On the Origin and Domestication History of Barley (*Hordeum vulgare*)

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Remains of barley (*Hordeum vulgare*) grains found at archaeological sites in the Fertile Crescent indicate that about 10,000 years ago the crop was domesticated there from its wild relative *Hordeum spontaneum.* The domestication history of barley is revisited based on the assumptions that DNA markers effectively measure genetic distances and that wild populations are genetically different and they have not undergone significant change since domestication. The monophyletic nature of barley domestication is demonstrated based on allelic frequencies at 400 AFLP polymorphic loci studied in 317 wild and 57 cultivated lines. The wild populations from Israel-Jordan are molecularly more similar than are any others to the cultivated gene pool. The results provided support for the hypothesis that the Israel-Jordan area is the region in which barley was brought into culture. Moreover, the diagnostic allele I of the homeobox gene *BKn-3,* rarely but almost exclusively found in Israel *H. spontaneum,* is pervasive in western landraces and modern cultivated varieties. In landraces from the Himalayas and India, the *BKn-3* allele IIIa prevails, indicating that an allelic substitution has taken place during the migration of barley from the Near East to South Asia. Thus, the Himalayas can be considered a region of domesticated barley diversification.

Introduction

Barley (*Hordeum vulgare* L.) is one of the founder crops of Old World agriculture. Archaeological remains of barley grains found at various sites in the Fertile Crescent (Zohary and Hopf 1993; Diamond 1998) indicate that the crop was domesticated about 8000 B.C. (B.C. = calibrated dates and b.c. = uncalibrated dates, where calibration refers to normalization of radiocarbon age estimates based on trees' growth rings; Nesbitt and Samuel 1996). The wild relative of the plant is known as *Hordeum spontaneum* C. Koch. In modern taxonomy, *H. vulgare* L. and *H. spontaneum* C. Koch, as well as *Hordeum agriocrithon* Åberg, are considered subspecies of *H. vulgare* (Bothmer and Jacobsen 1985). For reasons given by Nevo (1992), we will follow the traditional nomenclature, which considers separate taxa. *Hordeum spontaneum* and *H. vulgare* are morphologically similar, with the cultivated form having broader leaves, shorter stem and awns, tough ear rachis, a shorter and thicker spike, and larger grains (Zohary 1969). The wild progenitor *H. spontaneum* is still colonizing its primary habitats in the Fertile Crescent from Israel and Jordan to south Turkey, Iraqi Kurdistan, and southwestern Iran (Harlan and Zohary 1966; Nevo 1992). In the same area, *H. spontaneum* also occupies an array of secondary habitats, such as open Mediterranean maquis, abandoned fields, and roadsides. Similar marginal habitats have been more recently colonized by *H. spontaneum* in the Aegean region, southeastern Iran, and central Asia, including Afghanistan and the Himalayan region (Zohary and Hopf 1993). On the map given by Bothmer et al. (1995), for example, *H. spontaneum* is reported in Greece, Egypt, southwestern Asia, and eastward as far as southern Tajikistan and the Himalayas. Indeed, the Himalayas, Ethiopia, and Morocco have occasionally been considered centers of barley domestication (Åberg 1938; Bekele 1983; Molina-Cano et al. 1987).

We revisited the domestication history of barley using the approach which proved successful in locating the site of Einkorn wheat domestication (Heun et al. 1997). The method assumes that (1) DNA markers allow a measure of genetic distances; (2) within a wild species, geographical populations are genetically different; (3) the localities in which wild accessions were collected are known; and (4) the progenitors of crop plants have not undergone significant genetic change during the past 10,000 years (Zohary and Hopf 1993). The last assumption can be verified by a careful morphological analysis to exclude cases of introgression of cultivated germplasm into wild accessions. Our ultimate goal was to determine whether barley was domesticated more than once and to pinpoint the region of barley domestication.

Materials and Methods

Plant Materials

Three hundred sixty-seven *H. spontaneum* accessions were obtained from the International Center for Agricultural Research in the Dry Areas, Aleppo, Syria; the Australian Winter Cereals Collection, Tamworth, New South Wales; the U.S. Department of Agriculture, Aberdeen, Idaho; the Institut für Pflanzengenetik und Kulturpflanzenforschung, Gatersleben, Germany; the Swedish University of Agricultural Sciences, Svalöv; the Institut für Pflanzenbau und Pflanzenzüchtung (FAL), Braunschweig, Germany; and the Aegean Agricultural Research Institute, Izmir, Turkey. The accessions were grown at the Max-Planck-Institut für Züchtungsforschung (MPIZ), Cologne, Germany. Wild lines, together with a set of 20 cultivated genotypes, were characterized with respect to 36 plant, ear, and seed characters. Ten of these were found to have superior capacity to discriminate wild from cultivated lines: PH—plant height (cm); LW—flag leaf width (cm); EL—length of the spike (cm); EW—width of the spike (cm); GL—glume length compared with kernel length (1 = longer; 2 =

Key words: barley domestication, *Hordeum vulgare*, *Hordeum spontaneum*, Fertile Crescent, DNA markers.

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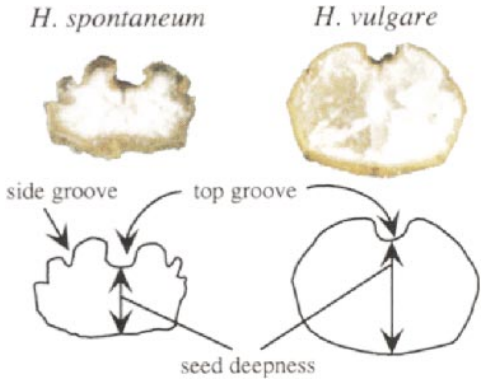

FIG. 1.—Cross sections of seeds from a typical *Hordeum spontaneum* line and from *Hordeum vulgare*. The side grooves were scored with 1 in a typical *H. spontaneum* line like that shown here, with 5 for *H. vulgare* and 2–4 for intermediate cases.

equal; 3 = shorter); AL—awn length (cm); NR—number of ear rows (1 = two; 2 = six); RF—fragility of ear rachis (1 = tough; 2 = fragile); SA—endosperm width (mm); and SB—depth of the lateral seed grooves (1 = *spontaneum* type as in fig. 1; 5 = *vulgare* type; 2–4 = intermediate). The last two characters were evaluated by computer scanning on median sections of seeds. Traits SA and SB were recorded for all 57 *H. vulgare* accessions included in group 14 of table 1. These were selected as representative of the genetic variability present in the cultivated gene pool. A discrimination index capable of correctly assigning the accessions to *H. spontaneum* or *H. vulgare* taxa was developed to be equal to LW + EW + GL + NR + SA + SB/2 − (PH/100 + EL/10 + RF). As in multivariate analysis, the index combines nine characters that, when used alone, already possess relevant discriminating capacities.

The *Hooded* (*K*) barley mutant phenotype is characterized by the formation of an ectopic flower at the lemma-awn interface, and it has been shown to be caused by a mutation in the *Bkn-3* gene, which belongs to the *Knotted-1*-like-homeobox (*Knox*) gene class (Müller et al. 1995).

