Abstract  Diversity was analyzed in wild and cultivated *Lactuca* germplasm using molecular markers derived from resistance genes of the NBS-LRR type. Three molecular markers, one microsatellite marker and two SCAR markers that amplified LRR-encoding regions, were developed from sequences of resistance gene homologs at the main resistance gene cluster in lettuce. Variation for these markers were assessed in germplasm including accessions of cultivated lettuce, *Lactuca sativa* L. and three wild *Lactuca* spp., *L. serriola* L., *L. saligna* and *L. virosa* L. Diversity was also studied within and between natural populations of *L. serriola* from Israel and California; the former is close to the center of diversity for *Lactuca* spp. while the latter is an area of more recent colonization. Large numbers of haplotypes were detected indicating the presence of numerous resistance genes in wild species. The diversity in haplotypes provided evidence for gene duplication and unequal crossing-over during the evolution of this cluster of resistance genes. However, there was no evidence for duplications and deletions within the LRR-encoding regions studied. The three markers were highly correlated with resistance phenotypes in *L. sativa*. They were able to discriminate between accessions that had previously been shown to be resistant to all known isolates of *Bremia lactucae*. Therefore, these markers will be highly informative for the establishment of core collections and marker-aided selection. A hierarchical analysis of the population structure of *L. serriola* showed that countries, as well as locations, were significantly differentiated. These differences may reflect local founder effects and/or divergent selection.

Key words  *Lactuca* · Resistance genes · LRR multigene family · Diversity · Microsatellite

Introduction

One of the major challenges in analyzing wild populations as sources of germplasm is in measuring relevant population diversity (Hawkes 1991). An important use of germplasm in crop improvement is as a source of disease resistance (Leppik 1970; Nevo et al. 1985; Dale 1991; Lenne and Wood 1991). However, measuring diversity in the genetic basis of resistance has until recently been difficult. Typically, diversity of resistance in natural populations or germplasm collections has been analyzed by assessing resistance phenotypes (Jana and Nevo 1991; Burdon 1996, 1997). However, the ability to distinguish between different resistances has been heavily dependent on the range of isolates used to assess resistance. Also, it has been impossible to distinguish between sources of resistance that are effective against all known isolates of a particular pathogen. The recent cloning of resistance genes provides the opportunity to assay variation specifically at resistance loci using molecular markers derived from sequences of the resistance genes.

Classical and molecular genetics have increasingly demonstrated that the resistance genes in diverse plant species are clustered in the genome either as genetically separable loci or as an apparent multiallelic series (Pryor and Ellis 1993; Hulbert 1997; Michelmore and Meyers 1998). On the basis of this clustered distribution, and by interference from other cell-cell recognition systems, resistance genes have been hypothesized to be functionally and evolutionary related (Michelmore et al. 1987; Pryor...
1997). Cloned resistance genes to diverse pathogens from a variety of species share common sequence motifs indicative of gene products involved in signal reception and transduction (reviewed in Hammond-Kosack and Jones 1997). The most prevalent class of resistance genes encode a nucleotide-binding site (NBS) and a leucine-rich repeat (LRR) region. Little is known about mechanisms generating variation at resistance loci. Recombination, duplication, divergent selection and transposition have all been implicated in the evolution of new resistance genes (Ellis et al. 1995; Hulbert 1997; Song et al. 1997; Meyers et al. 1998a, b). However, these studies have been based on the analysis of only one or a few haplotypes, usually in experimental or cultivated genotypes. The relative importance of these mechanisms in generating variation in natural populations has not been assessed.

Natural populations of Lactuca are widely represented around the world, with at least 100 species described in the genus (Lindqvist 1960a). The high levels of diversity of Lactuca species in the Mediterranean area and Southwest Asia indicates that this area may be a center of diversity for Lactuca spp. (Zohary 1991; De Vries 1997). Cultivated lettuce, Lactuca sativa, is part of a reproductively isolated group that includes the wild species Lactuca serriola, Lactuca saligna, Lactuca virosa (Lindqvist 1960a, b; Whitaker 1969; Ryder 1986). Other species have been described in this group, but they are less widespread and their status as distinct species is unclear (Ferakova 1977; Zohary 1991). L. sativa is fully interfertile with L. serriola, only partly cross-fertile with L. saligna and almost completely infertile with L. virosa (Zohary 1991). Cytogenetic and molecular marker data have confirmed the close taxonomic affinity between L. sativa and L. serriola and their more distant relationship to L. saligna and L. virosa (Lindqvist 1960b; Kesseli and Michelmore 1986; Cole et al. 1991; Kesseli et al. 1991; Hill et al. 1996; Witsenboer et al. 1998). L. serriola and, more rarely, L. saligna have been used as sources of resistance genes for introgression into L. sativa (Ochoa et al. 1987; Crute 1990). L. serriola is a common colonizer of disturbed habitats and has the most widespread global distribution of the Lactuca spp. (Ferakova 1977, Zohary 1991); extensive populations can be found on all continents; however, the genetic variation among populations has not yet been studied.

