4	37		10	2	16		3	4	38		5	4	40
	5	3	26		4	5	48		11	7	67		4	3	26		6	4	37
	6	1	1		5	5	48	e4033	1	2	16		5	1	5		7	2	19
	7	7	67		6	1	2		2	3	30		6	3	29	e4336	1	5	51
	8	4	38	e3643	1	6	58		3	4	36		7	3	28		2	2	17
	9	5	49		4	1	11	e4034	1	3	27	e4232	8	6	56		5	7	63
	10	2	22		6	2	24		2	6	54		1	2	15		7	1	8
e3532	2	4	34		8	1	1		3	2	21		2	1	1		8	1	7
	3	5	48		9	1	2		4	4	38		3	4	36		9	4	38
e3533	1	1	12		10	2	19		5	1	10		4	7	61		10	3	27
	2	1	12	e3644	1	4	34		6	1	6		5	3	33		11	2	19

(*continued*)

TABLE 3

(Continued)

PC[a]	BN[b]	Chr[c]	Position[d]	PC	BN	Chr	Position	PC	BN	Chr	Position	PC	BN	Chr	Position	PC	BN	Chr	Position
	3	4	38		2	7	60		7	1	20		6	3	33		12	1	3
	4	5	50		3	4	60		8	5	51		7	7	61		13	1	2
	5	5	49		4	7	63		10	1	9	e4233	1	1	2	e4338	1	7	61
	6	7	66		5	1	6		11	1	1		4	1	5		2	2	17
	7	1	8		7	3	25		12	2	18		2	4	43		3	5	52
	8	2	20		8	3	32		13	3	30		3	4	34		4	4	44
	9	4	44		9	5	52	e4036	1	7	59		5	5	46		6	4	44
	10	2	24		10	6	55		2	1	7	e4234	3	4	37		7	1	7
	11	4	40		11	7	65		3	1	2		4	5	50		10	3	27
	13	4	40		13	2	22		4	1	11		7	1	1		11	5	49
	14	4	35	e3646	1	1	5		5	2	18		9	5	48	e4340	2	7	63
	17	5	47		2	1	6		6	7	62		11	3	28		5	5	50
	18	1	12		3	7	63		7	1	5		12	7	63		6	3	32
e3534	1	3	27		4	3	29	e4038	1	3	31	e4236	2	3	30		7	5	51
	4	1	13		5	7	66		2	1	8		3	4	37	e4343	1	7	64
	5	7	68		6	3	25		3	7	62		4	1	6		2	1	13
e3536	1	7	59		7	7	59		4	7	67		5	7	61		3	3	27
	2	2	21		8	7	65		6	4	34		6	5	48		4	5	46
	3	7	60		9	4	39		7	7	66		7	5	47		5	3	25
	4	1	13	e3732	8	1	2		8	3	26		8	1	5		6	2	21
	5	6	56		1	2	22		9	7	59		9	7	61		7	2	19
	6	7	66		2	2	22		10	7	62		10	5	51		9	5	47
e3538	1	5	46		3	5	52	e4040	1	1	11		11	4	44		10	6	54
	3	1	2		4	6	54		2	1	7		12	4	44		11	1	4
	7	2	24		5	2	16		3	7	59		13	3	32		12	2	15
	8	2	24		6	1	2		4	7	61		14	7	66	e4344	1	1	7
	9	7	60		7	7	68		6	1	13		15	7	61		2	7	65
e3540	1	1	5	e3733	1	3	27		5	7	61	e4238	1	7	68		6	2	24
	2	1	9		3	2	22	e4043	1	1	12		2	7	67		7	3	26
	4	3	33		4	5	52		2	6	58		3	2	19		9	7	62
	5	2	19		5	2	23		5	3	33		4	5	50	e4346	1	7	67
	6	4	40		6	3	27		3	2	24		5	3	28		2	5	51
	8	7	63		7	2	15		7	2	18		6	3	25		4	7	66
e3543	1	2	18		8	4	35	e4044	1	5	47		7	2	20		5	2	18
	3	5	51		9	3	31		3	1	6		8	4	35		6	3	30
	4	2	17		10	3	31		5	2	18		9	5	52		7	1	5
	5	3	32	e3734	1	4	37		6	5	51		10	7	64		8	2	22
e3544	1	7	63		2	2	18		7	5	52		11	7	62		9	1	6
	2	1	7		3	1	7	e4046	1	1	13		12	3	25				
	3	3	26		4	2	20		4	7	63	e4240	1	7	67				
	4	7	67		5	4	40		6	1	8		2	7	66				
	6	7	59		6	3	32		7	7	63		7	6	55				
	9	2	22		7	6	58		8	6	54		3	7	61				
	10	7	68		8	4	45		9	7	68		4	1	7				
e3546	1	1	6		10	5	48	e4132	1	4	42		5	7	62				
	2	1	7		11	3	32		2	1	6		6	1	6				
	4	7	60		12	1	5		7	2	24	e4243	1	3	29				
	6	3	28		14	5	46		3	2	16		2	1	7				
					16	1	5		4	3	27		3	1	11				
				e3736	1	7	62		5	7	66		4	7	65				

[a] PC, primer combination.
[b] BN, band number according to the figure displayed at the Web site cited in materials and methods.
[c] Linkage group assignment.
[d] Linkage subgroup assignment.

relative distances were recalculated from the Proctor × Nudinka RFLP/AFLP integrated map of Becker *et al.* (1995)]. The mapping populations are numbered in Figure 1 as follows: (1) Steptoe × Morex (Kleinhofs *et al.* 1993); (2) Harrington × TR306 (Tinker *et al.* 1996); (3) Blenheim × E24/3 (Thomas *et al.* 1995); (4) Franger × Rupee (De Scenzo *et al.* 1994); (5) T. Prentice × V. Gold (Kjaer *et al.* 1995); (6) Betzes × Golden Promise (Laurie *et al.* 1993); (7) Captain × *H. spontaneum* (Laurie *et al.* 1993); (8) Steffi × Atlas (Schweizer *et al.* 1995); (9) Igri × Triumph (Laurie *et al.* 1995); (10) *H. spontaneum* × SE16 (Sherman *et al.* 1995); (11) Dicktoo × Morex (Hayes and Meszaros 1997); (12) Chebec × Harrington (Langridge *et al.* 1996a); (13) Igri × Franka (Graner *et al.* 1994); (14) Vada × *H. spontaneum* (Graner *et al.* 1991); (15) Galleon × Haruna nijo (Langridge *et al.* 1996c); (16) Proctor × Nudinka (Liu *et al.* 1993); (17) Proctor × Nudinka (Röder *et al.* 1993); (18) Bonus *lax-a*^{1} × *H. spontaneum* (Laurie *et al.* 1996); (19) Clipper × Sahara (Langridge *et al.* 1996b); and (20) Integrated map (Qi *et al.* 1997).