The study of the geographic distribution of *Bkn-3* alleles was based on 304 of the 317 wild accessions analyzed by AFLP fingerprinting and placed at least to a country. In addition, 5 accessions collected from natural stands in the Himalayan region corresponding to the wild barley *H. agriocrithon* were analyzed, along with an additional 11 *H. spontaneum* lines, all from Israel. The *H. vulgare* lines included the 57 mentioned above, to which another 17 were added. Of these 74 lines, 21 were landraces from the Himalayan-Indian region, 24 were landraces and old varieties from Mediterranean, Balkan, and African locations—including a few from central Asia—and 29 were modern Western varieties. Thirty-five *Hooded* genotypes were examined (two K^e [*Elevated Hood*]). The strains BGS152 (*K*) and BGS153 (K^e) were obtained from the Barley Genetics Cooperative, Tucson, Ariz. The strain *K*-Atlas was obtained from L. Stebbins, University of California at Davis. Other *K* and K^e lines were selected from the collection of 5,842 barley accessions available in the gene bank at Braunschweig, Germany. Lines used in this study are described on the Internet site http://www.mpiz-koeln.mpg.de/salamini/salamini.html.

Genotype Fingerprinting

The AFLP procedure of Zabeau and Vos (1993) was adopted. A total of seven primer combinations were used to amplify *Eco*RI- and *Mse*I-digested DNA. Autoradiographs were scored for presence versus absence of amplified DNA fragments at a total of 400 positions which were polymorphic in the genotypes considered.

DNA Sequencing and PCR Analyses

Figure 2*A* depicts the genomic structure of the *BKn-3* gene, along with regions sequenced and primers used in PCR amplifications. The complete sequence of the K^e allele (allele IIIb) of *BKn-3* is reported in figure 3. The primers used for amplifying parts of the promoter were p32 (AGC TTT GTT AAT GAA GCA GAA TCG) and p33 (TTC GCC TTG GAC ATG AAT ATG), and those used for amplifying parts of intron IV were p4199 (TGA AGA CGA TGA TTC ATG CCA GC) and p2312 (GAA ACT CGT GAT ATC TGT GTC C). PCR reactions were carried out according to standard protocols using *Taq* DNA polymerase (1 U per reaction) and buffer (+$MgSO_4$) and 20 pmol of each primer per reaction. The annealing temperatures ranged from 56°C to 61°C, and barley DNA concentrations varied from 50 to 200 ng per reaction.

PCR products of amplified *BKn-3* regions were purified on Qiagen columns and sequenced using an AB1377 DNA sequencer (Applied Biosystems). Sequences were compared with the aid of the PILEUP program in the GCG software package (Wisconsin Package, version 9.0, Genetics Computer Group, Madison, Wis.).

PCR procedures were used to discriminate between *BKn-3* alleles based on the sizes of amplified products. A nested PCR procedure was used to discriminate between wt (I, II, IIIa) and *K* (IIIb, IIIc) alleles based on primers pA (TTC TTT GTG TGT GTT CTG GGG A), pB (AGG TTT GAA CTT GGA CTC GCC), and pC (GCT TTC CAA GGG AGT TCT GAC). The test revealed the presence, size, and position of the 305-bp duplication in intron IV of *BKn-3*. Primers pA and pB are located 5′ and 3′ of the 305-bp DNA element (fig. 2*A*). The 5′ sequence of pC corresponds to the final 11 bp of the 305-bp element, while its 3′ sequence is identical to the first 10 bp. Products of 649 and 335 bp were expected for allele IIIc (fig. 2*B*). For allele IIIa, a single fragment of 344 bp was amplified. Allele II has a 2-bp insertion, leading to the amplification of a 346-bp fragment. Primers p32 and p33 allowed the identification of lines with allele I. The PCR amplification products derived from this allele were 422 bp long; all other alleles gave rise to products of 441 bp (fig. 2*C*). The primers p32 and pCA (CGC TCC GTT GCA GTT GG) differentiate allele II from alleles I and IIIa. No amplification product is expected for alleles IIIa, IIIb, and IIIc, while alleles I and II generated PCR fragments of 346 and 327 bp, respectively (fig. 2*D*).

Table 1
Mean Values of Morphological Characters Measured for Wild (*Hordeum spontaneum*) and Cultivated (*Hordeum vulgare*) Accessions of Barley

Location	Abbreviation	*N*[a]	PH[b]	LW[c]	EL[d]	EW[e]	GL[f]	AL[g]	NR[h]	SA[i]	SB[j]	RF[k]	Index[l]	Min[m]	Max[m]
Israel-Jordan	I-J	104	1.18	1.31	1.05	0.60	0.47	1.63	1	0.91	0.79	2	0.86	−0.13	1.94
Israel, unknown location	I-u	28	1.20	1.35	1.03	0.60	0.43	1.67	1	0.95	0.82	2	0.92	0.14	2.00
Lebanon–western Syria	L-WS	12	1.19	1.03	1.10	0.62	0.44	1.54	1	0.88	0.83	2	0.52	−0.21	1.60
Turkey near Gaza	T-G	31	1.22	1.15	1.14	0.57	0.43	1.58	1	0.85	1.20	2	0.85	−0.42	1.88
Turkey near Diyarbakir and northern Syria	T-NS	12	1.21	1.23	1.07	0.61	0.44	1.70	1	0.80	0.90	2	0.69	−0.06	1.72
Northern Iraq and western Iraq	Iq-In	22	1.16	1.24	0.98	0.64	0.43	1.43	1	0.92	1.10	2	1.19	0.46	1.98
Iraq, unknown location	Iq-u	11	1.10	1.17	0.93	0.68	0.45	1.45	1	0.82	1.27	2	1.37	0.60	1.85
Southwestern Iran	In	26	1.13	1.28	1.00	0.58	0.43	1.31	1	0.86	0.93	2	0.95	0.14	1.87
Iran, unknown location	In-u	27	1.15	1.28	1.02	0.60	0.41	1.18	1	0.91	0.99	2	1.02	−0.04	2.01
Mediterranean and north Africa	Me	9	1.06	1.04	0.89	0.66	0.50	1.11	1	0.97	0.97	2	1.06	0.51	1.41
Central Asia	Asia	23	1.16	1.20	0.98	0.56	0.40	1.27	1	0.89	1.02	2	0.93	0.08	2.00
Himalayas	Him	4	0.97	1.78	1.08	0.60	0.33	0.15	1	0.91	0.69	2	1.25	1.10	1.47
Unknown location	UK	8	1.18	1.16	1.08	0.60	0.44	1.45	1	0.84	0.72	2	0.50	0.09	1.18
Cultivated	Cult	57	1.10	1.60	0.93	1.02	0.33	1.42	1.7	1.16	2.25	1	5.30	3.95	6.13

[a] Number of lines measured.
[b] Plant height (cm × 10^{-2}).
[c] Flag leaf width (cm).
[d] Spike length (cm × 10^{-1}).
[e] Spike width (cm).
[f] Glume length compared with kernel length (1 = longer; 2 = equal; 3 = shorter).
[g] Awn length (cm × 10^{-1}).
[h] Number of ear rows (2 = six rows; 1 = two rows).
[i] Endosperm width (mm) (see fig. 1).
[j] Depth of endosperm grooves (× 0.5) (see fig. 1).
[k] Fragility of the rachis (1 = tough rachis; 2 = fragile rachis).
[l] The index is defined in *Materials and Methods*.
[m] Min and Max indicate the range of variability of the index.

