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Cytoplasmic DNAs and nuclear rDNA restriction fragment length polymorphisms in commercial witloof chicories

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Abstract Restriction fragment length polymorphisms of cytoplasmic DNAs and nuclear rDNA were analyzed in several Cichorium intybus genotypes, comprising four white inbred lines, eight red witloof experimental lines, and a number of F₁ hybrids derived from two white parents. Chloroplast and mitochondrial restriction patterns led to the distinction between two different cytoplasts, called I and II. Southern hybridization using a nuclear rDNA probe revealed that all the lines possessed two types of rDNA repeat units. The shortest unit was 10 kb and was common to all lines. The largest rDNA repeat unit was 10.5 kb in lines I and 10.4 kb in lines II. In addition, a sequence heterogeneity between the 10.5- and 10.4-kb rDNA repeat units was revealed by SacI digestion. A 10-kb rDNA unit was successively cloned, mapped, and used as a probe to check the genetic purity of F₁ hybrid seeds between line I and II white parents. We found a 30% average percentage of impurities, originating both from selfing and full-sib crossing, in different open-pollinated hybrid samples.

Key words Chloroplast DNA · Mitochondrial DNA · rDNA · RFLP · Witloof chicory

Introduction

The diploid species Cichorium intybus (2n = 2x = 18, genus Cichorium, family Compositae), widespread in Europe and in temperate areas of Asia, has long been cultivated for food and for its medicinal properties. The species contains three groups distinguishable by different morphologies. The first group includes some Italian chicories (Treviso, Verona, Chioggia), whereas the second group contains varieties cultivated for their sweet roots, which when roasted supply a coffee substitute. These coffee chicories represent new industrial crops for the production of both fructose syrup and polyfructanes as food fibers. The third group comprises the witloof chicories which have been bred for their root capacity to develop white tuberised buds (chicons) in artificial conditions. Coffee chicories and witloof chicories are thought to derive from Magdebourg chicory, an ancestor of coffee chicory characterized by large roots.

In western European countries, the witloof chicories represent one of the most important vegetable crops. They are mainly allogamous, but seeds can be obtained from about 20% of plants in most varieties by selfing. Several factors, such as unfavourable floral morphology and the occurrence of both autoincompatibility and pollen competition systems, limit selfing. The latter system is responsible for higher growth rates of allopollen tubes than of autopollen tubes. Chicory breeders are, therefore, actively involved in a search for: (1) cytoplasmic male sterility to eliminate selfing and to obtain 100% F₁ hybrids, (2) a haploidization technique to accelerate the breeding of pure lines and subsequently of new F₁ hybrids, (3) non-selective herbicide resistances to control weeds during chicory culture (Millecamps 1989; Vermeulen et al. 1992), and (4) diversification by crossing witloof with Italian chicories.

Despite its popular success, very little genetic and molecular data is available on witloof chicory (Vermeulen et al. 1994). Strong inbreeding depression, long generation time, and the cross-pollinating nature of chicories are responsible for this lack of knowledge. As a result, the scientific management of chicory breeding is poorly developed in comparison with that of other important vegetable crops. In the present study, we report variations in cytoplasmic and nuclear DNA restriction patterns from several witloof chicory genotypes. The aim of our analysis was varietal identification, the protection of breeder’s rights, and the determination of parentage in witloof chicories.
Materials and methods

Plant materials

Heads of the different witloof lines and F₁ hybrid seeds were provided by INRA (Centre de Versailles). Heads from four white lines (A, B, C and D genotypes) and eight red lines were analyzed (Table 1). Red lines originated by crossing white witloof chicories and wild red Verona salads, followed by four backcrosses with the white parent. Two types of white F₁ hybrid seeds were also analyzed: (1) three F₁ hybrid seeds of Flash (A × C), Turbo (B × C) and Bea (B × D) obtained under strictly controlled conditions, i.e. hand-emasculating of the parent used as female, in a greenhouse, and (2) seven seed samples of the Flash F₁ hybrid, representing 2800 plants, produced under open-field conditions.

Isolation of chloroplast and mitochondrial DNAs

Chloroplasts were isolated from green leaves in a medium of high ionic strength (Bookjans et al. 1984). Lysis of chloroplasts and chloroplast (ct) DNA purification by CsCl ultracentrifugation were performed as described previously (Till-Bottraud et al. 1992).

