Phytochromes belong to a family of photoreceptors that perceive, interpret, and translate light signals that effect plant growth and development. The objectives of this study were to use a heterologous phytochrome cDNA probe from oat to (1) identify phytochrome loci in barley, (2) determine the chromosomal and genomic location of phytochrome gene(s) in barley, and (3) assess the extent of genetic variation at phytochrome loci in cultivated barley (*Hordeum vulgare*). Restriction fragment length polymorphism (RFLP) analysis detected four distinct phytochrome loci in barley, arbitrarily designated *phyl*, *phy2*, *phy3*, and *phy4*. Wheat-barley addition line analysis indicated that *phyl*, *phy2*, and *phy3* are located on barley chromosomes 7, 4, and 5, respectively, while *phy4* represents duplicate loci located on barley chromosomes 2 and 7. Linkage mapping confirmed our results using wheat-barley addition line analysis and located the *phyl* and *phy3* loci to the short arm of barley chromosome 7 and to the centromeric region of barley chromosome 5, respectively. Two to three alleles were detected at each of the five phytochrome loci examined. Genetic diversity values for the phytochrome loci were near previous estimates for RFLPs. Division of the barley samples based on spike morphology indicated that the level of diversity in two- and six-rowed barley is about equal, although significant differences in allelic frequencies were detected between the subgroups.

Light is a critical environmental factor influencing plant growth and development. Light provides the radiant energy necessary for photosynthesis and is the primary stimulus for many changes in plant growth patterns and developmental events, such as leaf primordia formation, plastid development, shade avoidance, and flowering induction (Sage 1992). Perception and interpretation of light stimuli are modulated by several endogenous photoreceptors, including phytochromes (red and far-red light receptors), blue light receptors, ultraviolet-A receptor(s), and ultraviolet-B receptor(s). Of the different photoreceptor systems, the phytochromes are the best characterized (Furuya 1993; Quill et al. 1995).

Phytochromes are cytosolically localized dimers composed of two polypeptides, each containing a covalently attached tetrapyrrole chromophore. The photosensory capacity of the molecule is derived from its ability to exist in two known forms that are reversibly interconvertible by red and far-red light: the red-absorbing (Pr) form, and the far-red absorbing (Pfr) form. Photointerconvertibility of the two forms functions as a switch that regulates gene expression (Quill 1991). The exact molecular nature by which phytochromes regulate signal pathways and gene expression is not known. However, photoregulation by phytochromes has been implicated in the expression of a number of nuclear genes, including the small subunit of ribulosebisphosphate carboxylase and chlorophyll a/b binding protein (Silverthorne and Tobin 1984), protoclorophyllide reductase (Apel 1981), ribosomal RNA (Thien and Schopfer 1982), and several unidentified mRNAs (Thompson et al. 1983).

Multiple phytochrome genes have been identified, sequenced, and compared for several plant species using recombinant DNA technology [reviewed by Quill (1991) and Quill et al. (1995)]. Complete phytochrome amino acid sequences have been isolated from a variety of plant species, including oat, zucchini, pea, rice, and corn. Recently, Clack et al. (1994) and Sharrock and Quill (1989) reported the isolation of five structurally distinct but related phytochrome genes from *Arabidopsis*, which they designated as *PhyA*, *PhyB*, *PhyC*, *PhyD*, and *PhyE*.
PhyD, and PhyE. Analytical investigations of these phytochrome variants suggested that each phytochrome type has a specialized photosensory function, which may explain the diversity of responses attributed to the phytochrome system (Quail et al. 1995).

Despite significant biochemical, physiological, and molecular research efforts, information on the genomic location and genetic diversity of the phytochrome gene(s) in agronomic crops is limited. To date, the chromosomal location of only the phytochrome PhyA locus has been identified in wheat and rice (Caussé et al. 1994; Oghara et al. 1994). In this article we used a heterologous phytochrome cDNA probe from oat to (1) identify multiple phytochrome loci in barley, (2) determine the chromosomal and genomic location of phytochrome gene(s) in barley using linkage analysis and wheat-barley chromosome addition lines, and (3) assess the extent of genetic variation at phytochrome loci in cultivated barley (Hordeum vulgare).