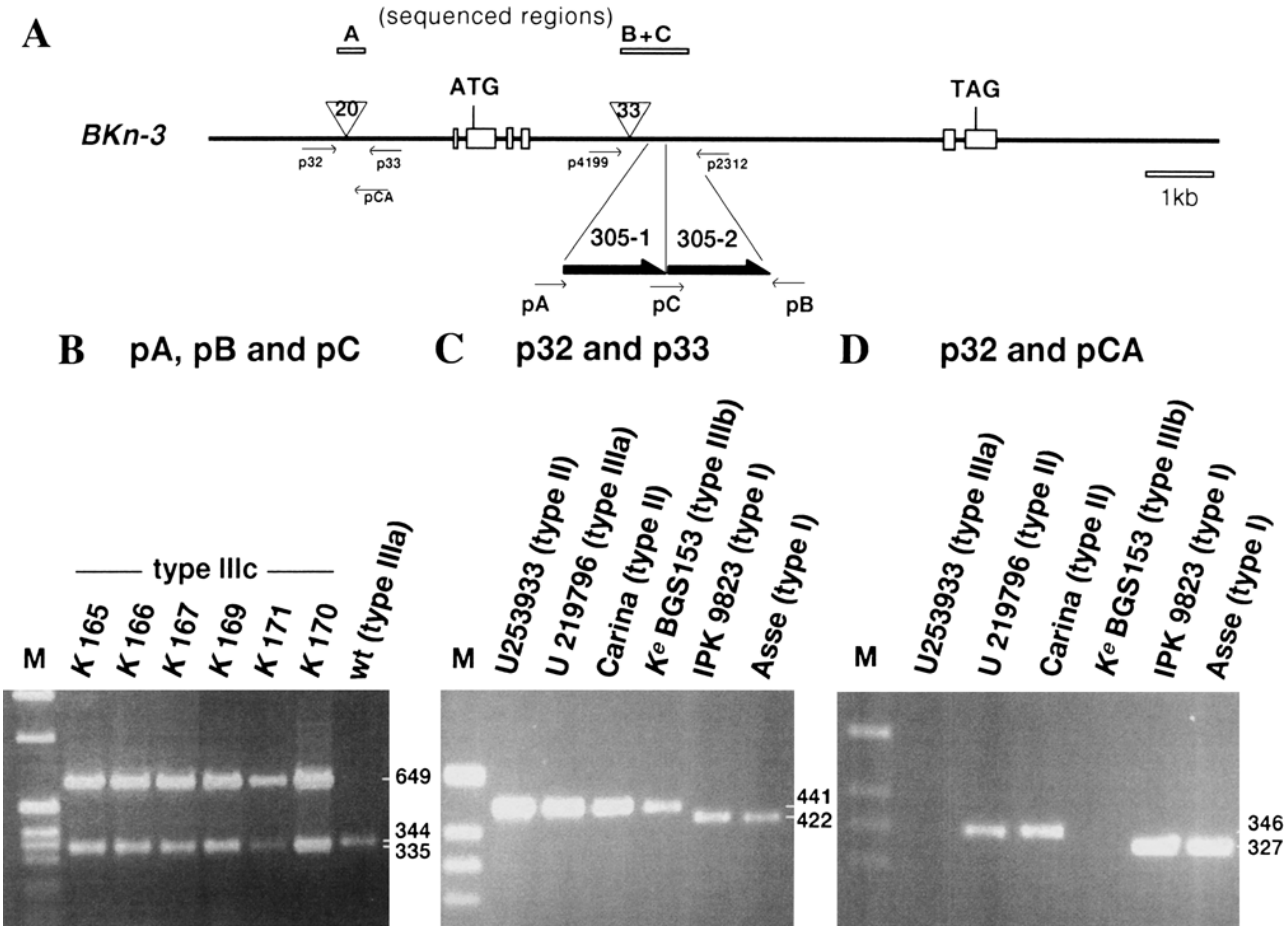

FIG. 2.—*A,* Genomic structure of the *BKn-3* gene with regions sequenced and primers used in this study. Open boxes represent the six exons of the gene. Translational start and stop codons are depicted. Triangles mark major DNA insertion polymorphisms characteristic for certain alleles of *BKn-3* (see also fig. 3 and table 2). The 305-bp duplication and the locations of the primers used in the PCR experiment are also indicated. *B,* Nested PCR analysis using primers pA, pB, and pC. The DNAs were from six *K* (allele IIIc) lines and one wt line. The sizes of the PCR products were 649 and 335 bp for the six *K* lines and 344 bp for the wt line (this line has a type IIIa allele of *BKn-3*; see also table 2). *C,* Results of a PCR experiment carried out with primers p32 and p33. The test was designed to individuate allele I within the *Hordeum spontaneum* accessions. *Hordeum spontaneum* accession IPK 9823 has allele II. The sizes of the PCR products were 422 bp for allele I and 441 bp for the other alleles. *D,* Results of a PCR experiment based on primers p32 and pCA. *Hordeum spontaneum* accessions U253933 and U2219796 have the 20-bp promoter insertion in *BKn-3* (see table 2; they have identical amplification patterns in *C*). The pCA primer allows the amplification of only allele I (327 bp) and allele II (346 bp).

Methods for Phylogenetic Analysis

Molecular fingerprinting generated a database listing the presence or absence of an amplified fragment at each of 400 AFLP loci. The results were used to calculate genetic distances between lines. When groups of lines were compared, frequencies of AFLP bands within each group were used. Genetic distance algorithms used were DICE (Dice 1945), Roger-W (Wright 1978), NEI72 (Nei 1972), the average taxonomic distance DIST, and the Euclidean distance (as in Rohlf 1982). Trees were constructed by neighbor-joining (NJ; Saitou and Nei 1987), FITCH (Fitch and Margoliash 1967), restricted maximum-likelihood (REML; Felsenstein 1981) and unweighted–pair group (UPGMA; Sokal and Michener 1958) methods. All phylogenetic trees, as well as the consensus tree summarizing the relative group positions in 10 different phylogenetic trees (Margush and McMorris 1981), were computed with the PHYLIP program (Felsenstein 1989). The relationship of single *H. spontaneum* line to a consensus *H. vulgare* molecular idiotype was assessed based on AFLP data by calculating the genetic distance DICE (Dice 1945). With DICE, depending on whether fragment alleles exist (+) or not (−) at AFLP loci, two genotypes will be considered similar (++ = a; −− = d) or different (+− or −+ = b and c, respectively). The algorithm for genetic similarity is DICE = 2a/2a + b + c; that is, the component d is excluded as a case of similarity, while a double weight is assigned to a. The consensus idiotype of *H. vulgare* was based on scoring within the *H. vulgare* group of 57 lines, with band frequencies of less than 0.5 scored as 0 and all other frequencies scored as 1.