Mitochondria were isolated as reported by Chetrit et al. (1985) with some modifications. White leaves from heads were homogenized at 4 °C in a Waring blender for 3 × 5 s at high speed in 6 ml/g of fresh weight buffer A (0.5 M mannitol, 50 mM Tris-HCl pH 8.0, 3 mM EDTA, 0.1% BSA, 1 mM β-mercaptoethanol). The homogenate was filtered through a 35-μm nylon net and the filtrate centrifuged twice with some modifications. White leaves from heads were homogenized at 4 °C in a Waring blender for 3 × 5 s at high speed in 6 ml/g of fresh weight buffer A (0.5 M mannitol, 50 mM Tris-HCl pH 8.0, 3 mM EDTA, 0.1% BSA, 1 mM β-mercaptoethanol). The homogenate was filtered through a 35-μm nylon net and the filtrate centrifuged twice with some modifications. White leaves from heads were homogenized at 4 °C in a Waring blender for 3 × 5 s at high speed in 6 ml/g of fresh weight buffer A (0.5 M mannitol, 50 mM Tris-HCl pH 8.0, 3 mM EDTA, 0.1% BSA, 1 mM β-mercaptoethanol). The homogenate was filtered through a 35-μm nylon net and the filtrate centrifuged twice with some modifications. White leaves from heads were homogenized at 4 °C in a Waring blender for 3 × 5 s at high speed in 6 ml/g of fresh weight buffer A (0.5 M mannitol, 50 mM Tris-HCl pH 8.0, 3 mM EDTA, 0.1% BSA, 1 mM β-mercaptoethanol). The homogenate was filtered through a 35-μm nylon net and the filtrate centrifuged twice with some modifications. White leaves from heads were homogenized at 4 °C in a Waring blender for 3 × 5 s at high speed in 6 ml/g of fresh weight buffer A (0.5 M mannitol, 50 mM Tris-HCl pH 8.0, 3 mM EDTA, 0.1% BSA, 1 mM β-mercaptoethanol). The homogenate was filtered through a 35-μm nylon net and the filtrate centrifuged twice with some modifications. White leaves from heads were homogenized at 4 °C in a Waring blender for 3 × 5 s at high speed in 6 ml/g of fresh weight buffer A (0.5 M mannitol, 50 mM Tris-HCl pH 8.0, 3 mM EDTA, 0.1% BSA, 1 mM β-mercaptoethanol). 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MtDNAs were also distinguished by specific 20.9- and 33.0-kb SalI-specific fragments respectively. BanHI, BglII, EcoRI, EcoRV, HindIII, HpaII and SalI enzymes failed to distinguish between the different lines (data not shown).

Two types of mtDNA restriction patterns were found among the four white witloof lines with the BanHI, BglII, SalI, ClaI, HindIII and KpnI enzymes. Lines A and B possessed an identical type-I mtDNA (Fig. 2) distinct from that of lines C and D (type II). HindIII patterns revealed the presence of specific 6.6-, 4.4- and 4.0-kb fragments in type-I mtDNAs and of specific 7.1- and 3.2-kb fragments in type-II mtDNAs. A 8.0-kb BglII fragment was specifically observed in A and B lines, while three BglII fragments of 38.5, 5.9 and 2.5 kb appeared to be specific to lines C and D. Type-I and type-II mtDNAs were also distinguished by specific 20.9- and 33.0-kb SalI fragments, respectively. The eight red witloof lines contained one or the other mtDNA type, but in accordance with the ctDNA type (Table 1).

Results

Analysis of cytoplasmic DNAs

The ctDNA isolated from the four white witloof lines showed two distinctive types of restriction patterns with the SalI enzyme only. Type I was found in lines A and B and type II in lines C and D (Fig. 1). Both ctDNA types were equally distributed among the eight red witloof lines analyzed (Fig. 1 and Table 1). Type-I and type-II ctDNAs possessed 49.2- and 15.5-kb SalI-specific fragments respectively. BanHI, BglII, EcoRI, EcoRV, HindIII, HpaII and SalI enzymes failed to distinguish between the different lines (data not shown).

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