Materials and Methods

Genetic Materials

Three sets of genetic materials were studied in this experiment. The first set of materials was a barley mapping population consisting of 138 double haploid (DH) lines developed from the cross between the barley cultivars Steptoe and Morex. The mapping population used in this experiment has a well-developed molecular map consisting of 434 molecular markers, spanning 1430 cM, and is one of the principal mapping populations of the North American Barley Genome Mapping Project (Kleinhofs et al. 1993). The second set included a series of wheat-barley chromosome addition lines (Islam 1983). These materials included a barley cultivar (Betzes), a wheat cultivar (Chinese Spring), and six addition lines representing chromosomes 1, 2, 3, 4, 6, and 7 of barley. Each addition line contains the 21 pairs of wheat chromosomes from Chinese Spring plus one pair of barley chromosomes from the barley cultivar Betzes. The wheat-barley addition lines were used to confirm the chromosomal location of phytochrome loci and to determine chromosomal locations of phytochrome loci that did not segregate between the parents of the mapping population. The third set of genetic materials was used to determine genetic diversity levels at phytochrome loci; this was composed of a broad sample of 96 accessions of cultivated barley (H. vulgare) representing all major barley growing areas of the world.

RFLP Analysis

DNA isolation and RFLP procedures were as described by Saghai Maroof et al. (1984) and Zhang et al. (1993). An initial screening of a subset of barley lines was used to determine which restriction enzyme detected the highest level of polymorphism. Briefly, 8 μg of barley DNA was digested with each of six restriction enzymes (HindIII, EcoRV, XbaI, Dral, SacI, and EcoRI) and electrophoresed on 0.8% agarose gels, followed by standard Southern transfer to nylon membranes. Blots were hybridized overnight with a randomly primed 32P-dCTP-labeled phytochrome probe (see following section). Following hybridization, blots were washed three times, twice for 5 min at room temperature with 1× SSC and 0.1% SDS and once for 15 min at 65°C with 0.5× SSC and 0.1% SDS. After washing, blots were covered with plastic wrap and exposed to X-ray film for 7-10 days. The restriction enzyme XbaI detected the highest level of variation among the barley lines, and was therefore used to evaluate the level of genetic diversity among all 96 barley accessions at the phytochrome loci.

Phytochrome Clone

The oat cDNA phytochrome clone utilized in this experiment was kindly donated by S. Kay (University of Virginia) and has been fully described by Hershey et al. (1984). The phytochrome probe was prepared by digesting the cDNA clone with the restriction enzymes KpnI and SalI, which yielded a 3.0 kbp fragment representing the phytochrome coding region. This fragment was purified from agarose gel slices and labeled with 32P-dCTP by a random primer protocol (Feinberg and Vogelstein 1983). Since multiple phytochrome loci were detected with this probe, each phytochrome locus was arbitrarily designated with an arabic numeral corresponding to the decreasing size of the restriction fragment containing each phytochrome locus (e.g., phy1 > phy2 > phy3 > phy4; see also Figure 1).

Data Analysis

Segregation ratios for each polymorphic phytochrome locus in the double haploid barley population were tested for goodness-of-fit to an expected 1:1 genotypic ratio using the computer program Linkage-1 (Suiter et al. 1983). Segregation data for the phytochrome loci were then combined with the existing linkage data for the Steptoe × Morex cross (Kleinhofs et al. 1993). Linkage analysis was performed using the computer program MapMaker 3.0b (Lander et al. 1987) at LOD = 4.0, with a maximum Haldane distance of 50 cM.

Diversity values (h) for each phytochrome locus were calculated using the genetic diversity index, h = 1 − Σp2, where pi is the frequency of the ith allele. Since the total sample of barley accessions can be divided between two groups based on spike morphology (two- or six-rowed), the diversity values for the total sample were further partitioned into their respective components as described by Nei (1973), Hw = Hv + Gwr, where Hw is the total diversity, Hv is the within-group diversity component, and Gwr is the between-group differentiation.

Results

Chromosomal Assignment of Phytochrome Loci in Barley Using Wheat-Barley Addition Lines

RFLP analysis using the phytochrome probe detected four distinct DNA fragments (Figure 1). Initial mapping of these four fragments by wheat-barley addition lines analysis unambiguously assigned each of these DNA fragments to separate barley chromosomes, indicating that each of these DNA fragments represents a distinct barley phytochrome locus. Hence, we will refer to each fragment as a specific phytochrome locus and label them phy1, phy2, phy3, and phy4, where phy1 represents the largest restriction fragment detected and phy4 represents the smallest restriction fragment detected. Figure 1 shows the RFLP banding pattern produced in the wheat-barley addition line analysis when probed with the phytochrome probe. The barley parent of the addition lines (Betzes, lane 8) shows four distinct DNA fragments (phy1, phy2, phy3, and phy4), and is completely different from the banding pattern observed in the wheat parent (Chinese Spring, lane 1). Analysis of the wheat-barley addition lines (lanes 2-7) show that the phy1 and phy2 loci are located on barley chromosome 7 (lane 7) and 4 (lane 5), respectively. Two bands corresponding to the phy4 fragment were detected on barley chromosomes 2 (lane 3) and 7 (lane 7), suggesting that the phy4 gene is represented as a duplicate locus in the barley genome. None of the six wheat-barley addition lines showed a corresponding band for the phy3 locus. Since
the wheat-barley addition line that carries barley chromosome 5 is sterile and is not represented in the analysis, we infer that the phy3 locus is located on barley chromosome 5. In summary, the wheat-barley addition lines analysis indicates that phytochrome loci phy1, phy2, and phy3 are located on chromosomes 7, 4, and 5, respectively, while phy4 represents a duplicate locus presumably located on chromosomes 2 and 7 of barley (Figure 1, Table 1).