Results

Morphological Attributes of *H. spontaneum*

Lines of *H. spontaneum* that have been introgressed by cultivated germplasm—i.e., feral forms—will appear to be more closely related than others to the *H. vulgare* gene

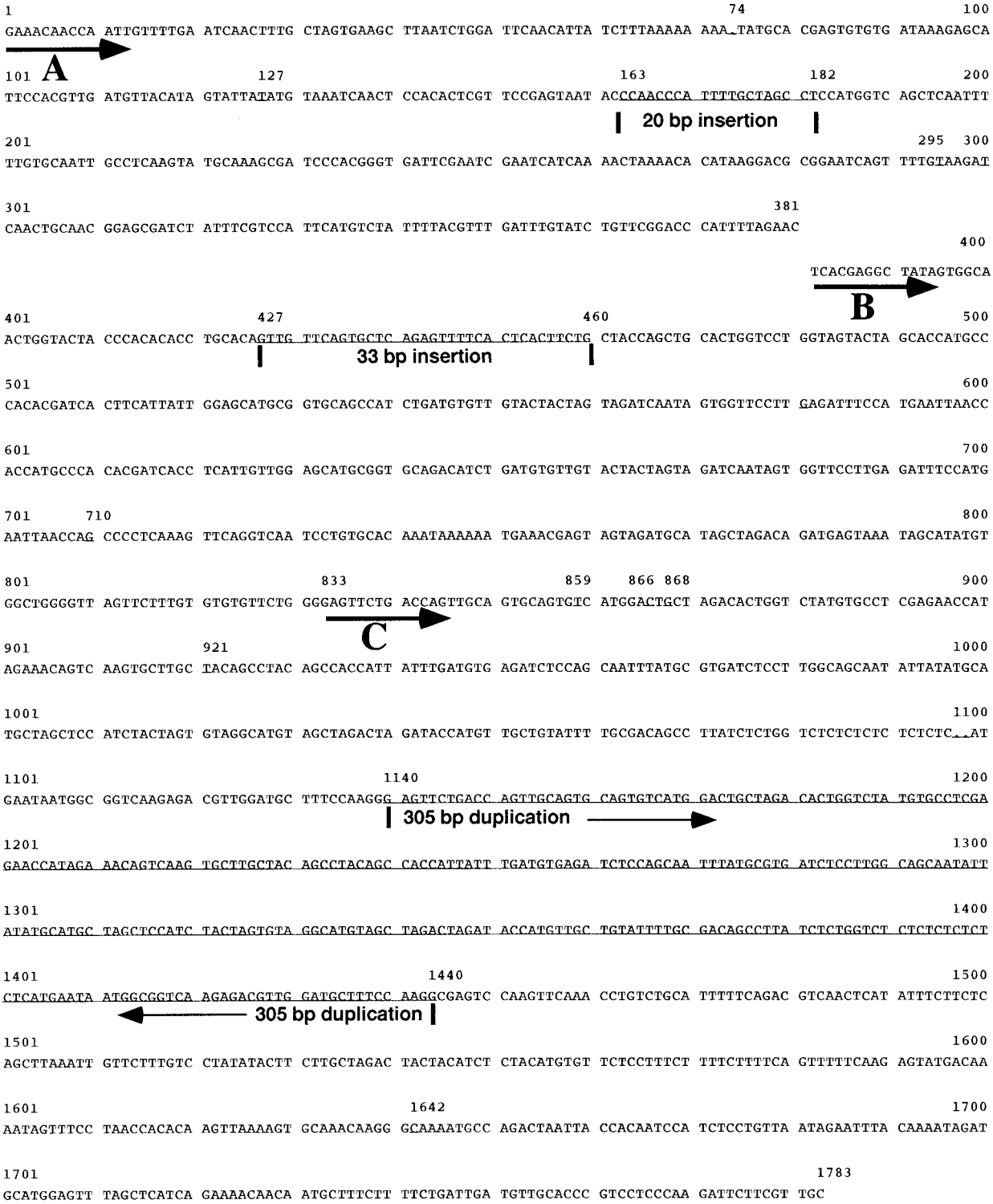

FIG. 3.—Sequence of the *BKn-3 K*^{e} allele in the regions indicated by A, B, and C in figure 2*A*. Major structural polymorphisms between *K*^{e} and other alleles of *BKn-3* are underlined. Differences among alleles are detailed in table 2. The sequence is deposited at EMBL under accession number X83518.

pool when analyzed molecularly and will thus be erroneously considered as putative progenitors of cultivated barley. To avoid this possibility, a careful morphological analysis is necessary. In this study, 50 of the 367 wild accessions were discarded based on the scoring of the morphological traits described in table 1. Of those remaining, 207 were collected from primary habitats at known (within 10 km) sampling points. These locations were in Israel and Jordan (group 1 in table 1; also included were four lines collected in southwestern Syria near the border with Jordan), in Lebanon and western Syria (group 3), in the vicinity of Gaza in Turkey (group 4), in the region of Di-

yarbakir in Turkey and in northern Syria (group 5), in northern Iraq and western Iran (group 6), and in southwestern Iran (group 8). For an additional 66 wild accessions from the Fertile Crescent, only the country of sampling was known (Israel [group 2], Iraq [group 7], or Iran [group 9]). *Hordeum spontaneum* lines from secondary habitats were included in the groups of Mediterranean and North African origin (group 10) and those from Central Asia (group 11) and the Himalayas (group 12). Group 13 consisted of eight wild accessions of unknown origin. Group 14 contained the 57 cultivated lines of *H. vulgare.*

Table 1 presents the morphological characterization of the 14 groups of lines considered. Compared with the wild forms, the plants of the cultivated lines were shorter (PH/100), they had larger flag leaves (LW) and ear widths (EW) and shorter glumes (GL), their ears frequently formed six rows (NR), their seeds were broader (SA), and their endosperm grooves were less pronounced (SB/2). The discrimination index had a mean value of 5.30 for the sample of cultivated lines and varied between −0.42 and 1.10 in the 13 groups of wild accessions. Among single wild accessions, the index reached a maximum value of 2.01, while for the group of 20 *H. vulgare* control lines the minimum value was 3.95. It was concluded that the 317 *H. spontaneum* accessions selected for molecular fingerprinting did not show evident signs of genetic introgression from *H. vulgare*. The only exceptions were the four *H. spontaneum* lines sampled from natural stands in the Himalayan region (group 12): although most of their morphological traits fell within the range of those typical for wild groups, they had ears that virtually lacked awns.

Cultivated Barley is of Monophyletic Origin

Castiglioni et al. (1998) selected from a collection of 5842 accessions 67 *H. vulgare* lines characterized by large differences in ear, grain, and plant characters and in geographic area of cultivation. The lines were either landraces or old cultivated varieties. Of those lines, 57 were considered in this study. They were cultivated in the Himalayan region (3), in India, Yemen, and Pakistan (5), in Afghanistan, Turkestan, and central Asia (6), in the Mediterranean area (7), in Ethiopia and central Africa (8), in the Balkans (4), in southern Europe (4), in central Europe (8), in northern Europe (5), and in America and Australia (7). The AFLP fingerprinting data for the 57 cultivated lines and the 317 wild accessions were subjected to phylogenetic analyses using different procedures. One tree is reported in figure 4*A*; the 57 cultivated genotypes (red) cluster together, excluding all of the 317 wild accessions (blue). All other trees based on different algorithms gave similar results. This finding allows us to consider the cultivated gene pool as a single taxonomic entity.