RESULTS

Mapping of AFLP markers and ISTRs in the Proctor × Nudinka cross: Proctor and Nudinka were analyzed with 72 AFLP primer combinations and each combination yielded on average 7.1 polymorphic AFLP markers. Of 6299 readable bands (87.5 per primer combination), 833 (14.0%) were polymorphic. The 116 AFLP markers mapped by Becker *et al.* (1995) by using 113 DHs were considered together with the 395 new AFLP loci. In total, 511 AFLP markers and 32 ISTRs were added to the RFLP map of Heun *et al.* (1991). In total, 57,743 AFLP data points were produced, with 12% missing data. There were slightly more Nudinka than Proctor alleles (51% *vs.* 49%). In performing MAPMAKER analysis, the backbone markers assigned to linkage groups were not ordered. By means of the ASSIGN command (LOD 3.0 and 2.5), all other markers were placed. The LINKS and ATTACH commands were used to attribute markers to the most likely chromosome in a few cases. To order all markers on the assigned chromosomes, three-point data analysis was performed at LOD 3.0, with a maximum distance of 50 cM. The ORDER command was given twice for each chromosome using 100 as the minimum number of informative DH lines. When the program failed to find a starting order, this number was decreased to 50. The ORDER command was given also at LOD 2.0 to map markers that could not be placed at LOD 3.0. The TRY command was given to place all those markers for which the program was unable to find a location. Figure 1 shows the combined RFLP/AFLP/ISTR map (on the right-hand side) of each linkage group. Since the order of markers in dense clusters cannot be precisely established with a population of the size that we have used (see discussion), we divided the seven linkage groups into 68 subgroups, within which the most probable (although not definitive) order of markers is given in Figure 1. The order and the relative distance between the backbone markers were in good agreement with the data of Becker *et al.* (1995). Minor changes were observed in marker-dense regions, especially when flanked by gaps (map regions extending for long distances without intervening markers). Such changes concerned chromosome *2* (subgroup 19), chromosome *4* (in a region spanning the subgroups 36–39), and the telomeric region of chromosome *7*. The inversion of marker order on chromosome *2* was also observed by Sherman *et al.* (1995). The rearrangement on chromosome *4* affects a cluster containing many AFLP markers; an inverted order of RFLPs is reported here by Langridge *et al.* (1996a,b). The finding of AFLP markers beyond the putative telomeric marker XcsuBG141 (Röder *et al.* 1993) on chromosome *1* is in agreement with Sherman *et al.* (1995). The backbone markers on chromosome *6* are in agreement with Becker and Heun (1995). On chromosome *2*, markers e4238-3 and e4133-1 are inverted as compared to Becker *et al.* (1995). Some gaps present on the Becker *et al.* (1995) map have been filled: on chromosome *3* by the subgroup 26 markers; on chromosome *3* by ISTR9 between subgroups 26 and 27; on chromosome *1* by subgroup 3 markers; on chromosome *6* by ISTR34.

The RFLP/AFLP/ISTR data were also analyzed using the ERROR DETECTION option of MAPMAKER. This option considers the probability at each locus that its allelic configuration with respect to flanking markers arises in part from typing errors. Significant corrections in the total length of the map resulted, leading to a reduction from 2673 to 1597 cM (see discussion). Other changes were also observed: chromosome *1* was shortened by less then 10%, with markers e4040-2 and e4138-3 being inverted; chromosome *3* was shortened about 10-fold within each subgroup and 1.5-fold in the intervals between subgroups; chromosome *4* was 4-fold shortened mainly in the region spanning subgroups 36–38, resulting in a placement of markers XcnlWG181 and XcnlWG232 in agreement with the original Proctor × Nudinka map; chromosome *5* was shortened by 3-fold on average within subgroups, and by a factor of two in the intervals; chromosome *7* was shortened within subgroups 59 (10×), 60–63 (4×), 65 (3×), 66 (5×), and 67–68 (2×). In the latter case, a drastic rearrangement of marker order occurred. When the JoinMap program was used, the total length of the map resulted in 1264 cM.

Mapping mutant alleles of loci that control phenotypic traits to the AFLP map: The *brc-5* mutation is recessive and conditions the elongation of the rachilla, which is the second-order ramification axis of the barley ear. The elongated rachilla develops as an ear rachis, thus generating a ramified ear phenotype (Figure 2, A–C). The *brc-5* mutation was mapped using 45 F_2 *brc-5*/*brc-5* plants derived from the cross *brc-5* × Nudinka, together with 5 WT F_2 plants. Primer combination E36M36 produced one AFLP band (e3636-2) linked to the *brc-5* allele. Two out of 45 homozygous *brc-5* F_2 plants were recombinants, which corresponds to a linkage of 2.5