The genetic and molecular bases of disease resistance in lettuce have been investigated with a primary focus on downy mildew caused by the fungus Bremia lactucae. Parallel genetic studies on host and pathogen demonstrated that at least 15 dominant genes for resistance (Dm genes) in lettuce were matched by avirulence genes in B. lactucae in a gene-for-gene interaction (Flor 1956; Crute and Johnson 1976; Hulbert and Michelmore 1985; Farrara et al. 1987). Many other resistant accessions have been identified but few have been characterized genetically (e.g. Farrara and Michelmore 1987; Bonnier et al. 1992, 1994). The Dm genes characterized so far are clustered in four linkage groups along with resistance to other pathogens (Kesseli et al. 1993; Maisonneuve et al. 1994; Robbins et al. 1994; Witsenboer et al. 1995). The major cluster determines at least 11 Dm specificities including Dm3 as well as resistance to root aphid. This cluster has been saturated with molecular markers using several approaches (Michelmore et al. 1991; Paran and Michelmore 1993). Recently, resistance-gene candidates (RGCs) encoding NBS and LRR motifs have been identified using PCR with degenerate oligonucleotide primers designed from sequences conserved between resistance genes cloned from other species (Shen et al. 1998). One family of over 24 members, RGC2, is localized in the major cluster of resistance genes and contains the Dm3 gene (Meyers et al. 1998a; K. Shen et al., unpublished).

In the present study, we used molecular markers to analyze diversity at the major resistance gene cluster in Lactuca spp. Molecular markers were developed from the sequences of RGC2 members. Variation was analyzed in a broad collection of L. sativa and the three wild relatives, L. serriola, L. saligna and L. virosa, that had previously been characterized for resistance to downy mildew. In addition, a detailed analysis of diversity was conducted within and between wild populations of L. serriola from two climatically similar regions, Israel and California, the former being close to the center of diversity for Lactuca and the latter an area of more recent colonization (Zohary 1991). This analysis provides the first molecular data on the level and distribution of resistance gene variation within and between natural populations. The large number of haplotypes detected in wild species provides a basis for decisions on the conservation and exploitation of Lactuca germplasm as well as the experimental basis for future studies on the evolution of disease resistance genes.

Materials and methods

Plant material

Two sets of materials were examined. The first set included 74 accessions of the cultivated species, L. sativa, and 74 accessions of wild Lactuca species. The cultivated accessions were selected to represent diversity within the species and had been previously studied with a range of molecular markers (Kesseli et al. 1991; Hill et al. 1996). The cultivated samples included the diversity for downy mildew resistance genes (Dm genes; Farrara et al. 1987) as well as genotypes of importance to US agriculture. The wild Lactuca spp. comprised a total of 16 accessions of L. serriola, 47 accessions of L. saligna, eight accessions of L. virosa. These wild accessions had previously been shown to be resistant to all isolates of B. lactucae tested up to 1996 (O. Ochoa, unpublished). Single accessions of Lactuca augustana, Lactuca indica and Lactuca perennis, Cichorium endivia (endive) and Helianthus annuus (sunflower), all members of the Compositae family, were included as outgroups. Cultivars and wild accessions were obtained from germplasm collections at the Plant Introduction Center, Pullman, Wash., USA, the Centre for Genetic Resources, CPRO-DLO, P.O. Box, 16, 6700 AA Wageningen, The Netherlands, and the Department of Vegetable Crops, UC Davis, USA.

The second set of genotypes was composed of 505 samples of L. serriola collected as individual plants from two regions, Israel and California (Fig. 1). In each region, collections were made...