Relationships Between Wild and Cultivated Gene Pools

The relationships between groups of wild barley and the cultivated gene pool were assessed based on AFLP allele frequencies calculated for each group of lines. The phylogenetic tree in figure 4*B* illustrates the position of the cultivated gene pool with respect to six groups of wild lines sampled from precisely known locations within the Fertile Crescent. The wild accessions that are most closely related, as a group, to *H. vulgare* were sampled in Israel and Jordan. The genetic distances between cultivated and wild groups increase from the southern parts to the northern Fertile Crescent. The lines sampled in southern Iran were, in fact, also closer to cultivated genotypes than were the lines sampled in more northerly locations. Figure 4*C* shows the result of a similar analysis considering three additional groups of lines also sampled in the Fertile Crescent. Two groups of lines from the Israel and Jordan area—one group sampled at precisely known locations and the second from less well defined areas—map together, both showing the closest relationship with the *H. vulgare* gene pool. The two Iranian groups also map together, as do the two from Iraq. In figure 4*D*, wild *H. spontaneum* lines from secondary habitats were added to the analysis of figure 4*B*. Lines sampled in Himalayan and Mediterranean locations appeared to be closely related to *H. vulgare*, while wild lines from central Asia were genetically closer to *H. spontaneum* genotypes from the eastern part of the Fertile Crescent. The consensus tree in figure 4*E* indicates that the relationships described in figure 4*B–D* remained almost unchanged when studied with different methods.

A further analysis aimed to localize, within the Israel-Jordan area, sites at which the wild lines are more closely related to the cultivated gene pool. First, the genetic distance between *H. vulgare* and each of the 317 lines of *H. spontaneum* was calculated based on the genetic distance DICE (Dice 1945). In this process, the AFLP data for each line were used and compared with a consensus molecular idiotype of *H. vulgare.* Genetic distances varied between 0.219 and 0.608. An arbitrary group of 45 wild *H. spontaneum* accessions that appeared to be more closely related to cultivated barley (distance values of between 0.219 and 0.300) was selected. These included, as expected (see *Discussion*), 9 lines sampled from secondary habitats—4 in the Himalayan region and 5 in Mediterranean locations (1 in Cyrenaica and 4 in Cyprus)—and 34 accessions from the Fertile Crescent. Of the latter, 2 were from Turkey-Northern Syria, 5 were from Iran and 1 was from an unknown sampling point. Of the remaining 26 wild *H. spontaneum* genotypes from Israel-Jordan, 20 were collected from the sites reported in figure 5*A*. These 20 lines did not originate from a single geographical area; they belong to loose southern (around 33°40′N, 35°15′W) and northern (around 31°45′N, 35°W) clusters.

The relationships between the accessions of the two Israel-Jordan clusters, the five Iranian lines, and the groups of lines sampled in secondary habitats of the Mediterranean and Himalayan regions were also studied with a phylogenetic analysis carried out with AFLP data from single accessions (fig. 4*F*). The tree obtained shows (1) that the Iranian and Israeli lines belong to two separate wild gene pools; (2) that the northern and southern Israeli clusters tend to remain separate in this analysis; and (3) that the lines from secondary habitats in the Mediterranean are related to the Israeli lines, while the Himalayan accessions are genetically related to the Iranian group of wild lines.