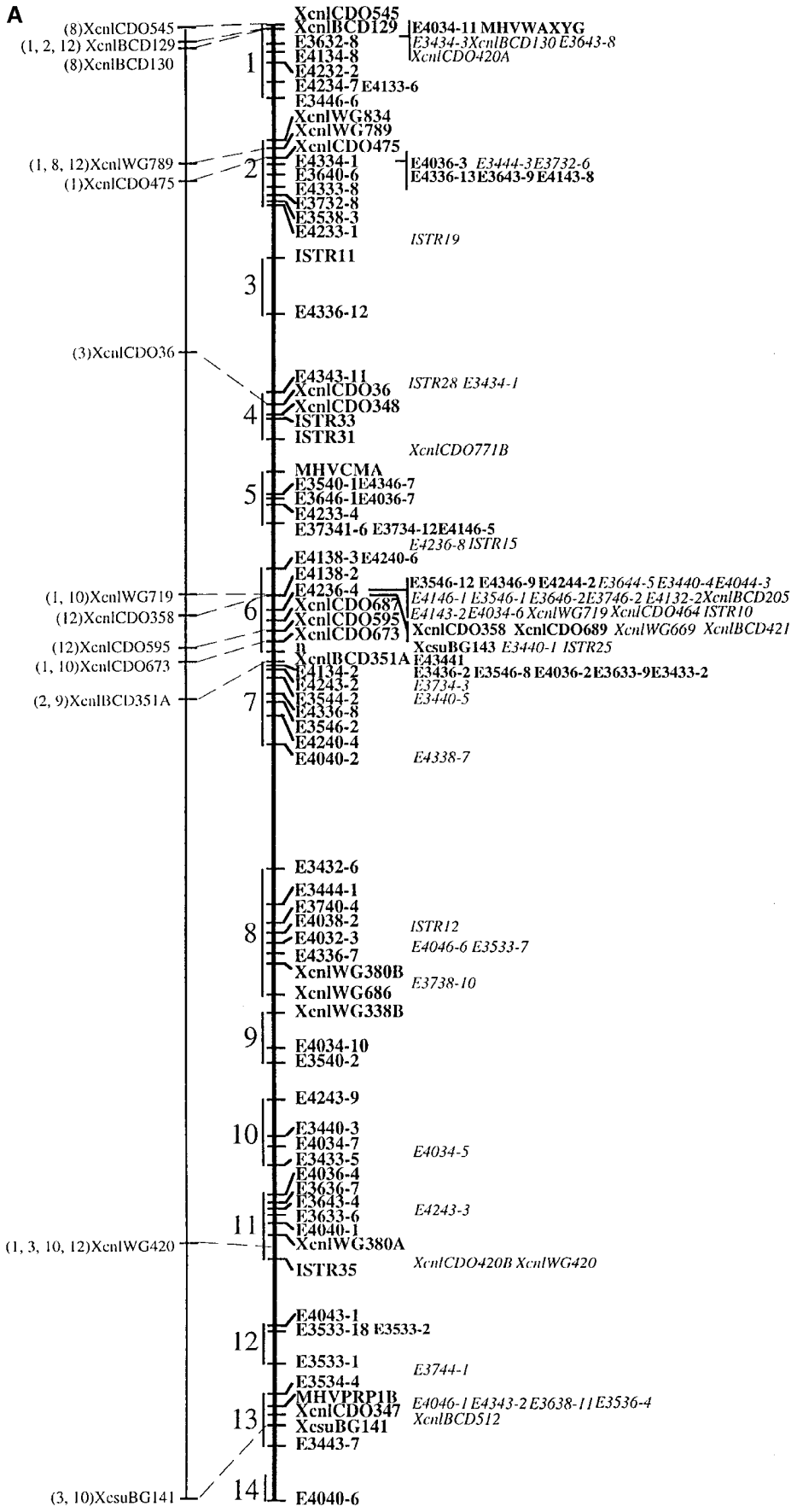

Figure 1.—Linkage maps of the seven barley chromosomes based on 113 DH lines derived from the cross Proctor × Nudinka. (A) Chromosome *1*, (B) chromosome *2*, (C) chromosome *3*, (D) chromosome *4*, (E) chromosome *5*, (F) chromosome *6*, (G) chromosome *7*. For each chromosome, a backbone RFLP map is given on the left (see text for details). On the right, the backbone map is integrated with AFLP and ISTR loci. The RFLP/AFLP/ISTR map is completed by indications of linkage subgroups (1–68). Markers represented in large boldface type have been placed at LOD 3.00 in the framework of each chromosome; markers in a smaller boldface type have been placed in unique positions at LOD 2.00; markers in italics have been assigned to an interval; and markers with an asterisk have been placed with the TRY command of MAPMAKER. Numbers in parentheses correspond to references cited in materials and methods, scoring and mapping section.