**Linkage Analysis of Phytochrome Loci**

The linkage relationships for two of the four phytochrome loci were determined using segregation data for 434 molecular marker loci from a double haploid mapping population. The parents of the mapping population (Steptoe and Morex) differed in phytochrome alleles for only two (phy1 and phy3) of the four phytochrome loci (Figure 2). Chi-square tests revealed that the segregation of the phy3 locus fit the expected 1:1 segregation ratio, while the phy1 locus deviated significantly from the expected 1:1 ratio (chi-square values were 8.83 and 0.26 for phy1 and phy3, respectively). Interestingly, Causse et al. (1994) reported significant distortion of several molecular marker loci mapping near a phytochrome (PhyA) locus on rice chromosome 3 and suggested that the segregation distortion observed in this genomic region in rice may be associated with the presence of a closely linked genetic factor(s) regulating fertility. Whether a similar genetic phenomenon is affecting the segregation of this phytochrome locus in barley is not known.

Linkage analysis located the phytochrome locus phy1 to the short arm of barley chromosome 7 at 21.6 cM from marker ABG316B and 10.1 cM from ABG705 (Figure 2). Phytochrome locus phy3 mapped near the centromeric region on the long arm of barley chromosome 5, 9.2 cM from marker ABC160 and 11.3 cM from ABG464 (Figure 2). The genetic mapping of phy1 and phy3 to barley chromosomes 7 and 5, respectively, confirmed the inferences obtained from wheat-barley addition lines. The map location of these two phytochrome loci are of particular interest because both loci are positioned in relatively large gaps within the barley molecular map (Figure 2). For example, previous to the

**Table 1. Sample size, number of alleles, diversity values, and differentiation component for each locus in the total sample and in each subgroup of cultivated barley**

<table>
<thead>
<tr>
<th>Locus</th>
<th>Chromosomal location*</th>
<th>Total sample</th>
<th>No. of alleles</th>
<th>Diversity</th>
<th>N</th>
<th>No. of alleles</th>
<th>Diversity</th>
<th>N</th>
<th>No. of alleles</th>
<th>Diversity</th>
<th>N</th>
<th>Gm (%)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>phy1</td>
<td>7</td>
<td></td>
<td>3</td>
<td>0.573</td>
<td>67</td>
<td>2</td>
<td>0.308</td>
<td>21</td>
<td>3</td>
<td>0.626</td>
<td>46</td>
<td>4.5</td>
</tr>
<tr>
<td>phy2</td>
<td>4</td>
<td></td>
<td>2</td>
<td>0.366</td>
<td>83</td>
<td>2</td>
<td>0.497</td>
<td>26</td>
<td>2</td>
<td>0.188</td>
<td>57</td>
<td>8.0</td>
</tr>
<tr>
<td>phy3</td>
<td>5, 7</td>
<td></td>
<td>3</td>
<td>0.549</td>
<td>83</td>
<td>3</td>
<td>0.560</td>
<td>27</td>
<td>3</td>
<td>0.523</td>
<td>56</td>
<td>1.4</td>
</tr>
<tr>
<td>phy4</td>
<td></td>
<td></td>
<td>2</td>
<td>0.323</td>
<td>89</td>
<td>2</td>
<td>0.500</td>
<td>24</td>
<td>2</td>
<td>0.123</td>
<td>61</td>
<td>8.2</td>
</tr>
</tbody>
</table>

* Chromosomal locations were determined by wheat-barley addition line analysis and/or linkage analysis.
* Differentiation component between the two- and six-rowed barley groups.
* Only those samples that could be scored unambiguously were used in the diversity analysis.
positioning of phy1, the molecular map of barley contained a 31.7 cM gap on chromosome 7. After the addition of phytochrome locus phy1 the size of this gap has been reduced to 21.6 cM.