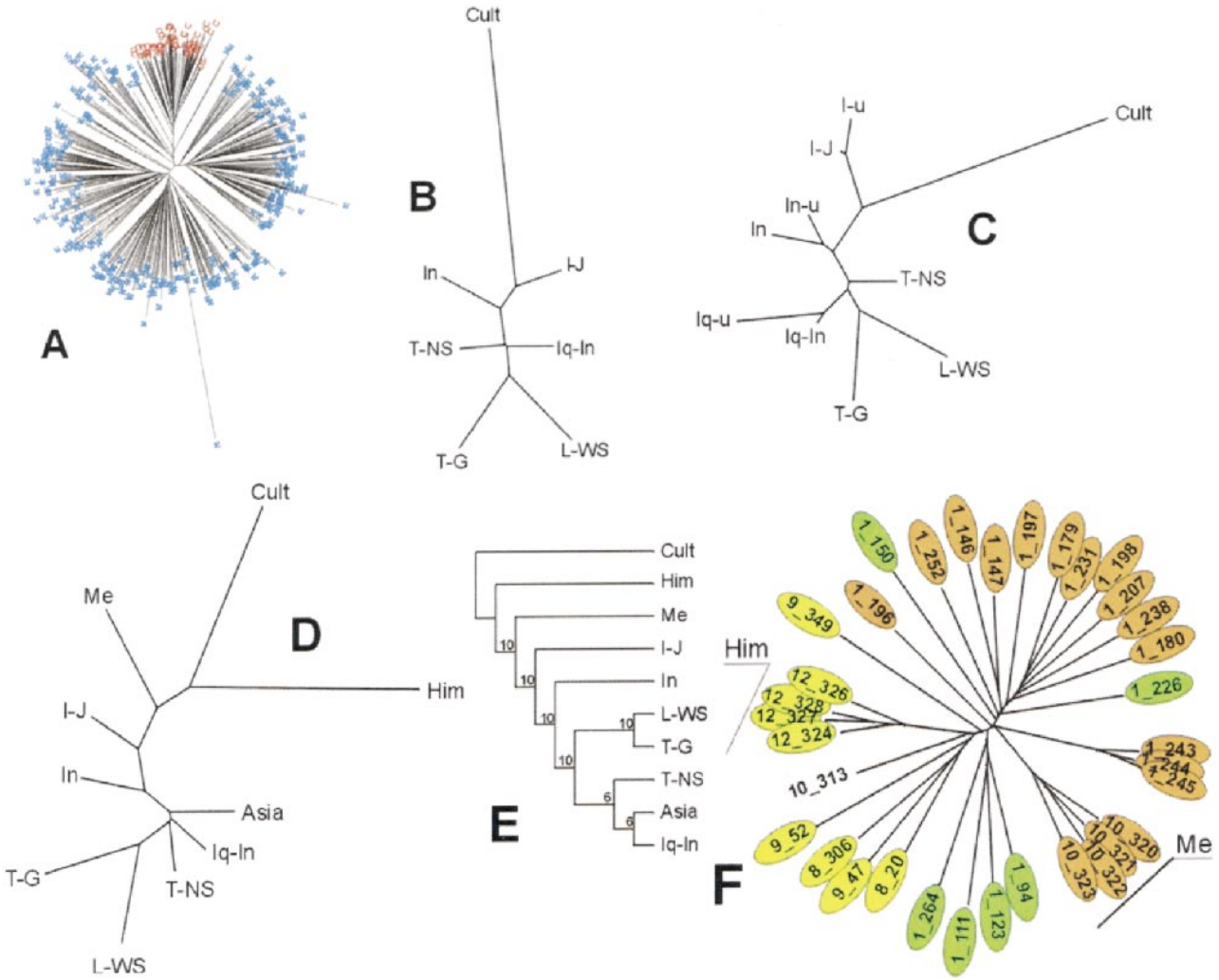

FIG. 4.—*A,* Unrooted polygenetic tree reporting AFLP-based genetic relationships among 317 *Hordeum spontaneum* accessions (blue) and 57 cultivated lines of *Hordeum vulgare* (red). The DICE genetic distances as computed by SAS (SAS Institute Inc. 1989) served as the input for the neighbor-joining tree-building method implemented in the PHYLIP package (see *Materials and Methods*). *B–D,* Unrooted phylogenetic trees derived from AFLP band frequencies in groups of wild and cultivated barley accessions. Groups of wild lines considered in *B* were from six primary habitats in the Fertile Crescent, those in *C* were from nine primary habitats, and those in *D* were from six primary and three secondary habitats. Tree building was based on the neighbor-joining method and NEI72 genetic distances. *Hordeum spontaneum* lines sampled from known locations in the Fertile Crescent are included in groups I-J (from Israel and Jordan; 104 lines), L-WS (Lebanon and western Syria; 12 lines), T-G (Turkey around Gaza; 31 lines), T-NS (Turkey around Diyarbakir and northern Syria; 12 lines), Iq-In (northern Iraq and western Iran; 22 lines), and In (southwestern Iran; 26 lines). Groups I-u (28 lines), Iq-u (11), and In-u (27) include *H. spontaneum* genotypes sampled in unknown locations in Israel, Iraq, and Iran, respectively. Groups Me (9 lines), Asia (23 lines), and Him (4 lines) consist of *H. spontaneum* accessions sampled in secondary habitats located in the Mediterranean–north Africa region, the Himalayas and central Asia, respectively. Group Cult includes 57 lines of *H. vulgare. E,* Consensus tree summarizing the relative positions of accession groups in 10 different phylogenetic trees constructed by various combinations of tree-building methods and genetic-distance algorithms. AFLP band frequencies were used as the basis for tree building. A number at a fork is the number of times that the assemblage consisting of the groups to the right of that fork occurred among the 10 trees considered. *F,* Unrooted tree based on AFLP data, showing the phylogenetic relationships between lines selected from among the 45 *H. spontaneum* accessions that were more closely related to *H. vulgare.* Twenty lines were from known locations in Israel-Jordan (group 1: green, north cluster; brown, South cluster). The yellow lines (group 9) were from Iran. The group 10 lines were from Cyprus (Me; brown). Line 10-313 (uncolored) was from Cyrenaica. The four lines of group 12 (Him; yellow) were from the Himalayan region.

The Himalayan Region as a Center of Cultivated Barley Diversification

The AFLP data indicate the Israel-Jordan area as a possible site of barley domestication (see *Discussion*). In our AFLP experiments, cultivated landraces were included from other putative centers of barley domestication, such as the Himalayas, Ethiopia, and Morocco. These genotypes unequivocally had a common monophyletic origin with the other cultivated lines analyzed. This excludes the possibility that the regions mentioned were centers of domestication, although they may have been centers of diversification of cultivated barley. This is particularly true for the Himalayan region, where not only local landraces, but also two- and six-rowed wild forms (*H. spontaneum* and *H. agriochriton,* respectively) have been sampled.

Müller et al. (1995) reported that the barley mutant Hooded (*K*), a cultivated form introduced to Western

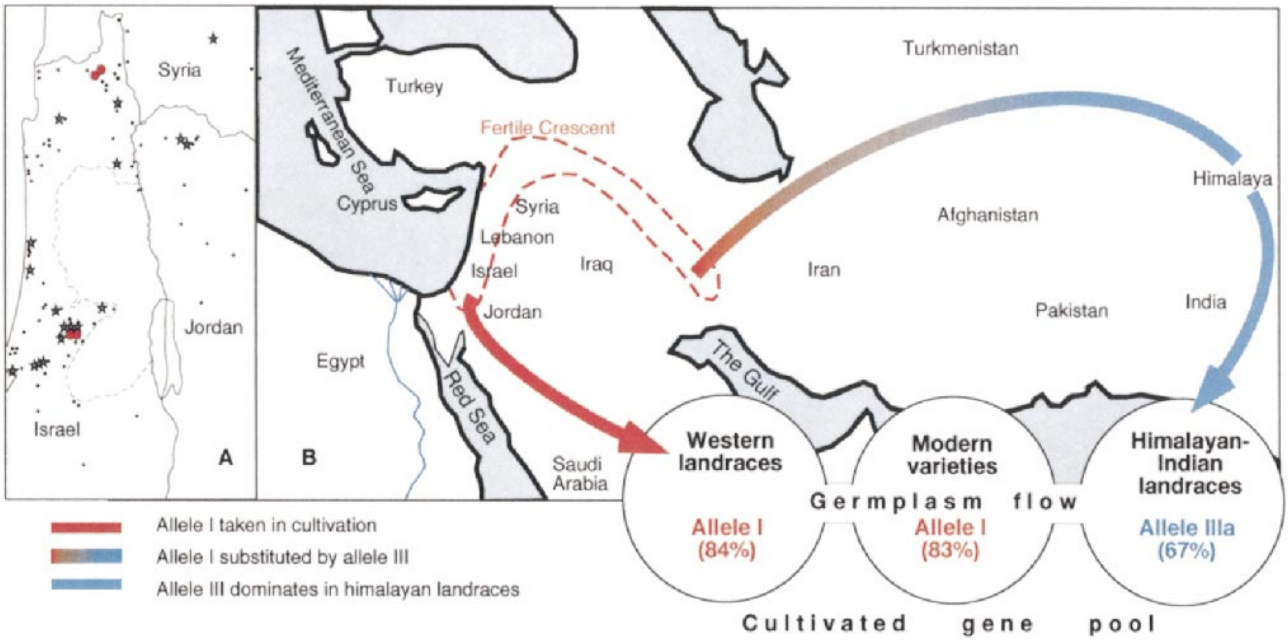

FIG. 5.—*A*, Sampling sites of 104 *Hordeum spontaneum* lines collected in Israel and Jordan and near the Jordan-Syria border. Asterisks indicate lines with genetic distances to the cultivated gene pool between 0.219 and 0.300 DICE units (see *Materials and Methods*). Red dots indicate sites of collection of *H. spontaneum* lines with *BKn-3* allele I. *B*, Flow of alleles of the *BKn-3* gene from wild *H. spontaneum* populations to cultivated germplasm. The borders of primary habitats of *H. spontaneum* (according to Harlan and Zohary 1966) are represented by the dotted red line and correspond to the Fertile Crescent.

countries about 200 years ago from the Himalayan region (Harlan 1931), has a mutation in the homeobox gene *BKn-3*. Three alleles of the gene were characterized molecularly at the time: wt, *K*, and K^e. The *K* allele has a 305-bp duplication in intron IV (region C in fig. 2*A*). The allele K^e has a 33-bp insertion at positions 427–460 in intron IV (region B in fig. 2*A*), as well as the duplication of 305 bp. We used the *BKn-3* alleles as diagnostic markers to follow the flow of germplasm from wild to cultivated lines. The molecular analysis was also extended to a part of the *BKn-3* promoter (region A, positions 1–380 in fig. 2*A*).