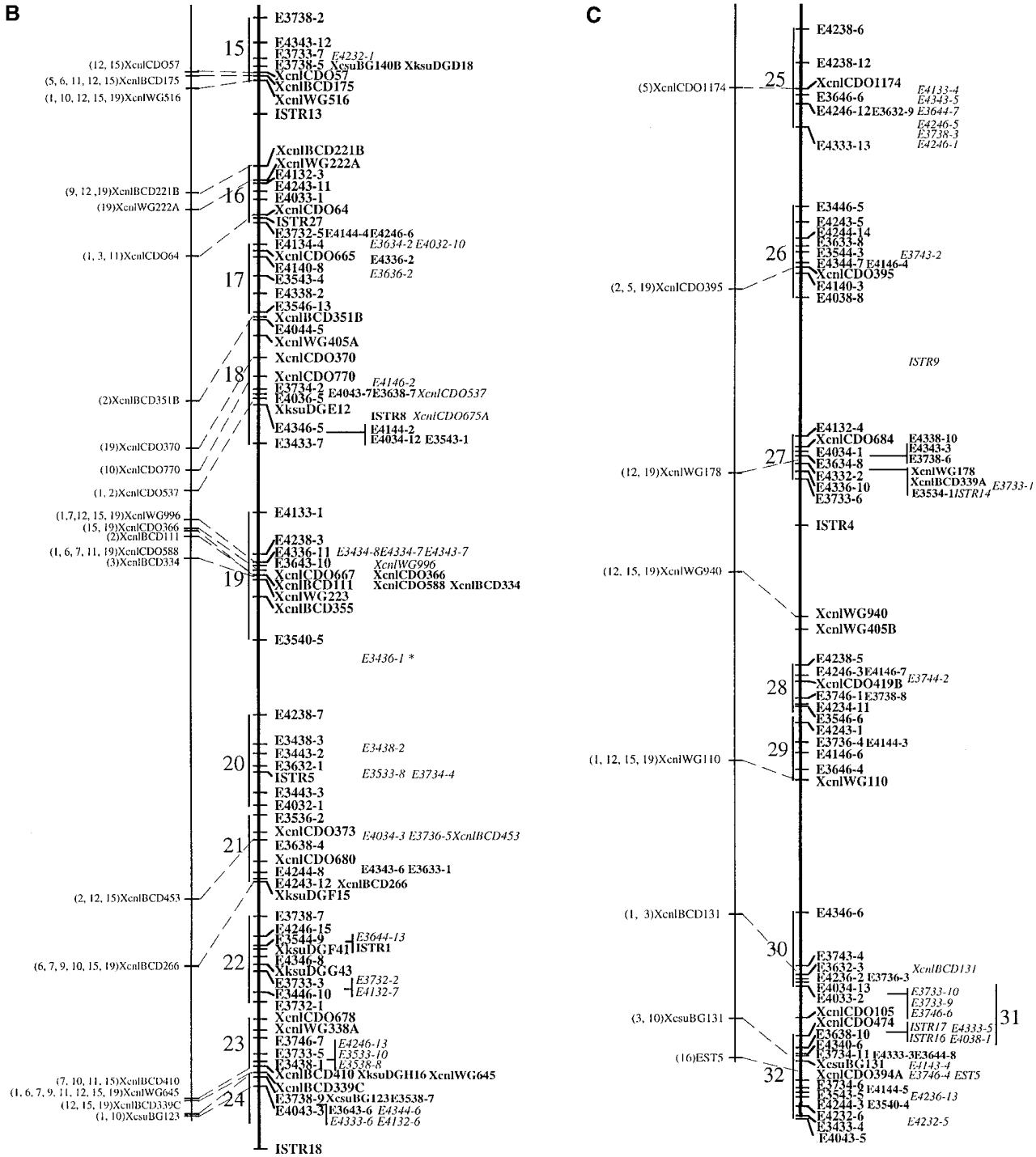

Figure 1.—*Continued.*

cM ± 1 ($P = 0.05$). The primer combinations E40M32 and E43M38 revealed linkage of *brc-5* with e4032-10 and e4338-2, two markers that map on chromosome *2* close to e3636-2. Linkage mapping, which considered 36 segregating bands obtained with 11 AFLP primer combinations, positioned the locus on chromosome *2* between markers e4338-2 and e3636-2, in a region spanning 8 cM. Figure 2E shows the segregation of AFLP band e3636-2 in 45 homozygous *brc-5* F_2 plants, while Figure 2D depicts the region of chromosome *2* where the *brc-5* locus maps. AFLP amplifications with primers E42M46, E41M40, E43M36, E35M46, E37M32, E41M34, and E41M44 were also carried out to confirm that associations between mutant phenotype and other segregating AFLP bands were not caused by linkage, but by distorted or chance segregation. Data derived from a small F_2 population of 15 *brc-5* plants from the cross Proctor × *brc5* confirmed the map location and allowed the scoring of markers that were previously uninformative in theNudinka cross, such as e4246-6 (repulsion; 2 recombi-

