Isolation and characterization of safflower (Carthamus tinctorius L.) chloroplast DNA

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Summary

Safflower (Carthamus tinctorius L.) chloroplasts were isolated and purified with the aid of commercially available nylon mesh, differential centrifugation, and DNase I treatment. These chloroplasts were free of nuclei as determined by light microscopy of aceto orcein stained preparations, and similar to those observed by electron microscopy in spinach and many other higher plants, being bounded by a double membrane layer and characterized by the presence of a lamellar system surrounded by embedding matrix, and stacked membranes or grana lamallae. DNA was isolated and purified from such chloroplasts, and characterized with respect to cesium chloride density gradient isopycnic centrifugation, denaturation, renaturation kinetics and restriction enzyme analysis. These studies show that safflower chloroplast DNA is similar to many other higher plant DNAs having a density of 1.700 g/cm³ (G + C = 40.8%), a Tₘ of 86 °C (G + C = 40.7%) and a molecular complexity and genome size of about 10⁸ daltons.

Introduction

Although safflower (Carthamus tinctorius L.) has been cultivated for thousands of years in the Near and Middle East where it has been used as a source of dye for coloring cloth and as a source of oil, it was not introduced on a commercial scale in the U.S.A. until the 1940s. Production has been limited not only by competition with other commercially important crops but also by continuing problems with root rot, various foliar diseases and insects well adapted to survival on other Compositae (see reviews by Knowles) (1, 2). Plant breeding programs have centered around developing greater disease resistance (2), changing lipid and amino acid composition (3, 4), removal of objectional bitter monoglucosides and the cathartic 2-hydroxyarctin so the meal can be used as a feed for monogastric animals and man (5), cold tolerance, male sterility (6), polyploids (7) and cytogenetic studies (8). There are two major types of safflower oil in commercial production. The first is the standard polyunsaturated oil used in margarines, salad oils, and surface coatings. The second is a high quality oleic acid oil used for frying and for various industrial purposes. The meal is used as a feed for livestock (1).

Recently considerable progress has been made in developing a high oil content male sterile variety (9). This has stimulated a greater interest in biochemical studies on safflower. To date such studies have been primarily centered around various aspects of lipid biochemistry (10, 11, 12). We are particularly interested in the molecular biology of cytoplasmic gene expression in safflower, the first step of which requires the availability of well char-
acterized DNA. In this communication we report the isolation and characterization of DNA from safflower chloroplasts which were purified with the aid of commercially available nylon mesh (13), differential centrifugation (14), and DNase I treatment (15). Nucleic acid studies on higher plants and algae have shown chloroplast DNA to differ from nuclear DNA, particularly with respect to the lack of 'odd' bases (16) and ease of renaturation (17). Renaturation characteristics have been used as a criterion of purity (18) and for calculating genomic complexity of chloroplast DNA (14). Under controlled conditions of salt and temperature, the rate of DNA renaturation is inversely proportional to its nucleotide sequence complexity. Such rates are conveniently monitored spectrophotometrically by observing the loss of hyperchromicity, usually at 25 °C below the transition temperature (Tm). Comparison with DNA of known complexity under identical conditions provides a standard and a means of calculating sequence complexity (19). Chloroplast DNAs are found to be in the range of 10^8 daltons, which in most cases agrees reasonably well with values calculated from electron microscopic and restriction endonuclease data (13). Although kinetic heterogeneity has been observed during renaturation of higher plant chloroplast DNA (15), more recent work would tend to dispute this claim (14).

While there is excellent agreement as to the densities of algal chloroplast DNAs, higher plant chloroplast DNAs appear to be less similar. This has been attributed to their similarity in density to nuclear DNA; and in spite of attempts to clarify the situation, which resulted in the view that higher-plant chloroplast DNAs have a density of 1.697 g/cm^3 in neutral cesium chloride (16), chloroplast DNAs of various densities have been reported (13).

The DNA from safflower chloroplasts reported in this communication was characterized with respect to cesium chloride isopycnic centrifugation, denaturation, renaturation kinetics, and restriction enzyme analysis. These studies show that this DNA is similar to that of many other higher plant chloroplasts (20) having a density of 1.700 g/cm^3 (G + C = 40.8%), a Tm of 86 °C (G + C = 40.7%), and a kinetic complexity and molecular weight of about 10^8 daltons.

### Experimental procedures

**Plant Growth:** Safflower seedlings were grown in high density in tapered plastic dishes (9 × 12 and 6 inches deep) filled with vermiculite, and containing four cotton wicks to draw nutrient (Ortho Multipurpose Garden and Lawn Food, 16–16–16) from a lower reservoir. A Sherer plant growth chamber containing a combination of fluorescent and incandescent lights was used to maintain plant growth conditions (18 hr days, 75 °C, and min. humidity).

**DNA Isolation:** The procedure was modified from Wells and Birnstiel (15) and Kolodner and Tewari (14). In a typical isolation, two-week old seedlings were placed in the dark for two to three days. Leaves and coleoptiles were removed and homogenized in ice-cold Buffer A (4 ml/g fresh weight) containing 0.3 M mannitol, 0.05 M Tris, 0.003 M EDTA, 0.001 M mercaptoethanol, 0.1% bovine serum albumin, with two 5 sec bursts in a Waring blender at medium setting. Homogenates were filtered through two layers of cheesecloth and then successively through Nytex nylon mesh (Tetko, Inc., NY) of 74, 44 then 20 microns. Fibers retained by the cheesecloth were again homogenized in one-half of the original volume of Buffer A, and filtered as above. The two filtrates were combined and centrifuged for 10 min at 40 × g at 4 °C. The supernatant was decanted, centrifuged at 1020 × g for 15 min at 4 °C, and the resulting crude chloroplast pellet suspended in Buffer A (0.2 ml/g fresh weight). MgCl₂ (0.01 M) and DNase I (50 μg/ml) were added and the suspension incubated for 1 hr at 4 °C. Following this incubation, 3 volumes of Buffer B (0.6 ml/g fresh weight) containing 0.3 M sucrose, 0.05 M Tris, 0.02 M EDTA, pH 8.0 were added and the suspension centrifuged at 1500 × g for 15 min at 4 °C. The pellet was washed twice by suspending it in the same volume of Buffer B and recentrifuging as above. The two filtrates were combined and centrifuged for 10 min at 40 × g at 4 °C.

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