Regions A, B, and C were sequenced for 12 cultivated barley lines (8 modern Western varieties and 4 lines from the Himalayan region), for 2 *H. spontaneum* accessions, for a single *H. agriocrithon* accession from the Himalayas, for 18 *K* lines, and for 2 K^e lines. The three known *BKn-3* alleles were detected, as were two new ones (table 2). The two K^e lines had allele IIIb, with the 305-bp duplication in region C. *BKn-3* alleles

Table 2
Polymorphisms Observed Between *Bkn-3* Alleles from Various Sources

	Insertions in Promoter					Insertions in Intron IV of *Bkn-3*											
	Position No. in Region A[b]					Position No. in Region B[c]				Position No. in Region C[d]							
DNA Source[a]	74	127	163[e]	295	300	427[f]	460	581	710	859	866	868	921	1097[g]	1140[h]	1642	Allele Type
Hordeum spontaneum line 369	T	C	−	C	C	+	G	G	A	G	C	G	C	−	−	T	I
Hordeum vulgare Europe	T	C	−	C	C	+	G	G	A	G	C	G	C	−	−	T	I
H. vulgare Carina	−	T	+	T	C	+	G	−	A	T	T	C	T	+	−	C	II
H. spontaneum line 382	−	T	+	T	T	+	G	G	G	T	C	G	T	−	−	C	IIIa
H. vulgare Himalayas	−	T	+	T	T	+	G	G	G	T	C	G	T	−	−	C	IIIa
H. vulgare K^e	−	T	+	T	T	+	G	G	G	T	C	G	T	−	+	C	IIIb
H. vulgare K	−	T	+	T	T	−	C	G	G	T	C	G	T	−	+	C	IIIc

[a] Description and origin of *H. spontaneum* lines available from http://www.mpiz-koeln.mpg/salamini/salamini.html.
[b] Corresponds to positions 1–380 in figure 3.
[c] Corresponds to positions 381–832 in figure 3.
[d] Corresponds to positions 883–1783 in figure 3.
[e] Indicates presence (+) or absence (−) of a 33-bp insertion at positions 427–459.
[f] Indicates presence (+) or absence (−) of a 20-bp insertion at positions 163–182.
[g] Indicates presence (+) or absence (−) of a 2-bp insertion (TC) at positions 1097–1098.
[h] Indicates presence (+) or absence (−) of a 305-bp insertion at positions 1140–1444.

Table 3
Frequency of *Bkn-3* Alleles in 320 Lines of *Hordeum spontaneum* Collected in Primary and Secondary Habitats of the Species and in 109 Cultivated *Hordeum vulgare* Strains

Germplasm and Groups[a]	*N*[b]	Frequency of *Bkn-3* Alleles[c]				
		I	II	IIIa	IIIb	IIIc
Wild primary habitats						
I-J + Iu	143	4.2	74.2	21.6	0	0
L-WS	12	0	75.0	25.0	0	0
T-G	28	3.5	71.5	25.0	0	0
T-NS	12	0	66.7	33.3	0	0
Iq-In + Iq-u	33	0	33.3	66.7	0	0
In + In-u	51	0	45.1	54.9	0	0
Wild secondary habitats						
Me	9	0	66.7	33.3	0	0
Asia	23	0	34.8	65.2	0	0
Him	9	0	22.2	77.8	0	0
Cultivated						
Western landraces	24	83.3	16.7	0	0	0
Modern varieties	29	82.8	10.3	6.9	0	0
Himalayan-Indian landraces	21	19.0	14.3	66.7	0	0
K^e	2	0	0	0	100	0
K	33	0	0	0	0	100

[a] Group abbreviations as in table 1.
[b] Number of lines tested.
[c] Expressed as a proportion of all alleles found.

from *K* genotypes were similar to the K^e allele but with a deletion of 33 bp in region B (allele IIIc). A line of *H. spontaneum* and one *H. agriocrithon* accession from the Himalayan region had allele IIIa. The cultivated Himalayan landraces also had *BKn-3* allele IIIa, which can thus be considered the progenitor sequence of K^e and *K*, from which it differs by the absence of the 305-bp duplication. A line of *H. spontaneum* from Israel had the same allele as the European barley varieties. This allele (allele I) has a deletion of 20 bp at positions 163–182 in region A of the K^e sequence and other differences scattered along its DNA sequence. Allele II was found first in the European *H. vulgare* variety Carina. Its sequence is devoid of the 305-bp duplication and different in several respects from alleles I, IIIa, IIIb, and IIIc (table 2).

Allelic assignment to 320 wild (*H. spontaneum* and *H. agriocrithon*) and 109 cultivated *Hordeum* genotypes—originating from several countries or geographical areas—was completed based on the PCR amplifications described in *Materials and Methods*. All wt genotypes had alleles that lacked the 305-bp duplication. K^e and *K* lines had, as expected, alleles IIIb and IIIc, respectively. In all of the *Hooded* lines considered, the 305-bp duplication starts and ends at the same positions. Other PCR amplifications discriminated between wt genotypes that carried alleles I, II, and IIIa.

Table 3 and figure 5*B* summarize all PCR data concerning *BKn-3* allele assignment. In the wild species, the prevailing alleles were II and IIIa. The frequencies of these alleles varied from east to west and from north to south: allele IIIa prevailed in the northeastern part of the Fertile Crescent, while II dominated in southwestern locations. Allele I was rare in wild species and present only in *H. spontaneum*. The seven instances found were in lines from Israel (6) and Turkey (1). Of the six Israeli-Jordan lines, four were sampled at precise locations (fig. 5*A*).

Among cultivated lines, allele IIIa largely prevailed in the Himalayan region, while allele I was largely dominant among landraces from Europe, Africa, and western Asia. It also prevailed in modern *H. vulgare* cultivars. In these cultivars, the finding of *BKn-3* alleles different from I is easily explained by the use of *H. spontaneum* in barley breeding as a donor of disease resistance genes (Nevo 1992). Allele IIIb was characteristic for the K^e strains, and IIIc was typical for the *K* genotypes. In summary, allele I, found almost exclusively (but rarely) in the Israel-Jordan region, characterized the wild progenitor which generated, monophyletically, the cultivated Western gene pool of today. In the cultivated barleys of the Himalayas, allele I was replaced by allele IIIa. Allele IIIa in the Himalayan region gave rise to the mutant forms corresponding to genotypes K^e and *K*.

Discussion

Figure 4*A* closes the long-lasting debate on the origin of barley: landraces from Ethiopia, Mediterranean regions, and the Himalayas form a single taxonomic entity with other cultivated lines. This entity branches off at a precise and unique point from a phylogenetic tree of 317 wild lines. The possible origin of cultivated Moroccan (Molina-Cano et al. 1987) and Ethiopian (Bekele 1983) forms from *H. spontaneum* had already been rendered less likely by the finding that Moroccan wild populations of *H. spontaneum* are of hybrid origin (Giles and Leftkovitch 1984, 1985) and, for Ethiopia, by the possible mutational origin of the flavonoid variants found there (Fröst, Holm, and Asker 1975). The Hi-

malayan region, in contrast, has stimulated much more discussion (e.g., Zohary 1959; Staudt 1961).