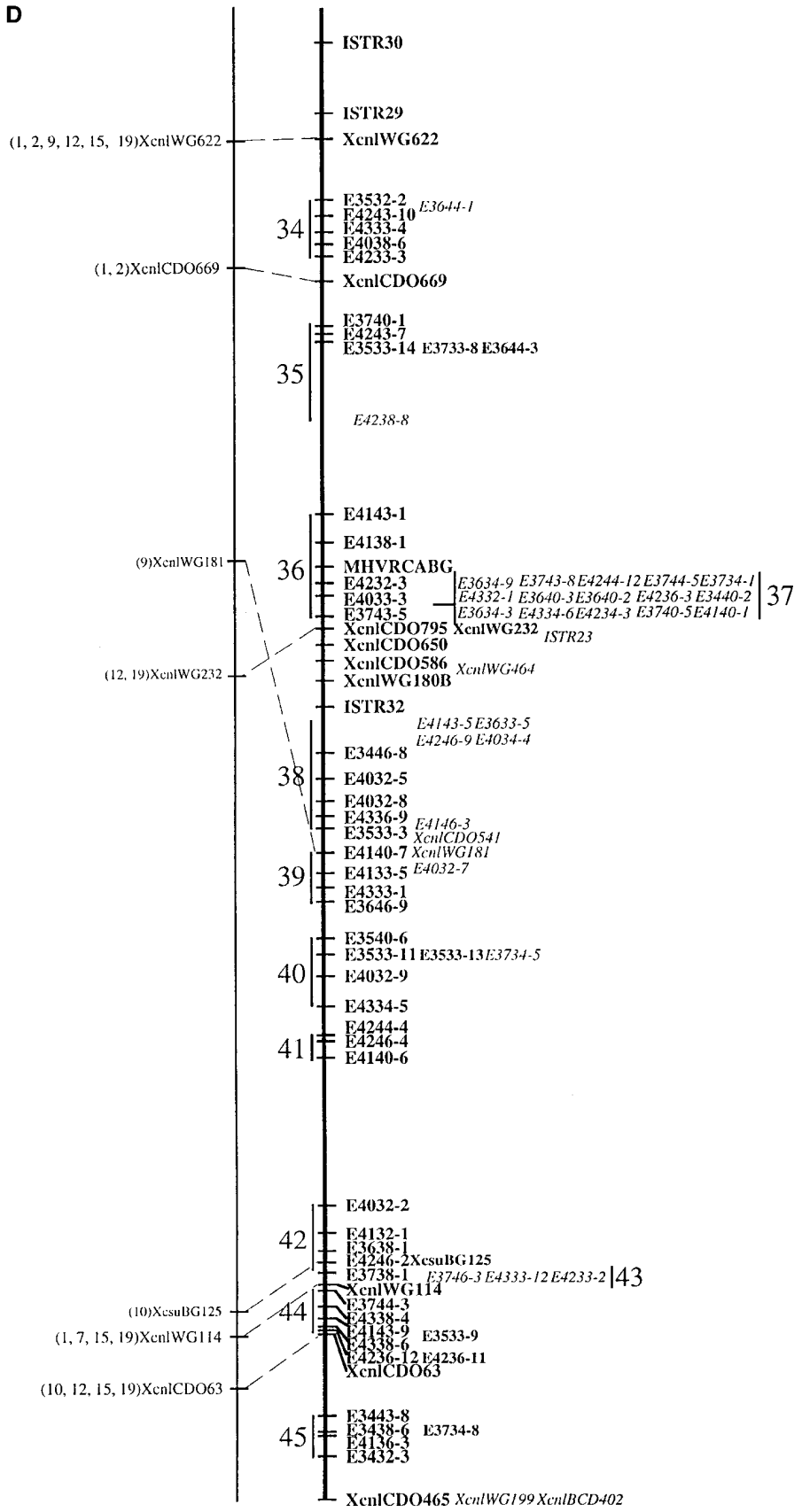

Figure 1.—*Continued.*

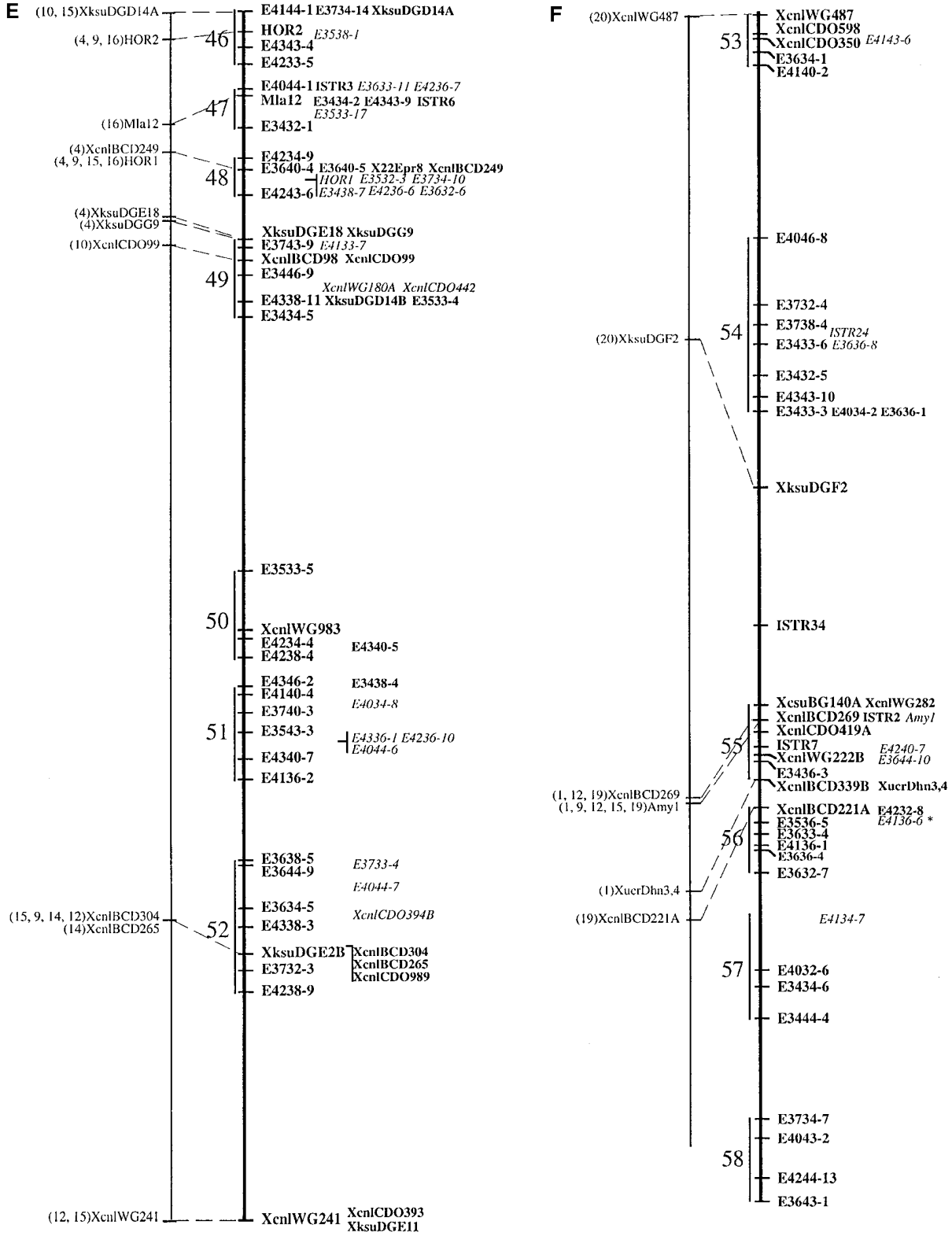

Figure 1.—*Continued.*

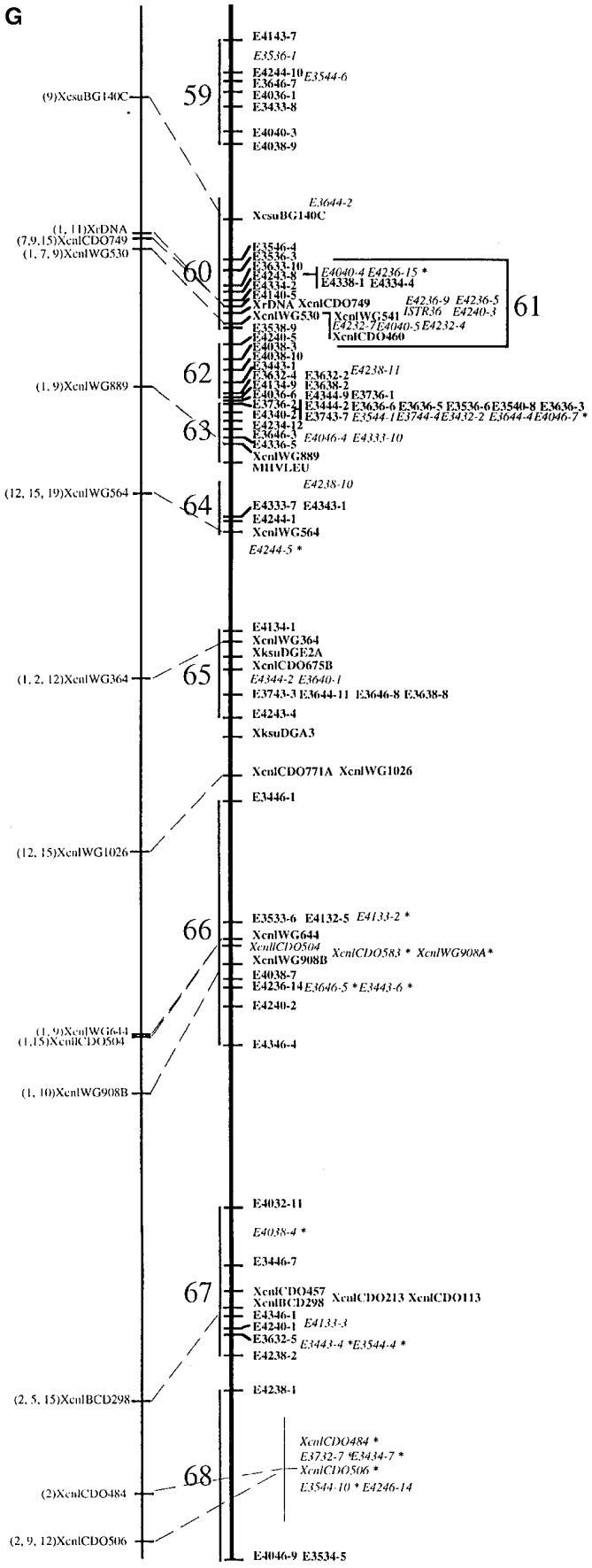

Figure 1.—*Continued.*

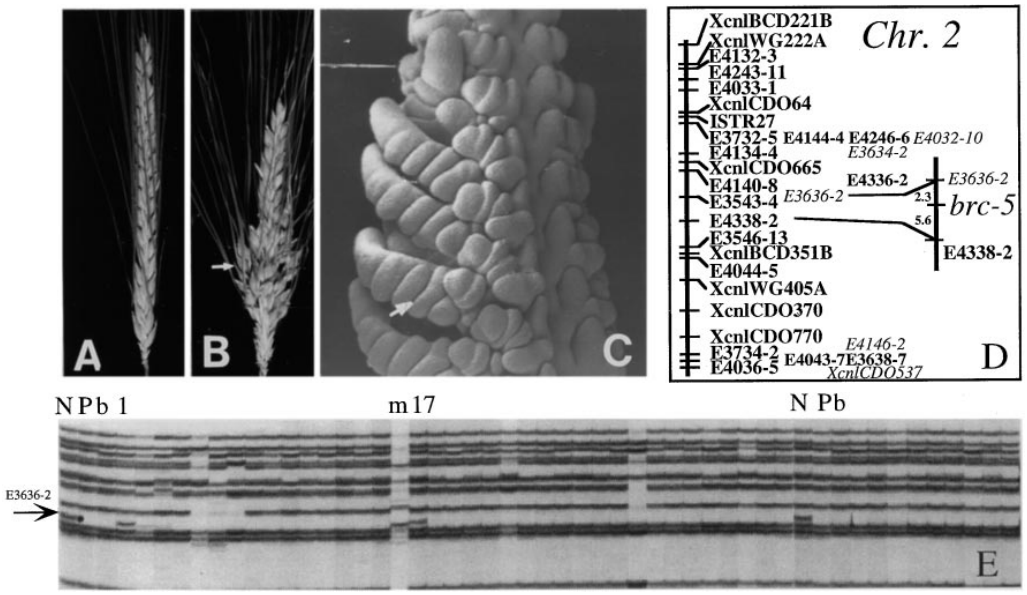

Figure 2.—Assignment of the *brc-5* locus to barley chromosome *2*. (A) Phenotype of WT and (B) *brc-5* plants. (C) Scanning electron microscope (SEM) image of the ear primordia of a *brc-5* plant (1.5 cm = 500 μm). The rachilla (the axis of the spikelet) is elongated, giving the ear a ramified habitus. An ectopic ear is indicated by the arrow. SEM was performed according to Bowman *et al.* (1989). (D) Region of chromosome *2*, subgroup 17, where the *brc-5* locus was mapped. (E) AFLP mapping of the *brc-5* locus. N, Nudinka; P, Proctor; b, *brc-5*; m, missing datum. The other lanes refer to the 45 F_2 M plants from the *brc-5* × Nudinka cross. The AFLP band e3636-2, present in Nudinka and absent in *brc-5*, is present only in the F_2 M plants 1 and 17.

nants), e3732-5 (repulsion; 1 recombinant), e4336-2 (repulsion; 0 recombinants), and e4140-8 (repulsion; 0 recombinants).