Genetic Diversity
Ninety-six accesses of cultivated barley were assayed for genetic diversity at each phytochrome locus. Polymorphism was detected at all four phytochrome loci. Three distinct allelic variants were detected for phytochrome loci phy1 and phy3, while two variants were detected for phytochromes phy2 and phy4 among all the barley accessions examined. The predominant allele for phy1, phy2, phy3, and phy4 had frequencies of 0.55, 0.57, 0.60, and 0.64, respectively. A total of 10 alleles were observed in the entire sample of 96 barley accessions for all four phytochrome loci. Phytochrome locus phy1 exhibited the highest level of genetic diversity (0.57) followed by the phy3 locus (0.55), while phy2 and phy4 showed the lowest diversity values (0.37 and 0.32, respectively; Table 1).

The 96 accesses of cultivated barley can be partitioned into two groups on the basis of the possession of two- or six-rowed spikes. The number of alleles and diversity values calculated for each barley group are presented in Table 1. Although the number of alleles and the diversity values appeared to be different at many of the phytochrome loci between the groups, the overall difference was not significant and is largely the result of different sample sizes between the groups. However, partitioning of the barley accessions showed that the frequency of the alleles at specific phytochrome loci differed dramatically between the two subgroups. For example, the most frequent allele (number 2) detected at the phy2 locus (P = .54) in the two-rowed group was rarely observed (P = .11) in the six-rowed barley group. The Csr statistic, which measures the amount of differentiation between groups, had an average value of 5.53%, ranging from 1.4% at the phy3 locus to 8.2% at the phy2 locus (Table 1).

Discussion
We used a heterologous phytochrome cDNA probe from oat to detect five barley phytochrome loci, arbitrarily designated as phy1, phy2, phy3, and phy4 (duplicate locus). The identification of five phytochrome loci suggests that phytochrome is represented as a small multigene family in barley. This is consistent with previous studies by Hershey et al. (1985) who reported the existence of a small family of at least four phytochrome genes in oat (Avena sativa) and by Clack et al. (1994) and Sharrock and Quail (1989) who reported the existence of a small phytochrome gene family in Arabidopsis that consists of at least five structurally related phytochrome genes (PhyA, PhyB, PhyC, PhyD, and PhyE). Quail et al. (1995) suggested that the large diversity of responses attributed to the phytochrome system is the result of photosensory specialization of individual phytochrome variants. Indeed, photomorphogenic mutant analysis indicates that phytochromes PhyA and PhyB have contrasting and specific photosensory functions in controlling hypocotyl elongation in etiolated Arabidopsis seedlings (Dehesh et al. 1993; McCormac et al. 1993).

While the identification of five distinct phytochrome loci in barley supports the conclusion that phytochrome genes are encoded as small multigene families in higher plants, the photosensory specialization of these particular loci in barley is not known. Equally unclear is the relationship among the barley phytochrome loci and the phytochrome loci identified in Arabidopsis (which has become the model system for the study of photoreceptors in higher plants) and other plant species. Interestingly, however, since several specific homologous chromosomes are conserved among barley, wheat, and rice (Kurata et al. 1994; Saghal Maroof et al. 1996), and since previous genetic mapping has localized a phytochrome locus (PhyA) to wheat chromosome 4 (Oghihara et al. 1994) and two phytochrome loci (PhyA and PhyB) to rice chromosome 3 (Causse et al. 1994; Lin et al. 1995), one might infer that the barley phytochrome phy2 locus on barley chromosome 4 represents a homolog of one of these phytochrome loci, based solely on chromosomal homologies among these grass species (barley chromosome 4, wheat chromosome 4, and rice chromosome 3 are homologous chromosomes). An equally intriguing observation is the similarity in the chromosomal location of the phytochrome phy3 locus and a major photoperiod response gene (Ppd3) in barley (Blake T, personal communication). The phytochrome phy3 locus maps to a genomic region of chromosome 5 flanked by the same RFLP markers as the photoperiod response gene Ppd3 (Blake T, personal communication). A more detailed mapping study will be necessary to determine the exact genetic relationship between this phytochrome locus (phy3) and the photoperiod response gene Ppd3 and to determine the relationships among the phytochrome loci identified in this study and those reported in other plant species.

In addition to identifying the chromo-
The genomic location of phytochrome loci on chromosomes 7 and 5 of barley, respectively. Only a portion of the chromosomes containing the phytochrome loci are shown. Scale is shown at the left in centiMorgans. The approximate location of centromere regions are indicated by the C.

In this investigation we have identified five phytochrome loci that represent a small multigene family in barley. These loci have been described by their chromosomal and genomic locations and genetic diversity levels. The identification and description of phytochrome loci are important steps in the understanding of the photosensory circuitry of higher plants and in the eventual manipulation of the photosynthetic system at the molecular level.

References


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