The finding of wild forms with six-rowed ears and brittle rachises (*H. agriocrithon*) in wild stands (Åberg 1940) was previously viewed as support for the hypothesis that this region was a barley domestication center. This hypothesis was later rejected because the six-row strains were explained as feral forms contaminating cereal varieties (Zohary 1959; Staudt 1961; Takahashi 1964). More recent data, however, indicate that true wild *H. spontaneum* is present in Tibet, Nepal, India, Pakistan, and Afghanistan (Yang and Yen 1985; Shao 1987; Corke and Atsmon 1990). These new findings were the basis for Xu (1987) to conclude that the six-row naked forms of cultivated Chinese barleys may derive from genotypes domesticated in Tibet, starting from a wild species. Our study indicates that the *BKn-3* alleles IIIb and IIIc, which were already present in cultivated landraces at the time of their discovery (Harlan 1931), originated from the wt allele IIIa. This allele differs in several parts of its sequence (table 2) from those present in Western *H. vulgare* varieties, while it is predominant in the cultivated landraces in the Himalayas. These data forced us to reconsider the Himalayas as a possible center of barley domestication.

The AFLP data now strongly support the monophyletic domestication of barley. A solution to the apparent discrepancy between the two sets of data is provided by the diffusion of *BKn-3* allele IIIa in *H. spontaneum* populations. This allele is, in fact, predominant in the eastern part of the Fertile Crescent and in related secondary habitats of the wild species. Apparently, during its migration from the western Fertile Crescent to central Asia, cultivated barley was introgressed by wild Eastern germplasm, generating the cultivated—frequently naked—varieties of Tibet and surrounding countries. A second product of these hybridizations was, most probably, the appearance in Himalayan wild stands of *H. agriochriton,* a feral form of clear hybrid origin (Zohary 1969). Very many data support the occurrence of natural crosses between wild and cultivated *Hordeum* taxa (Zohary 1960; Kobyljanskij 1967; Kamm 1977; Brown et al. 1978; Allard 1988).

A second aim of this study was to pinpoint the site at which barley may have been domesticated in the Fertile Crescent, according to the procedure successfully used for einkorn (Heun et al. 1997). This method makes use of AFLPs, markers which have been shown to be useful in the evaluation of the genetic variability present in wild and cultivated barleys (Pakniyet et al. 1997; Schut, Qi, and Stam 1997; Castiglioni et al. 1998). The assumption that genetic diversity exists in the gene pool of *H. spontaneum* and that it is at least in part dependent on the geographic origins of the wild populations considered has also been demonstrated (Nevo et al. 1979, 1986*a*, 1986*b*; Snow and Brody 1984; Jana et al. 1987; Chalmers et al. 1992; Dawson et al. 1993). The assignment of a wild state to all the collected accessions of *H. spontaneum* proved to be more difficult. Unlike einkorn, barley has a long history of cultivation in the Fertile Crescent. Some races of wild barley, moreover, have a strong tendency to behave as weeds. Helbaek (1959) wrote, ''I never saw a field of any crop in Kurdistan in which wild barley was not to be found growing as a weed.'' Thus, the possibility that molecular markers from the cultivated gene pool were introgressed, via natural crosses, into *H. spontaneum* populations is very real.

Indeed, based on morphological analyses, 50 wild lines were discarded from our sample because they showed signs of introgression by *H. vulgare.* Of the remaining 317, two groups of lines, sampled in secondary habitats in Mediterranean countries and in the Himalayas, were closely related molecularly to the cultivated gene pool (fig. 4*C*). However, the locations at which these lines were collected must be excluded as sites of barley domestication for the following reasons: (1) The Himalayan lines reveal a hybrid origin with respect to at least one character—awn length. (2) Some of the Aegean accessions can be considered feral forms or even wild barleys which may have, in the sixth and fifth millennia b.c. (Zohary and Hopf 1993), followed the migration of cultivated landraces of cereals during their spread from the Near East to the Aegean region. Being present as weeds in cultivated fields, they may have exchanged genetic materials with *H. vulgare.* (3) As shown by archeological data, two-rowed forms of *H. spontaneum* with brittle rachises, apparently collected from the wild (Zohary and Hopf 1993), were already being harvested by humans in the Fertile Crescent prior to the appearance of agriculture. Such forms have been found at Ohalo II (Sea of Galilee; 17000 b.c.), Tell Abu Hureyra (North Syria; 9000 b.c.), Mureybit (North Syria; 8000 b.c.), and Tell Aswad (east of Damascus; 7800 b.c.). (4) Unmistakable remains of nonbrittle barley, i.e., cultivated forms, date from Tell Abu Ureyra (7500 b.c.; Hillman 1975), Tell Aswad (6900 b.c.; van Zeist and Bakker-Heeres 1985) and Jarmo, Iraq (7000 b.c.; Helbaek 1959). All of these sites, like those at which six-row cultivated types have been discovered (Tell Abu Hureyra, Ali Kosh, Tell-es-Sawwan, Catal Hüyük, Hacilar; Zohary and Hopf 1993), are in the Fertile Crescent. The archeological data allow us to conclude that the Fertile Crescent is the place of origin of cultivated barley, as indicated by the fixation of nonbrittle mutations and, subsequently, by the emergence of the six-rowed, hulled, and naked types. But the question remains: where exactly in this area did domestication take place?

The data provided here direct us to the southern parts of the Fertile Crescent, specifically to the Israel-Jordan area; *BKn-3* allele I, typical of the cultivated Western landraces, was found in only seven *H. spontaneum* lines, and six of these were sampled in Israel, four at known locations. In the Israel-Jordan area, moreover, the frequencies of molecular alleles at 400 AFLP loci are most similar to those of the cultivated gene pool. The analysis of the 45 wild lines that are more tightly related to *H. vulgare* based on AFLP frequencies shows that 26 were sampled in the Israel-Jordan area. However, in contrast to the case for einkorn wheat (Heun et al. 1997), they do not originate from a single restricted geo-

graphic district. Two loose clusters nevertheless emerge, as reported in figure 3*A*.

Similar phenotypes occur in samples of *H. spontaneum* from southwestern Iran (Nevo et al. 1986*a*). Moreover, out of the 45 *H. spontaneum* lines found to be more related to *H. vulgare,* 5 were collected in southwestern Iran. In this area, however, *BKn-3* allele I was not found. Moreover, the five Iranian *H. spontaneum* types that are more related to *H. vulgare* are phylogenetically separated from the 20 Israeli-Jordan genotypes (fig. 4*F*). Thus, they should not have contributed to early barley domestication in the western Fertile Crescent for geographical and genetic reasons. A likely interpretation of their role in cultivated barley diversification based on their genetic similarity to introgressed forms of Himalayan *H. spontaneum* (yellow in fig. 4*F*) assumes that (1) some wild Iranian forms donated, via natural crosses, their *BKn-3* allele IIIa to the cultivated Himalayan forms, and (2) in the process, they contributed other molecular marker alleles to cultivated Himalayan varieties, thus maintaining some genetic links with the cultivated gene pool of the Himalayas.

In conclusion, our suggestion is that the Israel-Jordan area in the southern part of the Fertile Crescent has the highest probability of being the geographical area within which wild barley was domesticated. Wild populations found in the southern part of the Fertile Crescent in western Iran have also contributed germplasm to the cultivated barley on its way to the Himalayas. In the present state of our research, it is possible to pinpoint only with loose precision two geographic areas within the Israel-Jordan region in which the first domestication of barley may have taken place.

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