Mapping DNA probes on the Nudinka × Proctor AFLP map: The incidence of RFLPs in 67 barley stocks was assayed using genomic and cDNA probes. Genomic probes, in part obtained from A. Graner (Institut für Pflanzengenetik und Kulturpflanzenforschung, Gatersleben, Germany), revealed various levels of polymorphism. Probe MWG58 was polymorphic when tested on *Taq*I or *Alu*I digests. The MWG611-*Alu*I probe-enzyme combination allowed the detection of polymorphisms in 20% of the barley stocks; when the combination *Bkn*3 promoter probe and *Alu*I was tested, 15% of lines were polymorphic. MWG634, tested on *Msp*I- and *Rsa*I-digested DNAs, revealed different allelic states in 6 and 25% of genotypes, respectively. The degree of polymorphism detected was lower when cDNA probes were used, particularly when cDNAs for barley homeobox genes were tested (2.5% of the lines resulted polymorphic for c*Bkn*3 when tested on *Alu*I-digests). In some cases, no polymorphism was observed for these genes, even when using genomic probes. For MADS-box genes and *Adh* cDNAs, the level of polymorphism (between 4 and 20%) was relatively high, similar to the results reported for the hordein genes (Kanazin *et al.* 1993), a barley embryo desiccation-induced gene, and the thiamin gene (Pecchioni *et al.* 1993). The AFLP-based mapping procedure for DNA probes was tested with a homeobox (*Bkn-2*) and a MADS box-containing (*BM-7*) genes.

The *Bkn2* gene contains a homeodomain and codes for a putative transcriptional activator. In Southern analysis with the enzyme *Rsa*I, a 1.5-kb genomic *Pst*I/*Sal*I fragment revealed a polymorphism between the barley line *vulgare hybernum* and Nudinka. The polymorphism consisted of a 320-bp fragment in *vulgare hybernum* that was absent in Nudinka. Sixty F_2 plants were classified on the basis of their RFLP pattern and fingerprinted with the AFLP primer combinations E37M38, E40M38, E42M32, E37M33, E41M34, E42M44, E42M36, E35M46, E40M44, E35M40, E43M43, and E36M36. The primer combination E40M44 amplified a band, e4044-1, linked in coupling to the presence of the 320-bp RFLP fragment. Primers E43M43 and E42M36, which amplify bands linked to e4044-1, were also tested on the same 60 F_2 plants. The *Bkn2* gene was mapped to chromosome *5* on linkage subgroup 47, close to markers e4044-1, e4236-7, e4343-9, and e4343-4. The mapping of *Bkn2* was thus possible by testing 14 AFLP primer combinations, allowing the detection of about 98 polymorphisms (7 per primer combination). A similar approach carried out with RFLP markers would have been much more demanding.

The second probe mapped was the MADS box-containing gene *BM-7*. A cDNA clone of 600 bp revealed RFLP between Nudinka and *v.h.* isthmos (Figure 3A). The analysis of 45 F_2 plants derived from a cross between these two lines revealed three genotypic groups. Group 1 was homozygous for the 450-bp Nudinka fragment, group 2 was homozygous for the 410-bp *v.h.* isthmos fragment, and group 3 was heterozygous (Figure 3B). DNA from each of these F_2 plants was analyzed using the

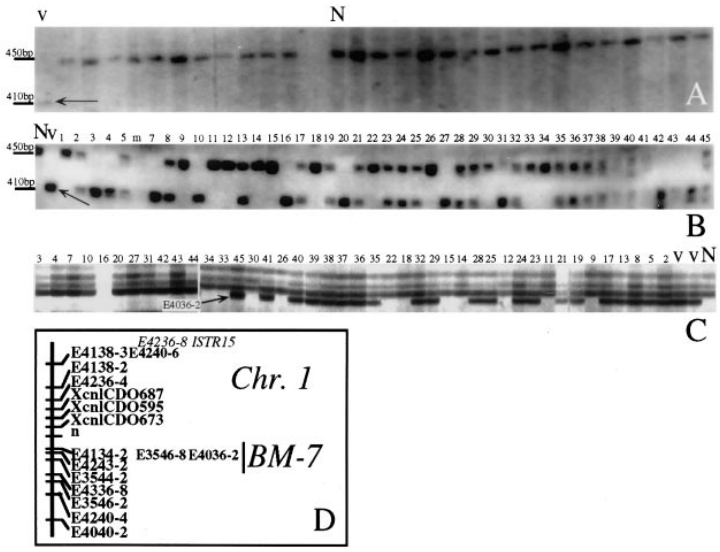

Figure 3.—Assignment of the *BM-7* gene to barley chromosome *1*. (A) Southern analysis of 38 WT barley lines (only some of those listed in Table 1). Only the variety *v.h.* isthmos (v) revealed a polymorphism between Nudinka (N) when *Rsa*I-digested DNAs were hybridized with the *BM-7* probe. (B) Southern blot of F_2 plants from the cross Nudinka × *v.h.* isthmos probed with *BM-7*. v, *v.h.* isthmos; N, Nudinka; m, missing datum. Arrows in A and B indicate *v.h.* isthmos-specific bands. (C) AFLP analysis of the 45 F_2 plants with the primer combination E40M36. Genotype no. 1 was missing in the AFLP analysis. Note that all plants missing the AFLP band e4036 (arrow) are homozygous for the 450-bp RFLP Nudinka fragment, indicating a close linkage between the RFLP and AFLP loci. (D) Chromosome *1* linkage map in the region where the *BM-7* gene was mapped.

AFLP primer combinations E43M38, E36M36, E40M32, E40M40, E40M36, E42M43, E40M38, E35M46, E37M34, and E37M40. The primer combination E40M36 amplified a fragment (e4036-2) missing in the 11 plants homozygous for the 450-bp RFLP fragment of Nudinka (Figure 3, B and C), suggesting a close linkage for the two markers. The data obtained allowed the gene *BM-7* to be placed on chromosome *1* in subgroup 7 (Figure 3D). The gene mapped near *nudum (n)*, a locus mapping approximately 3 cM from the *multiovary* (Tazhin 1980), which is a putative mutant for a MADS box-like gene resulting in transformation of stamens into female organs (Mena *et al.* 1986). The *BM-7* DNA sequence is available at the Web site cited in materials and methods.

DISCUSSION

Knowledge of the precise position of mutant loci on molecular maps can lead to their association with specific genes, when these are also precisely mapped. This approach was followed by Müller *et al.* (1995) to associate the barley-*Hooded* phenotype with a mutation in the homeobox-encoding gene *Knox3*. This strategy requires a dense linkage map. To increase the number of mapped loci, 511 AFLP markers were placed on the Proctor × Nudinka map. As the genetic background of existing barley mutants was different from those of Proctor and Nudinka, F_2 populations from crosses with each of these two parental lines were generated. It was expected that a monomorphic AFLP allele identified in a "mutant × Proctor" cross would have been polymorphic in the "mutant × Nudinka" cross. These F_2 populations were used for AFLP mapping experiments, where linkage of an AFLP fragment to the mutant locus was revealed by significant deviations from the expected Mendelian ratio of 3:1. When the AFLP fragment was present in the wild type (coupling configuration), its presence in 75% of the F_2 plants homozygous for the mutant allele indicated independent segregation, while a frequency of 0% indicated tight linkage. On the other hand, the incidence of F_2 mutant plants having the AFLP marker in repulsion configuration varied from 75% for the absence of linkage to 100% for complete linkage. The estimate of linkage in repulsion was thus less secure than that of the coupling configuration. For this reason, in scoring AFLP markers in F_2 populations, more reliance was placed on bands linked in repulsion to a mutation.

AFLP bands closely linked to a given mutation can be identified in the figure reported at the Web site http://www.mpiz-koeln.mpg.de/salamini/salamini.htm/. Their positions on the linkage map can be found by consulting Table 3. The use of this table allowed the identification of further primer combinations capable of generating other polymorphisms at linked AFLP loci. In the best case of mutant mapping so far encountered, data from a few AFLP gels were sufficient to enable a single experienced scientist to map the mutation *brc-5* on chromosome *2* at a distance of 2.3 cM from each of the nearest flanking markers. This was possible because several AFLP markers were scored in each gel, thus leading to more rapid mapping of mutations than described, for example, in Arabidopsis for the RFLP-based method by Fabri and Schäffner (1994). Several other PCR methods for rapid mutation mapping in Arabidopsis are reported by Williams *et al.* (1993) and Konieczy and Ausubel (1993). However, these methods are only extensions of the bulk segregant analysis procedure described by Michelmore *et al.* (1991). This method is useful to enrich for PCR markers in the vicinity of a given genetic locus but does not assign the locus to a specific linkage group. Our efforts will now concentrate on the production of F_2 populations from crosses with mutant lines not yet listed in Table 2.

The mapping of DNA probes required, in addition to AFLP analysis, an RFLP step. Once an RFLP was found between Proctor or Nudinka and 1 of the 67

barley lines chosen as representative of the genetic variability present within the species, the corresponding F_2 population was selected. F_2 plants were classified according to their allelic state at the RFLP locus and AFLP analysis was carried out on the same materials. The combined RFLP and AFLP data allowed the detection of linkage between the RFLP and AFLP loci, as shown for genes *Bkn2* and *BM-7*.

The method proposed avoids some of the problems encountered when mapping DNA probes to barley chromosomes. Barley has a low degree of DNA polymorphism (Graner *et al.* 1990; Heun *et al.* 1991). Based on Southern data, the RFLP probes of Heun *et al.* (1991), as well as RFLP probes mapped in other crosses, revealed polymorphisms in only a limited number of genetic stocks (Laurie *et al.* 1992; Pecchioni *et al.* 1993). It follows that in crosses involving the lines Proctor and Nudinka, a considerable fraction of the RFLP loci revealed by random probes are monomorphic. Using the RFLP technique, nevertheless, one has a sufficient probability of finding at least 1 polymorphic line among the 67 listed in Table 1. In crosses between this line and Proctor and Nudinka, F_2 plants can be classified genotypically by using the RFLP probe. The AFLP analysis performed on the same F_2 plants exploits the very-high-diversity index of these markers (Russell *et al.* 1997). The combination of the two marker techniques, in conclusion, is capable of overcoming the cited drawbacks. Thus, mapping of almost any DNA probe can be achieved using a single restriction enzyme for Southern analysis.

The second problem that has been encountered concerns map expansion and marker order in dense linkage maps. When 511 AFLP polymorphisms were added to the Heun *et al.* (1991) map, a substantial increase in map length from 1096 to 2673 cM was observed. Typing errors are proposed to be, in part, responsible for map expansion (Lincoln and Lander 1992). Säll and Nilsson (1994) designate as "singletons" those cases of single markers that recombine in a chromosomal region where flanking markers have a parental allelic state. Singletons, in addition to originating from scoring errors, are also the products of double crossover events, which are increasingly detected when maps are enriched with more markers. For barley, a map density-dependent increase in detection of double crossovers contradicts the finding that the number of crossovers estimated from RFLP data of medium-density maps is already significantly higher than the number of chiasmata observed in cytological studies (Nilsson *et al.* 1993; Säll and Nilsson 1994). This suggests that meiotic products, which are assumed to derive from double crossover events, may have a different origin. For example, singletons could be the products of meiotic gene conversion as predicted by the double-strand break repair model of recombination in yeast (Szostak *et al.* 1983), where 50% of products of the resolution of the Holliday junction retain parental flanking sequences. Data from maize (Civardi *et al.* 1994; Xu *et al.* 1995; Okagaki and Weil 1997) and barley (Büschges *et al.* 1997) also support the occurrence of double-strand break repair in plants. Our data demonstrate that, regardless of their origin, singletons increase map length and influence gene order in dense maps. When we used the ERROR DETECTION option of MAPMAKER, a reduction in map length from 2673 to 1597 cM was observed and the marker order within linkage subgroups was also modified. Similar conclusions were drawn from the analysis of the same set of data with the JoinMap program, which also seems to eliminate the products of noncanonical recombination events. In the latter case, the contraction of the map length was even more drastic.

A known phenomenon related to dense linkage maps is the clustering of markers in specific chromosomal regions, as reported for barley (Becker *et al.* 1995; Powell *et al.* 1997; Qi *et al.* 1997), wheat (Hart 1994), tomato (Tanksley *et al.* 1992), rice (Nandi *et al.* 1997), and potato (Van Eck *et al.* 1995). Although no unequivocal explanations for clustering have been found, the suggested hypotheses have considered centromeric suppression of recombination (Tanksley *et al.* 1992; Frary *et al.* 1996), amplification of polymorphic centromeric repetitive sequences (Qi *et al.* 1997), and preferential amplification of the AT-rich region by *Mse*I-based primers, as possible mechanisms (Rouppe van der Voort *et al.* 1997). It is interesting to note that some of the linkage gaps present in the RFLP Proctor × Nudinka map are still devoid of markers after AFLP analysis. As the linkage gaps present in different molecular maps of barley (for references see materials and methods) are in part located in different chromosomal regions, it is tempting to speculate that they may in part correspond to regions of genetic similarity between the chromosomal DNAs of the two strains used to construct a given map. We are currently approaching the problem by developing dense AFLP maps in different mapping populations.

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